CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

022271Orig1s000

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: 22-271
Submission date: 25 July, 2011 (resubmission)

Drug: alogliptin
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 alogliptin for the intended indication. The previous Complete Response letter requested that the applicant provide data from an embryofetal development study in rats that included separate alogliptin and metformin arms in addition to the combination groups. This information was considered important because it was anticipated that alogliptin would be widely used together with metformin and because other information suggested the possibility for a developmental effect from interaction of the two drugs. The applicant included such a combination study in the resubmission. This study did not indicate any unexpected synergistic increase in developmental toxicity with the combination of alogliptin and metformin.

Several other nonclinical studies were also included in the resubmission. These were reviewed but are not considered critical to the approval decision.

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. 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

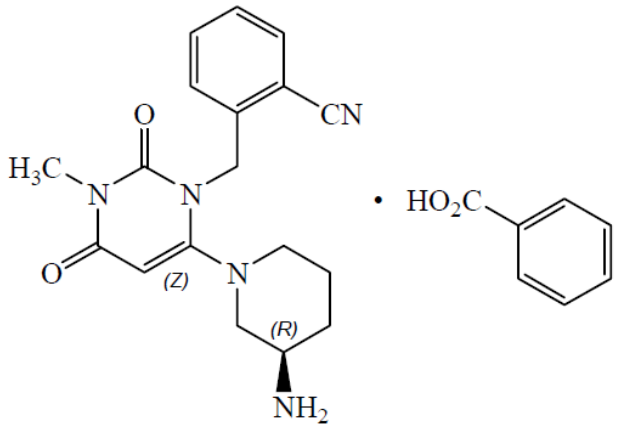
2.1.5 Molecular Formula/Molecular Weight

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)

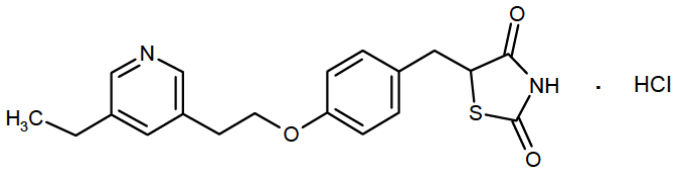
Pioglitazone – $C_{19}H_{20}N_2O_3S$ • HCl / 392.90 g/mol (HCl salt); 356.43 g/mol (free base)

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

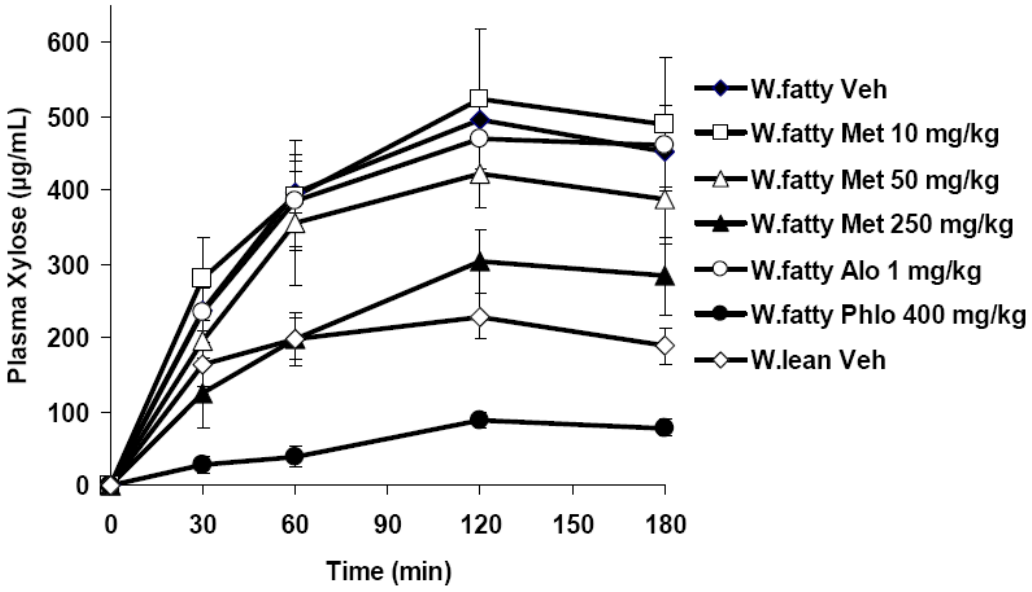
Effect of alogliptin and metformin on intestinal xylose absorption (Study SD1AD2007-KT-184)

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)

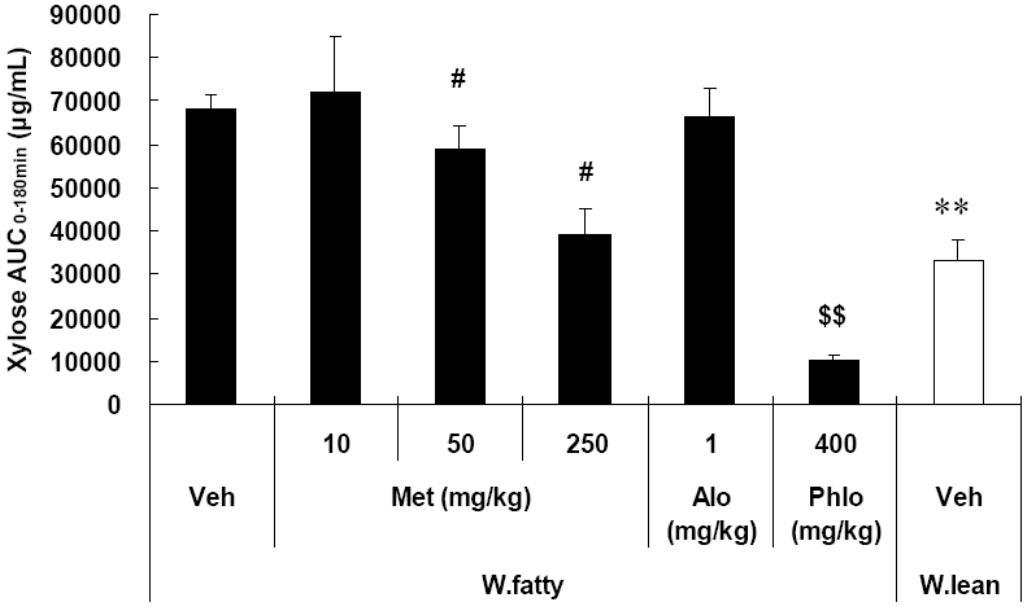


Figure Legend – Rats were fasted for 18 h, and administered 10, 50, and 250 mg of metformin (Met), or 1 mg of alogliptin (Alo) 1 hr before an oral xylose load (0.8 g/kg). Phlorizin (Phlo) (400mg/kg) was simultaneously administered with xylose. Plasma xylose levels (A) and $AUC_{0\text{-}180min}$ of plasma 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

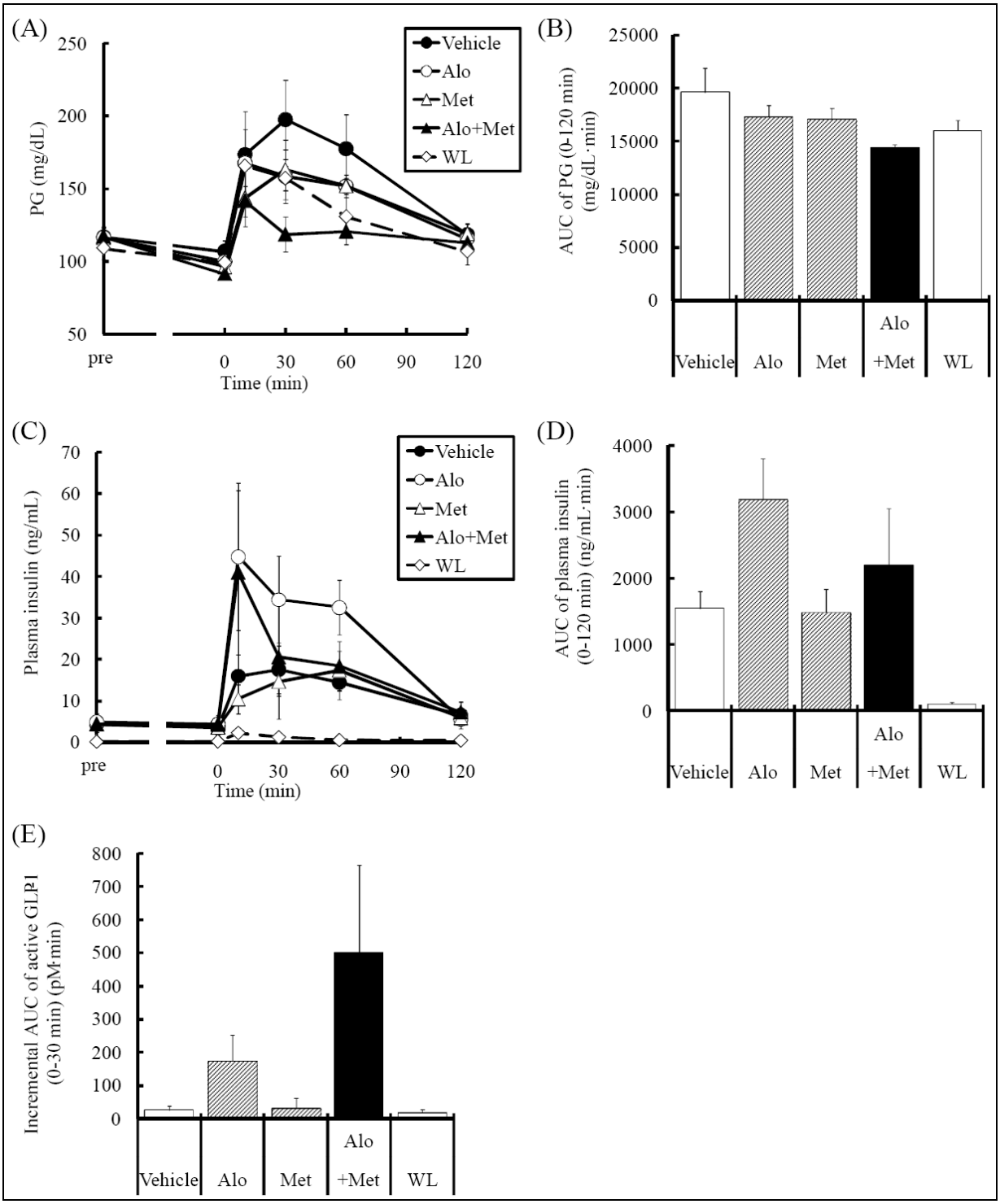


Figure Legend – Combined effects of alogliptin and metformin on plasma glucose, insulin levels and active GLP-1 levels in female WF rats (A) and (C) show time-dependent changes of PG and plasma insulin after 1 g/kg glucose challenge, respectively. Data in (B) and (D) represent AUC0-120min of PG shown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

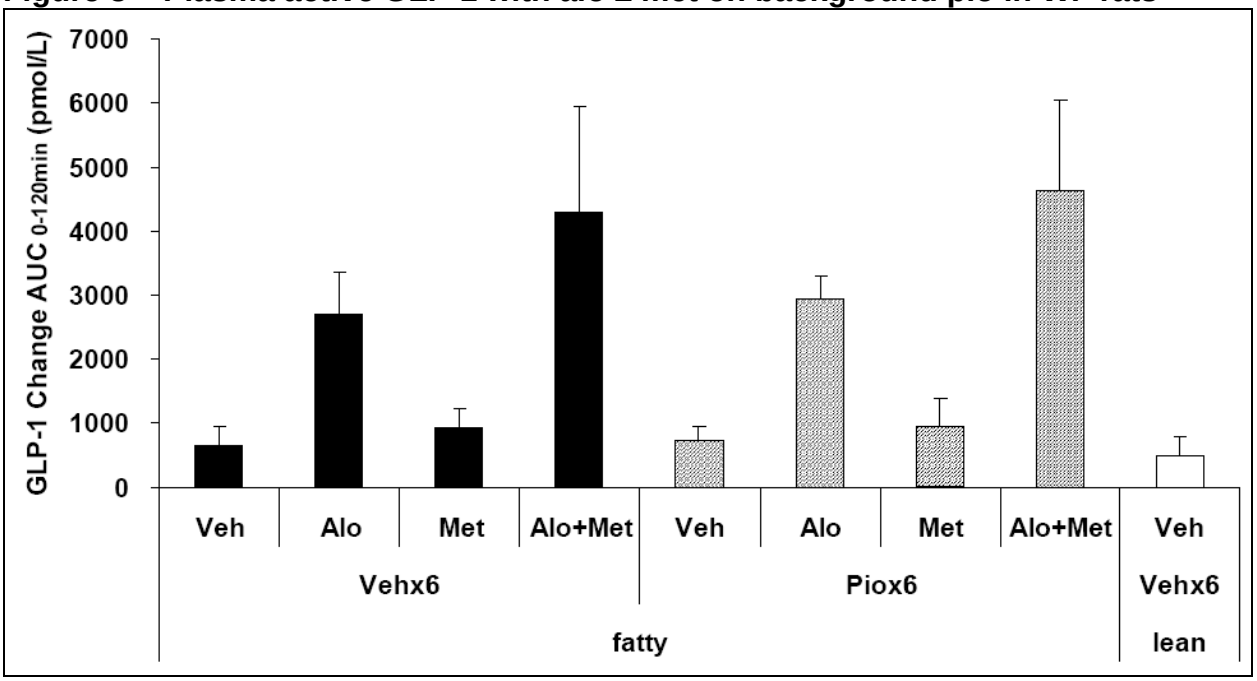


Figure legend – AUC of $AUC_{0\text{-}120min}$ of incremental active GLP-1. Values are mean ± SD (n=6 for Wistar fatty, n=5 for Wistar lean rats). Veh, vehicle; Alo, alogliptin; Met, metformin; Pio, pioglitazone.

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

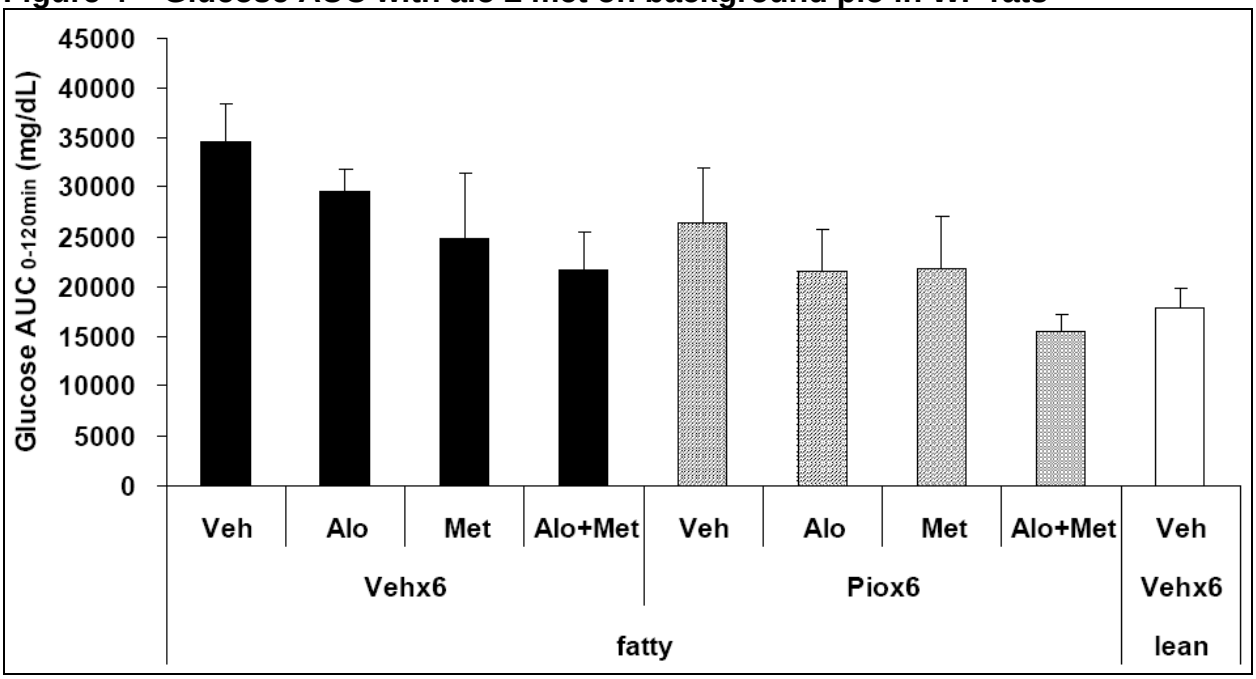


Figure legend – Effect of triple combination of alogliptin, pioglitazone, and metformin on plasma glucose $AUC_{0\text{-}120\ min}$ after OGTT in Wistar fatty rats. Values are mean ± SD (n=6 for Wistar fatty, n=5 for Wistar lean rats). Veh, vehicle; Alo, alogliptin; Met, metformin; Pio, pioglitazone.

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

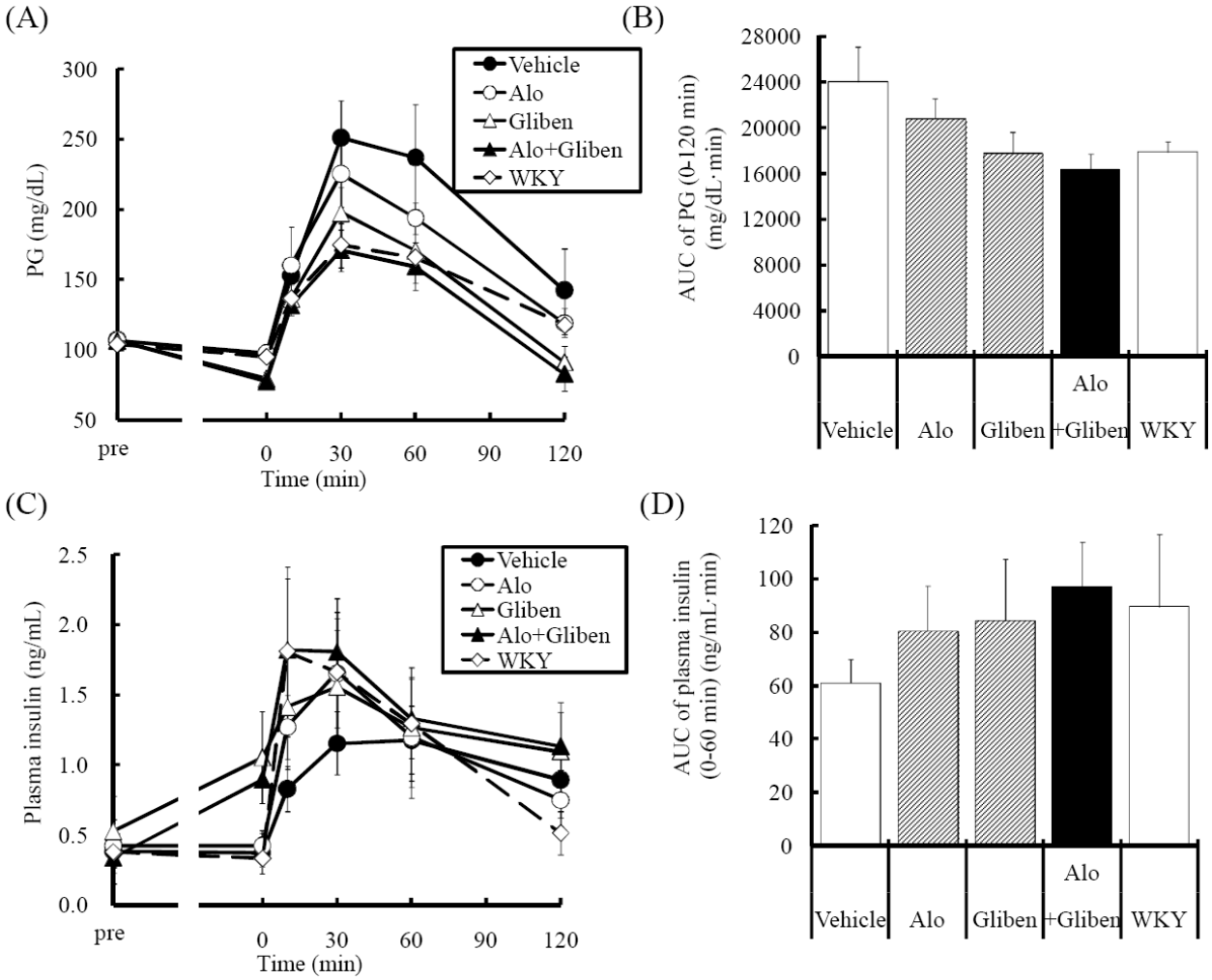


Figure Legend – Combined effects of alogliptin and glibenclamide on PG and insulin levels in male N-STZ-1.5 rats (A) and (C) show time-dependent changes of PG and plasma insulin after 1 g/kg glucose challenge, respectively. Data in (B) and (D) represent $AUC_{0\text{-}120min}$ of PG shown in (A) and $AUC_{0\text{-}60min}$ 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

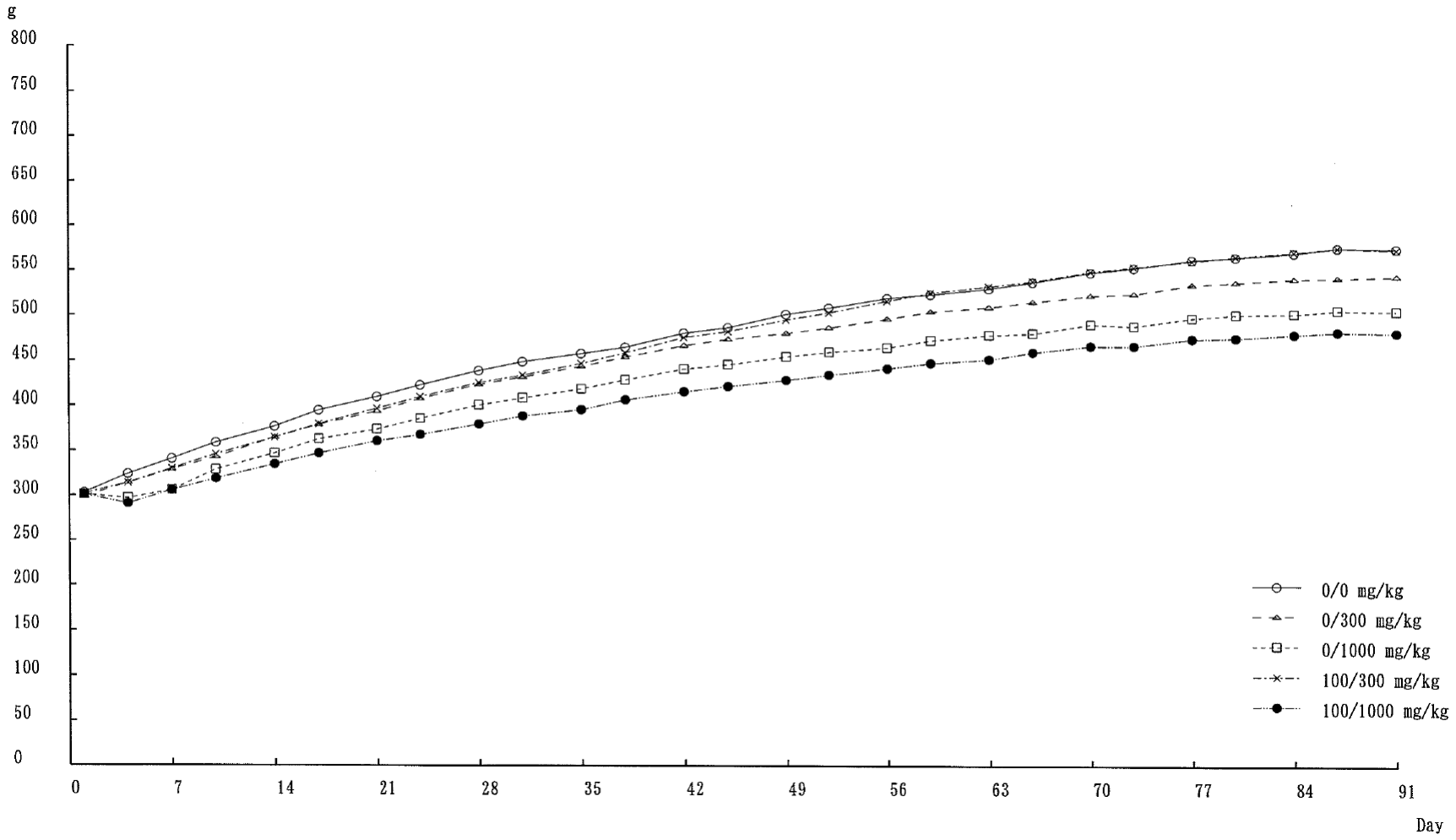
Mortality – A single HD metformin male (1/10) died on day 7 after clinical signs of decreased feces and decreased spontaneous movement. The decedent had very low food consumption, lost weight throughout the dosing period, and was "undernourished" at necropsy. Death was attributed to metformin-related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

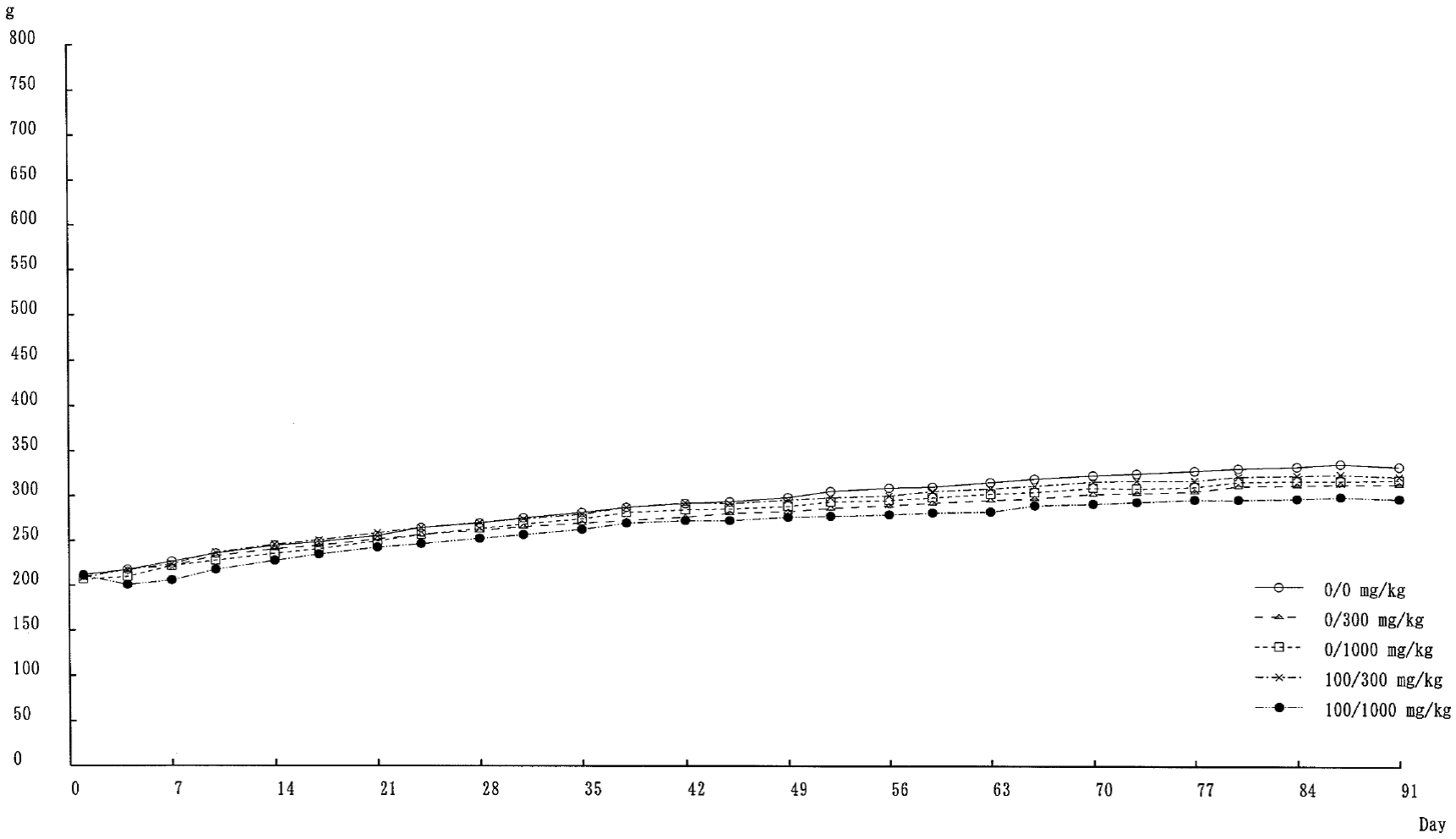
Body Weights (twice weekly) – Final body weights (↓10-20%; ss) and body weight gain (↓29-34%; ss) were reduced in HD metformin + alogliptin male and female groups compared to vehicle controls. Male LD (300 mg/kg) and HD (1000 mg/kg) metformin only groups had decreased body weight (↓5% and 15%) and body 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
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450
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300
250
200
150
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7
14
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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: 22-271
Submission date: 12/27/2007

Drug: alogliptin
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 both found the nonclinical information adequate to support approval of alogliptin for the intended indication. Relatively minor fetal effects were only noted at doses that produced exposures approximately 100- to 200-fold the expected human exposure and that caused maternal toxicity. Carcinogenicity findings considered statistically significant by the executive carcinogenicity assessment committee were combined thyroid C-cell adenomas and carcinomas in male rats. However, these occurred at a large multiple (≥ 288X) of the expected maximum human exposure and are, therefore, expected to represent minimal risk to humans. No drug-related neoplasms were seen at a lower dose that provided 32-fold higher exposure than the expected maximum human exposure.

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 B appears to be appropriate and that some of the carcinogenicity findings should be addressed in labeling. Specific wording can be addressed at a later time.

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/s/

Paul Brown
6/25/2009 05:49:05 PM
PHARMACOLOGIST

SUPERVISOR'S MEMO

Date:	16 June 2009
RE:	Amended memo to NDA 22-271, need for rat embryofetal development study
Sponsor:	Takeda Pharmaceuticals
Drug/Indication	Alogliptin, DPP4 inhibitor Type 2 Diabetes

The Division recently received information regarding a potential teratogenic interaction between another DPP4 inhibitor and metformin in an embryofetal development study in rats. If approved, alogliptin would be commonly prescribed as an add-on therapy to metformin; therefore, findings of potential teratogenicity with DPP4 inhibitors in combination with metformin are concerning and are relevant to the alogliptin monotherapy NDA.

Takeda has been already been informed that an embryofetal development study in rats evaluating the alogliptin/metformin combination is required to support development of their fixed-dose alogliptin/metformin combination product. Based on the recently heightened concern for potential teratogenicity with the combination, *the Division additionally requests that the results of the rat embryofetal development study with the alogliptin/metformin combination be submitted to support appropriate labeling of the alogliptin monotherapy NDA 22-271*.

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/s/

Todd Bourcier
6/17/2009 05:39:33 PM
PHARMACOLOGIST
Amended memo regarding rat embryofetal development study for alogliptin + metformin

SUPERVISOR MEMO

Date:	21 Aug 2008
RE:	NDA 22-271
Sponsor:	Takeda Pharmaceuticals
Drug/Indication	Nesina (alogliptin, DPP4 inhibitor) / Type 2 diabetes

Takeda pharmaceutical is seeking marketing approval for alogliptin, proposed trade name Nesina, as a treatment option for Type 2 diabetes. 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. Among many in development, one is approved for US and global markets (Januvia), one is approved for non-US markets (Galvus), and one other is the subject of an NDA currently under review by the Division (Onglyza).

Dr. David Carlson, the primary non-clinical reviewer, concludes that the pharmacology and toxicology data support approval of alogliptin (25mg q.d.). *I concur with Dr. Carlson's assessment.*

Dr. Carlson's decision is based on the benign toxicological profile of alogliptin in rats, dogs, mice, and monkeys over a considerable range of drug exposure relative to anticipated clinical exposure. Very large exposures (≥ 200 fold excess vs. clinical exposure) were required to identify target organs in animals, which included kidney, lung, liver, and male reproductive organs. Cutaneous toxicity in monkeys associated with some DPP4 inhibitors was not observed with alogliptin. Findings in the carcinogenicity assessment in rats and mice were similarly observed only at high drug levels that pose a negligible clinical risk. Findings from the reproductive toxicology studies did not reveal relevant toxicities without also producing signs of maternal toxicity. Given the large exposure multiples, even moderate increases of drug exposure in susceptible patients, for example those with renal insufficiency, presents a negligible risk of reproducing animal toxicities in human subjects at clinically relevant doses.

Concerns over cardiovascular safety have arisen from review of the clinical data. Despite careful review, no correlative or suspect cardiovascular toxicity was apparent in the non-clinical data. Nevertheless, and as Dr. Carlson rightly observes in his review, non-clinical studies are conducted in healthy animals and are unable to directly address potential cardiovascular liabilities in the context of existing co-morbidities common to the Type 2 diabetic population.

Dr. Carlson predicts that hypersensitivity or pseudo-allergy responses will be seen in susceptible individuals should alogliptin gain marketing approval. This prediction is based on rash-type

findings in dogs and to a limited extent in patients enrolled in controlled clinical trials. This response has been observed with some other DPP4 inhibitors in development, and also in post-marketing experience with sitagliptin (Januvia). Some cases with Januvia were severe, prompting a change in product labeling. Of note, hypersensitivity-type responses were not observed with Januvia in animals prior to approval, but were rather quickly observed once prescribed to masses of patients post-market. Given that alogliptin clearly produces hypersensitivity reactions in animals and humans at the pre-market stage, I agree with Dr. Carlson's prediction and additionally suggest that such reactions will occur with greater frequency and severity than experienced with Januvia.

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

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/s/

Todd Bourcier
8/27/2008 10:59:26 AM
PHARMACOLOGIST

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

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER:	**22-271**
SERIAL NUMBER:	**000**
DATE RECEIVED BY CENTER:	**12/27/07**
PRODUCT:	**Nesina™ Tablets (Alogliptin)**
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): 21 August, 2008

TABLE OF CONTENTS

EXECUTIVE SUMMARY

I. Recommendations

A. Recommendation on approvability

Approval. Pharmacology/Toxicology recommends approval of NDA 22-271 (Nesina)

B. Recommendation for nonclinical studies

No additional studies needed.

C. Recommendations on labeling

Issues to be addressed in product labeling:

- Reproductive toxicity
  - Not a teratogen
  - Category B (Sponsor proposes Cat. B)
  - Include additional language in Section 8.1 about maternal toxicity and reproductive effects (fetal growth/development effects only at maternally toxic doses) and male reprotoxicity (testes atrophy, sperm findings)?
  - Alogliptin concentration in milk is 2-times maternal plasma concentration in rats
    - Fetal exposure confirmed in rat
    - Fetal exposure expected in human

- Carcinogenicity
  - Increased thyroid C-cell adenomas + carcinomas in male rats (ECAC review/concurrence)
    - Need some labeling explanation (i.e., potential change of draft label)
      - 32X margin at NOAEL, tumors at high multiple of human exposure (188X MRHD)

- Section 13.2 'Animal Toxicology and/or Pharmacology' probably not necessary

II. Summary of nonclinical findings

A. Brief overview of nonclinical findings

[*note* – **MRHD** (maximum recommended human dose) estimates based on proposed maximum 25 mg QD ($AUC_{0\text{-}24} \approx 1.5$ μg*h/ml, $C_{max} \approx 140$ ng/ml)]

Alogliptin was well tolerated in animals up to doses on the order of tens- to hundreds-fold higher than expected human exposures. Drug-related mortality was evident only in multiple dose studies and only at high exposure multiples in mice, rats, and rabbits (generally > 50-fold MRHD). Body weights were typically lower than controls after repeat dosing at very high exposure multiples (generally > 100-fold MRHD).

Major target organs identified at high multiples of human exposure are testes, kidney, liver, urinary bladder, and lung. Toxicity in target organs seen in chronic studies was seen at high doses in short or moderate duration studies (up to 13 weeks), with the exception of lung histiocytosis seen only in six month and two year studies. Target organs were typically identified in rodent studies, as clinical signs and decreased body weight were the only notable findings in dogs (up to > 250-times human exposures) and no remarkable toxicity was seen in monkey studies up to 31-times expected human exposures. Kidney and bladder toxicity were the only toxicities that contributed to mortality in animals found dead on study (mice, rats, rabbits). See tabulated target organ summary, below.

Dogs showed consistent but intermittent clinical signs of reddened/flushing ears and face, along with body and facial swelling. Signs in dogs were tolerated throughout multiple dosing for up to nine months and did not prove to be dose-limiting. The observations may be evidence of a hypersensitivity or pseudoallergy-type response. Takeda did not investigate the mechanism of reddening/edema, but investigative studies with another DPP4 inhibitor showed the reactions are characterized by increased histamine release, strongly suggesting pseudoallergy and not a true, immunoglobulin-mediated allergic reaction. Alogliptin does bind to melanin and accumulate in pigmented tissues in rats, but there was no evidence of phototoxicity in a dedicated study. The skin reactions in dogs do not seem to be related to the necrotic skin lesions seen with other DPP4 inhibitors. Alogliptin did not cause any remarkable skin lesions in mice, rats, dogs, or monkeys.

Tumor findings were limited to increased incidence of combined thyroid C-cell adenomas and carcinomas in males rats (≥ 288X MRHD). Notable tumor findings in mice were limited to a 5% incidence of benign hepatocellular adenomas in 300 mg/kg/day females (74X MRHD), which was within the historical range of some studies. There were no malignant hepatocellular carcinomas or evidence of

hyperplasia or pre-neoplastic lesions. The mouse tumors were not considered alogliptin-induced.

There was no non-neoplastic evidence that thyroid was a target organ and there was no drug-related increase in thyroid C-cell hyperplasia in rats. Exenatide (Byetta) and liraglutide, two GLP-1 analogs, increase thyroid C-cell adenomas in rats, but there is no evidence to suggest the finding with alogliptin (which increases GLP-1) is due to a common mechanism. The GLP-1 analogs markedly increase c-cell hyperplasia, which is not seen with alogliptin. In addition, there is no evidence of increased c-cell tumors with three other DPP4 inhibitors (sitagliptin/Januvia, vildagliptin/Galvus, saxagliptin/Onglyza). At the high doses of alogliptin in the carcinogenicity studies, GLP-1 was likely increased 5-fold or more, which suggests a common endocrine mechanism between DPP4 inhibition and synthetic GLP-1 treatment would be apparent in animals treated throughout their lifetime. In addition, there were no common carcinogenic effects with the four different DPP4 inhibitors, which argues against any mechanism-related carcinogenic effect of DPP4 inhibition.

Thus, alogliptin poses minimal carcinogenic risk to humans based on high exposure multiples at the NOAEL (32X) for rat thyroid C-cell tumors, very high exposure multiples (≥ 288X) at doses that caused increased combined thyroid C-cell adenomas and carcinomas in male rats, and absence of any other drug-related tumors in rats (> 400X female MRHD) or mice (60X MRHD).

Reproductive toxicity: Alogliptin was not teratogenic at doses greater than 200-fold higher than expected human exposure. There were no remarkable effects on pregnancy or fetal development except at maternally toxic doses that were generally greater than 200-fold higher than expected human exposure. The major notable finding from reproductive toxicity studies was slightly increased percentage of sperm abnormalities in males (NOAEL ≈ 67x MRHD). The male findings were consistent with sporadic male reproductive toxicity seen in other non-clinical toxicity studies at high alogliptin doses. Nevertheless, rat sperm abnormalities did not affect fertility.

Fetal findings were limited to decreased body weight and minor skeletal variations (incomplete ossification of sternebrae, skull bones, and hyoid) at maternally toxic doses. Decreased body weight persisted throughout the life of the F_1 generation and there were slight delays in postnatal development and maturation and slightly decreased numbers of implantation sites and viable embryos in pups from F_1 matings. There were no effects at doses that didn't cause maternal toxicity, which provided approximately 100- to 200-fold exposure margins at the NOAELs compared to expected human exposure.

Target organ summary:

- **Kidney** (≥ 32X MRHD; > 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', combined hepatocellular adenoma + carcinoma
- **Testes** (≥ 32X MRHD at NOAEL; > 200X MRHD at LOAEL)
  - Rat: testes atrophy/degeneration, small seminal vesicles, oligospermia and abnormal sperm, epididymides 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

B. Pharmacologic activity

Alogliptin is an orally active DPP4 inhibitor indicated for treatment of type 2 diabetes mellitus as an adjunct to diet and exercise. 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. 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, an incretin mimetic, is also marketed in the U.S. Exenatide is an analog of glucagon-like peptide 1 (GLP-1), which is cleaved and inactivated by DPP4, thus the intended pharmacodynamic effect of increased GLP-1 activity is similar for exenatide and DPP4 inhibitors.

In support of the intended pharmacodynamic effect and *in vivo* clinical data, alogliptin has been shown to inhibit DPP4 enzymatic activity and lower blood glucose in a variety of *in vitro*, *ex vivo* (e.g. DPP4 plasma activity), and *in vivo* studies in healthy and diabetic animal models. DPP4 inhibition *in vivo* persisted for several hours in all species tested. Alogliptin shows an excellent selectivity profile (~ 15,000-fold) for DPP4 compared to other 'DASH' family enzymes. Alogliptin (SYR-322) showed no inhibition of DPP2, DPP8, DPP9, or related DASH enzymes (PREP, FAP/seprase, tryptase). As shown in the table below, alogliptin DPP4 selectivity is similar to sitagliptin (Januvia) and superior to vildagliptin (Galvus). Edema and necrotic skin lesions seen with several DPP4 inhibitors may be due to off-target inhibition of DPP8 and/or DPP9, thus the high selectivity of alogliptin for DPP4 predicts a limited risk for off-target toxicity. Human plasma C_{max} at the proposed high dose of 25 mg is approximately 400 nM, which is at least 250-times lower than maximum *in vitro* concentration that failed to inhibitor other DASH family enzymes.

DPP4 Inhibition Selectivity Comparison †			
Enzyme	**IC_{50} (nM)**		
	Alogliptin (Nesina)	**Vildagliptin (Galvus)**	**Sitagliptin (Januvia)**
DPP4	7	24	12
DPP2	$>1x10^5$	$>1x10^5$	$>5x10^4$
DPP8	$>1x10^5$	1400	$1.9x10^4$
DPP9	$>1x10^5$	82	$6.2x10^4$

† Approximate mean of *in vitro* inhibition of DPP4 and related 'DASH' family dipeptidyl peptidase recombinant human enzymes

C. Nonclinical safety issues relevant to clinical use

1. Hypersensitivity and/or pseudoallergy are predicted in sensitive individuals in the clinical population based on dog findings. The reaction in dogs seemed to be separate from DPP4-inibitor induction of necrotic skin lesions. 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. Takeda did not investigate mechanisms of dog hypersensitivity/pseudoallergy, but evidence from other DPP4 inhibitors suggest a histamine-related response rather than an immunoglobulin-mediated allergic response.

2. The overall non-clinical toxicity profile suggests minimal target organ risks in humans. However, since DPP4 cleaves substrates other than the targeted incretin hormones, inhibition of DPP4 may have unintended consequences with prolonged dosing. As noted in the Januvia review "Effects on human immunity, specifically recall responses to antigens and immune cell trafficking, may be adversely affected by DPP4 inhibition. This risk is an unavoidable characteristic of…the drug class."

3. Alogliptin concentrations were high in kidney and renal medulla (~7-fold higher than plasma) during the first 4 h post-dose in rats. Relatively high drug concentration (~10% of C_{max}) persisted in kidney and renal medulla for 48 h. Kidney is a target organ and a major route of excretion. Clinical chemistry biomarkers and histopathology showed kidney toxicity occurred at very high concentrations in animals (≥ 200-times the MRHD), thus risks of kidney toxicity in humans is considered minimal. Slightly higher kidney exposure in patients with renal impairment is not likely to significantly increase the risk of kidney toxicity. With respect to exposure in patients with renal impairment, Takeda offered the following summary:

   "Alogliptin is primarily renally excreted; therefore, exposure is increased in patients with moderate and severe renal impairment/ESRD, requiring dose adjustments in these patients. Even if these patients were to inadvertently receive alogliptin 25 mg, the predicted exposure would be similar to that of patients receiving alogliptin 100 mg daily, a dose which was shown to be safe and well tolerated for 12 weeks."

4. No cardiac toxicity in animals that would be predictive of human toxicity (no signal for ischemia/thrombosis in animals). In the NDA mid-cycle review meeting, the medical officer noted potential drug-related cardiac toxicity signals in clinical trials. A comprehensive review of animal data did not uncover any specific signals suggesting any cardiac risks or any potential mechanisms for preliminary clinical cardiac findings. It is worth noting that healthy animals are used in standard toxicology studies, thus any risks and co-morbidity specific to the type 2 diabetic patient population cannot be adequately assessed in non-clinical studies.

5. Alogliptin readily crosses the placenta and is secreted in milk in rats at approximately 2-times higher concentrations than maternal plasma. Fetal exposure was confirmed in rats and assumed in nursing rats based on good overall oral bioavailability and the absence of evidence that alogliptin would be retained in milk and not absorbed in nursing pups. No specific risks to fetuses, neonates, or nursing infants are predicted from reproductive toxicity studies, nevertheless, animal data support a conclusion that human fetuses and nursing infants will be exposed to alogliptin from maternal drug use.

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

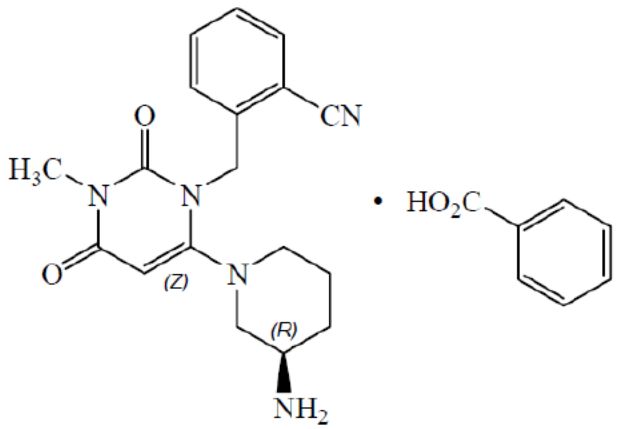
2.6.1 INTRODUCTION AND DRUG HISTORY

NDA number: 22-271
Review number: 1
Sequence number/date/type of submission: N-000/27 December, 2007/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: (b) (4)

Reviewer name: David B. Carlson, Ph.D.
Division name: Metabolism and Endocrinology Products
HFD #: 510
Review completion date: 20 August, 2008

Drug:

Trade name: Nesina™
Generic name: Alogliptin Benzoate (USAN); Alogliptin (INN; free base)
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; 2-[[6-[(3*R*)-3-Amino-1-piperidinyl]-3,4-dihydro-3-methyl-2,4-dioxo-1(2*H*)-pyrimidinyl]methyl]benzonitrile monobenzoate
CAS registry number: 850649-62-6
Molecular formula/molecular weight: $C_{18}H_{21}N_5O_2 \cdot C_7H_6O_2$ / 461.51 g/mol (benzoate salt); 339.30 g/mol (free base)
Structure:



Relevant INDs/NDAs/DMFs: IND 69,707 (alogliptin); IND 73,193 (SYR322 + pioglitazone); IND 101,628 (SYR322 + metformin); NDA [(b) (4)]; > 20 DPP4 INDs

Drug class: Dipeptidyl peptidase IV inhibitor (DPP4 inhibitor) (also abbreviated DPP-4 and DPP-IV)

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

Clinical formulation: Tablets; film-coated tablets containing [(b) (4)] 6.25 mg, 12.5 mg, or 25 mg SYR-322 benzoate. Tablets are the same size, distinguished by color ([(b) (4)] light pink, yellow, light red). 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 Alogliptin Tablets

Ph.Eur., 95/45/EC Designation	USP/NF Designation	Quality Standards
Mannitol	Mannitol	Ph.Eur., USP
Cellulose, microcrystalline	Microcrystalline cellulose	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
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
Macrogol [(b) (4)]	Polyethylene glycol [(b) (4)]	Ph.Eur., NF
[(b) (4)]		Ph.Eur., USP
		NF

Route of administration: Oral

Disclaimer: Some of the sponsor's tables and figures from the electronic NDA submission have been included in this review. All drug-related trends are discussed in relation to concurrent vehicle control groups in each study 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.

Studies reviewed within this submission: All studies were previously reviewed under IND 69,707. Summaries of reviews are included in this NDA review. Various written

summaries, figures, and tables from IND reviews are reproduced and cited throughout the text.

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

2.6.2 PHARMACOLOGY

2.6.2.1 Brief summary

Alogliptin (SYR-322; Nesina™) is indicated for treatment of type 2 diabetes mellitus as an adjunct to diet and exercise. It is an orally active dipeptidyl peptidase IV (DPP4) inhibitor. 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. 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, an incretin mimetic, is also marketed in the U.S. Exenatide is an analog of glucagon-like peptide 1 (GLP-1), which is cleaved and inactivated by DPP4, thus the intended pharmacodynamic effect of increased GLP-1 activity is similar for exenatide and DPP4 inhibitors. Alogliptin is currently proposed as a monotherapy as an adjunct to diet and exercise and as add-on therapy to other oral diabetic therapies (metformin, sulfonylurea, thiazolidinedione, or insulin) when glycemic control becomes inadequate. Use of alogliptin in clinical trials has shown no effect on body weight and no apparent increase in hypoglycemic events or effect on lipids.

As described in the original IND review,

> "SYR-322 is described as a potent, selective, orally available inhibitor of dipeptidyl peptidase IV (DPP IV) which is responsible for degrading and inactivating the gastric incretins GLP-1 and GIP. These incretins have short half-lives (~2-5 minutes) and are released in response to food intake. GLP-1 and GIP exert several anti-diabetic actions, including enhanced insulin biosynthesis and secretion, induced glucose competency, delayed gastric emptying, and reduced plasma free fatty acids. The potential benefits of the incretins for Type II diabetic patients may be enhanced and prolonged by slowing their degradation via inhibition of DPP IV, the target of SYR110322."
>
> "DPP IV (also known as CD26) is a homodimeric, cell surface serine protease. Widely distributed, highest levels of DPP IV are found in the kidney, liver, pancreas, thymus, spleen, epithelial and endothelial cells, and lymphoid and myeloid cells. A soluble form of DPP IV is shed to the circulation, and is considered by the sponsor to be the therapeutically relevant target pool. DPP IV is one member of the 'DPP-IV activity and structural homologue' (DASH) family of serine proteases, with fibroblast activation

> protein α (FAPα) sharing the highest level of homology to DPP IV. DPP8 and DPP9 are also closely related cytoplasmic enzymes differentially expressed in skeletal muscle, heart, liver, and activated T-cells. The function of DPP8 and DPP9 are unknown. The relative selectivity of new compounds for inhibition of DDP IV versus closely related enzymes may thus bear importantly on the safety and efficacy profile of drug candidates."
>
> "Substrate specificity of DPP IV is somewhat promiscuous; however, the number of 'true' endogenous substrates identified thus far is limited. GLP-1 and GIP are established endogenous and biologically relevant substrates of DPP IV. Nevertheless, DPP IV is reported to cleave neuropeptides, growth hormones, and T-cell and macrophage-derived chemokines. Substrate promiscuity of DPP IV, if present in vivo, may affect the safety and efficacy profile of DPP IV inhibitor drug candidates."

While the physiological role of DPP8 and DPP9 are still unknown, inhibition of DPP8/9 have been associated with animal toxicity including alopecia, histopathological changes in multiples organs, gastrointestinal toxicity, and blood and immune system effects (thrombocytopenia, reticulocytopenia, enlarged spleen) which may be mediated by inhibition of T-cell proliferation (Lankas GR et al., Diabetes (2005) 54(10):2988). Skin, immune, and GI-related toxicity have been observed with some DPP4 inhibitors and, based on the similar toxicity profile with selective DPP8 and/or DPP9 inhibition, some toxicity attributed to DPP4 inhibition may be due to off-target inhibition of DPP8/9.

Alogliptin and other DPP4 inhibitors are not expected to have any effect in the absence of a glucose load because the target incretin hormone GLP-1 is released after meals. In support of that hypothesis, alogliptin treatment did not increase plasma insulin or reduce plasma glucose in fasted rats. Risks of hypoglycemic events due to DPP4 inhibition are expected to be lower than some diabetic therapies such as sulfonylureas or insulin, which do lower plasma glucose in fasted rats. In addition, alogliptin did not inhibit intestinal sugar absorption in a fatty rat model (in contrast to metformin, which inhibits intestinal sugar absorption).

The sponsor briefly summarized the pharmacodynamic effects in human clinical trials as follows:

> "The efficacy, safety, pharmacokinetics, and pharmacodynamics of alogliptin have been studied at doses ranging from 6.25 to 800 mg. Single-dose administration of alogliptin to healthy subjects and multiple-dose administration to subjects with T2DM produce rapid inhibition of DPP-4, with no dose-limiting adverse events (toxicities). Peak DPP-4 inhibition exceeded 93% for most doses, and inhibition at 24 hours exceeded 80% for alogliptin doses of 25 mg and higher. In healthy subjects, peak and total exposure to GLP-1 across all doses are 2- to 4-times greater than placebo, and dose-related elevations in GLP-1 persist 24 hours after dosing. In T2DM, levels of GLP-1 are reduced and the glucose-lowering actions of GLP-1, but not GIP, are

preserved; therefore, alogliptin potentially restores glycemic homeostasis by increasing and prolonging GLP-1 levels through DPP-4 inhibition, thus enhancing the effects of endogenous insulin."

In support of the intended pharmacodynamic effect and *in vivo* clinical data, alogliptin has been shown to inhibit DPP4 enzymatic activity and lower blood glucose in a variety of *in vitro*, *ex vivo* (e.g. DPP4 plasma activity), and *in vivo* studies in healthy and diabetic animal models.

A brief summary of selected important *in vivo* efficacy studies in animals and representative graphical summaries is highlighted here (all alogliptin treatments administered orally unless noted). Additional *in vivo* results related to primary pharmacodynamics are summarized below in Section 2.6.2.2.

- **Wild-type C57BL/6 mice** – single alogliptin dose (30 mg/kg) improved oral glucose tolerance (~25% ↓ glucose AUC) and increased plasma insulin by approximately 50%
- **Male diabetic Zucker rats** – single alogliptin dose (≥5 mg/kg) increased peak and sustained plasma GLP-1 levels consistent with DPP4 inhibition, but efficacy for lowering blood glucose and increasing insulin was not clearly established in the model
- **Male Wistar N-STZ-1.5** – improved glucose tolerance and insulin response
  - Dose-dependent decreased plasma DPP4 activity and increased plasma GLP-1 levels (Figure 1, below)
  - Single alogliptin dose (≥ 0.3 mg/kg) improved glucose tolerance and insulin response after glucose challenge
  - 4-week alogliptin treatment showed only modest improvement in $HbA1_c$ (↓ 0.3%) and pancreatic insulin (modest long term efficacy)
  - Single alogliptin dose (1 mg/kg) improved glucose tolerance and plasma insulin levels in N-STZ-1.5 rats pushed to secondary sulfonylurea (glibenclamide) failure
- **Diabetic (7-wk old) *ob/ob* mouse** – improved diabetes metabolic parameters
  - 2-day dietary: dose-dependent reduced plasma DPP-4 activity (≥ 80% reduction) and increased intact GLP-1 (~ ≥ 5-fold) (Figure 1, below)
  - 4-week dietary exposure (mean 2.8 and 14.1 mg/kg/day or 8.4 and 42.3 mg/m^2) improved metabolic parameters, including increased plasma GLP-1
  - 4-week dietary high dose (42.2 mg/kg/day or 126.6 mg/m^2) decreased plasma DPP4 activity ≥ 80% and decreased $HbA1_c$ (0.9%) and improved metabolic parameters including glucose tolerance after DPP4 activity returned to baseline
  - 4-week dietary treatment improved insulin positive staining in β-cells, supporting a preservation of β-cell function (no effect on α-cell morphology or glucagon positive cells)
- **Female Wistar fatty rats** – improved diabetes metabolic parameters
  - Single oral dose, dose-dependent improved glucose tolerance and insulin response and dose-independent increased plasma GLP-1

- 8-week treatment improved total cholesterol and GLP-1 levels

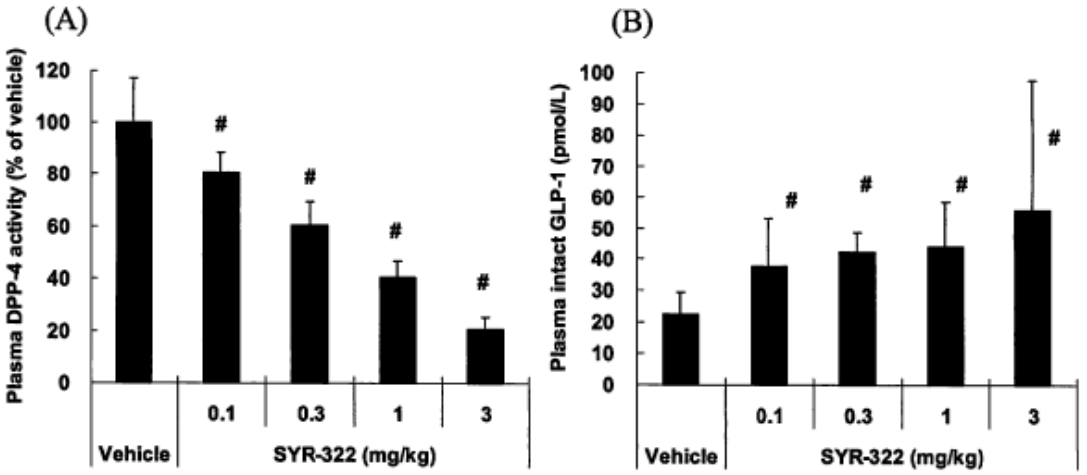


Figure 1 Acute effects of SYR-322 on the plasma levels of DPP-4 activity and GLP-1. Plasma DPP-4 activity (A) and intact GLP-1 concentrations (B). SYR-322 was dosed to fasted 41-week-old N-STZ-1.5 rats, and plasma DPP-4 activities were measured 1.5 h after the compound dosing. Then, the rats were gavaged with liquid meal 2 h after the compound dosing, and plasma intact GLP-1 concentrations were measured 5 min after the meal challenge. Data are presented as means and S.D. (n = 8).

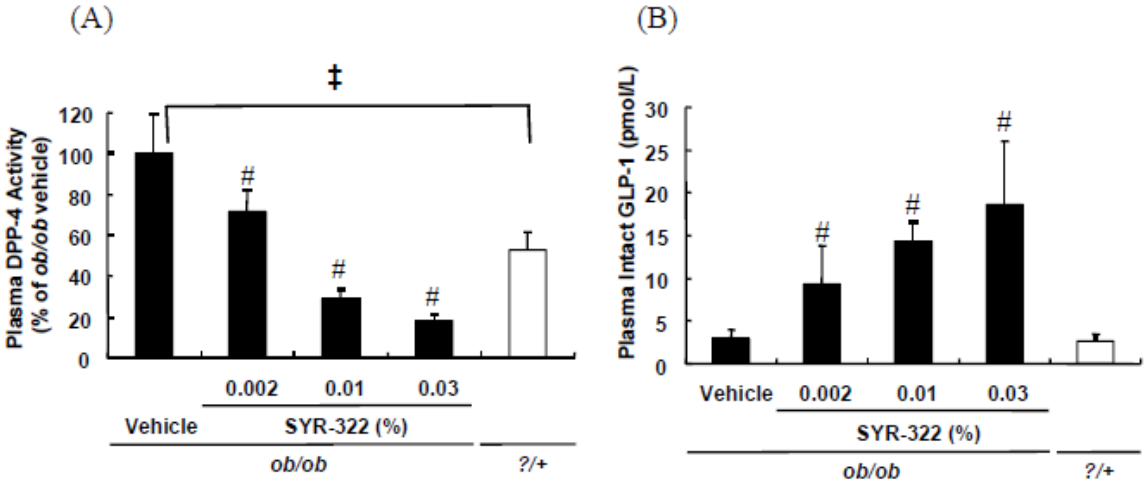


Figure 1 Short-term effects of SYR-322 on the plasma levels of DPP-4 activity and GLP-1.

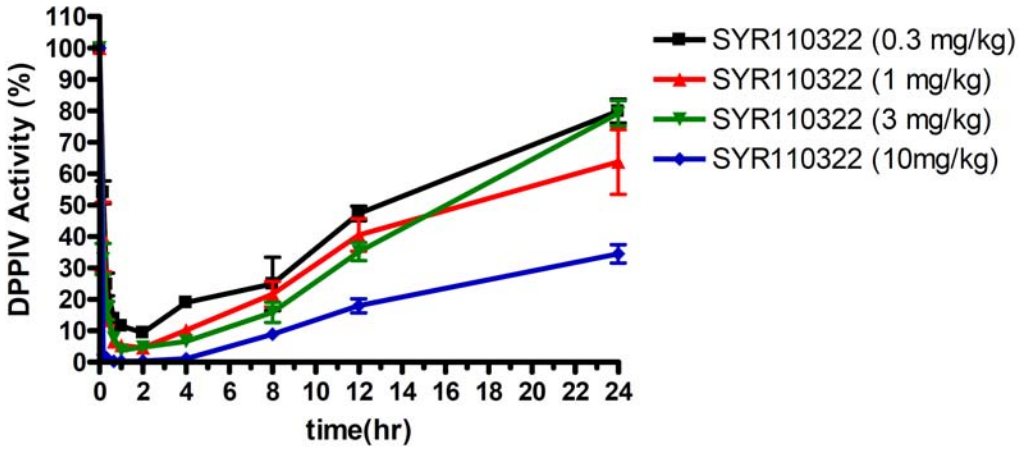
Plasma DPP-4 activity (A) and intact GLP-1 concentrations (B). SYR-322 was dosed to 7-week-old *ob/ob* mice at indicated doses as diet admixture for 2 days, and DPP-4 activity and intact GLP-1 concentrations were determined. # $p \leq 0.025$ compared with vehicle with one-tailed Shirley Williams test. ‡ $p \leq 0.01$ by Aspin-Welch test. Values are means and S.D. (n = 8 for *ob/ob*, n = 5 for ?/+).

DPP4 inhibition *in vivo* persisted for several hours in all species tested. The sponsor hypothesized that relatively long DPP4 inhibition after a single dose (or single daily dosing) is due to a therapeutically relevant pool of DPP4 remaining in circulating blood. SYR-322 did show relatively high association with blood cells in rats and dogs, with approximately 20-40% of administered dose distributed with blood cells for at least 8-24 h post-dose (see Section 2.6.4.4, below). Representative dose- and time-course curves of DPP4 inhibition and plasma glucose and insulin trends (after oral glucose tolerance test) from the sponsor's study reports are shown below.

Representative *In Vivo* DDP4 Inhibition Curve (Male ZDF Rat)

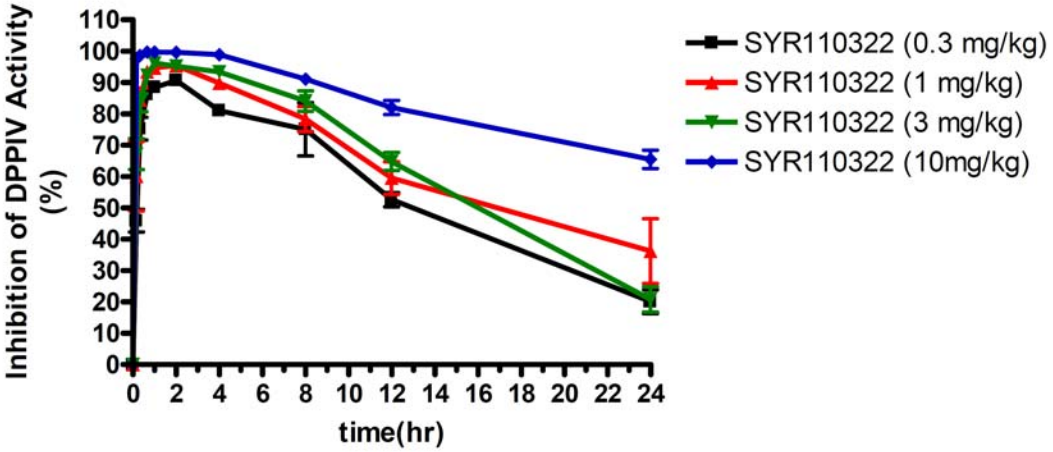
Figure 5 DPP IV Activity (a) and Inhibition (b) in Male Zucker fa/fa Rats after Oral Administration of SYR110322 at 0.3-10 mg/kg

(a)



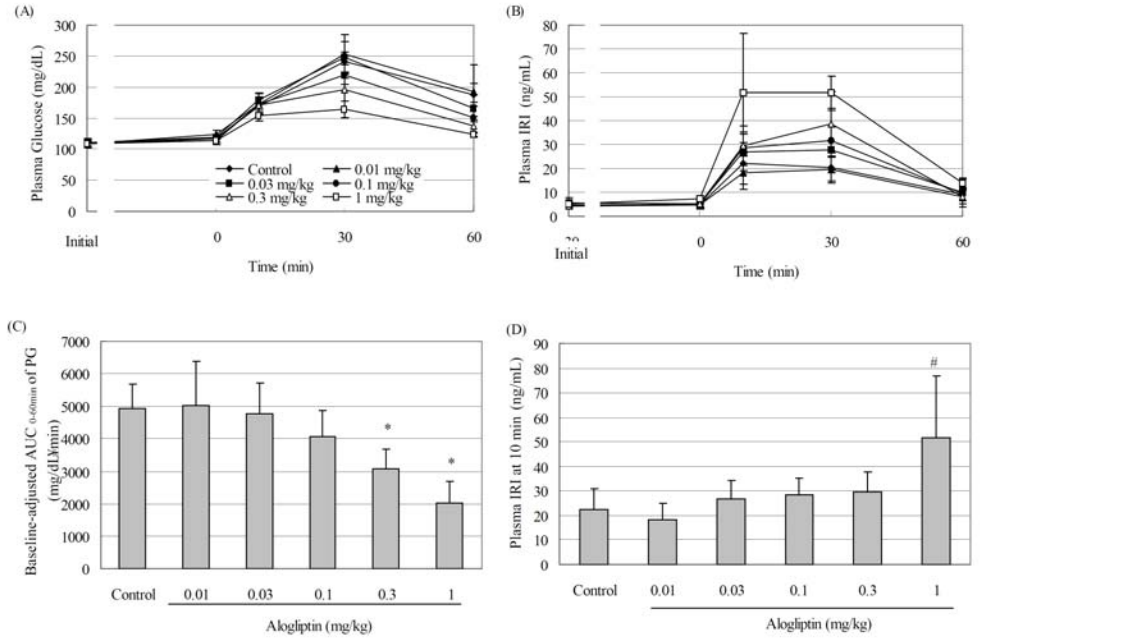
Mean ± SE, n=4

(b)



Representative *In Vivo* Plasma Glucose and Insulin Following Glucose Challenge (Female ZDF Rat)

Figure 2.g Effects of Alogliptin on Plasma Glucose and Insulin Levels in Female Wistar Fatty Rats



(A) Effects on plasma glucose.
(B) Effects on plasma IRI.
(C) Effects on adjusted plasma glucose AUC(0-60 min).
(D) Effects on plasma IRI at 10 minutes postdose.
*P≤0.025 vs control by 1-tailed Williams test.
#P≤0.025 vs control by 1-tailed Shirley-Williams test.

2.6.2.2 Primary pharmacodynamics

Mechanism of action: Dipeptidyl peptidase IV (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. There is some evidence that GLP-1 contributes to delayed progression of T2DM by slowing declines in β cell function which lead to the non-insulin-dependent diabetes characteristic of T2DM.

DPP4, also identified as the leukocyte antigen CD26, has many substrates, including neuropeptides (e.g. neuropeptide Y, substance P), growth factors (e.g. growth hormone releasing hormone (GRH)), and various chemokines. Inhibition of DPP4 could lead to side effects related to immune function, chemotaxis, and cell growth.

Inhibition of DPP4 is expected to increase postprandial GLP-1 concentrations, which in turn should enhance insulin secretion and glucose metabolism.

Drug activity related to proposed indication:

The (R)-enantiomer of SYR-322 is the active drug substance and it has approximately 150-fold higher DPP4 inhibitory activity than the (S)-enantiomer. The drug substance and drug product have been sufficiently characterized to show the (S)-form contributes an insignificant amount (b) (4) to the final product. In addition, the sponsor characterized the potential for chiral inversion and there was no apparent *in vivo* conversion of the (R)- to the (S)-enantiomer (see Section 2.6.4.5). Thus, based on the limited concentration and low relative DPP4-inibitory activity compared to (R)-SYR-322, the (S)-enantiomer does not contribute to *in vivo* alogliptin activity.

There are two minor metabolites of alogliptin in humans. The *N*-demethylated SYR-322 (M-I; SYR-324) is present in plasma at <1% of parent concentration and the *N*-acetylated form (M-II; SYR-135457) contributes approximately 4-6% of the parent drug equivalents after human oral exposure. The M-I form is a potent DPP4 inhibitor (approximately equipotent to SYR-322) while the M-II form is inactive against DPP4.

Inhibitory activity of SYR-322 and metabolites has been extensively tested *in vitro* and confirmed in various species *in vivo*. The IC_{50} for SYR-322 DPP4 inhibition is approximately 7 nM. At the proposed recommended maximum human dose of 25 mg QD, the mean human C_{max} is 140 ng/ml or 413 nM, which is well above the *in vitro* IC_{50}.

Trends for *in vitro* inhibition of DPP4 and related enzymes by alogliptin enantiomers and metabolites and selected other DPP4 inhibitors are shown in the reviewer's summary table below. The sponsor's summary table is also shown below. As shown, alogliptin (SYR-322) showed no inhibition of DPP2, DPP8, DPP9, or related DASH enzymes (PREP, FAP/seprase, tryptase).

DPP4 Inhibition Selectivity Comparison †						
Enzyme	**IC_{50} (nM)**					
	SYR-322	**(S)-322**	**M-I**	**M-II**	**Vildagliptin (Galvus®)**	**Sitagliptin (Januvia™)**
DPP4	7	1045	5	$>5x10^4$	24	12
DPP2	$>1x10^5$	$>1x10^5$	$>1x10^5$	$>1x10^5$	$>1x10^5$	$>5x10^4$
DPP8	$>1x10^5$	$>1x10^5$	$>1x10^5$	$>1x10^5$	1400	$1.9x10^4$
DPP9	$>1x10^5$	$>1x10^5$	$>1x10^5$	$>1x10^5$	82	$6.2x10^4$
PREP	$>1x10^5$	$>1x10^5$	$>1x10^5$	$>1x10^5$	$>5x10^4$	$>1x10^5$
FAP/ seprase	$>1x10^5$	$>1x10^5$	$>1x10^5$	$>1x10^5$	$7.3x10^4$	$>1x10^5$
Tryptase	$>3.9x10^5$	--	$>3.9x10^5$	--	$>2x10^5$	$>4x10^5$

† Approximate mean of *in vitro* inhibition of DPP4 and related 'DASH' family recombinant human enzymes
-- not evaluated
SYR-322 (alogliptin), (S)-enantiomer of SYR-322 (SYR111475), metabolites (M-I, M-II)

Sponsor Table

Table 2.a In Vitro Inhibitory Activity of Alogliptin, M-I, M-II, and (*S*)-Alogliptin

Enzyme (source)	IC50 (nmol/L)				Reference
	Alogliptin	M-I	M-II	(*S*)-alogliptin	
DPP-4 (rat plasma)	8, 18	-	-	-	322/00005, 322/00071
DPP-4 (dog plasma)	6, 16	-	-	-	322/00005, 322/00071
DPP-4 (human plasma)	10	-	-	-	322/00071
DPP-4 (human recombinant)	6.9, 6.7	5	>50,000	1045	322/00001, 322/00009 322/00187
DPP-4 (human Caco2 cells)	15	-	-	-	322/00071
DPP2 (rat kidney, human recombinant)	>100,000	>100,000	>100,000	>100,000	322/00001, 322/00009, 322/00072, 322/00187
DPP8 (human recombinant)	>100,000	>100,000	>100,000	>100,000	322/00001, 322/00009, 322/00072, 322/00187
DPP9 (human recombinant)	>100,000	>100,000	>100,000	>100,000	322/00010.001R, 322/00072, 322/00187
PREP (rat brain, human recombinant)	>100,000	>100,000	>100,000	>100,000	322/00001, 322/00009, 322/00072, 322/00187
FAPα/seprase (human recombinant)	>100,000	>100,000	>100,000	>100,000	322/00001, 322/00009, 322/00072, 322/00187
Tryptase (human recombinant)	>390,000	>390,000	-	-	322/00001, 322/00009

-=not evaluated.

A brief summary of DPP4 activity from plasma of various animal models after oral alogliptin is highlighted here.

- ***In vivo* rat-** DPP4 activity in plasma decreased ≥ 85% in a variety of assays in healthy rats by 15 min post-dose. DPP4 activity recovered to 50% of normal activity by **10 h** and normal activity by 24 h after a single oral dose.
  - ***Diabetic Zucker (fa/fa) rat***- A single oral SYR-322 dose followed by glucose challenge 30 min post-dose in male Zucker *fa/fa* rats caused dose-dependent increases in GLP-1 (up to 2.5-fold) and blood glucose. Plasma drug concentrations of approximately 16 nM effectively inhibited plasma DPP4 activity > 80%. Maximum DPP4 inhibition reached 99%, with inhibition of DPP4 activity as early as 10 min post-dose and partial inhibition (50-80%) continuing through 12 h post-dose without full recovery at 24 h post-dose.
- ***In vivo* dog-** DPP4 activity in plasma decreased ≥ 95% in a variety of assays by 15-30 min post-dose. DPP4 activity recovered to 50% normal activity by **14-16 h** and normal activity by 24 h after a single oral dose.
- ***In vivo* monkey-** Single oral dose in male monkeys caused > 80% DPP4 inhibition by 30 min post-dose and inhibition continued to at least 24 h post-dose, however, effects on insulin, glucagon, and GLP-1 were marginal compared to vehicle.
- ***In vivo mouse***- Two day dietary treatment of *db/db* diabetic mice inhibited DPP4 plasma activity (up to ~85% decrease) and increased intact plasma GLP-1 concentrations (approximately 5-fold).

As noted above, DPP4 is found in both membrane bound, cell-surface (e.g. as 'CD26' on T-cells) and soluble (e.g. plasma) forms. Alogliptin and M-I inhibit both the membrane

bound and free (soluble) DPP4. A representative tabular summary of DPP4 inhibition by metabolites M-I and M-II in Caco-2 cell culture (membrane bound) and plasma of human and animals are shown below (sponsor's Tables 1 and 2).

Table 1 Inhibitory activities of SYR-322 M-I and M-II against Caco-2 DPP-IV

	IC_{50} (nmol/L) (95% confidence interval) Caco-2
SYR-322 M-I	21.4 (20.9-21.8)
SYR-322 M-II	>30000

Table 2 Inhibitory activities of SYR-322 M-I and M-II against plasma DPP-IV

	IC_{50} (nmol/L) (95% confidence interval)		
	human plasma	dog plasma	rat plasma
SYR-322 M-I	13.6 (12.8-14.5)	17.1 (16.8-17.4)	19.0 (16.4-22.0)
SYR-322 M-II	>30000	>30000	>30000

2.6.2.3 Secondary pharmacodynamics

The ability of SYR-322 to affect enzyme activity or radioligand receptor binding was assessed in a screen of 89 (b) (4) assays. Initial screens showed significant inhibition of non-selective rat opiate receptor (65% inhibition) and phosphodiesterase 4 (PDE4, 75% inhibition) at 10 μM SYR-322. Confirmatory assays showed < 10% inhibition of human opiate δ, κ and μ receptors and ≤ 19% inhibition of PDE4 (three confirmatory assays up to 10 μM). There was no explanation for high variability in PDE4 assays, but the potential for PDE4 inhibition *in vivo* should be considered low based on ≤ 19% inhibition at 10 μM in the majority of assays.

SYR322 showed little interaction or interference with a battery of 94 receptors and enzymes in *in vitro* binding and functional screening assays up to 10 μM. An interaction with opiate receptor (not subtype specific) was noted, with an approximate IC_{50} of 10 μM, but the potential for *in vivo* effects was considered minimal based on a 25-fold lower maximum expected human concentration. No other binding sites or enzymes tested were inhibited greater than 50% by SYR-322.

SYR-322 treatment for eight weeks in female Wistar fatty rats at up to 10 mg/kg had no effect on body weight gain, food consumption, and 10 mg/kg caused a slight attenuation increases in plasma total cholesterol from baseline (-19% compared to controls in week 8).

2.6.2.4 Safety pharmacology

Neurological effects: Central nervous system risks from SYR-322 are considered minimal based on results from single and repeat dose safety pharmacology observations in rats, distribution studies, and the *in vivo* non-clinical toxicology program.

No treatment-related effects were observed on CNS measures in a functional observational battery (FOB) study in rats after a single oral dose or after 25 days of dosing up to 300 mg/kg SYR-322. Distribution studies in rats showed drug does not readily cross the blood:brain barrier.

Cardiovascular effects: Cardiovascular risks from SYR-322 are considered minimal based on results from a series of *in vitro* safety pharmacology studies and the *in vivo* non-clinical toxicology program.

SYR-322 did not inhibit hERG channel activity *in vitro* at concentrations up to 30 μM. Effects on hERG channels expressed in several cell types (CHO, CHO-K1, HEK293) were investigated and maximum hERG inhibition was approximately 10% at 30 μM SYR-322 in HEK293 cells (IC_{50} > 30 μM). SYR-322 did not prolong action potential duration (APD), resting membrane potential, action potential amplitude, or the maximum rate of depolarization in isolated canine Purkinje fibers. A slight shortening of APD by 8 to 34 msec at 1 μM and 30 μM, respectively, was seen in the dog Purkinje fiber assay.

The non-clinical toxicology studies in dogs confirmed an absence of remarkable cardiovascular findings after chronic SYR-322 exposure. A single dose safety pharmacology study showed 25 mg/kg SYR-322 caused a transient, reversible drop of 10-20 mm Hg in mean blood pressure and an equivocal, modest increase in mean QT duration of 10 msec. No changes in QT duration were noted in a one month toxicology study in dogs (blood pressure was not assessed in the one month study). No abnormal ECG findings were seen in 3- and 9-month dog studies. The weight of evidence from *in vivo* dog studies suggests there are no clear cardiovascular findings in dogs that would readily predict cardiovascular risks to the diabetic target population.

Pulmonary effects: Pulmonary risks from SYR-322 are considered minimal based on results from an *in vitro* safety pharmacology study and the *in vivo* non-clinical toxicology program. No treatment-related effects on the pulmonary system were seen in a safety pharmacology study up to a single oral dose of 100 mg/kg SYR-322 in rats.

Incidence and severity of lung alveolar histiocytosis increased in rats exposed to high doses of SYR-322 for six months or more. Severity was limited to minimal to mild histiocytosis with the exception of two males treated chronically with 800 mg/kg/day (469-times the maximum recommended human dose (MRHD)). Findings were considered minimal with respect to human risk, based on high exposure margins (32-times the MRHD at the NOAEL, 283-times the MRHD at the LOAEL) and an absence of known adverse effects of lung phospholipidosis in humans.

Renal effects: Kidney risks from SYR-322 are considered minimal based on results from the *in vivo* non-clinical toxicology program. No dedicated renal safety pharmacology studies were conducted. The only notable kidney finding seen in the non-clinical toxicology studies was an increase in chronic progressive nephropathy in female rats. The finding was considered a modest increase in spontaneous disease and occurred only at doses approximately 279-times the MRHD or higher.

Gastrointestinal effects: Gastrointestinal (GI) risks from SYR-322 are considered minimal based on results from the *in vivo* non-clinical toxicology program. No dedicated gastrointestinal safety pharmacology studies were conducted. No GI organs were identified as target organs in the battery of non-clinical toxicology studies.

Abuse liability: The potential for abuse was not specifically assessed. An IC_{50} on the order of 10 μM was estimated in an *in vitro* assay for non-selective binding to opiate receptors. Non-selective opiate receptor binding occurred at approximately 25-times the MRHD. SYR-322 showed no significant inhibition of selective human δ, κ, and μ opioid receptors heterologously expressed in cell culture. The potential for abuse is considered minimal.

Other: No other safety pharmacology risks were identified and no additional dedicated safety pharmacology studies were conducted.

2.6.2.5 Pharmacodynamic drug interactions

Potential for interaction with other DASH family enzymes was considered minimal and discussed above (see Section 2.6.2.3). Neither SYR-322 or major metabolites showed any inhibitory potential against DPP2, DPP8, DPP9, or DASH family enzymes. SYR-322 selectivity for DPP4 inhibition is approximately 15,000-fold greater than for other DPP and DASH enzymes. Various *in vivo* studies, summarized below, also confirmed the absence of any clear potential for drug interactions.

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 (Table 1). 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.

Table 1 Effects of the combination of SYR-322 and pioglitazone on metabolic parameters.

parameter	Analysis point	Control	Pioglitazone	SYR-322	Combination	*db/+m*
Body weight (g)	day 26	33.0 ± 1.8	38.1 ± 2.2	33.4 ± 2.0	40.6 ± 2.4	25.4 ± 1.0
	% of Control #	*100*	*115*	*101*	*123*	*77*
Food intake (g/day)	daily	7.4 ± 0.7	6.9 ± 0.6	7.7 ± 0.6	6.5 ± 0.5	5.4 ± 1.0
	% of Control	*100*	*93*	*103*	*88*	*73*
Plasma insulin (ng/mL)	day 22	6.5 ± 2.3	15.6 ± 10.3	7.7 ± 3.3	24.5 ± 15.7	2.2 ± 1.6
	% of Control	*100*	*241*	*120*	*379*	*33*
Plasma glucagon (pg/mL)	day 24	363 ± 79	303 ± 109	355 ± 77	214 ± 69	83 ± 36
	% of Control	*100*	*84*	*98*	*59*	*23*
DPP-IV activity (% of control)	day 22	100 ± 7	93 ± 8	21 ± 3	22 ± 3	107 ± 15
Fasting plasma glucose (mg/dL)	day 27	352 ± 110	253 ± 93	346 ± 94	175 ± 78	81 ± 8
	% of Control	*100*	*72*	*99*	*50*	*23*
Fasting plamsa triglyceride (mg/dL)	day 27	73 ± 22	53 ± 9	63 ± 38	24 ± 8	41 ± 8
	% of Control	*100*	*72*	*87*	*33*	*57*
Fasting insulinogenic index (ng/mg)	day 27	3.4 ± 2.0	7.3 ± 6.5	4.1 ± 3.9	10.4 ± 4.5	0.6 ± 0.1
	% of Control	*100*	*217*	*121*	*309*	*18*
Fasting DPP-IV activity (% of control)	day 27	100 ± 5	88 ± 11	85 ± 12	77 ± 21	75 ± 14

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.

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 (see representative sponsor's Figures 3 & 5, below).

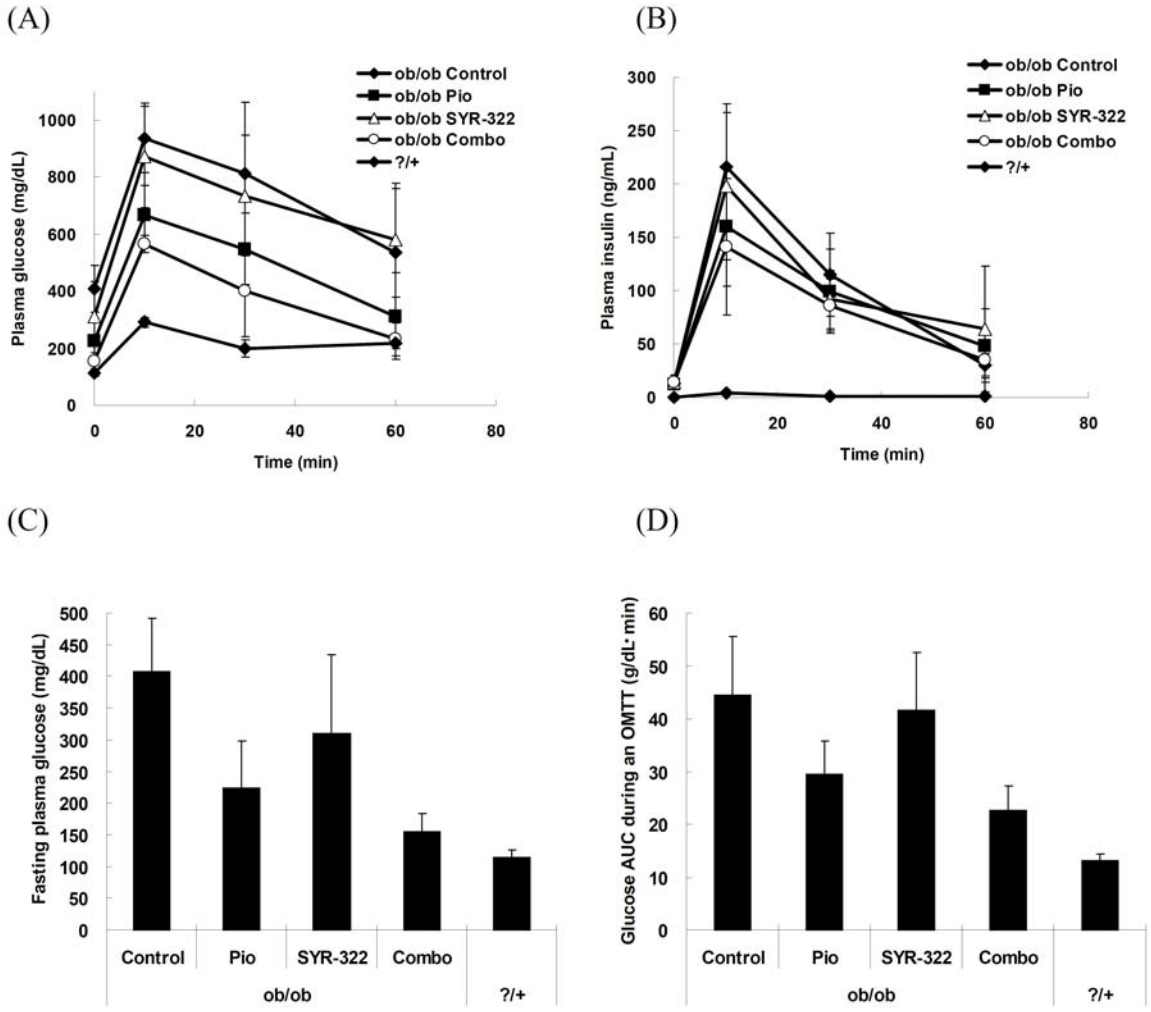


Figure 3 Effects of combined treatment with SYR-322 and pioglitazone in OMTT.
Time course of plasma glucose (A) and insulin (B), fasing plasma glucose before a meal load (C) and glucose AUC (D).
The mice were fed CE-2 diet containing 0.03 % SYR-322 or 0.003 % pioglitazone alone or in combination for 4 weeks. After chronic dosing, mice were fasted overnight and an OMTT were performed. Two-way ANOVA showed significant effects of pioglitazone ($P \leq 0.01$) and SYR-322 ($P \leq 0.01$) on fasting glucose levels, a significant effects of pioglitazone ($P \leq 0.01$) but no significant effect of SYR-322 ($P = 0.13$) on glucose AUC, and a significant effect of pioglitazone ($P \leq 0.01$) but no significant effect of SYR-322 on plasma insulin at 10 min. No interactions were shown between pioglitazone and SYR-322 on these parameters. Data are presented as means and SD (N=8 in *ob/ob* mice, N=5 in *?/+* mice).

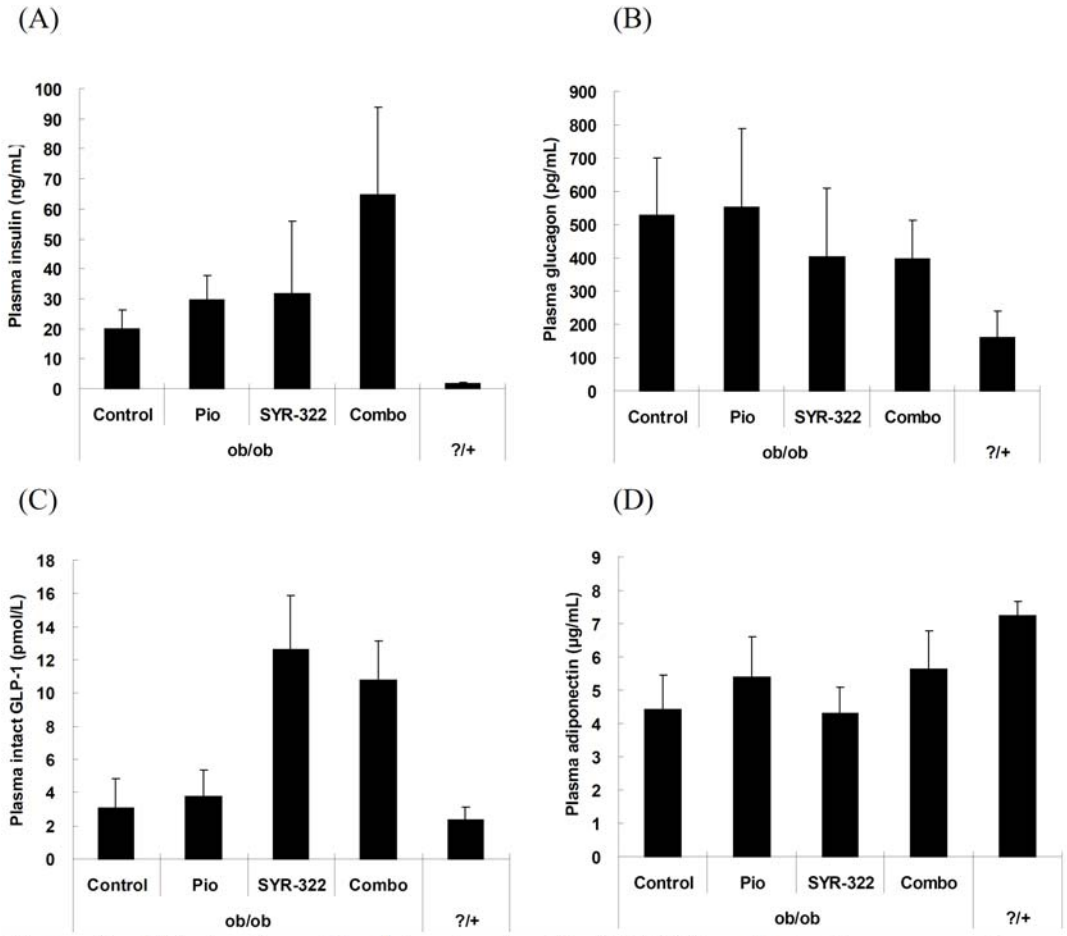


Figure 5 Effects of combined treatment with SYR-322 and pioglitazone on plasma levels of insulin (A), glucagon (B), intact GLP-1 (C) and adiponectin (D) after 4 weeks treatment.

The mice were fed CE-2 diet containing 0.03% SYR-322 or 0.003% pioglitazone alone or in combination for 4 weeks. Plasma samples were collected after 4 weeks treatment and analyzed for metabolic parameters. Two-way ANOVA showed significant effects of pioglitazone ($P \leq 0.01$) and SYR-322 ($P \leq 0.01$) on plasma insulin levels, a significant effect of SYR-322 ($P \leq 0.05$) but no significant effect of pioglitazone on plasma glucagon levels, a significant effect of SYR-322 ($P \leq 0.01$) but no significant effect of pioglitazone on plasma intact GLP-1 levels, and a significant effect of pioglitazone ($P \leq 0.01$) but no significant effect of SYR-322 on plasma adiponectin levels. No interactions were shown between pioglitazone and SYR-322 on these parameters. Data are presented as means and SD (N=8 in *ob/ob* mice, N=5 in *?/+*mice) .

The ability of SYR-322 to prevent the onset of diabetes in pre-diabetic *db/db* mice was assessed in three to four week combination dietary treatment with the α-glucosidase inhibitor voglibose. Combination treatment showed modest improvements in glycemic control and plasma and pancreatic insulin levels, and seemed to prevent the onset of diabetes compared to either treatment alone. See sponsor's Figure 2, below.

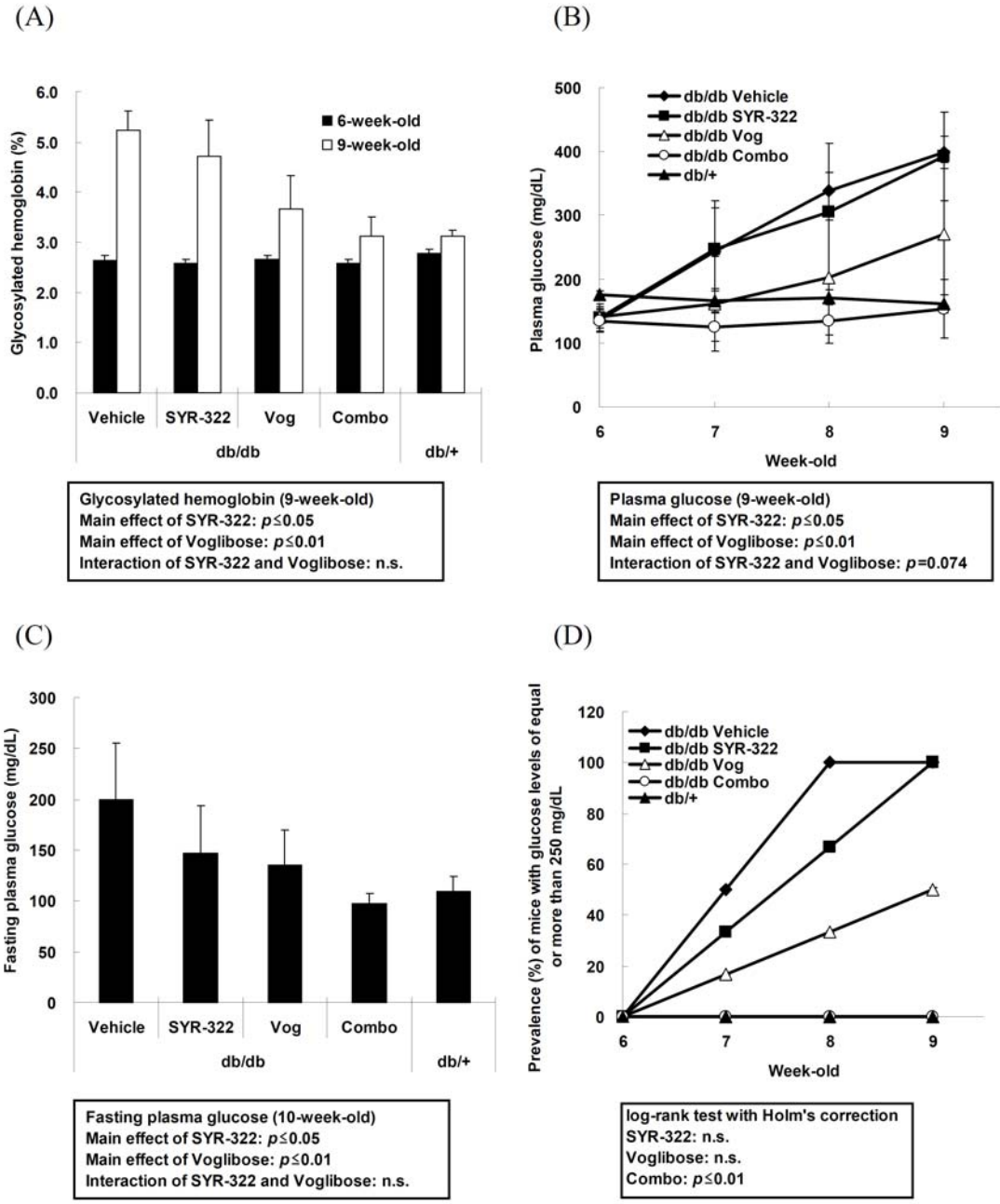


Figure 2 Chronic effects of SYR-322 plus voglibose combination treatment on glycemic parameters.

The levels of glycosylated hemoglobin (A), fed plasma glucose (B), fasting plasma glucose (C), and percent prevalence of mice with glucose levels of equal or more than 250 mg/dL (D). Analysis points and results of two-way ANOVA are indicated in insets in Fig. 2(A) to (C). Time (weeks of age)-to-event (glucose levels of equal or more than 250 mg/dL) variable was analyzed with the log-rank test with Holm's correction, and a result is indicated in the inset in Fig. 2(D). Data are presented as means and SD (n=6 for *db/db* mice, n=5 for *db/+* mice). Vog, Voglibose; Combo, Combination.

2.6.3 PHARMACOLOGY TABULATED SUMMARY

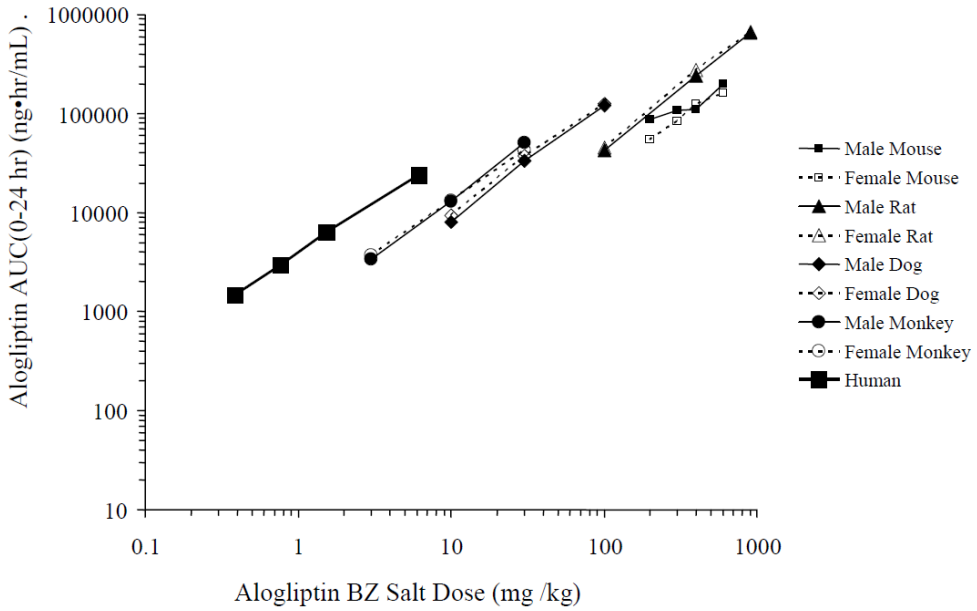
None (intentionally left blank).

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

2.6.4.1 Brief summary

Pharmacokinetic and toxicokinetic parameters were assessed in a variety of *in vitro* and *in vivo* studies. Alogliptin is rapidly absorbed after oral dosing in all species tested, with apparent bioavailability of 60% to 90% and T_{max} generally 1 to 2 h, but ranging from 1 to 5 h in various studies. Bioavailability profiles vary somewhat depending on the alogliptin salt form. The sponsor determined the benzoate salt profile was superior, thus this review focuses on the benzoate form chosen as the drug substance. Exposure increases with increasing dose, generally in proportion with dose. Repeated exposures generally resulted in moderate drug accumulation (≤ 2-fold). At very high dose exposures, drug accumulation was more pronounced, supporting decreased metabolism of parent at very high drug concentrations (e.g. ≥ 500-fold higher than expected human exposure). There were no remarkable sex differences in exposure after repeat dosing. The sponsor summarized the relationship of alogliptin dose (benzoate salt form) to total exposure ($AUC_{0\text{-}24\ h}$) in various species in Figure 3.a, which shows human exposure is slightly higher than monkey and dog at similar doses.

Figure 3.a Summary Plot of AUC Values for Alogliptin Versus Dose for Mice, Rats, Dogs, Monkeys, and Humans



Reviewer's note – the sponsor did not indicate whether human exposure values were from healthy volunteers, type 2 diabetes patients, or mean of healthy and diabetic individuals

Alogliptin is widely distributed in animals but elimination in most tissues is rapid and distribution to brain and CNS is minimal. Protein binding is moderate in all species, with less than 40% protein binding in all species. At the approximate human plasma drug C_{max}, protein binding was similar in humans (32%) and the animal models used in non-clinical toxicology studies (27-36%). Alogliptin shows moderate association with blood cells, with an estimated 30 to 50% bound to blood cells in various species. Drug concentrations were highest (≥ 7-fold higher than plasma) in bile, kidney, renal medulla, liver, and lacrimal glands during the first 4 h post-dose. Relatively high drug concentration (~10% of C_{max}) persisted in kidney and renal medulla for 48 h. Alogliptin and M-I remained in the sclera and uveal tract of the eyes of pigmented rats (but not albino rats) for at least 8 weeks after a single oral dose. The sponsor hypothesized that alogliptin bound to melanin, but a dedicated phototoxicity study showed no evidence of drug-induced phototoxicity.

Overall metabolism is modest in animals and humans and no significant human-specific metabolites have been identified. The majority of administered drug is excreted as parent compound in urine or conjugated alogliptin in feces. Only two notable *in vivo* metabolites were identified in humans, both of which represent less than 10% of administered drug. The drug substance is predominantly the (R)-enantiomer of alogliptin with no apparent *in vivo* chiral inversion to the (S)-enantiomer and the contribution of the (S)-form to the pharmacodynamic activity of alogliptin is insignificant.

As noted in section 2.6.2, M-I but not M-II showed DPP4 inhibitory potential. Alogliptin is N-demethylated by CYP 2D6 to give M-I. As drug development progressed, it became apparent that the metabolite M-I was a potent inhibitor of DPP4 and contributed a significant amount of pharmacodynamic activity in certain species. M-I was measured retrospectively from plasma TK samples in a variety of non-clinical experiments and it was then included in many standard TK analyses. Ultimately, it was clear that M-I exposure was low in humans (approximately 1%) and adequately covered in all animal species, thus measurement of M-I in all non-clinical studies was not considered a regulatory requirement. Nevertheless, M-I concentrations were measured in many non-clinical studies and M-I toxicokinetic data are summarized along with parent SYR-322 where data were available.

Alogliptin is rapidly eliminated after oral exposure in rats and dogs, with up to 98% of administered dose excreted within 24 h. Urinary excretion of drug, M-I, M-II, and unidentified metabolites ranged from about 30% to 50% of administered oral dose. Biliary excretion generally accounted for 20% to 30% of the balance of drug equivalents that were excreted in feces. There is evidence of enterohepatic circulation of a small fraction of absorbed dose, with enterohepatic reabsorption of up to approximately 20% of the biliary fraction which would account for less than 10% of total administered dose. Alogliptin is also secreted in milk of lactating rats, with approximately 2-fold higher levels of drug equivalents in milk than maternal plasma. Rat fetuses were also exposed to drug via placental transfer, with fetal blood levels 2- to 5-fold lower than dams. Thus,

some exposure to human fetuses and nursing infants from mothers taking alogliptin is likely.

Alogliptin showed limited potential to inhibit major human cytochrome P450 enzymes at the expected human dose. Potential for clinical drug-drug interactions with alogliptin relative to Phase I metabolism is considered low based on *in vitro* findings.

The maximum recommended human dose for diabetes treatment is 25 mg. All comparisons to human doses in this review are based on a maximum 25 mg daily dose and approximate mean PK estimates of $AUC_{0\text{-}24\ h}$ = 1.5 µg*h/ml and C_{max} =140 ng/ml (413 nM).

2.6.4.2 Methods of Analysis

Methods for quantification and stability of SYR-322 and metabolites or drug equivalents in plasma were validated in all experimental animal species used in pivotal pharmacology and toxicology studies. Methods of analysis were reviewed under the IND and key conclusions such as lower limit of quantification (LLOQ) and stability times are summarized here (sodium heparin anticoagulant unless noted).

- ***CD-1 mouse plasma*** – validated for plasma LC/MS/MS quantification of free SYR-322, M-I, and M-II (sodium heparin anticoagulant) with a 0.5 ng/ml (M-II only) or 2 ng/ml LLOQ. Recovery in mouse plasma was ≥72%. Stability was shown for 3 freeze-thaw cycles, up to 8 h at room temperature, 48 h at 10°C, and 3 months at ≤ -15°C.
- ***Monkey plasma*** – validated for plasma LC/MS/MS quantification of free SYR-322, M-I, and M-II with a 0.5 ng/ml (M-I and M-II) or 5 ng/ml LLOQ (SYR-322). Recovery was ≥65% in monkey plasma. Stability was shown for up to 8 h at room temperature and 3 months ≤ -15°C.
- ***Rat and dog plasma*** – validated for plasma LC/MS/MS quantification of free SYR-322, M-I, and M-II with a 0.5 ng/ml (M-I and M-II) or 2 ng/ml LLOQ (SYR-322).. Recovery in rat plasma was between approximately 75-90% and approximately 49-71% in dogs. Stability was shown for 3 freeze-thaw cycles, up to 8 h at room temperature, 48 h at 10°C, and 30 days at ≤ -15°C (updated to 3 months in rat plasma ≤ -15°C).
    - SYR-322 and M-I stability in rat plasma up to 306 days and dog plasma up to 331 days at -80°C
    - (S)-SYR-322 in rat (LLOQ= 10 ng/ml) and dog plasma (LLOQ = 2 ng/ml)
    - Pioglitazone (AD-4833) and metabolites M-II, M-III, M-IV with a 50 ng/ml LLOQ in rat. Stability was shown for up to 28 days at 5°C and 24 h at room temperature.
- ***Fetal rat serum*** – validated for serum LC/MS/MS quantification of SYR-322 and M-I with a 0.5 ng/ml (M-I) or 1 ng/ml LLOQ (SYR-322).
- ***Rat milk*** – validated for milk LC/MS/MS quantification of SYR-322 and M-I with a 0.5 ng/ml (M-I) or 1 ng/ml LLOQ (SYR-322).

- ***Rabbit plasma*** – validated for plasma LC/MS/MS quantification of SYR-322 and M-I with a 0.5 ng/ml (M-I) or 1 ng/ml LLOQ (SYR-322). Stability was shown for up to 218 days at -80°C

2.6.4.3 Absorption

Oral bioavailability was assessed *in vivo* in GLP-compliant studies. Bioavailability after a single oral dose was estimated at 61% in male rats ($t_{1/2}$ of 4.9 h and T_{max} at 5 h). Bioavailability was higher in monkey and dog, with approximately 80% bioavailability in monkey and 89% in male dog ($t_{1/2}$ = 6.7 h) and T_{max} of 1-2 h in monkeys and dogs. Similar to dogs, bioavailability Lymphatic absorption was essentially non-existent (< 0.1%) in rats. Drug distribution to blood cells was estimated in dogs, with 38% blood cell distribution at 1 h decreasing to 23% at 8 h post-dose. In rats, approximately 97% of the absorbed dose was excreted by 24 h post-dose (50% in urine, 47% in feces), while 98% of the absorbed dose was excreted by 72 h in dogs (40% urine, 58% feces).

Intestinal absorption was estimated directly in rat portal plasma after administration to the jejunal loop. Intestinal absorption was approximately 30% during the first 2 h after radiolabel dosing (71.5% radioactivity remained in jejunal loop).

In exploratory, non-GLP studies, oral bioavailability ranged from approximately 42 to 73% in rats and dogs and > 75% in monkeys after treatment with a variety of SYR-322 salts. Bioavailability was highest from the benzoate salt, which led to the benzoate salt as the choice for the drug substance.

Summaries of GLP-compliant studies showing oral bioavailability and summary PK parameters in rat and dog following a single oral dose of SYR-322 are shown below.

Table 1 Pharmacokinetic parameters of radioactivity in the plasma of male rats after single oral and intravenous administrarion of [^{14}C]SYR-322

Administration route	Pharmacokinetic parameter				
	C_{max} (μg equiv./mL)	T_{max} (hour)	$t_{1/2}$ (hour)	$AUC_{0\text{-}24\ h}$ (μg equiv. · h/mL)	F_{app} (%)
Oral	0.145 ± 0.019	5.0 ± 1.7	4.9 ± 0.2	1.235 ± 0.030	61.1 ± 4.1
Intravenous	-	-	3.4 ± 0.3	0.674 ± 0.042	-

Each value represents the mean ± S.D. for three animals.
- : Not determined.
Oral dose : 3 mg/kg as free base
Intravenous dose : 1 mg/kg as free base

Table 1 Pharmacokinetic parameters of radioactivity in the plasma of male dogs after single administration of [^{14}C]SYR-322

Pharmacokinetic parameter	Administration route	
	Oral	Intravenous
C_{max} (μg equiv./mL)	0.653 ± 0.148	-
T_{max} (hour)	2.0 ± 0.8	-
$t_{1/2}$ (hour)	6.7 ± 0.7	5.3 ± 0.1
$AUC_{0\text{-}24\ h}$ (μg equiv. · h/mL)	4.785 ± 1.012	1.800 ± 0.061
F_{app} (%)	88.6 ± 19.0	-

Each value represents the mean ± S.D. for four animals.
- : Not determined.
Oral dose : 3 mg/kg (as free base)
Intravenous dose : 1 mg/kg (as free base)

2.6.4.4 Distribution

Alogliptin was widely distributed in rat after a single oral [^{14}C]SYR-322 dose. There was limited radioactivity in brain and CNS, showing limited potential for drug or metabolites to cross the blood-brain barrier. Tissue T_{max} was approximately 4 h for most tissues. In the rat, 35-41% of administered dose was localized to blood cells at 24 h post-dose. As noted above, similar distribution to blood cells was observed in dogs, with a maximum 38% of dose in blood cells at 1 h post-dose and 23% remaining in dog blood cells at 8 h post-dose.

Drug was moderately protein bound in all species, with binding dependent on plasma drug concentration (24 – 52%). Human protein binding ranged from 28-32% from 10 μg/ml to 0.01 μg/ml [SYR-322]. See reviewer and sponsor summary tables, below.

SYR-322 Plasma Protein Binding †	
Species	**Protein Binding**
Human	32%
Rat	36%
Dog	27%
† SYR-322 plasma protein binding determined *in vitro* (ultrafiltration method) based on protein binding at 0.1 μg/ml (approximate C_{max} = 140 ng/ml at human dose of 25 mg QD)	

Table 4.c In Vitro Plasma Protein Binding of Alogliptin and M-I

Species	Alogliptin Bound %		M-I Bound %		
	10 μmol/L	100 μmol/L	1 μmol/L	10 μmol/L	100 μmol/L
Mouse	29.6 (b)	24.1 (b)	23.1 (b)	12.9 (b)	11.7 (b)
Rat	40 (a)	24 (a)	21.7 (b)	11.7 (b)	21.3 (b)
Dog	24 (a)	23 (a)	27.6 (b)	13.4 (b)	13.4 (b)
Human	24 (a)	15 (a)	12.2 (b)	13.8 (b)	32.3 (b)

Source: (a) 322/00016 and (b) 322/00030

PK parameters were similar in both sexes. Drug was rapidly absorbed with $T_{max} \leq 0.5$ h. Approximately 35-40% of drug equivalents were associated with blood cells during the first 24 h during the major exposure period. Clearance from blood cells was similar, but slightly slower, than from plasma. Nearly 95% of drug equivalents were excreted within 24 h, with $t_{1/2} \leq 4$ h.

Drug concentrations were highest (≥ 7-fold higher than plasma) in bile, kidney, renal medulla, liver, and lacrimal glands during the first 4 h post-dose. No drug was apparent in CNS (brain or spinal cord). Relatively high drug concentration (~10% of C_{max}) persisted in kidney and renal medulla for 48 h. Drug accumulated in eye and uveal tract and elimination from skin was slow in pigmented Long Evans rats (see summary table from original IND review, below). Accumulation in sclera of the eye in pigmented, but not albino, rats was notable. Drug remained in sclera for at least 8 weeks, which suggests the compound or metabolite has a high affinity for melanin. The sponsor conducted a follow-up phototoxicity study to help characterize potential risks of melanin binding and results showed there was no evidence of drug-induced phototoxicity (see Section 2.6.6.8).

ng Equivalents of ^{14}C-SYR110322 in eye of long evans rats									
hours post-dose	0.5h	1h	2h	4h	8h	24h	48h	96h	168h
Eye	2310	2630	3770	2510	5120	5210	5440	4390	4510
Uveal tract	9250	12400	19900	18200	18100	22100	23100	22300	19000
Skin	3000	2010	2070	1620	1240	461	568	611	BLQ

2.6.4.5 Metabolism

Alogliptin is relatively stable in humans and extent of metabolism is low in *in vitro* microsome and hepatocyte incubations. Eight metabolites (M1-M8) were identified in all species, *in vitro* and in plasma, with MI being the predominant metabolite. Relative distribution of SYR-322 metabolites from incubations of hepatocytes from various species are shown in sponsor's Table 1.

Metabolism is more extensive in animals *in vivo* compared to *in vitro* trends. *In vivo* metabolism is lowest in humans (7-8%) and monkey (10%), intermediate in rat (33%) and greatest in dog (63%), with respect to total metabolism of parent drug.

Table 1: Relative distribution of SYR110322 metabolites in hepatocytes (10 µM SYR110322 incubations for 3 hr)

Species	Relative % Distribution[a]								
	SYR110322	M1	M2	M3	M4	M5	M6	M7	M8
Human	99.48	0.46	0.01	0.00	0.00	0.03	0.00	0.01	0.00
Rat	79.55	19.60	0.23	0.28	0.00	0.01	0.16	0.14	0.04
Dog	80.07	17.75	0.65	0.61	0.00	0.00	0.44	0.04	0.44
Monkey	94.76	4.53	0.05	0.02	0.00	0.26	0.26	0.04	0.07

[a]The peak area of the individual metabolite was divided by the sum of the peak areas from all the metabolites in a sample, and multiplied by 100 to give the relative percent distribution of the individual metabolite.

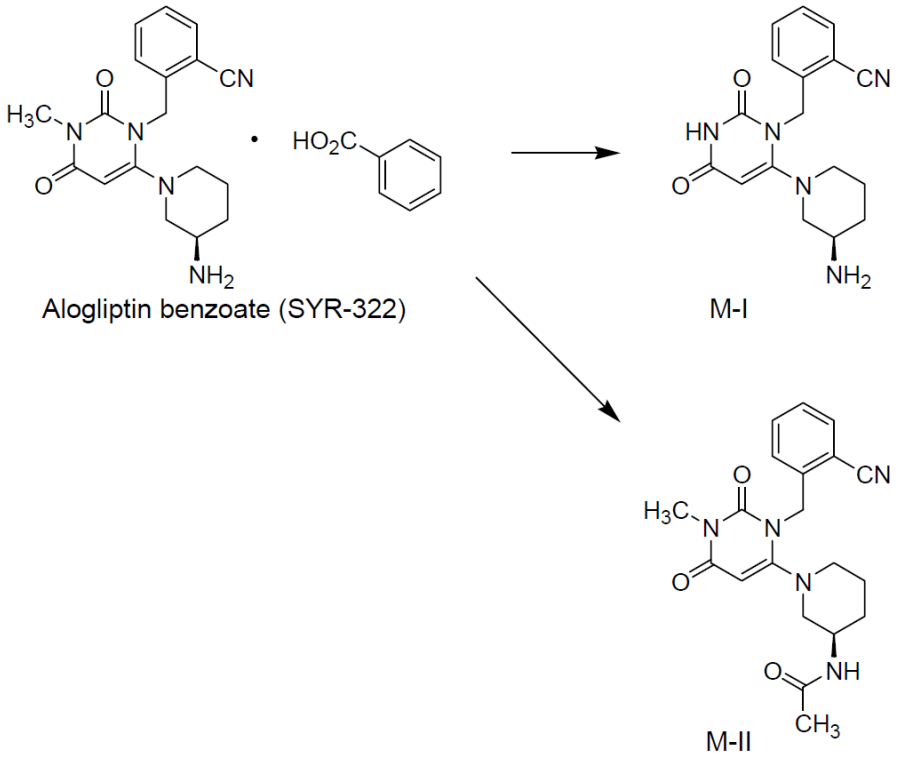
Production of M-I (SYR-324) was assessed compared to parent drug after repeat dose alogliptin treatment in dogs and rats. M-I accounts for 50-60% of total exposure to alogliptin ($AUC_{0\text{-}24\ h}$) and for 30-40% of the C_{max} in rats and dogs. M-I metabolism in female rats was approximately half that in males. Monkey metabolism was limited (similar to human), with M-I accounting for about 3% of AUC and 2% of C_{max} of parent, although the half-life is about two times longer in monkey (8 h) than in dogs or rats (3-4 h). See sponsor's summary in Table 7, below.

Table 7 Comparison of SYR110324 and SYR110322 in Rats, Dogs, and Monkeys

Species/Sex	SYR110322		SYR110324		Ratio of 324/322 (%)	
Rat (Day 28, 30 mg/kg)	C_{max} ng/mL	$AUC_{0\text{-}24}$ h • ng/mL	C_{max} ng/mL	$AUC_{0\text{-}24}$ h • ng/mL	C_{max} ng/mL	$AUC_{0\text{-}24}$ h • ng/mL
Male	994	5266	400	2911	40.2	55.3
Female	2417	9878	547	2651	22.6	26.8
Dog (Day 26, 25 mg/kg)						
Male	4980	32077	1583	16850	31.8	52.5
Female	6643	29282	2347	19374	35.3	66.2
Monkey (Day 1, 25 mg/kg)						
Male	9600	50795	178	1694	1.9	3.3

The sponsor's proposed metabolic pathway for alogliptin is shown in Figure 3.b.

Figure 3.b Proposed Metabolic Pathways of Alogliptin



No unique human metabolites have been identified. A species comparison on metabolites is shown in the sponsor's Table 3.d.

Table 3.d Comparison of Metabolites Across Species

Compound	Human	Rat	Dog	Monkey
alogliptin	P, U, F	P, U, F, B	P, U, F	P (a)
M-I	P, U, F	P, U, F, B	P, U, F	P (a)
M-II	P, U, F	P, F	P	P (a)
Reference	322-ADME-014	322/00113.002A, 322/00129, 322/00131 322/00132.001A, 322/00180.001A 322/00182, 322/00022	322/00134 322/00181.001A 322/00183 322/00022	322/00022

P=plasma (serum used for human metabolite analysis), U=urine, F=feces, B=bile.
(a) Only plasma samples were profiled for metabolite identification.

Alogliptin was incubated with a mixture of CYPs and with purified individual recombinant human CYP450 enzymes to characterize individual enzymes responsible for drug metabolism. CYP2D6 was found to be primarily responsible for the major MI metabolite. CYP3A4 was responsible for production of the other main metabolite, MII, and for minor metabolites M3, M6, and M8 (see sponsor's summary table, below).

Summary of Cytochrome P450 Enzyme(s) Responsible for Generating SYR110322 Metabolites

Metabolite	Primary CYP enzyme*	Minor CYP enzymes
M1 [MH-14]+	**2D6**	**3A4, 2C19, 2E1**
M3 [MH-2]+	**3A4**	2C19, 2E1, 2D6
M8 [MH+14]+	----	3A4, 2C19, 2D6
M2, M6 [MH+16]+	3A4	

Potential for chiral inversion of alogliptin (R-enantiomer of SYR-322) was investigated in rats and dogs given a single oral gavage dose (3 mg/kg) containing [^{14}C]SYR-322. Formulation purity was noted as 100% (R)-SYR-322 by the sponsor, although the study report showed 99.7% purity. Nevertheless, SYR-322 was confirmed to be nearly 100% (R) enantiomer. No (S)-SYR-322 was detected in plasma or urine of animals, suggesting no quantifiable chiral inversion *in vivo*. In addition to the dedicated single dose study, the sponsor submitted supplemental toxicokinetic data from 13-week rat and dog studies and a single-dose study in humans. Exposure to (S)-SYR-322 was ≤ 0.1% in rat, dog, and human. Generally, the level of (S)-enantiomer seen in plasma was equivalent to the amount of (S)-enantiomer impurity in SYR-322 production lots, which provided further evidence that chiral inversion was not significant *in vivo*. Such low exposure to the (S)-enantiomer compared to the more active (R)-form is not expected to contribute to the pharmacological activity of the drug substance.

To investigate which drug equivalents have high affinity for melanin, pigmented rats were given a single oral gavage dose (3 mg/kg) of [^{14}C]SYR-322. Radioactivity in the sclera of the eye was quantified and drug- and metabolites were characterized in sclera (in which drug-accumulation was seen in previous studies). Parent SYR-322 accounted for 74% and 67.5% of total administered radioactivity at 24 and 168 h post-dose, respectively. M-I metabolite was approximately 7.5% at 24 and 168 h post-dose, with limited M-II present.

PK parameters for alogliptin and metabolites M-I and M-II were assessed in rats given a single oral (3 mg/kg) or *iv* (1 mg/kg) dose of radiolabeled SYR-322. Majority of radioactive dose was unchanged SYR-322 for both exposure routes. M-I was the major metabolite after oral dosing, whereas neither M-I nor M-II were detected after *iv* dosing. F_{app} apparent absorption ratio (oral) was ~46% (with 61% of total ^{14}C absorbed).

Table 1 Pharmacokinetic parameters of SYR-322 and its metabolites in the plasma of male rats after single oral and intravenous administration of [^{14}C]SYR-322

Component	Pharmacokinetic parameter				
	T_{max} (hour)	C_{max} (μg equiv./mL)	$AUC_{0\text{-}24\ h}$ (μg equiv. · h/mL)		F_{app} (%)
			Oral	Intravenous	
Total ^{14}C	5.0	0.145	1.235 (100.0)	0.674 (100.0)	61.1
SYR-322	3.0	0.055	0.627 (50.8)	0.451 (66.9)	46.3
M-I	6.0	0.037	0.429 (34.7)	NC (NC)	-
M-II	ND	ND	NC (NC)	NC (NC)	-

ND : Not detected.
NC : Not calculated.
Oral dose : 3 mg/kg (as free base)
Intravenous dose : 1 mg/kg (as free base)
Pharmacokinetic parameters of total ^{14}C were obtained in the study numbered SYR-322 (8).
Figures in parentheses denote % of total ^{14}C.

PK parameters for parent and metabolites M-I and M-II were also assessed in male dogs given a single oral (3 mg/kg) or *iv* (1 mg/kg) dose of radiolabeled SYR-322. The majority of the radioactive dose was unchanged SYR-322 for both exposure routes. In the *iv* group a small fraction was metabolized to M-I (~5%), but M-II was not detected. Neither M-I nor M-II were detected after oral dosing. F_{app} apparent absorption ratio (oral) was ~68% (with ~89% of total ^{14}C absorbed).

Table 1 Pharmacokinetic parameters of SYR-322 and its metabolite in the plasma of male dogs after single oral and intravenous administration of [^{14}C]SYR-322

Component	Pharmacokinetic parameter				
	T_{max} (hour)	C_{max} (μg equiv./mL)	$AUC_{0\text{-}24\ h}$ (μg equiv. · h/mL)		F_{app} (%)
			Oral	Intravenous	
Total ^{14}C	2.0	0.653	4.785 (100.0)	1.800 (100.0)	88.6
SYR-322	1.0	0.404	1.259 (26.3)	0.613 (34.1)	68.5
M-I	ND	ND	NC (NC)	0.093 (5.2)	-
M-II	ND	ND	NC (NC)	NC (NC)	-

ND : Not detected.
NC : Not calculated.
Oral dose : 3 mg/kg (as free base)
Intravenous dose : 1 mg/kg (as free base)
Pharmacokinetic parameters of total ^{14}C were obtained in the study numbered SYR-322 (13).
Figures in parentheses denote % of total ^{14}C.

Metabolism was also investigated after administration of [^{14}C]-SYR-322 into the jejunal loop in rat. Similar to results noted in Section 2.6.4.4, radiolabeled equivalents were absorbed into the portal plasma. Unchanged SYR-322 accounted for 91% and 100% of radioactivity at 0.5 and 2 h post-dose. No M-I or M-II metabolites were detected. Overall approximately 33% of the administered dose was absorbed from the jejunal loop by 2 h post-dose and metabolism of parent compound was limited.

2.6.4.6 Excretion

The sponsor summarized overall excretion from single dose rat and dog studies in Table 3.e.

Table 3.e Excretion of Total Radioactivity Across Species Following a Single PO Dose of [^{14}C]Alogliptin BZ

Species	Sex	Dose (mg/kg freebase)	Percent of Dose Excreted (%)			Report Number
			Urine	Feces	Total	
Rat	M	30	32.1	62.7	96.2	322/00116
Rat	F	30	37.5	53.8	95.5	322/00116
Rat	M	3	34.3	64.4	98.7 (a)	322/00131
Dog	M	3	40.4	58.8	99.2	322/00134

(a) The excretion into the expired air was not detected.

Excretion was investigated in a series of *in vivo* studies in dog and rat.

A single oral dose study with radiolabeled [^{14}C]SYR-322 in dogs showed 60% of drug equivalents were excreted at 24 h post-dose (38% in urine and 22% in feces). By 72 h post-dose, approximately 97% of administered dose was excreted with 40% in urine and 58% in feces. Metabolites (M-I, M-II, and other unidentified) and parent drug were quantified from urine and feces collected over 72 h. Unidentified metabolites were the predominant compounds found in urine (20% administered) and feces (45% administered). Unchanged SYR-322 and M-I were also excreted in urine (13% and 7%, respectively) and feces (both 6%) in relation to administered dose. M-II was not detected in either urine or feces.

PK and elimination of radiolabeled SYR-322 equivalents were also determined in intact and bile-duct cannulated male and female dogs given a single oral capsule of [^{14}C]SYR-322). Consistent with other studies, drug equivalents showed moderate association with blood cells (up to 50%) in intact and bile-duct cannulated animals. Urinary elimination was the main excretion route in both groups. Approximately 53% of administered dose was found in urine of intact dogs, compared to 27-35% in feces over 168 h (84-92% of total dose recovered) in intact dogs. Approximately 51% of administered dose was found in urine in bile-duct cannulated dogs, compared to 23-35% in bile and 11-21% in feces over 96 h (96-99% of total dose recovered). Elimination was essentially complete by 48 h post-dose in intact dogs and 24 h post-dose in bile-duct cannulated dogs. Total absorption was estimated at 86% in males and 74% in females, with $T_{max} \approx$ 1-2 h.

In separate single oral dose studies in rats with radiolabeled [^{14}C]SYR-322) approximately 98% of the administered dose was recovered by 24 h post-dose, with 34-50% in urine, 47-64% in feces. Radioactivity was undetectable in expired air and there was no appreciable radioactivity (< 0.1%) detected in lymph over a 24 h post-dose period or in gastric contents at 24 h post-dose. Minimal radioactivity was found in intestinal contents (1.9%) at 24 h post-dose

Rats were also given a single intraduodenal administration of [^{14}C]SYR-322) to directly examine intestinal excretion trends. At 24 h post-dose, 100% of drug was accounted for, with the majority excreted in urine (44%) and the balance distributed to bile (30%) and feces (26%) in bile duct-cannulated rats. Unchanged SYR-322 and M-I were the main compounds in urine (17% and 9% of administered, respectively) and feces (47% and 8% of administered, respectively). A minor amount of M-II (1%) was excreted in feces, but none was excreted in urine. Unidentified metabolites accounted for 8% of administered dose in both urine and feces. In bile, the majority of radiolabeled drug equivalents consisted of unidentified metabolites (20%), plus SYR-322 (6%) and M-I (4%), with no M-II collected.

Drug equivalents were partially reabsorbed by enterohepatic circulation, as determined by intraduodenal administration of naïve bile-duct cannulated rats with bile from rats previously exposed to radiolabeled SYR-322. At 24 h post-dose, radiolabeled equivalents were found in bile (11%) and urine (11%), showing at least 22% of SYR-322 or metabolites were reabsorbed and entered into enterohepatic circulation. At 2 h post-dose, 73% of radiolabel from the administered bile was excreted in feces.

Secretion of SYR-322 in milk was assessed in lactating rats given a single oral (3 mg/kg) dose of [^{14}C]SYR-322. SYR-322 equivalents were secreted in milk, with a C_{max} of 0.5 h and a two-phase elimination from milk (rapid for 24 h, slower between 24-48 h). Maximum milk concentrations were higher than maximum plasma concentrations and elimination curves were similar (Figure 1, below). Milk concentrations were approximately 2-fold higher than maternal plasma throughout the elimination phase until nominal SYR-322 remained in milk and plasma at 48 h post-dose.

Metabolite composition in maternal plasma and milk was investigated in a separate, similar study. M-I accounted for approximately 30 to 40% of drug equivalents at 4 and 8 h post-dose. M-II was not detected in milk. Neither M-I nor M-II were found in maternal plasma, while approximately 88% and 100% of plasma equivalents of the administered dose were parent drug after 0.5 h and 4 h.

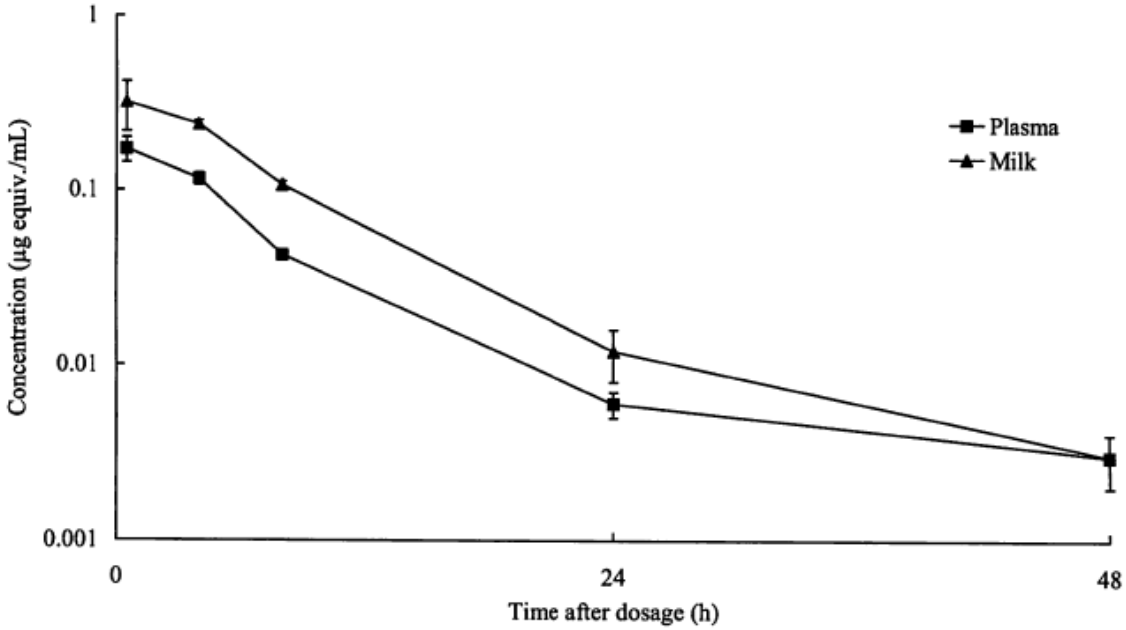


Figure 1 Concentrations of radioactivity in the plasma and milk of lactating rats after a single oral administration of [^{14}C]SYR-322 at a dose of 4.08 mg/kg (3 mg/kg as free base).

Each point with a vertical line represents the mean ± S.D. for three animals.

2.6.4.7 Pharmacokinetic drug interactions

The potential for SYR-322 or metabolites (generated by liver microsomes) to inhibit human cytochrome P450 (CYP) enzyme activity was assessed *in vitro* in pooled human liver microsomes. SYR-322 showed no inhibitory potential at concentrations up to 100 μM with the exception of CYP3A4/5. The IC_{50} was 78 μM for CYP3A4/5 inhibition, showing low potency inhibition.

SYR-322 does not inhibit human CYP enzymes at clinically relevant doses. The approximate C_{max} is 400 nM at the maximum recommended human dose of 25 mg, which is 195-fold lower than the *in vitro* IC_{50} for CYP3A4/5 inhibition.

CYP induction potential of alogliptin was assessed *in vitro* with primary human hepatocyte cultures and CYP-specific substrates. Alogliptin increased CYP3A4/5 activity approximately 2- and 6-fold at 2- and 200-times the MRHD, respectively. Alogliptin did not induced other standard CYPs in hepatocytes at up to 200-times the MRHD.

Co-incubations with other anti-diabetic agents (glyburide, glipizide, and pioglitazone) in human liver microsomes showed no effects on alogliptin half-life or intrinsic clearance.

Potential for clinical drug-drug interactions with alogliptin relative to Phase I metabolism is considered low based on *in vitro* findings.

2.6.4.8 Other Pharmacokinetic Studies

Pregnant rats were given a single oral gavage dose of [^{14}C]SYR-322 to investigate maternal transfer to the fetus. Radiolabeled drug equivalents were readily absorbed and circulated in maternal plasma, with placental and fetal drug exposure following similar trends as maternal plasma. Placental concentrations were highest and fetal plasma levels were 2- to 5-fold lower than maternal plasma. Results showed SYR-322 readily crosses the placenta, resulting in fetal exposure (see Figure 1). The major metabolite in maternal plasma was M-I, which reached up to 41% administered at 8 h post-dose. M-II was not identified in maternal plasma. In contrast, M-II was the major metabolite identified in fetal plasma, reaching 30% at 4 h but absent by 8 h post-dose. M-I was not detected in fetal plasma.

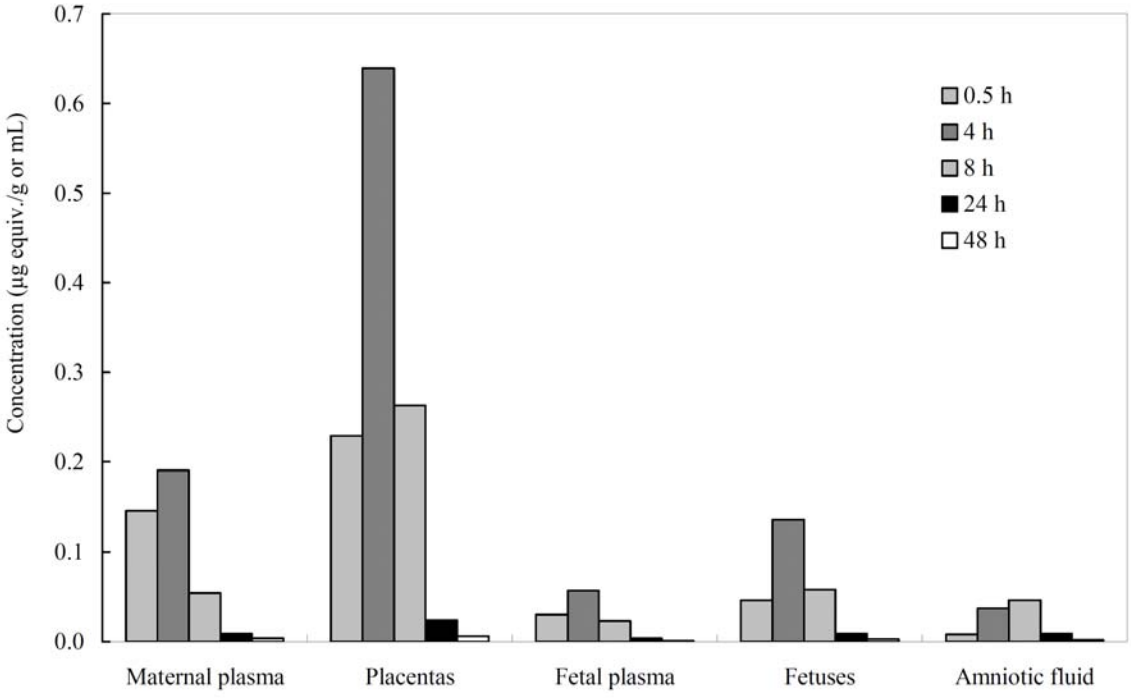


Figure 1 Concentrations of radioactivity in pregnant rats after single oral administration of [^{14}C]SYR-322 at a dose of 4.08 mg/kg (3 mg/kg as free base).

Each value represents the mean for three animals.

2.6.4.9 Discussion and Conclusions

See PK/TK summary in Section 2.6.4.1 above.

2.6.4.10 Tables and figures to include comparative TK summary

The sponsor summarized pharmacokinetic parameters from the wide variety of *in vivo* animal studies after oral (Table 3.a) and *iv* (Table 3.b) exposure.

Table 3.a Pharmacokinetic Parameters of Alogliptin Following PO Administration

Species	Sex	Dose (a) (mg/kg)	AUC(0-inf) (ng·hr/mL)	Cmax (ng/mL)	Tmax (hr)	T1/2 (hr)	BA (%)	Report No. [Reference]
Rat	M	8.1 (b,c)	1240±115	393±47.3	2.3±0.6	2.77±2.50	45.3	322/00019
Rat	M	20 (b,c)	2821±250	1192±189.5	1.7±0.3	1.04±0.15	41.8	322/00019
Dog	M	2.9 (d,e)	1612±509	658±207	0.417±0.14	3.04±0.748	66.8	322/00017
Dog	M	2.7 (b,f)	950±584	447±205	0.4±0.14	1.5±0.41	84.2	322/00018
Dog	M	2.3 (f,g)	819±216	430±140	0.4±0.14	1.75±0.39	85.2	322/00018
Dog	M	2.1 (b,h)	699±493	278±175	0.75±0.50	1.75±0.50	71	322/00020
Dog	M	2.1 (g,h)	722±229	293±113	0.9±0.55	3.48±1.87	73	322/00020
Monkey	M	1.7 (f,i)	2331±198	467±70	1±0.4	6.97±0.74	72	322/00008
Monkey	M	10 (f,i)	16633±2519	3208±1430	1±0.4	5.66±0.23	87	322/00008
Monkey	M	32 (f,i)	53638±3631	9600±4159	2.1±1.1	5.46±1.34	88	322/00008

BA=bioavailability, M=male.
(a) As active moiety.
(b) Vehicle was 4% ethanol in water.
(c) Administered as the TFA salt.
(d) Vehicle was 4% ethanol in saline.
(e) Administered as the HCl salt.
(f) Administered as the BZ salt.
(g) Administered in a gelatin capsule.
(h) Administered as the TS salt.
(i) Aqueous solution.

Table 3.b Pharmacokinetic Parameters of Alogliptin Following IV Administration

Species	Sex	Dose (a) (mg/kg)	AUC(0-inf) (ng·hr/mL)	Vd (mL/kg)	CL (mL/kg/hr)	T1/2 (hr)	Report No. [Reference]
Rat	M	2 (b,c)	623±39	3560±92	3287±179	1.14±0.03	322/00019
Rat	M	0.78 (b,d)	263±30	3516±650	2972±304	1.42±0.52	322/00019
Dog	M	0.94 (c,e)	786±273	3307±198	1295±422	2.93±0.84	322/00017
Dog	M	0.89 (b,f)	372±68.0	3853±347	2435±384	1.50±0.04	322/00018
Dog	M	0.62 (b,g)	291±49.0	3930±480	2165±333	1.65±0.13	322/00020
Monkey	M	1.1 (f,h)	2103±250	2602±561	528.7±64.2	5.74±1.70	322/00008

CL=clearance, M=male, Vd=apparent volume of distribution.
(a) As active moiety.
(b) Vehicle was 4% ethanol in water.
(c) Administered as the HCl salt.
(d) Administered as the TFA salt.
(e) Vehicle was 4% ethanol in saline.
(f) Administered as the BZ salt.
(g) Administered as the TS salt.
(h) Vehicle was sterile saline for injection.

Throughout this review exposure margins are shown based on FDA reviewer calculations. Exposure margin estimates may differ slightly between FDA values and the sponsor's values, likely due to calculations using approximate values (e.g. rounded off) or values with different numbers of significant figures. The sponsor's own calculations of

exposure margins in various species treated for 13- to 39-weeks were based on the following summary table (Table 4.a).

Table 4.a Mean Plasma AUC(0-24) Levels of Alogliptin at Nonclinical Doses Used in the Calculations of Exposure Margins

Species	Dose	Dosing Duration	AUC(0-24) (a) (µg·hr/mL)	Source Data Report No.
Mouse	400 mg/kg/day PO	13 weeks	117	322/00046.002A
Mouse	600 mg/kg/day PO	13 weeks	180	322/00046.002A
Rat	250 mg/kg/day PO	13 weeks	165 (b,c)	322.00045.002A
Rat	400 mg/kg/day PO	26 weeks	259	322/00088.002A
Rat	500 mg/kg/day PO	13 weeks	274 (b,d)	322/00045.002A
Rat	500 mg/kg/day PO	13 weeks	330 (c,d)	322/00045.002A
Rat	900 mg/kg/day PO	26 weeks	664	322/00088.002A
Rat	1000 mg/kg/day PO	13 weeks	605	322/00045.002A
Rat	1000 mg/kg/day PO	13 weeks	661 (c)	322/00045.002A
Dog	200 mg/kg/day PO	39 weeks	400	322/00089.002A
Monkey	30 mg/kg/day PO	13-weeks	47	322/00243

(a) Unless specified, values are for combined sexes.
(b) Extrapolated from AUC(0-24) values measured at 1000 mg/kg/day.
(c) Females only.
(d) Males only.

A brief summary and tables of SYR-322 and M-I exposure from single oral dose studies in monkey and human are included here. Exposure to M-I in monkey was approximately 2-4% that of parent drug after a single oral dose. Human M-I exposure accounted for approximately 1% of parent drug exposure after a single oral dose. Monkey M-I exposure was similar to mice and slightly higher than humans. Sponsor summary tables for single oral dose monkey and human SYR-322 and M-I exposure are shown below.

Sponsor's SYR-322 to M-I Comparison in Monkey

Species/Sex	SYR110322		SYR110324		Ratio of 324/322 (%)	
	C_{max} ng/mL	AUC_{0-24} h • ng/mL	C_{max} ng/mL	AUC_{0-24} h • ng/mL	C_{max} ng/mL	AUC_{0-24} h • ng/mL
Monkey (Day 1, 25 mg/kg)						
Male	9600	50795	178	1694	1.9	3.3

Sponsor's SYR-322 to M-I Comparison in Human

Table 11. Systemic Exposure ($AUC_{0-\infty}$) Estimates for SYR110322 and Metabolite (SYR110324)

	Dose of SYR110322 (N=5)					
Parameter	25 mg	50 mg	100 mg	200 mg	400 mg	800 mg
SYR110322 AUC[a] (mean)	1327	3139	5040	13711	22816	49595
Metabolite (SYR110324) AUC[a] (mean)	14.5	42.2	47.5	192.9	132.8	414.2

[a]AUC estimates for both SYR110322 and its active metabolite (SYR110324) were determined over the time interval 0 to infinity and are expressed as ng•h/mL.

Summary tables showing dose relationships for PK parameters for SYR-322, M-I, and M-II after a single oral administration in rat and dog are shown below. Exposure to SYR-322 and metabolites (C_{max} and AUC) increased dose-proportionally at low doses in dogs (0.3 to 3 mg/kg range) and greater than dose-proportionally in rats (3 to 100 mg/kg range) and at high doses in dogs (3 to 30 mg/kg range).

Table 1 Pharmacokinetic parameters of SYR-322Z, M-I, and M-II in rat plasma after single oral administration of SYR-322

Dose (mg/kg, as SYR-322Z)	Compound	T_{max} (h)	C_{max} (ng/mL)	$t_{1/2}$ (h)	AUC_{0-24h} (ng·h/mL)	AUC/Dose (10^{-6} kg·h/mL)
3	SYR-322Z	0.7 ± 0.3	68.1 ± 9.8	3.4 ± 0.3[#1]	368 ± 57	123 ± 19
	M-I	2.0 ± 1.7	33.6 ± 1.4	---	287 ± 28	95 ± 9
	M-II	1.8 ± 1.9	1.63 ± 0.42	---	6 ± 2	2 ± 1
30	SYR-322Z	0.8 ± 0.3	1430 ± 380	3.4 ± 0.4[#2]	7484 ± 1159	250 ± 39
	M-I	2.3 ± 1.5	499 ± 42	---	4461 ± 612	149 ± 20
	M-II	1.0 ± 0.0	36.1 ± 4.9	---	205 ± 52	7 ± 2
100	SYR-322Z	0.8 ± 0.3	6330 ± 1250	4.1 ± 0.6[#2]	40501 ± 7410	405 ± 74
	M-I	2.7 ± 0.6	991 ± 33	---	11072 ± 720	111 ± 8
	M-II	1.3 ± 0.6	145 ± 37	---	990 ± 199	10 ± 2

Mean value±S.D. (n=3). ---; Not estimated.
$t_{1/2}$; #1: T_{max}-8 h., #2: T_{max}-24 h.

Table 1 Pharmacokinetic parameters of SYR-322Z and its metabolite in the plasma of dogs after single oral administration of SYR-322

Dose (mg/kg as SYR-322Z)	Compound	T_{max} (h)	C_{max} (ng/mL)	$t_{1/2}$ (h)	$AUC_{0-24\,h}$ (ng · h/mL)	AUC/Dose (10^{-6} kg · h/mL)
0.3	SYR-322Z	1.0 ± 0.7	25.2 ± 5.74	1.9 ± 0.4[#1]	89 ± 22	298 ± 74
	M-I	1.3 ± 0.5	10.2 ± 2.81	3.6 ± 1.1[#2]	63 ± 25	211 ± 83
	M-II	NC	NC	NC	NC	NC
3	SYR-322Z	0.9 ± 0.3	244 ± 97.6	3.6 ± 3.8[#3]	991 ± 121	330 ± 40
	M-I	1.8 ± 1.5	115 ± 48.4	4.2 ± 0.7[#4]	754 ± 96	252 ± 32
	M-II	NC	NC	NC	NC	NC
30	SYR-322Z	2.8 ± 0.5	3470 ± 1060	3.6 ± 0.8[#4]	32896 ± 6057	1097 ± 202
	M-I	3.3 ± 0.5	1600 ± 389	5.6 ± 1.4[#4]	19018 ± 1916	634 ± 64
	M-II	NC	NC	NC	NC	NC

NC: Not calculated.
Each value represents the mean ± SD for four animals.
Time points used for the calculation of $t_{1/2}$ were as follows.
$t_{1/2}$; #1: T_{max}-8 h. (Animal No. 1, 2), T_{max}-6 h. (Animal No. 3, 4), #2: T_{max}-24 h. (Animal No. 1), T_{max}-8 h. (Animal No. 2, 3, 4), #3: T_{max}-8 h., #4: T_{max}-24 h.

The sponsor used archived plasma samples from one dose in one month rat and dog and single dose monkey studies to quantify M-I. The summary data are shown in the sponsor's table, below.

Table 1: Toxicokinetic Parameters for SYR110324 in Male and Female Rats and Dogs and in Male Monkeys After Oral (Gavage) Administration of SYR110322

Parameters	Gender	Rats - Day 28* 30 mg/kg	Dogs - Day 26 25 mg/kg		Monkeys – Day 1 30 mg/kg	
			Mean	CV%	Mean	CV%
Cmax (ng/mL)	Male	400	1583	17.1	178	42.8
	Female	547	2347	14.5	NA	NA
	Combined	NA	1965	25.5	NA	NA
Tmax** (hr)	Male	1.00	2.00 (2.00-4.00)		1.75 (1.50-4.00)	
	Female	1.00	2.00 (2.00-2.00)		NA	NA
	Combined	NA	2.00 (2.00-4.00)		NA	NA
T½ (hr)	Male	3.60	4.68	4.3	7.66	15.1
	Female	3.07	3.93	9.5	NA	NA
	Combined	NA	4.31	11.4	NA	NA
AUC(0-24) (ng·hr/mL)	Male	2911	16850	11.9	1694	42.2
	Female	2651	19374	7.4	NA	NA
	Combined	NA	18112	11.5	NA	NA

*PK parameters were determined from the composite profile for male and female rats.
**Median and range are reported for Tmax.
NA = Not Applicable
n = 3 per gender in dogs and rats
n = 4 for monkeys (all male)

2.6.5 PHARMACOKINETICS TABULATED SUMMARY

None.

2.6.6 TOXICOLOGY

2.6.6.1 Overall toxicology summary

General toxicology: All pivotal non-clinical toxicology studies used to support clinical safety were verified to be GLP-compliant. All studies discussed in this review were orally administered in Sprague Dawley rat, Beagle dog, CD-1 mouse, or *Cynomolgus* monkey unless otherwise noted. Various alogliptin salt forms including hydrochloride, trifluoracetate, and tosylated alogliptin salts were used in early non-clinical studies. The sponsor concluded the benzoate salt form was best suited for clinical development and all pivotal toxicology studies were conducted with the alogliptin benzoate salt formulation.

Alogliptin was well tolerated up to doses on the order of tens- to hundreds-fold higher than expected human exposures. Drug-related mortality was evident only in multiple dose studies and only at high exposure multiples (generally > 50-fold MRHD). Clinical signs were not consistent across species, but sporadic signs in alogliptin treated rodents included salivation, emesis, discolored fur, alopecia, and unkempt appearance. Dogs showed consistent but intermittent clinical signs of reddened ears, face, and body and facial swelling/flushing. Signs in dogs were tolerated throughout multiple dosing for up to nine months and may be evidence of a hypersensitivity or pseudoallergy-type response. Body weights were typically lower than controls after repeat dosing at very high exposure multiples (generally > 100-fold MRHD). There were no treatment-related deaths attributed to decreased body weight, although clinical chemistry biomarkers indicative of general stress were seen in some groups with decreased body weight.

Major target organs identified at high multiples of human exposure are testes (and male reproductive tissues), kidney, liver, urinary bladder, and lung. With the exception of lung histiocytosis, toxicity in target organs seen in chronic studies was seen at high doses in short or moderate duration studies (up to 13 weeks). Target organs were all identified in rodent studies, as clinical signs and decreased body weight were the only notable findings in dogs (up to > 250-times human exposures) and no remarkable toxicity was seen in monkey studies up to 31-times expected human exposures. There was unexplained treatment related mortality in mice at approximately 50-times human exposure in one month and 13 week studies, but no target organs were identified in animals found dead or in mice treated with up to 60-times expected human exposure in the two year carcinogenicity study.

Testes abnormalities were seen sporadically at high doses in short duration rat studies and confirmed in chronic studies. In rat one month rat studies, low incidences of severe oligospermia were observed at doses of 300 mg/kg (low dose study) but not at doses of 1333 mg/kg and higher (high dose study). A single incidence of moderate seminiferous tubule degeneration/atrophy was observed in the 13 week rat study at 1000 mg/kg. Severe oligospermia was observed again at 400 and 900 mg/kg doses in the six month rat study. Results from the rat fertility and two year carcinogenicity studies showed clear testes-

related toxicity. Sperm production increased concomitant with an increase in abnormal sperm and increased epididymal weight, but did not affect fertility in rats treated with 1000 mg/kg alogliptin (> 200-fold exposure margin). In the two year rat study at 400 mg/kg or greater doses (283-fold exposure margin), there were gross increases in testes size and increased epididymal oligospermia and germ cell debris (0.75- to 2.5-fold), seminal vesicle depletion (2.5-fold), and testes bilateral seminiferous tubule degeneration/atrophy (up to 3-fold). Initial reviews of the short term rat studies considered the testes findings to be sporadic and not likely related to treatment, but findings from the two year study suggest sporadic testes toxicity in shorter term studies were likely drug-related. No male reproductive toxicity was seen in dogs but prostate weight increased in the four week monkey study and there was a possible, albeit unconfirmed, decrease in testes weight in high dose (31-times human exposure) male monkeys in the 13 week study.

Tubular degeneration and mineralization were evidence of kidney toxicity in the high dose 28-day study in rats. In 13- and 26-week rat studies there were increases in chronic progressive nephropathy, but the lesion is common in toxicity studies and relationship to treatment was unclear. The two year rat study confirmed up to a 2-fold increase over background rates in chronic progressive nephropathy (approximately 50% of animals), but only in females. There were also increases in tubular degeneration/regeneration and transitional cell hyperplasia in the two year rat study. Finally, while mortality did not increase in alogliptin treated groups in the two year rat study, mortality attributable to kidney toxicity was increased in treated groups. Increased kidney related mortality was consistent with kidney-related mortality in the high dose one month rat study. Increased biomarkers of kidney toxicity and kidney-related mortality in pregnant rabbits in the embryofetal development study further support kidney as a target organ. Overall, kidney toxicity in two year rat study occurred at doses that caused approximately 280-times the expected human exposure.

Bladder toxicity trends were similar to kidney trends. Bladder erosion/ulcer and hemorrhage were seen in the high dose one month rat study but not in other studies up to 26 weeks. In the two year rat study there were increases in gross calculus/calculi and simple transitional cell hyperplasia. Bladder findings were noted as the cause of death in rats found dead in both the high dose one month and two year rat studies.

Liver hypertrophy and increased liver weights were seen at high doses (generally ≥60-fold exposure margins) in rats exposed to alogliptin for four weeks or longer. In the two year rat study there was also evidence of periportal vacuolation and basophilic foci in liver. Liver trends generally seemed to be adaptive in nature and liver function enzymes were not markedly increased in the chronic rat studies, suggesting limited effects on liver function.

Lung findings were noted in six month and two year rat studies, but not in shorter term studies. Increased incidences of histiocytosis were seen histologically in both chronic rat studies and gross discoloration and focus/foci were noted in the two year study. Lung findings occurred at greater than 170-times expected human exposure.

Some DPP4 inhibitors have been shown to cause edema and necrotic skin lesions in various species. The FDA has required repeat dose monkey studies of at least 13 weeks duration to characterize the potential for drug-induced necrotic skin lesions in all investigational DPP4 inhibitor drugs. Edema and reddened skin in dogs seen early in the non-clinical development program suggested potential skin-related toxicity. Longer duration dog studies allayed concerns to some degree, since dogs tolerated the clinical signs and dosing was not limited by the skin-related signs. No remarkable skin lesions or skin-related toxicity were noted in rodent studies. Four- and thirteen-week monkey studies were designed specifically to examine the potential for drug-induced skin lesions. There were no was no evidence of drug-related skin lesions in clinical observations, macroscopic analyses at necropsy, or histological analyses at necropsy in either monkey study. The NOAEL for skin-related toxicity in the thirteen week monkey study was 30 mg/kg/day, which provided approximately 31-times expected human exposure.

It was evident from investigative and reproductive studies in rats and rabbits that alogliptin readily crosses the placenta and is secreted in milk. Fetal exposure was confirmed in rats and assumed in nursing rats based on good overall oral bioavailability and no evidence that alogliptin would not be absorbed from milk. Reproductive toxicity findings *in utero* and from post-natal exposure were minimal in animals, nevertheless, animal data support a conclusion that human fetuses and nursing infants will be exposed to alogliptin from maternal drug use.

Overall, toxicity was unremarkable up to at least 30-times the expected human exposure in all species tested, including mice, rats, and dogs treated chronically with alogliptin.

Genetic toxicology: Potential for alogliptin genotoxicity was assessed in a standard battery of *in vitro* and *in vivo* genetic toxicity assays. Results showed no apparent risk of genotoxicity. Alogliptin was negative for mutagenic activity in an Ames assay and for clastogenic activity *in vivo* in a mouse micronucleus assay.

In an initial mouse lymphoma L5178Y $TK^{+/-}$ assay, SYR-322 induced 2- to 3-fold increases in small colonies and equivocal increases in induced mutant frequency in a 4 h incubation without a metabolic activation system. Results were negative in a 4 h incubation with a rat liver S9 metabolic activation system and in a follow-up 24 h incubation assay without metabolic activation. The sponsor conducted a new, supplemental assay consisting solely of a 4 h incubation without a metabolic activation system and results were clearly negative. It was not clear why results of one mouse lymphoma assay were positive, nevertheless, the weight of evidence in a series of assays supported the conclusion that alogliptin was negative for mutagenic potential in the mouse lymphoma system.

Metabolites M-I and M-II were not specifically tested for genetic toxicity. Metabolite concentrations are low in humans and no specific genetic toxicity risk has been determined. Nevertheless, rat liver S9 metabolic activation system used in *in vitro* Ames and MLA assays should be sufficient to produce N-demethyl and N-acetyl metabolites. In

addition, M-I and M-II were detected in rat plasma under similar conditions as those used in the *in vivo* micronucleus assay, thus *in vivo* genotoxicity assessment should have been adequate to evaluate the contribution of M-I and M-II.

Based on negative results in the *in vitro* Ames and MLA and *in vivo* mouse micronucleus assays, alogliptin was considered negative for genotoxic potential and no further genetic toxicity assays were required.

Carcinogenicity: Results of 2-year oral carcinogenicity studies in mice and rats are summarized below. The conclusions are based on concurrence after a review of the data by the Executive Carcinogenicity Assessment Committee.

Mouse – NOAEL = 300 mg/kg/day (non-neoplastic and neoplastic findings); 60X MRHD. The high dose (300 mg/kg/day) in a 2-year oral carcinogenicity study in mice did not result in remarkable toxicity, however, exposure at the high dose provided 45-fold to 74-fold exposure margins (male and female, respectively) over the MRHD. Notable tumor findings were limited to a 5% incidence of benign hepatocellular adenomas in 300 mg/kg/day females (74X MRHD). There were no similar tumors in any other group (up to 45X MRHD in males, 34X MRHD in females). The data suggest the increase in female hepatocellular adenomas was drug-related, but based on an absence of malignant hepatocellular carcinomas in the high dose group and the fact that tumor incidence was within the historical range of some studies in CD-1 mice, the finding was not considered evidence of treatment-induced tumors. In addition, there were no increases in malignant hepatocellular carcinoma or additional histopathology correlates to suggest hyperplasia, pre-neoplastic lesions or extensive hepatotoxicity. SYR-322 poses minimal carcinogenic risk to humans based on the absence of drug-related tumors at high exposure multiples in mice compared to maximum recommended human exposure.

Rat – NOAEL = 75 mg/kg/day; exposure margins of 32X (NOAEL) or 288X (LOAEL). Body weight decreases in a 2-year oral rat carcinogenicity study in high dose males (-22%) and females (-18%) at scheduled termination confirmed dose selection based on high dose MTD predictions. There was increased incidence of combined thyroid C-cell adenomas and carcinomas in males rats. There were no other tumors that were considered drug-related in males and there were no drug-related tumors in any female group. There was no non-neoplastic evidence that thyroid was a target organ and there was no drug-related increase in thyroid C-cell hyperplasia. At the doses in males which seemed to cause increased thyroid C-cell tumors, exposures were 288- and 533-fold higher than predicted maximum human exposure. The high dose in females, which did not have any drug-related tumors, provided more than 400-fold higher drug exposure than the maximum human dose. SYR-322 poses minimal carcinogenic risk to humans based on high exposure multiples at the NOAEL (32X) and very high exposure multiples ($\geq$ 288X) at doses that caused increased combined thyroid C-cell adenomas and carcinomas in males.

Reproductive toxicology: Reproductive toxicity of alogliptin was assessed in a standard battery of assays that covered the period from pre-mating and fertility, through

embryonic and fetal development, and postnatal development and reproduction of animals exposed *in utero* and during nursing. Alogliptin was not teratogenic at doses greater than 200-fold higher than expected human exposure. There were no remarkable effects on pregnancy or fetal development except at maternally toxic doses that were generally greater than 200-fold higher than expected human exposure. The major notable finding from reproductive toxicity studies was slightly increased percentage of sperm abnormalities in males. The male findings were consistent with sporadic male reproductive toxicity seen in other non-clinical toxicity studies at high alogliptin doses. Nevertheless, sperm abnormalities did not affect fertility.

Fetal findings were limited to decreased body weight and minor skeletal variations at maternally toxic doses. Decreased body weight persisted throughout the life of the F_1 generation and there were slight delays in postnatal development and maturation and slightly decreased numbers of implantation sites and viable embryos in pups from F_1 matings. There were no effects at doses that didn't cause maternal toxicity, which provided approximately 100- to 200-fold exposure margins at the NOAELs compared to expected human exposure.

Fertility and early embryonic development in rat: Male and female rats were treated with up to 1000 mg/kg SYR-322 to assess male fertility and effects on early embryonic development. The high dose was toxic to the parents, particularly males, causing 22% decreased weight gain concomitant with decreased food consumption. Increased sperm production and an increased percentage of abnormal sperm contributed to 10% increased caudal epididymal weight in males. Sperm abnormalities did not affect male fertility. Findings in most females were modest and there were no effects on female fertility or reproductive parameters with the exception of a single high dose female which had a single implantation site and no embryos. The NOAEL was considered 500 mg/kg for both males and females, which corresponds to exposures of approximately 67- to 167-fold higher than maximum human exposure in males and females (respectively).

Embryofetal development in rat and rabbit: Rat body weight and body weight gain were reduced in mid and high dose dams, which was concomitant with decreased food consumption. No pregnancy parameters were altered by treatment, but fetal body weights were reduced 8% in the high dose. Fetal developmental anomalies were limited to 2-fold increased skeletal variations of sternebrae and skull incomplete ossifications. There were no visceral variations or malformations in fetuses. TK data confirmed that alogliptin and M-I cross the placenta and drug and metabolite plasma concentrations were generally similar in dams and fetuses. Fetal skeletal ossification and decreased fetal weight were observed only at a dose that caused > 10% decreased maternal body weight gain, suggesting a potential effect of maternal stress or toxicity on the developing fetus. The NOAEL was considered 500 mg/kg for fetal rat effects, which provided 215-fold higher than maximum human exposure.

Embryofetal development was assessed in rabbits up to a dose that caused approximately 500-times greater exposure than the MRHD and which was lethal to approximately 35% of dams. Surviving dams showed signs of severe toxicity manifest as decreased activity,

breathing difficulty, 75% decreased body weight gain, and evidence of kidney toxicity and hematological signs of stress. Maternal toxicity had an affect on delivery in some animals, including early delivery and one incidence of total resorption. Fetal findings were limited to 15% decreased body weight and a single skeletal variation of increased unossified hyoid body. Mild maternal toxicity but no pregnancy or fetal abnormalities was seen at the next lower dose considered the NOAEL. At the NOAEL for maternal and fetal toxicity, 200 mg/kg, maternal exposure was 207-fold higher than expected human exposure.

Pre- and post-natal development in rat: Effects of SYR-322 on pre- and post-natal development, including maternal toxicity, were assessed in rats ('Segment III' reproductive toxicity study). The NOAEL was 250 mg/kg/day for maternal and developmental toxicity, which was 95-fold higher than the MRHD. Maternal toxicity was apparent at the mid and high doses (500 and 1000 mg/kg/day), based on decreased body weight gain and decreased food consumption during F_0 dosing. Maternal body weight decreases were consistent with findings from the embryofetal development toxicity study ('Segment II'). F_1 pup body weights were lower at the maternally toxic doses and low body weight persisted throughout weaning and into adulthood. There were no gross abnormalities in F_1 pups. Various decreases in F_0 delivery parameters were apparent at maternally toxic doses (e.g. ↑ stillborn index and ↓ pup survival). There were also slight developmental, sexual maturation, and behavioral abnormalities noted in high dose F_1 animals. There were no remarkable findings in F_1 mating, fertility, or pregnancy parameters. Modest delivery trends (not statistically significant) in high dose F_1 females included slightly lower implantation sites and viable embryos, and increased postimplantation loss, but results were confounded by lower maternal body weights and not considered remarkable. The NOAEL for F_1 reproduction and their fetuses (F_2) was the high dose of 1000 mg/kg, which was over 500-fold higher than expected human exposures (based on exposure estimates from other rat studies).

Special toxicology:

Local tolerance (Rabbits) – (1) Intravenous: There were no macroscopic signs throughout the study and there were no macroscopic or histological lesions at scheduled necropsies. NOAEL = 2.5 mg/ml concentration of SYR-322 (as free base). (2) Paravenous: Gross findings were limited to slight erythema in all animals starting day 1, which persisted for 2 to 4 days (absent day 5). Histological analysis showed slight subcutis hemorrhage in 2/3 animals on day 2 but none on day 14. Results were considered modest and tolerable in the animals. NOAEL = 2.5 mg/ml.

Hemocompatability – SYR-322 did not cause hemolysis or effects on plasma (flocculation, precipitation, or coagulation) as compared to saline *in vitro*. NOAEL = 2.5 mg/ml SYR-322 (1:10 in blood, 1:100 in plasma).

Phototoxicity – No evidence of skin reactions in a standard single oral dose phototoxicity study at SYR-322 doses up to 800 mg/kg in hairless mice.

2.6.6.2 Single-dose toxicity

A variety of single dose studies in animals were conducted to support the non-clinical development program. Only GLP-compliant studies are summarized in this review.

Acute GLP oral toxicity study of SYR-322 in rats

As summarized in the IND review,

> "No mortality, increase in clinical signs, change of body weight, or abnormal findings on necropsy were reported up to 14 days following a single oral dose of SYR110322 over a dose range of 500-2000mg/kg."

The maximum non-lethal dose of 2000 mg/kg in rat was 24-times higher than the maximum single human dose tested based on body surface area (mg/m^2) comparisons.

Acute GLP oral toxicity study of SYR-322 in dogs

As summarized in the IND review,

- "No mortality or abnormal findings on necropsy were reported up to 15 days following single oral dosing with SYR110322."
- "Reddening and warming of the facial area occurred at all dose groups and in 5/6 animals. Transient at the low-dose, prolonged and more severe at mid- and particularly high-doses."
- "High-dosed female experienced some weight loss, decreased activity, and emesis."

The maximum non-lethal dose of 500 mg/kg in dog was 20-times higher than the maximum single human dose tested based on body surface area (mg/m^2) comparisons.

Intravenous single dose toxicity study of SYR-322 in rats

A single dose *iv* study in rats was conducted to support clinical *iv* dosing for PK analysis. There were no notable effects in any animal up to the highest dose of 25 mg/kg. The study supported the sponsor's planned maximum clinical *iv* exposure of 15 mg per person.

2.6.6.3 Repeat-dose toxicity

A variety of repeat dose studies in animals, including exploratory (non-GLP) range-finding studies, were conducted to support the non-clinical development program. Only GLP-compliant studies are summarized in this review.

28 Day oral mouse

A 28 day oral toxicity study of SYR322 in CD-1 mice
30, 100, 300, 400 mg/kg
*6, 22, 66, 60 μg*h/ml*
NOAEL = 300 mg/kg (44X MRHD)

Mice were treated by oral gavage for four weeks at up to 400 mg/kg. The GLP-compliant study was used to support dosing in a 13-week mouse (which in turn would be used for 2-year carcinogenicity study dose selection). No dose-limiting toxicity was observed up to the high dose. There was mortality in a total of eight animals (four per sex), only in the high dose group. Cause of death was not determined (only one necropsy performed), but there were no drug-related histological lesions in high dose mice at study termination. The relationship between treatment and mortality was not clearly established.

Other potentially drug-related findings were limited to modest, dose-dependent decreases in various white blood cell types. While hematology findings were seen in both sexes, trends were not consistent across sexes. There were no clear drug-related hematology trends in the 13-week mouse study or the pivotal chronic, two-year carcinogenicity study in mice (although there were sporadic marked changes in both red and white blood cell parameters in the 2-year study that were considered unrelated to treatment).

Key study findings[1]:

- Mortality in 8% of males and 7% of females given 400 mg/kg SYR110322; cause of death not determined.

- Decrement in body weight gain of 32% in males given 400 mg/kg. Females not effected. Food consumption is not decreased in males or females.

- Decreases in lymphocyte, eosinophil, and monocyte counts in males and females in the 300 and 400 mg/kg groups; only decreased lymphocytes in males is statistically significant.

- Gross pathology and histology are generally unremarkable; the two microscopic findings found to be drug-induced in rats (i.e., nephropathy and thymic atrophy) occur in control and high dose mice with the same incidence.

NOAEL Determination

The Sponsor suggests a NOAEL of 400 mg/kg; however, based on unexplained mortality in both sexes at 400 mg/kg, and 32% decrease in body weight gain in high dose males, the reviewer suggests a NOAEL of 300 mg/kg.

[1] Key study findings from original IND review

TK analysis showed the active M-I metabolite (SYR-324) accounted for approximately 2% to 4% of total drug exposure. Drug and M-I exposure increased dose proportionally and there was no evidence of drug accumulation. The sponsor's TK summary for parent drug SYR-322 and a comparison of parent to M-I (SYR-324) are shown in the tables below.

Sponsor's SYR-322 TK Summary

		Day 1				Day 28			
Parameters	Gender	30 mg/kg	100 mg/kg	300 mg/kg	400 mg/kg	30 mg/kg	100 mg/kg	300 mg/kg	400 mg/kg
Cmax	Male	2670	12500	19617	29933	2243	8043	19067	17133
(ng/mL)	Female	3200	16900	22667	26267	1830	9897	18400	17433
Cmax/Dose	Male	89.0	125	65.4	74.8	74.8	80.4	63.6	42.8
($x10^{-6}$ kg/mL)	Female	107	169	75.6	65.7	61.0	99.0	61.3	43.6
Tmax[a]	Male	1.00	0.50	0.50	0.50	0.50	0.50	0.50	0.50
(hr)	Female	0.50	0.50	0.50	0.50	0.50	0.50	1.00	0.50
T½	Male	3.66	3.76	5.19	3.49	8.50	3.63	.	.
(hr)	Female	.	3.84	5.18	.	.	.	.	4.262
AUC(0-24)	Male	6566	31565	71209	112305	5781	20024	65921	58266
(ng·hr/mL)	Female	6822	29490	70490	101576	6265	25752	66776	62663
AUC(0-24)/Dose	Male	219	316	237	281	193	200	220	146
($x10^{-6}$ hr*kg/mL)	Female	227	295	235	254	209	258	223	157
Accumulation Ratio (R)	Male	NA	NA	NA	NA	0.88	0.63	0.93	0.52
	Female	NA	NA	NA	NA	0.92	0.87	0.95	0.62

NA = Not Available
"." = Missing
[a] Tmax was presented with the median value.

Table 5. Comparison of Toxicokinetic Parameters (AUC, C_{max}) for SYR110324 and SYR110322 in CD-1 Mice after 28 Days of Oral (Gavage) Administration of SYR110322

	SYR110322		SYR110324		Ratio of 324/322 (%)	
Species (Dose level)/Sex	C_{max} ng/mL	AUC_{0-24} (ng•h/mL)	C_{max} ng/mL	AUC_{0-24} (ng•h/mL)	C_{max} ng/mL	AUC_{0-24} (ng•h/mL)
Mice (30 mg/kg, Day 28)						
Male	2243	5781	56	171	2.5	2.9
Female	1830	6265	56	225	3.0	3.6
Mice (100 mg/kg, Day 28)						
Male	8043	20024	175	469	2.2	2.3
Female	9897	25752	234	708	2.4	2.7
Mice (300 mg/kg, Day 28)						
Male	19067	65921	250	978	1.3	1.5
Female	18400	66776	239	914	1.3	1.4
Mice (400 mg/kg, Day 28)						
Male	17133	58266	224	927	1.3	1.6
Female	17433	62663	266	880	1.5	1.4

Thirteen week oral mouse

A 13 week oral toxicity study of SYR322 in CD-1 mice
200, 300, 400, 600 mg/kg (day 1 only 200, 400, 800, 1200 mg/kg)
*71, 96, 117, 180 µg*h/ml*
NOAEL = 200 mg/kg (47X)

A thirteen week oral (gavage) toxicity study in CD-1 mice was conducted primarily to determine appropriate doses for a 2-year oral carcinogenicity study.

Key study findings[2]:

- Numerous deaths at 800 and 1200 mg/kg on Day 1 of dosing required reducing the dose to 300 and 600 mg/kg.

- Combining main and TK study animals, mortality started at 300 mg/kg (1/67 males, 1/67 females), progressed at 400 mg/kg (2/67 males, 4/67 females) and at 600 mg/kg (6/67 males, 7/67 females). Causes of death were not determined, although a few dead animals had red fluid in the thoracic cavity and had reddish lungs, indicative of gavage errors.

- Body weight gain and food consumption in treated groups did not change substantially from control values.

- Hematology, clinical chemistry, and gross pathology unremarkable in males and females.

- Gross pathology and histology are generally unremarkable; nephropathy, and hyperplasia of thymic lymphoid tissue and stomach epithelia occur in control and high dose mice with the same incidence and severity.

NOAEL Determination:

A NOAEL of 200 mg/kg is established by this study, based on unexplained mortality at 300 mg/kg in males and females. The number of deaths increase at higher doses, and there were no deaths at lower doses, suggesting that the observed mortality is related to drug treatment. Histopathology was unremarkable, and did not inform as to the cause of death or a NOAEL determination.

Mortality seemed to increase with treatment. With the exception of 3 males and 3 females with clear signs of gavage error and 1 female death attributed to "mishandling", no cause of death was determined for most animals. Histopathology was not conducted on 800 and 1200 mg/kg mice that died after a single dose. See summary table 1, below.

[2] Key study findings from original IND review

Table 1 – Mortality (unexplained) †		
Dose (mg/kg)	**Male**	**Female**
0	0	0
200	0	0
300	2	4
400	3	2
600	4	11
800 [a]	1	4
1200 [a]	15	8

† Mortality of main study (n=15/group) and satellite TK (n=52/SYR-322 group) animals not attributable to gavage errors or mishandling

[a] Single dose, mortality day 1 (dose groups lowered to 300 and 600 mg/kg, respectively, and animals replaced)

Clinical signs were limited to males and generally mild in nature, including low incidences of yellow hair in control and treated groups and unspecified swelling in a few 400 mg/kg (4/15) and 600 mg/kg (1/15). No clinical signs were observed in females.

There were no treatment-related histopathology findings.

Alogliptin was rapidly absorbed in mice after oral exposure, with T_{max} = 0.5-1 h. Exposure increased with dose, with total exposure ($AUC_{0\text{-}24\ h}$) increased approximately in proportion with dose and maximum exposure (C_{max}) increased less than dose-proportionally. There was no remarkable drug accumulation over the course of the study, although exposure was slightly increased in some sex-specific groups on day 90.

TK results were consistent with results from the 28-day mouse study. Active metabolite M-I (SYR-324) accounted for approximately 2-4% of parent drug exposure after 13-week oral dosing. M-I is produced slightly more in mouse compared to humans and based on 13-week study data, M-I concentrations in mice in the 2-year carcinogenicity were predicted to be much higher than maximum clinical human exposure. There were no sex differences in exposure. Sponsor's summary TK tables are shown below.

Sponsor's 13-Week Mouse SYR-322 TK Summary

Parameters	Gender	Day 1				Day 90			
		200 mg/kg	300 mg/kg	400 mg/kg	600 mg/kg	200 mg/kg	300 mg/kg	400 mg/kg	600 mg/kg
AUC(0-24) (ng·hr/mL)	Female	55728.70	87534.87	97645.87	152377.00	54754.46	84362.98	124441.92	160675.67
	Male	106645.94	89093.99	110960.90	123672.13	87330.19	107300.92	109216.57	199019.92
	All	81187.32	88314.43	104303.38	138024.57	71042.33	95831.95	116829.24	179847.79
AUC(0-24)/Dose ($x10^{-6}$ hr*kg/mL)	Female	278.64	291.78	244.11	253.96	273.77	281.21	311.10	267.79
	Male	533.23	296.98	277.40	206.12	436.65	357.67	273.04	331.70
	All	405.94	294.38	260.76	230.04	355.21	319.44	292.07	299.75
Cmax (ng/mL)	Female	23600.00	25966.67	26833.33	34133.33	14366.67	22333.33	22466.67	27533.33
	Male	20566.67	25400.00	28100.00	31133.33	25200.00	21133.33	25200.00	35566.67
	All	22083.33	25683.33	27466.67	32633.33	19783.33	21733.33	23833.33	31550.00
Tmax (hr)	Female	0.50	1.00	0.50	0.50	0.50	0.50	1.00	0.50
	Male	1.00	2.00	0.50	0.50	1.00	0.50	1.00	1.00
	All	0.75	1.50	0.50	0.50	0.75	0.50	1.00	0.75
T½ (hr)	Female	4.15	2.69	3.01	3.44	1.75	3.59	3.72	2.79
	Male	3.97	.	3.71	4.03	.	7.58	1.74	2.97
	All	4.06	2.69	3.36	3.74	1.75	5.58	2.73	2.88
Accumulation Ratio (R)	Female	.	.	.	.	0.98	0.96	1.27	1.05
	Male	.	.	.	.	0.82	1.20	0.98	1.61
	All	.	.	.	.	0.90	1.08	1.13	1.33

"." = Not Available

Sponsor's 13-Week Mouse SYR-324 (M-I) TK Summary

Parameters	Gender	Day 1				Day 90			
		200 mg/kg	300 mg/kg	400 mg/kg	600 mg/kg	200 mg/kg	300 mg/kg	400 mg/kg	600 mg/kg
AUC(0-24) (ng·hr/mL)	Female	1213.90	1606.29	1589.78	1997.78	765.86	968.99	1460.31	1169.02
	Male	3434.83	2294.35	2117.93	1960.64	1523.38	1217.96	1184.77	1729.49
	All	2324.37	1950.32	1853.86	1979.21	1144.62	1093.47	1322.54	1449.25
AUC(0-24)/Dose ($x10^{-6}$ hr*kg/mL)	Female	6.07	5.35	3.97	3.33	3.83	3.23	3.65	1.95
	Male	17.17	7.65	5.29	3.27	7.62	4.06	2.96	2.88
	All	11.62	6.50	4.63	3.30	5.72	3.64	3.31	2.42
Cmax (ng/mL)	Female	590.00	496.33	600.67	574.00	265.67	306.33	279.00	315.33
	Male	525.33	849.67	707.67	614.67	442.33	239.67	390.00	313.33
	All	557.67	673.00	654.17	594.33	354.00	273.00	334.50	314.33
Tmax (hr)	Female	0.50	1.00	0.50	0.50	0.50	0.50	1.00	0.50
	Male	2.00	2.00	0.50	0.50	1.00	0.50	1.00	1.00
	All	1.25	1.50	0.50	0.50	0.75	0.50	1.00	0.75
T½ (hr)	Female	3.75	1.49	2.94	3.50	1.71	4.78	.	4.03
	Male	.	.	3.57	4.86	.	.	1.76	3.39
	All	3.75	1.49	3.25	4.18	1.71	4.78	1.76	3.71
Accumulation Ratio (R)	Female	.	.	.	.	0.63	0.60	0.92	0.59
	Male	.	.	.	.	0.44	0.53	0.56	0.88
	All	.	.	.	.	0.54	0.57	0.74	0.73

"." = Not Available

Four week oral rat

Four week oral toxicity in Sprague Dawley rats
30, 100, 300 mg/kg
*7.5, 29, 88 μg*h/ml*
NOAEL > 300 mg/kg (> 59X MRHD)

Key study findings[3]:

- Exposure to SYR110322 appears gender-specific, with females being exposed to ~2 fold greater level than males.
- 28-Day dosing up to 300 mg/kg did not cause mortality, increase frequency of clinical signs, or change body weight or food consumption in rats.
- No overt treatment-related effect was observed on hematology and clinical chemistry parameters, or on ophthalmoscopy and urinalysis parameters.
- No treatment-related macroscopic or microscopic change is reported for animals in the main or recovery periods. *(Reviewer's note – see discussion below of oligospermia findings in two high dose males)*
- No treatment-related changes of organ weight was reported from the main or recovery study periods.

There were no treatment related effects in male or female rats in a functional observational battery (FOB) screen for CNS toxicity. The NOAEL for the CNS safety pharmacology screen was 300 mg/kg after both single and repeat (25 days) dosing.

Notable histological findings were summarized in the IND review in the table below. In the original IND review, the finding of severe oligospermia/germ cell debris in epididymides in two high dose (300 mg/kg) rats was considered a sporadic finding. This reviewer notes that the finding may have been drug-related, based on findings of increased sperm production and increased percentage of abnormal sperm in the rat fertility study and findings in longer term rat studies. Male reproductive toxicity findings are discussed in the 'Overall toxicology summary' in Section 2.6.6.1

[3] Key study findings from original IND review

Findings present in the treated groups but absent in the control group are as follows:

			Incidence of finding		
Males	**Finding**	**Severity**	**LD**	**MD**	**HD**
epididymides	oligospermia/germ cell debris	severe			2/10
kidneys	basophilic tubules, cortex	mild		1/3	
	hydronephrosis, unilateral	minimal			1/10
	hydronephrosis, unilateral	mild		1/3	
liver	focal necrosis	minimal			2/10
lung	subacute inflammation	minimal			1/10
	eosinophilic perivasuclar infiltration	minimal			1/10
lymph node, mandibular	hyperplasia, lymphoid	severe	1/2		
spleen	lymphoid depletion	minimal			1/10
testes	hypoplasia	severe			2/10
	primary benign teratoma	n/a			1/10
thymus	atrophy	minimal			1/10
	hyperplasia, lymphoid	minimal			1/10
Females					
kidney	tubular mineralization	minimal			3/10
sciatic nerve	degeneration, axonal/myelin	minimal			1/10
uterus with cervix	cyst	mild			1/10
	keratin cyst	minimal			1/10

Female exposure was approximately 2-fold higher than males at the low dose but sex differences in mid and high doses were modest (maximum 38% higher in females). The sponsor's TK summary in the short term study are shown below. At a later date, M-I plasma levels were measured in archived samples from the low dose (30 mg/kg) and M-I concentrations were approximately 92% and 41% of parent concentrations in males and females, respectively. The contribution of M-I to total estimated DPP4 inhibitory activity (i.e. AUC of SYR-322 + M-I) was approximately 47% in males and 29% in females.

Results: Toxicokinetic parameters were calculated and are summarized below:

		Day 1			Day 28		
Parameters	Gender	30	100	300	30	100	300
Cmax (ng/mL)	Male	469	5460	12200	994	7680	11243
	Female	1380	6067	11157	2417	8817	13600
Tmax (hr)	Male	1.00	0.50	1.00	1.00	0.50	1.00
	Female	1.00	0.50	0.50	1.00	0.50	1.00
T½ (hr)	Male	3.99	2.65	3.58	3.78	2.55	2.69
	Female	3.12	3.04	2.16	3.04	2.61	2.68
AUC(0-24) (ng·hr/mL)	Male	3158	20511	79093	5266	27075	74078
	Female	6510	28755	104652	9878	31661	101255
Accumulation Ratio (R)	Male	NA	NA	NA	1.67	1.32	0.94
	Female	NA	NA	NA	1.52	1.10	0.97

NA = Not available

Four week oral rat (high dose)

A 4 week oral toxicity study of SYR322 in Sprague-Dawley rats
1333, 1666, 2000 mg/kg
NOAEL < 1333 mg/kg (No TK analysis)

Key study findings[4]:

- 40% mortality in males at 2000 mg/kg; cause of death unclear but possibly related to gastrointestinal and renal complications.

- Mortality in females: 10% at 1333 mg/kg, 30% at 1666 mg/kg, and 70% at 2000 mg/kg; cause of death unclear but some deaths possibly related to gastrointestinal and renal complications.
  Reviewer's note – female mortality at all doses was consistent with increased treatment-related deaths from urinary and/or kidney related toxicity in the two year rat carcinogenicity study

- Reduction of body weight gain exceeded 10% in males and females at all doses (Range, 13% to 41%). Food consumption decreased in groups receiving drug.

- Liver weight increased 75% in males and females at 2000 mg/kg (LFT elevations ≤ 2 fold). A reduction in weight of several other organs was observed, including heart, spleen, and thymus.

NOAEL Determination

No NOAEL could be determined based on dose-related reduced body weight gain, kidney toxicity, urinary bladder toxicity and markers of an immune response and inflammatory cell infiltration at all doses. Incidence and severity of toxicity increased with dose and resulted in drug-related mortality.

Histopathology findings were not included in the draft study report reviewed in the IND and they are summarized here (see reviewer's summary table, below). There was generalized, modest dose-related inflammation in various tissues; coupled with dose-related thymus and spleen lymphoid depletion, findings suggest a generalized treatment related immune response.

Kidney, liver, and urinary bladder were target organs. In kidney, low dose findings were limited to minimal tubular degeneration/regeneration in males and tubular mineralization in females, while incidence and severity of tubular toxicity increased with dose. Increased ↑ ALP in all treatments, ranging from 56-94% at the low dose to 70-144% in high dose (♂ and ♀ respectively) was consistent with kidney toxicity. Histological liver findings were consistent with adaptive hypertrophy, which confirmed modest LFTs and gross

[4] Key study findings from original IND review; NOAEL determination from NDA and IND review (histopathology data not included in draft report submitted to the IND)

hypertrophy. Urinary bladder findings were absent in controls but there were low incidences of bladder toxicity (erosion/ulcer, hemorrhage, hyperplasia) in all groups, although the relationship to dose was not strong.

The sponsor attributed a single female death in the low dose to urinary calculi and concluded the death was common in the rat strain and unrelated to treatment. However, urinary calculus was not found in any other animals, which argues against a common background incidence in the study. Also, the presence of clear treatment-related kidney and urinary bladder findings in all dose groups suggests the death was potentially drug-related. Kidney and bladder related findings were implicated in other animal deaths in this study and in the two year rat carcinogenicity study.

Histopathology, 4 Week Rat (High Doses) †										
Tissue [a]	Finding	Severity	Male (n=10/group)				Female (n=10/group)			
			0	1333	1666	2000	0	1333	1666	2000
Kidneys	Tubular, degeneration/ regeneration	minimal	--	10	1	1	--	--	--	1
		mild	--	--	9	8	--	--	--	1
	Tubular, regeneration	minimal	--	--	--	1	--	--	--	--
	Tubular, mineralization	minimal	--	--	--	--	1	3	4	4
Liver	Hypertrophy, centrilobular	minimal	--	10	4	1	--	2	1	1
		mild	--	--	6	2	--	--	2	2
	Hypertrophy, panlobular	minimal	--	--	--	3	--	7	1	2
		mild	--	--	--	4	--	--	6	5
Thymus gland	Depletion, lymphoid	minimal	--	3	3	3	--	--	2	5
		mild	--	--	--	3	--	--	1	1
		moderate	--	--	--	1	--	--	--	1
		severe	--	--	--	1	--	1	2	1
	Necrosis, lymphoid	minimal	--	--	--	1	--	--	--	1
		moderate	--	--	--	1	--	1	--	2
		severe					--	--	--	1
Urinary bladder	Erosion/ulcer	minimal	--	--	--	2	--	--	--	--
		moderate	--	--	--	1	--	--	1	--
	Hemorrhage	minimal	--	1	1	1	--	--	1	1
		moderate	--	--	--	1	--	--	--	--
	Hyperplasia, simple transitional cell	minimal	--	1	3	--	--	--	3	--
		mild	--	1	--	1	--	1[a]	1[a]	--

† Salient findings, combined from animals found dead and sampled at terminal necropsy; doses represent mg/kg/day

-- represents no finding

[a] Spleen lymphoid depletion, minimal to severe, was absent in all control animals but seen in 3, 2, 8 males and 1, 3, 6 females in respective groups

[b] papillary/nodular transitional cell

Original IND Review Clinical Signs Summary

CLINICAL SIGNS		
Dose Group	**MALES**	**FEMALES**
Control	none	none
1333 mg/kg	Salivation, 7/10 Hair discolored yellow, 5/10 Weeks 3-4	Salivation, 2/10 Hair discolored or sparse, 9/10 Weeks 2-4
1666 mg/kg	Salivation, 3/10 Hair discolored yellow, 6/10 Cold skin, 2/10 Weeks 3-4	Hair discolored or sparse, 7/10 Cold skin, 2/10 Weeks 2-4
2000 mg/kg	Salivation, 3/10 Hair discolored yellow, 7/10 Cold skin, 4/10 Righting reflex impaired, 2/10 Activity decreased, 4/10 Weeks 2, 3-4	Salivation, 3/10 Hair discolored or sparse, 10/10 Cold skin, 5/10 Righting reflex impaired, 4/10 Activity decreased, 5/10 Limb function impaired, 4/10 Breathing difficult, 4/10 Tremors, 3/10 Moribund, 2/10 Weeks 2-4

Sponsor's Clinical Chemistry Summary

Summary of Clinical Chemistry Changes[a]			
	1333 mg/kg/day	1666 mg/kg/day	2000 mg/kg/day
Sodium	↓2%(↓2%)	↓2%(↓4%^{NS})	↓2%(↓4%^{NS})
Chloride	↓4%	↓4%(4%^{NS})	(↓4%^{NS})
Phosphorus	↑11%(↑15%)	↑11%(↑28%)	↑12%(↑35%)
ALP	↑56%(↑94%)	↑88%(↑182%)	↑70%(↑144%)
AST			↑52%(↑54%)
ALT		(↑51%)	↑40%(↑100%)
Urea nitrogen			(↑30%^{NS})
Total protein		(↓10%)	
Albumin	↓5%(↓11%)	↓7%(↓17%)	↓6%(↓16%)
A/G	↓9%(↓14%)	↓9%(↓19%)	↓12%(↓24%)
Cholesterol	↑32%(↑73%)	↑51%(↑77%)	↑57%(↑97%)
Glucose	↑36%		

[a]Relative to controls for males and (females)
↑Increase ↓Decrease
^{NS}Not statistically significant
ALP: Alkaline phosphatase; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; A/G: Albumin to globulin ratio

Exploratory GLP eight week oral rat

The sponsor conducted a GLP-compliant, exploratory study to compare toxicity of different DPP4 inhibitors under development. The FDA review focused on alogliptin results. Rats were treated with a single dose level of 50 mg/kg compared to vehicle controls (and other DPP4 inhibitors).

Alogliptin findings were unremarkable and the single dose level of 50 mg/kg was considered the NOAEL. No histopathology analysis was performed, so conclusions were limited to gross findings and standard clinical monitoring (signs, hematology, clinical chemistry, etc.).

Thirteen week oral rat

A 13 week oral toxicity study of SYR322 in Sprague-Dawley rats
100, 400, 1000 mg/kg
*Male: 32, 89, 549 μg*h/ml; NOAEL = 400 mg/kg (59X MRHD)*
*Female: 58, 240, 661 μg*h/ml; NOAEL = 400 mg/kg (160X MRHD)*

Key study findings[5]:

- No mortality and relatively benign clinical signs at all doses, in males and females.
- Reduction in body weight gain of 17-19% at 1000 mg/kg in males and females; the next lowest dose, 400 mg/kg, showed essentially no change of body weight gain. Food consumption did not change over time.
- Increased liver weight (20% males, 50% females) at 1000 mg/kg with evidence of hepatocytic hypertrophy; no evidence of cellular necrosis.
- Chronic progressive renal nephropathy in males (5 of 15) and females (1 of 15) at 1000 mg/kg; similar incidence of thymic atrophy is observed.
- Drug accumulates 2-fold over time in females at all doses, and in males only at 1000 mg/kg; consequently, exposure in males at the low and mid dose is ~2 fold lower compared to females by day 91.

NOAEL determination

A tentative NOAEL of 400 mg/kg for males and females is supported by renal damage and excessive reduction in body weight gain at 1000 mg/kg. This NOAEL is subject to change pending evaluation of the final report, because microscopic evaluation of the low and middose animals was not done or not presented in this draft report. A dose less than 1000 mg/kg is indicated for the 2 year carcinogenicity study in rats. *Reviewer's notes – (1) NOAEL estimate did not change after review of the final toxicity study report; (2) M-I (SYR-324) exposure ranged from 11-19%, 4-7%, and 2% of parent at respective doses and were not added to alogliptin concentrations for MRHD estimates*

Clinical signs were modest with yellowish-greenish discolored hair and increased salivation (males only) shown in the summary table from the IND review, below.

[5] Key study findings from original IND review

CLINICAL SIGNS (treatment-related, # effected/total)		
Dose Group	**MALES**	**FEMALES**
Control	Hair sparse/absent, 3/15	Hair sparse/absent, 3/15
100 mg/kg	Salivation, 1/15 Hair sparse/absent, 3/15	
400 mg/kg	Salivation, 1/15 Hair discolored, 2/15 Hair sparse/absent, 3/15	Hair discolored, 2/15 Hair sparse/absent, 8/15
1000 mg/kg	Salivation, 8/15 Hair discolored, 13/15 Hair sparse/absent, 6/15	Hair discolored, 11/15 Hair sparse/absent, 4/15

BODY WEIGHT: MALES			
Study Time	Dose, mg/kg	BW gain (g) over study	% Decrement
13 Week Rat	control	265	--
	100	260	2
	400	262	1
	1000	215	19

BODY WEIGHT: FEMALES			
Study Time	Dose, mg/kg	BW gain (g) over study	% Decrement
13 Week Rat	control	108	--
	100	109	0
	400	106	2
	1000	89	17

Modest hematology findings were seen in 1000 mg/kg males and females, including decreases in total erythrocytes, hematocrit, hemoglobin, increased absolute reticulocytes (more pronounced in females), and decreased total leukocytes in females. See IND summary tables, below.

RBC Parameters, Male rats				
Dose, mg/kg	Hct (%)	Hb (g/dL)	RBC (M/mm^3)	Retic. (%)
	Day 88	Day 88	Day 88	Day 88
0	46	15	8.4	155
100	44	14	7.9	138
400	44	14	7.7	181
1000	42	14	7.4	200

RBC Parameters, Female rats				
Dose, mg/kg	Hct (%)	Hb (g/dL)	RBC (M/mm^3)	Retic. (%)
	Day 88	Day 88	Day 88	Day 88
0	44	14	7.9	132
100	42	14	7.5	170
400	42	14	7.4	176
1000	40	13	6.9	224

WBC Parameters, Females				
Dose, mg/kg	Neutro (10^3/µl)	Lympho (10^3/µl)	Eosin (10^3/µl)	Baso (10^3/µl)
	Day 88	Day 88	Day 88	Day 88
0	0.99	6.4	0.09	0.03
100	0.94	6.1	0.12	0.03
400	0.82	5.2	0.11	0.02
1000	0.66	4.6	0.06	0.01

Histopathology findings of hepatocyte hypertrophy in most high dose (1000 mg/kg) males and females were consistent with increased liver weights in males (+20%) and females (+50%). There was no evidence of hepatocellular necrosis, suggesting an adaptive response rather than a response to overt hepatic toxicity.

The original IND did not note any effects on male reproductive tissues. A review of the study report histopathology data showed one incidence (n=15) of moderate unilateral degeneration/atrophy of seminiferous tubules in a male treated with 1000 mg/kg.

Minimal chronic, progressive nephropathy and thymus atrophy were seen in approximately one-third of high dose males and rarely in females. Histopathology findings are shown in the IND summary table, below.

HISTOPATHOLOGY, 13 WEEK RAT STUDY				
Tissue- Males	**Finding**	**Severity**	**Control**	**1000 mg/kg**
Kidneys	Nephropathy, chronic progressive	minimal	1/15	5/15
Liver	Hepatocytic hypertrophy, centrilobular	minimal	0/15	11/15
		mild	0/15	3/15
Thymus gland	atrophy	minimal	0/15	4/15
		severe	0/15	1/15
Females	**Finding**	**Severity**	**Control**	**1000 mg/kg**
Kidneys	Nephropathy, chronic progressive	minimal	0/15	1/15
Liver	Hepatocytic hypertrophy, centrilobular	minimal	0/15	7/15
		mild	0/15	8/15
Thymus gland	atrophy	minimal	0/15	2/15

Drug was readily absorbed in rats, with time to maximum plasma exposure increased with increasing dose and estimated T_{max} range of approximately 0.5 to 4 h. Half life estimates at thirteen weeks of dosing were approximately 5 h. Drug exposure increased approximately in proportion with dose on day 1 with no apparent sex differences in exposure. After thirteen weeks of dosing, drug accumulation was evident and total exposure and dose-related exposure increases were more variable between males and females. Drug accumulation was approximately 2-fold at all female doses and in males only at the high dose. Consequently, female exposure was approximately equal to males at the high dose and greater than 2-fold higher than males at low and mid doses.

Sponsor's Thirteen Week Rat TK Summary

		Day 1			Day 91		
Parameters	Gender	100 mg/kg	400 mg/kg	1000 mg/kg	100 mg/kg	400 mg/kg	1000 mg/kg
Cmax (ng/mL)	Female	5716.67	12033.33	21500.00	7939.53	20866.67	44133.33
	Male	4110.00	11793.33	15500.00	6006.67	14566.67	39400.00
Tmax (hr)	Female	0.50	1.00	8.00	1.00	2.00	4.00
	Male	0.50	1.00	8.00	0.50	2.00	4.00
T½ (hr)	Female	2.75	2.71	7.59	2.82	5.04	5.19
	Male	3.30	2.80	12.81	4.04	3.23	13.87
AUC(0-24) (ng·hr/mL)	Female	20518.03	137811.33	299588.33	57597.08	240528.58	660854.50
	Male	18992.87	124223.60	241370.83	32331.31	89090.70	548521.67
AUC(0-24)/Dose ($x10^{-6}$ hr*kg/mL)	Female	205.18	344.53	299.59	575.97	601.32	660.85
	Male	189.93	310.56	241.37	323.31	222.73	548.52
Accumulation Ratio (R)	Female	.	.	.	2.81	1.75	2.21
	Male	.	.	.	1.70	0.72	2.27

"." = Not Available

Metabolite M-I was produced in rats, as expected based on 28-day study data. However, M-I exposure was lower at the higher doses tested in the 13-week study compared to the 28-day study which found 27% (females) to 55% (males) for M-I compared to parent. In this 13-week study, relative exposure to M-I decreased with increasing dose of SYR-322. M-I concentrations ranged from 11-19% (100 mg/kg), 4-7% (400 mg/kg), and approximately 2% (1000 mg/kg) of parent exposure (see sponsor's summary, Table 10, below). Rat M-I contributed more to the overall drug activity than human or mouse M-I, with the exception of an equivalent percentage to mice in the 1000 mg/kg rat group.

Table 10. Comparison of SYR110324 and SYR110322 in Sprague-Dawley Rats after 91 Days of Oral (Gavage) Administration of SYR110322 Benzoate salt

Species (Dose level)/Sex	SYR110322		SYR110324		Ratio of 324/322 (%)	
Rat (100 mg/kg, Day 91)	C_{max} ng/mL	AUC_{0-24} (ng•h/mL)	C_{max} ng/mL	AUC_{0-24} (ng•h/mL)	C_{max} ng/mL	AUC_{0-24} (ng•h/mL)
Male	6007	32331	694	6268	11.5	19.4
Female	7940	57597	718	6209	9.0	10.8
Rat (400 mg/kg, Day 91)						
Male	14567	89091	748	6237	5.1	7.0
Female	20867	240529	695	9180	3.3	3.8
Rat (1000 mg/kg, Day 91)						
Male	39400	548522	719	10604	1.8	1.9
Female	44133	660854	766	12876	1.7	1.9

Six month chronic rat

26 week oral toxicity study of SYR-322 in Sprague Dawley rats (+ 4 week recovery)
100, 400, 900 mg/kg
*44, 259, 664 μg*h/ml (32X, 173X, 443X MRHD)*
NOAEL = 400 mg/kg (173X MRHD)

Key study findings[6]:

- There were no drug-related deaths.

- Body weight and food consumption decreased in males and females at 900mg/kg; males were more affected than females despite similar drug exposures. Clinical signs at this dose include an 'unkempt' appearance, discolored hair, and salivation. *(See IND review summary tables, below)*

- Red cell mass decreased ≤ 8% at drug week 13 but recovered by drug week 26 in the 900mg/kg group, associated with a slight increase in reticulocytes.

- ALP increased ≤ 2 fold in 900mg/kg males and females, associated with minimal hepatocellular hypertrophy in females and increased liver weight (≤ 15%) in both sexes. These effects were reversible within 4 weeks of recovery.

- Serum inorganic phosphate increased 10-20% in 900mg/kg males and females which did not revert to control levels after 4 weeks recovery. The cause and significance of this finding is unclear.

- The only clear drug-related change in histology was reversible hepatocellular hypertrophy in high dose females.

- Histology changes of uncertain relation to drug include an increased incidence of alveolar histiocytosis and renal nephropathy in high dose females.

- Exposure as AUC accumulated up to 2.5 fold by drug week 26

NOAEL Determination

Results of the 26 week study are comparable to those of the 13 week study, specifically the identification of drug-related reductions in body weight and increased liver weight/hypertrophy. Renal nephropathy was also noted in the 13 week study. The NOAEL for the 26 week study is 400mg/kg, the same as the 13 week study, indicating that identified toxicities do not progress substantially with chronic treatment.

[6] Key study findings from IND review

MALES: Body Weight			
Study Time	Dose, mg/kg	BW (g)	% control
26 week Rat	0	632	100
	100	640	100
	400	635	100
	900	553	87
4 week recovery	0	624-621= 3g gain	
	900	600-557= 43g gain	

FEMALES: Body Weight			
Study Time	Dose, mg/kg	BW (g)	% control
26 week Rat	0	336	100
	100	345	100
	400	347	100
	900	318	95
4 week recovery	0	323-316= 5g gain	
	900	358-335= 23g gain	

The histopathology summaries from the IND review are shown below. When the study was reviewed, it was not clear whether the unusual findings of hard palate erosion and urinary bladder erosion could be connected to drug-related skin lesions seen with certain other DPP4 inhibitors. Studies conducted later in monkeys were negative for skin lesions and there is no reason to believe the two incidences of erosion/ulcer in rats are treatment related (see monkey review summaries, below).

In the original IND review, it was not clear whether severe testicular degeneration and epididymal oligospermia noted in three high dose and one mid dose males were due to drug because gross and histological analyses showed testes were normal in all other males. Based on data showing increased incidence of bilateral oligospermia and germ cell debris in the chronic 2-year carcinogenicity study, it's possible the testes toxicity was due to drug and apparent only in the most susceptible rats.

MALE HISTOPATHOLOGY, 26 WEEK RAT STUDY						
Tissue	Finding	Severity	0	100	400	900
			n=13	n=15	n=15	n=13
Epididymides	Oligospermia/germ cell debris	severe		0	1	3
Testes	Degeneration/atrophy	severe		0	1	3
Hard Palate	erosion/ulcer	moderate			0	1
Urinary Bladder	erosion/ulcer	moderate			0	1
	hyperplasia, papillary/nodular transitional cell	minimal			0	1
		mild				1

FEMALE HISTOPATHOLOGY, 26 WEEK RAT STUDY						
Tissue	Finding	Severity	0	100	400	900
			n=15	n=14	n=15	n=15
Kidneys	chronic progressive nephropathy	minimal	1	2	2	4
Liver	centrilobular hepatocellular hypertrophy	minimal			0	9
Lungs	alveolar histiocytosis	minimal	4	5	9	11
		mild				1

IND review notes – "The incidence of alveolar histiocytosis increased in 400 and 900 mg/kg females, but not in males. There were no lung findings in the recovery group. A relation to drug treatment is uncertain."

Drug was readily absorbed after single and repeated dosing, with a T_{max} of approximately 0.5 to 1 h. In contrast to short term repeat dose studies in rats, there was no sex difference in exposure in rats after chronic dosing. Exposure increased with increasing dose, with total exposure as AUC increasing greater than dose proportionally and maximum plasma exposure (C_{max}) increased less than dose proportionally. SYR-322 accumulated over the course of the study, achieving 50% to 2-fold greater AUC by day 90 and continuing through day 180. The sponsor's TK summary is shown below.

Sponsor's Six Month Rat TK Summary

		Day 1			Day 90			Day 180		
Parameters	Gender	100 mg/kg/day	400 mg/kg/day	900 mg/kg/day	100 mg/kg/day	400 mg/kg/day	900 mg/kg/day	100 mg/kg/day	400 mg/kg/day	900 mg/kg/day
Cmax (ng/mL)	Female	7643.33	13100.00	22000.00	7783.33	19266.67	36600.00	8870.00	27200.00	46166.67
	Male	5300.00	11746.67	20766.67	6506.67	17766.67	39466.67	7823.33	20166.67	56100.00
	All	6471.67	12423.33	21383.33	7145.00	18516.67	38033.33	8346.67	2368.33	51133.33
Tmax (hr)	Female	1.00	1.00	1.00	1.00	1.00	4.00	1.00	0.50	0.50
	Male	0.50	0.50	8.00	1.00	1.00	8.00	1.00	0.50	0.50
	All	0.75	0.75	4.50	1.00	1.00	6.00	1.00	0.50	0.50
T½ (hr)	Female	2.46	3.37	-	3.11	4.13	16.65	4.75	4.14	6.60
	Male	2.61	2.80	-	3.00	4.32	-	2.83	4.46	7.68
	All	2.54	3.08	-	3.05	4.22	16.65	3.79	4.30	7.14
AUC(0-24) (ng·hr/mL)	Female	27956.13	129339.67	242060.00	43925.11	204224.58	603780.00	45355.75	275256.75	672099.17
	Male	20868.95	128056.00	266203.33	36023.65	179051.50	591349.17	42392.53	241901.67	656320.83
	All	24412.54	128697.83	254131.67	39974.38	191638.04	597564.58	43874.14	258579.21	664210.00
AUC(0-24)/Dose ($x10^{-6}$ hr*kg/mL)	Female	279.56	323.35	268.96	439.25	510.56	670.87	453.56	688.14	746.78
	Male	208.69	320.14	295.78	360.24	447.63	657.05	423.93	604.75	729.25
	All	244.13	321.74	282.37	399.74	479.10	663.96	438.74	646.45	738.01
Accumulation Ratio (R)	Female	-	-	-	1.57	1.58	2.49	1.62	2.13	2.78
	Male	-	-	-	1.73	1.40	2.22	2.03	1.89	2.47
	All	-	-	-	1.64	1.49	2.35	1.80	2.01	2.61

"-" = Not Available

Four week oral dog

Four week oral toxicity study of SYR-322 in Beagle dogs
7.5, 25, 75 mg/kg
*5, 31, 117 µg*h/ml (SYR-322 AUC, does not include additional 60% active M-I exposure)*
NOAEL > 75 mg/kg (≥ 78X)

Key study findings[7]:

- Male beagles dosed 75 mg/kg, the high dose, presented warm and reddened skin daily for 1 to 2 weeks following administration of SYR110322. Females were unaffected; however, females experience this reaction at slightly higher dose levels (100 mg/kg, study # MPI 1063-010). *Reviewer's note – the finding was consistent with skin findings in a 7-day exploratory study*

- Increases of serum cholesterol were seen in females and appeared dose-related. Lipid levels reverted to vehicle group levels after a 2 week recovery period.

- Toxicokinetic parameters did not differ between males and females, unlike sex differences seen in rats.

[7] Key study findings from IND review

- 24h AUC and Cmax increase 30-fold and 15-fold in response to a 10-fold increase in dose level.

- The reviewer concurs with a NOAEL of 75 mg/kg, based on the mild and transient nature of the facial flushing at this dose level. *Reviewer's note – dogs tolerated higher doses in the chronic study, thus the 75 mg/kg dose in this study was considered less than the NOAEL*

There were no treatment-related ECG findings in males or females after four weeks of dosing. Troponins I and T were also assessed and there were no treatment related changes in the markers of ischemic cardiac toxicity.

Notable histological findings were summarized in the IND review in the table below.

Histopathology: Findings in male and female tissues were infrequent and described as minimal to mild in severity. Findings present in the treated groups but absent in the control group are as follows:

			Incidence of finding (no. / 3 dogs)		
Males	**Finding**	**Severity**	**LD**	**MD**	**HD**
adrenal	cysts	minimal	1/3		
epididymides	inflammation	minimal		1/3	
lymph node, mandibular	erythrocytosis/phagocytosis, sinus	minimal			1/3
parathyroid gland	cysts	minimal			1/3
kidneys	subacute inflammation	minimal	1/3		
Females					
kidney	infarct	mild		1/3	
	subacute inflammation	minimal			1/3
	subacute inflammation	mild		1/3	
salivary gland, mandibular	lymphocytic infiltration	minimal	1/3		1/3
tongue	subacute inflammation	minimal	2/3	1/3	
ureters	dilatation/inflammation/hyperplasia	mild		1/3	
urinary bladder	simple transitional cell hyperplasia	mild		1/3	
	subacute inflammation	mild		1/3	
vagina	subacute inflammation	minimal		1/3	

Drug exposure increased more than dose proportionally. Maximum plasma levels (C_{max}) increased approximately 15-fold and total AUC increased approximately 30-fold over the 10-fold dose range. Absorption time was variable, with approximate T_{max} of 2 h (ranged 1 to 8 h). Half life was consistent across doses and was approximately 2.5 to 3.5 h. There was no evidence of drug accumulation or sex differences in exposure. The sponsor's summary TK data (combined sexes) are shown in the table below.

Archived plasma TK samples were evaluated for M-I metabolite concentrations after the initial study was completed. M-I exposure was approximately 60% that of parent drug. Based on potent DPP4 inhibition by M-I, the active metabolite was expected to contribute about 37% of total pharmacodynamic activity in the 28-day dog study. Sponsor's table comparing parent to M-I exposure is shown below.

Sponsor's Four Week Dog TK Summary (Sexes Combined)

	Day 1			Day 26		
Parameters	7.5	25	75	7.5	25	75
Cmax (ng/mL)	1096.33 (14.6)	5753.33 (23.1)	15980.00 (19.9)	1165.33 (21.7)	5811.67 (19.6)	14918.00 (27.6)
Tmax (hr)	1.50 (1.00-2.00)	1.25 (0.50-2.00)	1.50 (0.50-4.00)	1.50 (1.00-4.00)	1.00 (1.00-4.00)	2.00 (0.50-8.00)
T½ (hr)	3.24 (14.3)	2.58 (17.8)	3.24 (18.9)	2.95 (5.7)	2.78 (6.1)	2.91 (12.9)
AUC(0-24) (ng·hr/mL)	3955.05 (15.3)	26025.83 (21.0)	115193.45 (15.6)	5105.66 (13.7)	30679.48 (15.1)	116612.61 (19.1)
Accumulation Ratio (R)	NA	NA	NA	1.31 (17.0)	1.20 (11.8)	1.02 (14.7)

Mean (CV%) values are presented, with the exception of Tmax, which has the median and range reported.

Sponsor's SYR-322 to M-I Comparison

Species/Sex	SYR110322		SYR110324		Ratio of 324/322 (%)	
	C_{max} ng/mL	AUC_{0-24} h • ng/mL	C_{max} ng/mL	AUC_{0-24} h • ng/mL	C_{max} ng/mL	AUC_{0-24} h • ng/mL
Dog (Day 26, 25 mg/kg)						
Male	4980	32077	1583	16850	31.8	52.5
Female	6643	29282	2347	19374	35.3	66.2

Exploratory GLP eight week oral dog

The sponsor conducted a GLP-compliant, exploratory study to compare toxicity of different DPP4 inhibitors under development. The FDA review focused on alogliptin results. Dogs were treated with a single dose level of 10 mg/kg compared to vehicle controls (and other DPP4 inhibitors). Groups were limited to 2 animals/sex, so results were preliminary (i.e. exploratory) in nature and variability was high.

Alogliptin findings were unremarkable and the single dose level of 10 mg/kg was considered the NOAEL. No histopathology analysis was performed for alogliptin animals, so conclusions were limited to gross findings and standard clinical monitoring

(signs, hematology, clinical chemistry, etc.). Plasma TK was conducted on day 1, day 28, and day 56, with sampling time course limited to 8 h post-dose. Results were variable, sample sizes were small, thus TK results were of limited predictive value compared to more comprehensive data from 28-day, 13-week, and 9-month dog studies. Nevertheless, the sponsor's summary TK data are provided in the table below for comparison.

Sponsor's eight week dog TK summary

Parameters	Gender	Day 1	Day 28	Day 56
AUC(0-8) (ng·hr/mL)	Male	4946.35 (26.42%)	8041.76 (13.98%)	3581.43 (8.39%)
	Female	4780.31 (18.40%)	4983.93 (36.85%)	11162.06 (52.99%)
	Combined	4863.33 (18.81%)	6512.85 (33.15%)	7371.74 (75.34%)
Cmax (ng/mL)	Male	1605.00 (32.16%)	2345.00 (2.11%)	1275.00 (24.96%)
	Female	1715.00 (16.08%)	1339.50 (44.40%)	2910.00 (34.02%)
	Combined	1660.00 (20.71%)	1842.25 (36.64%)	2092.50 (53.46%)
R1	Male	. (.%)	1.65 (12.68%)	. (.%)
	Female	. (.%)	1.10 (53.44%)	. (.%)
	Combined	. (.%)	1.37 (35.07%)	. (.%)
R2	Male	. (.%)	. (.%)	0.76 (34.43%)
	Female	. (.%)	. (.%)	2.26 (36.37%)
	Combined	. (.%)	. (.%)	1.51 (66.22%)
Tmax (hr)	Male	1.00 (1.00-1.00)	1.50 (1.00-2.00)	1.50 (1.00-2.00)
	Female	2.00 (2.00-2.00)	2.50 (1.00-4.00)	1.50 (1.00-2.00)
	Combined	1.50 (1.00-2.00)	1.50 (1.00-4.00)	1.50 (1.00-2.00)

Mean (CV%) values are presented, with the exception of Tmax, which has the median and range reported.

Thirteen week oral dog

A thirteen week oral toxicity study of SYR-322 in Beagle dogs
10, 30, 90 mg/kg
*SYR-322 (9, 35, 123 μg*h/ml); SYR-324 (5, 13, 26 μg*h/ml)*
NOAEL: Male = 90 mg/kg (99X MRHD); Female = 30 mg/kg (34X MRHD)

Key study findings[8]:

- No clear treatment-related change in histopathology, hematology, or clinical chemistry
- Facial swelling and reddening of skin in some females at 30 and 90mg/kg
- Reduced weight gain and body weight and females at 90mg/kg, with a corresponding 8.5% reduction in food consumption. Weight gain loss occurs in males, but the data is not conclusive as to the extent of loss (not dose-related). See sponsor's body weight summary table, below.
- The small number of dogs per group (4) and data variability precludes attributing weight changes in some organs (spleen, adrenal, thymus) to treatment with drug
- ***NOAEL Determination:*** The data support a NOAEL of 30mg/kg in females, and 90mg/kg in males. The moderate loss in weight gain, actual body weight, and reduced food consumption in females dosed 90mg/kg suggests a tolerability issue not identified in the current study. This issue may also affect males, but the data is too variable at 30 & 90mg/kg to draw firm conclusions. *Reviewer's note – male and female dogs tolerated higher doses in the chronic dog study.*

Sponsor's Body Weight Summary

Summary of Groups Mean Body Weight in Kilograms

Dose Level	Male			Female		
(mg/kg/day)	Pretest	Week 13	(%)	Pretest	Week 13	(%)
0 (Control)	8.8	11.0	(+25.0)	7.2	8.4	(+16.7)
10	8.4	10.3	(+22.6)	8.1	9.5	(+17.3)
30	8.0	8.9	(+11.3)	8.2	9.1	(+11.0)
90	8.6	10.4	(+20.9)	7.2	7.8	(+8.3)

% - Difference from pretest

Treatment-related clinical signs were limited to sporadic soft feces in males and females and facial swelling and skin reddening (ears, abdomen, face) in females.

[8] Key study findings from IND review; MRHD estimates based on total SYR-322 + SYR 324 (M-I)

IND Review Clinical Signs Summary Tables

CLINICAL SIGNS- MALES (n=4/group)								
	control		10mg/kg		30mg/kg		90mg/kg	
Sign	# dogs effected	# observ.	# dogs effected	# observ.	# dogs effected	# observ.	# dogs effected	# observ.
Soft Feces	4	29	4	12	4	27	4	38

CLINICAL SIGNS- FEMALES (n=4/group)								
	control		10mg/kg		30mg/kg		90mg/kg	
Sign	# dogs effected	# observ.	# dogs effected	# observ.	# dogs effected	# observ.	# dogs effected	# observ.
Soft Feces	4	14	4	17	4	29	4	24
Facial Swelling	0	0	0	0	2	3	3	4
Red skin of ears, abdomen, or face	3	10	1	1	2	3	3	11

There were no treatment-related cardiovascular effects based on ECG, clinical monitoring, and necropsy observations.

There were no clear treatment-related histological findings. Skin lesions were seen in two animals but lesions were not consistent with drug-related skin lesions seen with other DPP4 inhibitors. Notable findings, generally limited to minimal to mild lesions, are shown in the IND review summary table below.

IND Review Histopathology Summary Table

HISTOPATHOLOGY, 13 WEEK DOG STUDY						
Tissue- Males	**Finding**	**Severity**	**0**	**10**	**30**	**90**
			n=4	**n=4**	**n=4**	**n=4**
Skin	erosion/ulcer	minimal	0	0	0	1
	exudates, epidermal surface	minimal	0	0	0	1
Thymus	atrophy	minimal	0	2	1	3
		mild	2	0	2	0
Tissue- Females	**Finding**	**Severity**	**0**	**10**	**30**	**90**
			n=4	**n=4**	**n=4**	**n=4**
Skin	erosion/ulcer	moderate	0	0	1	0
	exudates, epidermal surface	moderate	0	0	1	0
	epidermal hyperplasia	mild	0	0	1	0
	chronic-active inflammation	mild	0	0	1	0
Thymus	atrophy	minimal	0	3	1	0
		mild	0	0	2	1

Plasma was analyzed on day 1 and day 89 for toxicokinetic analysis of alogliptin and M-I. Trends were similar to the 28-day dog study, with M-I accounting for approximately 50-60% of parent compound. Drug and M-I exposure generally increased in proportion to dose. There was modest drug accumulation over time, with greater accumulation (~40%) at the low dose compared to mid- and high doses (20-28%). Trends for M-I and combined SYR-322 + M-I did not accumulate over time. There were no sex differences in exposure. Sponsor's TK summary tables are shown below.

Sponsor's SYR-322 TK Summary

		Day 1			Day 89		
Parameters	Gender	10 mg/kg	30 mg/kg	90 mg/kg	10 mg/kg	30 mg/kg	90 mg/kg
AUC(0-24) (ng·hr/mL)	Female	6048.84	30790.05	106482.15	9455.24	37105.48	126350.50
	Male	6431.01	29251.03	114251.20	8090.83	33475.68	120529.01
	All	6239.92	30020.54	110366.68	8773.03	35290.58	123439.76
AUC(0-24)/Dose ($x10^{-6}$ hr*kg/mL)	Female	604.88	1026.34	1183.14	945.52	1236.85	1403.89
	Male	643.10	975.03	1269.46	809.08	1115.86	1339.21
	All	623.99	1000.68	1226.30	877.30	1176.35	1371.55
Cmax (ng/mL)	Female	1712.50	5805.00	17275.00	2545.00	7802.50	17325.00
	Male	1620.00	7760.00	17475.00	1727.50	6705.00	17225.00
	All	1666.25	6782.50	17375.00	2136.25	7253.75	17275.00
Tmax (hr)	Female	1.00	1.50	0.88	1.00	1.13	2.50
	Male	2.00	1.13	1.75	2.00	1.25	2.00
	All	1.50	1.31	1.31	1.50	1.19	2.25
T½ (hr)	Female	3.74	2.28	2.77	3.35	3.01	2.82
	Male	3.21	2.45	2.64	3.33	2.77	2.89
	All	3.47	2.37	2.70	3.34	2.89	2.86
Accumulation Ratio (R)[a]	Female	.	.	.	1.67	1.21	1.49
	Male	.	.	.	1.26	1.21	1.06
	All				1.47	1.21	1.28

"." = Not Available

[a] Mean R was calculated from the ratio of individual AUC(0-24) between Day 89 and Day 1.

Sponsor's SYR-324 (M-I) TK Summary

		Day 1			Day 89		
Parameters	Gender	10 mg/kg	30 mg/kg	90 mg/kg	10 mg/kg	30 mg/kg	90 mg/kg
AUC(0-24) (ng·hr/mL)	Female	5129.10	18614.29	39235.20	5303.93	13471.13	25415.25
	Male	4938.12	19152.46	48994.86	4331.48	11822.59	26450.36
	All	5033.61	18883.37	44115.03	4817.70	12646.86	25932.81
AUC(0-24)/Dose ($x10^{-6}$ hr*kg/mL)	Female	512.91	620.48	435.95	530.39	449.04	282.39
	Male	493.81	638.42	544.39	433.15	394.09	293.89
	All	503.36	629.45	490.17	481.77	421.56	288.14
Cmax (ng/mL)	Female	763.25	2125.00	3672.50	673.00	1435.00	1842.50
	Male	810.00	2325.00	4415.00	558.00	1240.00	1925.00
	All	786.63	2225.00	4043.75	615.50	1337.50	1883.75
Tmax (hr)	Female	2.25	3.00	3.25	1.75	2.50	4.50
	Male	2.50	2.00	4.00	2.50	2.50	4.00
	All	2.38	2.50	3.63	2.13	2.50	4.25
T½ (hr)	Female	4.26	3.31	4.48	4.09	4.57	5.88
	Male	3.79	3.46	4.25	4.30	3.88	5.04
	All	4.03	3.39	4.37	4.20	4.23	5.40
Accumulation Ratio (R) [a]	Female	.	.	.	1.08	0.72	0.71
	Male	.	.	.	0.88	0.62	0.56
	All				0.98	0.67	0.63

"." = Not Available

[a] Mean R was calculated from the ratio of individual AUC(0-24) between Day 89 and Day 1.

Nine month chronic dog

Nine month oral toxicity of SYR-322 in Beagle dogs with a four week recovery
30, 100, 200 mg/kg
*40, 200, 400 µg*h/ml*
NOAEL > 200 mg/kg (267X MRHD for SYR-322)

Key study findings[9]:

- Unexplained death of one male at 200mg/kg after 20 weeks of dosing. Moderate consolidation of lung lobes is the most likely cause, but the relationship to drug treatment is uncertain. *Reviewer's note – the death was not considered treatment related by the IND reviewer, based on estimation of a NOAEL > 200 mg/kg*

- Lymphocytic infiltration (minimal) of the lungs in 1 to 2 males at all doses is the only histopathological finding potentially related to drug treatment.

[9] Key study findings from IND review

- Skin redness of the ears/face/mouth area was seen in several males and females at 100 and 200mg/kg. Redness lasted from the first week of dosing to the end of the study, and only reversed in some dogs after 4 weeks recovery. Sporadic swelling of the face, eyes, neck, and foot were also noted in isolated cases.

- BW decreased ~10% in 200mg/kg males and females during the first month of dosing. BW recovered in males by the second month, but persisted in females to the end of the study. Reduced food consumption accompanied decreased BW during the first month. *(See sponsor's Figures 1 and 1a, below)*

- Total cholesterol increased 15% at all doses, due in part to a similar rise in HDL cholesterol.

- Spleen weight in females tended to decrease ~10% at 100 and 200mg/kg, but a relation to drug treatment is uncertain.

- ***NOAEL Determination*** – Redness of facial skin at 100 and 200mg/kg was the only finding clearly related to drug treatment. Such findings were also noted with SYR110322 in shorter duration studies at 75mg/kg. No new toxicities were identified in the 9 month study that were not present in the 13 week study.
    - Facial flushing has not been used as the basis for a NOAEL; transient flushing has occurred in clinical trials without serious consequences. Therefore, the NOAEL for this study is greater than 200mg/kg, the highest dose tested

Figure 1 Mean Body Weight Values – MALE

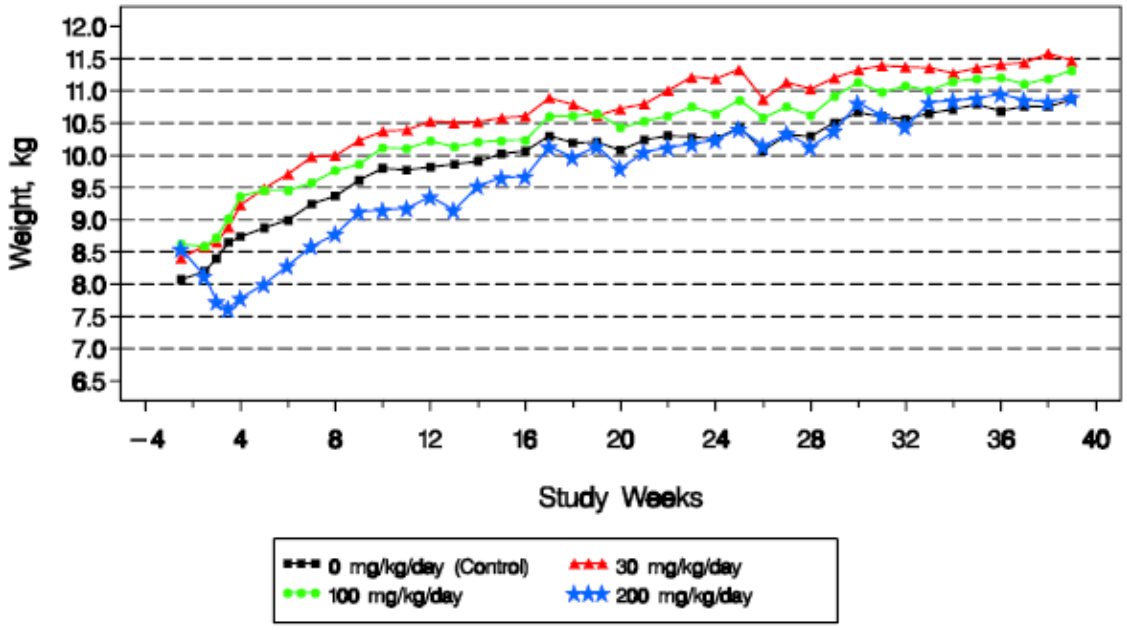
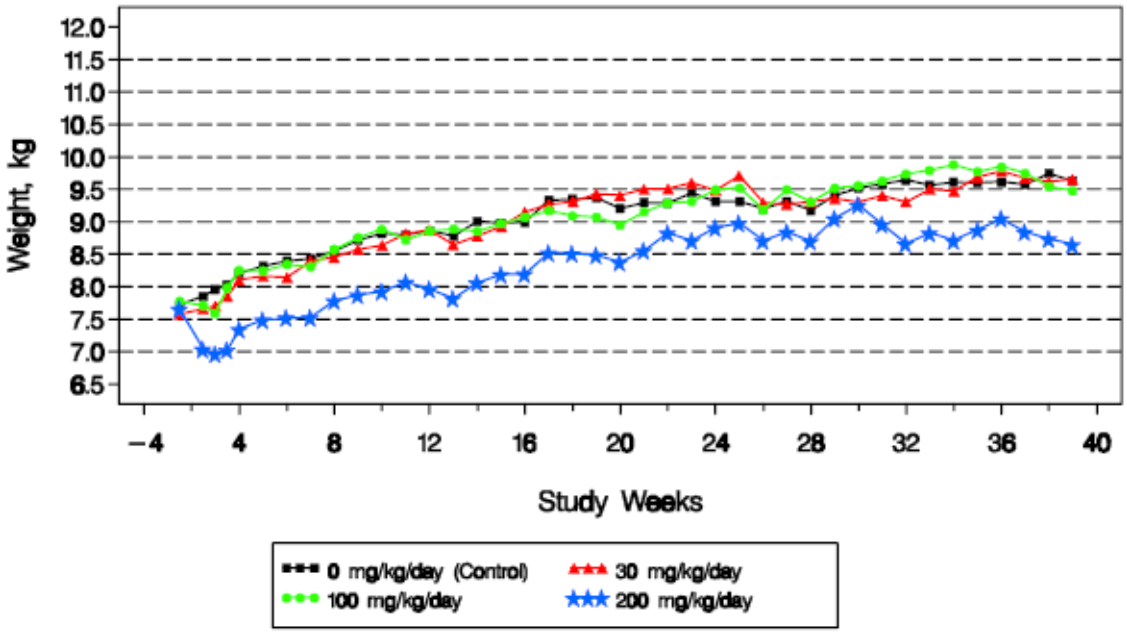


Figure 1A Mean Body Weight Values – FEMALE



There were no treatment-related ECG findings in males or females after nine months of dosing. Troponins I and T were also assessed and there were no treatment related changes in the markers of ischemic cardiac toxicity.

There were no clear treatment-related histopathology findings in dogs. Lung lymphocytic infiltration of minimal severity was seen in some males in all SYR-322 groups. The lesion was seen in 1/3 treated males after the drug-free recovery period. Incidence and severity were not dose-dependent and lymphocytic infiltration was not considered biologically significant.

Histological skin lesions in one male were consistent with gross skin abrasion in the animal, but were not considered indicative of skin lesions induced by other DPP4 inhibitors. See summary table from the IND review, below.

MALE HISTOPATHOLOGY, 39 WEEK DOG STUDY						
Tissue	Finding	Severity	0	30	100	200
			n=4	n=4	n=4	n=3
Lung	lymphocytic infiltration	minimal	0	2	2	1
Skin	Hyperkeratosis	moderate		0	1	0
	epidermal hyperplasia	moderate		0	1	0
	acute inflammation	mild		0	1	0
	necrosis	severe		0	1	0
Thymus	lymphoid depletion	minimal	1	3	1	3
		mild	3	1	1	0
		moderate			1	
		severe			1	

Reviewer's note – thymus depletion was not dose-dependent but lesions were documented in the IND review due to the 'severe' lesion in one 100 mg/kg male

Exposure increased with increasing dose, with total exposures as AUC from 100 to 200 mg/kg and maximum plasma exposure (C_{max}) increases roughly dose-proportional. SYR-322 accumulated over the course of the study, independent of dose, achieving roughly 30% to 2-fold greater AUC on day 272. Based on minimal human M-I (SYR-324) exposure (≤ 1% of parent drug), SYR-324 was not measured in the chronic dog study and total exposure to DPP4-inhibiting equivalents could be considered under-estimated based on trends limited to parent alogliptin exposure. The sponsor's TK summary is shown below.

Sponsor's Nine Month Dog TK Summary

SYR110322S Analysis – Males					
Day	Dose (mg/kg/day)	AUC (0-24) (ng·hr/mL)	AUC (0-24) Dose ($x10^{-6}$ hr*kg/mL)	Cmax (ng/mL)	Accumulation Ratio (R)
1	30	30896.23	1029.87	6797.50	-
	100	147082.30	1470.82	18650.00	-
	200	279737.64	1398.69	24542.86	-
272	30	41741.42	1391.38	8010.00	1.38
	100	187076.96	1870.77	19300.00	1.27
	200	396593.29	1982.97	38383.33	1.42

SYR110322S Analysis – Females					
Day	Dose (mg/kg/day)	AUC (0-24) (ng·hr/mL)	AUC (0-24) Dose ($x10^{-6}$ hr*kg/mL)	Cmax (ng/mL)	Accumulation Ratio (R)
1	30	29638.34	987.94	6442.50	-
	100	111674.13	1116.74	15707.50	-
	200	257856.88	1289.28	21342.86	-
272	30	40126.62	1337.55	8330.00	1.35
	100	206757.38	2067.57	23050.00	1.89
	200	403179.14	2015.90	39157.14	1.64

Four week oral monkey

Four-week oral gavage toxicity study of SYR-322 in monkeys
1, 10, 30 mg/kg
*1, 12, 31 µg*h/ml*
NOAEL = 30 mg/kg (21X MRHD)

Key study findings[10]:

- Doses of 1, 10, and 30 mg/kg (3:3 M:F per group, 0.4X, 5X, and 15X MRHD of 25 mg/kg (AUC basis)) were generally well tolerated by Cynomolgus monkeys.
- Hematology findings included 25% reduction in reticulocytes in HD males and a 20% increase in lymphocytes in HD females.
- All but one treated male showed a decrease in blood glucose, though the differences in the group averages were not significant.
- Males had significantly enlarged prostates (by 81%) at the highest dose, and females showed a dose-dependent increase in ovary and pituitary gland mass (HD females had increases of 70% and 50% respectively).

[10] Key study findings from IND review

- Histopathological examination of the skin from multiple locations prone to developing lesions does not appear to show any treatment-related adverse events at plasma drug concentrations up to 22μM (IC50's: DPP4 15nM, DPP8/9 ≥100μM) ➔ i.e. exceeds IC_{50} for DPP4 but below IC_{50} for DPP8 and DPP9.

- Cmax and AUC0-24h generally increased proportionally to increases in dose for SYR-322Z. Repeated dosing increased exposure for high dose females.

- Exposure to the M-I metabolite varied greatly between animals and tended to decrease after repeated dosing (as a fraction of SYR-322Z exposure).

Thirteen week oral monkey

Thirteen-week oral gavage toxicity study of SYR-322 in monkeys
3, 10, 30
*4, 13, 47 μg*h/ml*
NOAEL ≥ 30 mg/kg (31X MRHD)

Summary and NOAEL Determination **–**The study was designed to examine potential for drug-induced skin lesions, which have been observed with other drugs in the DPP4 class. There was no evidence of drug-related skin lesions in clinical observations, macroscopic analysis at necropsy, or histological analysis at necropsy. There were no remarkable treatment related findings. The NOAEL for the study was the high dose of 30 mg/kg/day, which provided 31-times expected human exposure.

Key Study Findings:

- No remarkable treatment-related findings

- Possible drug-related effect of reduced testes size at high dose. No conclusion was possible due to small sample sizes and the limited scope of the study to examine skin lesions (histopathology examination limited to skin samples)

Toxicokinetic summary: Drug was readily absorbed (T_{max} ≈ 1-2 h) and converted to metabolites M-I and M-II, with drug exposure increased approximately dose-proportionally. Metabolite concentrations were minor (≤ 10%) compared to parent drug, with C_{max} and AUC_{0-24} approximately 3-5% for M-I and 0.5% for M-II. There were no apparent sex differences and no evidence of drug accumulation.

Organ weights: Organ weight data show a potential effect on testes size at the high dose. Findings are likely related to sexual maturity of individual animals, but small size of testes in 2/3 high dose males that were **not** noted as grossly small in size suggest there may be a drug related. See sponsor's summary data, below.

Table 7 Organ weight - Summary					Male
Test article Dose	Animal number	Final body weight kg Day 92	Testis left g Day 92	Testis right g Day 92	Testes g Day 92
Control 0 mg/kg	10101	3.0	9.09	8.63	17.72
	10102	4.0	7.31	7.56	14.87
	10103	3.3	1.59	1.53	3.12
	Mean	3.43	5.997	5.907	11.903
	S.D.	0.51	3.919	3.828	7.739
SYR-322 3 mg/kg	10201	3.4	0.58	0.60	1.18
	10202	3.9	5.52	5.20	10.72
	10203	2.3	0.48	0.52	1.00
	Mean	3.20	2.193	2.107	4.300
	S.D.	0.82	2.881	2.679	5.561
SYR-322 10 mg/kg	10301	3.1	2.82	2.49	5.31
	10302	3.3	4.89	5.70	10.59
	10303	3.7	4.55	4.40	8.95
	Mean	3.37	4.087	4.197	8.283
	S.D.	0.31	1.110	1.615	2.702
SYR-322 30 mg/kg	10401	3.1	0.61	0.57	1.18
	10402	3.3	2.38	1.97	4.35
	10403	4.3	4.45	4.39	8.84
	Mean	3.57	2.480	2.310	4.790
	S.D.	0.64	1.922	1.933	3.849

Reviewer's note – 'small testes' were noted grossly for animal numbers 10201, 10203 (low dose) and 10401 (high dose)

2.6.6.4 Genetic toxicology

Ames Mutagenicity Assay

Alogliptin was negative for mutagenic potential in a standard battery of bacterial strains with and without a metabolic activation system. Positive controls induced mutant colony counts and confirmed the sensitivity of the assay. The sponsor's summary table from the definitive assay is shown below.

Sponsor's Mutagenicity Summary (Definitive Assay)

Test Article Id : SYR110322S
Study Number : AA89HC.503.BTL Experiment No : B2

Average Revertants Per Plate ± Standard Deviation

Liver Microsomes: None

Dose (µg/plate)	TA98	TA100	TA1535	TA1537	WP2 uvrA
Vehicle	10 ± 2	111 ± 24	12 ± 3	5 ± 2	11 ± 1
75	12 ± 2	126 ± 4	16 ± 4	3 ± 1	15 ± 4
200	12 ± 1	125 ± 7	13 ± 2	4 ± 1	11 ± 2
600	12 ± 1	125 ± 5	19 ± 3	6 ± 2	12 ± 1
1800	13 ± 3	112 ± 6	17 ± 1	5 ± 1	11 ± 2
5000	11 ± 3	120 ± 7	11 ± 2	4 ± 1	12 ± 1
Positive	63 ± 2	559 ± 15	243 ± 30	330 ± 21	89 ± 28

Liver Microsomes: Rat liver S9

Dose (µg/plate)	TA98	TA100	TA1535	TA1537	WP2 uvrA
Vehicle	20 ± 2	133 ± 16	13 ± 4	7 ± 3	11 ± 2
75	20 ± 5	140 ± 10	12 ± 3	4 ± 2	10 ± 2
200	25 ± 5	126 ± 19	9 ± 2	3 ± 2	12 ± 1
600	24 ± 4	134 ± 4	13 ± 6	5 ± 1	11 ± 3
1800	22 ± 2	142 ± 13	12 ± 2	4 ± 2	14 ± 2
5000	24 ± 2	127 ± 13	12 ± 4	6 ± 3	11 ± 2
Positive	752 ± 30	842 ± 180	111 ± 13	105 ± 4	490 ± 64

Vehicle = Vehicle Control
Positive = Positive Control (50 µL plating aliquot)
Plating aliquot: 100 µL

Mouse Lymphoma Assay

Alogliptin mutagenic and chromosomal aberration potential were assessed in a series of mouse lymphoma L5178Y $TK^{+/-}$ assays. Following an initial toxicity assay, SYR-322 induced 2- to 3-fold increases in small colonies and equivocal increases in induced mutant frequency in a 4 h incubation without a metabolic activation system. Results were negative in a 4 h incubation with a rat liver S9 metabolic activation system and in a follow-up 24 h incubation assay without metabolic activation.

FDA recommended additional testing to clarify the "equivocal" mutagenic response without metabolic activation. The sponsor conducted a new, supplemental assay consisting solely of a 4 h incubation without a metabolic activation system and results

were clearly negative at a concentration of 1000 µg/ml which resulted in approximately 12-16% survival of colonies. Positive control results confirmed the sensitivity of the assay system. Summary results from the supplemental assay are shown in the tables below.

It was not clear why results of one mouse lymphoma assay were positive. Nevertheless, the weight of evidence in a series of assays supported the conclusion that alogliptin was negative for mutagenic potential in the mouse lymphoma system.

CLONING DATA FOR L5178Y/TK$^{+/-}$ MOUSE LYMPHOMA CELLS TREATED WITH SYR110322S IN THE ABSENCE OF EXOGENOUS METABOLIC ACTIVATION
Supplemental Assay (4-hour exposure)

Test Article Concentration (µg/mL)		TFT Colonies Counts			Mean	VC Colonies Counts				Mean	Mutant Freq.[a]	Induced Mutant Freq.[b]	% Total Growth[c]
Solvent	1	45	65	60	57 ±8	208	199	194	200	±6	57		
Solvent	2	52	84	44	60 ±17	212	125	178	172	±36	70		
Mean Solvent Mutant Frequency= 63													
500	A	38	28	35	34 ±4	165	182	149	165	±13	41	-23	79
500	B	44	47	22	38 ±11	129	135	163	142	±15	53	-10	61
600	A	71	73	66	70 ±3	194	160	194	183	±16	77	13	71
600	B	54	67	78	66 ±10	171	170	175	172	±2	77	14	57
650	A	59	77	63	66 ±8	178	195	163	179	±13	74	11	74
650	B	64	70	39	58 ±13	194	207	218	206	±10	56	-7	60
750	A	65	59	45	56 ±8	169	127	148	148	±17	76	13	40
750	B	51	40	26	39 ±10	194	195	201	197	±3	40	-24	69
1000	A	50	47	50	49 ±1	189	176	205	190	±12	52	-12	16
1000	B	54	51	57	54 ±2	188	172	185	182	±7	59	-4	12
Positive Control - Methyl Methanesulfonate (µg/mL)													
15		191	172	94	152 ±42	44	52	86	61	±18	502	439	22
20		165	183	164	171 ±9	35	39	34	36	±2	948	885	10

Solvent = DMSO

TOTAL COMPOUND TOXICITY DATA FOR L5178Y/TK$^{+/-}$ MOUSE LYMPHOMA CELLS TREATED WITH SYR110322S
IN THE ABSENCE OF EXOGENOUS METABOLIC ACTIVATION
Supplemental Assay (4-hour exposure)

Test Article Concentration (µg/mL)		Cell Concentration (X 10^6)[a] Day 1	Day 2	Susp Growth Total[b]	%Cntl[c]	Cloning Growth Avg VC	%Cntl[d]	% Total Growth[e]
Solvent	1	1.416	1.508	23.7		200		
Solvent	2	1.403	1.454	22.7		172		
500	A	1.196	1.552	20.6	89	165	89	79
500	B	1.138	1.464	18.5	80	142	77	61
600	A	0.947	1.585	16.7	72	183	98	71
600	B	0.851	1.513	14.3	62	172	92	57
650	A	1.012	1.599	18.0	77	179	96	74
650	B	0.882	1.280	12.5	54	206	111	60
750	A	0.685	1.519	11.6	50	148	80	40
750	B	0.916	1.495	15.2	66	197	106	69
1000	A	0.229	1.066	3.6	15	190	102	16
1000	B	0.189	0.890	3.0	13	182	98	12
1250	A	0.052	0.196	0.0	0	++		
1250	B	0.036	0.116	0.0	0	++		
Positive Control - Methyl Methanesulfonate (µg/mL)								
15		0.973	1.430	15.5	67	61	33	22
20		0.904	1.165	11.7	50	36	19	10

Solvent = DMSO

In Vivo Micronucleus Assay in Mice

There was no evidence of increased micronucleated erythrocytes in male or female mice treated with a single 200 mg/kg oral dose of alogliptin. Summary results are shown the sponsor's Table 6, below.

Table 6: Summary of Bone Marrow Micronucleus Analysis Following a Single Dose of SYR110322S in ICR Mice

Treatment (20 mL/kg)	Sex	Time (hr)	Number of Mice	PCE/Total Erythrocytes (Mean +/- SD)	Change from Control (%)	Micronucleated Polychromatic Erythrocytes: Number per 1000 PCEs (Mean +/- SD)	Micronucleated Polychromatic Erythrocytes: Number per PCEs Scored[1]
Corn oil	M	24	5	0.514 ± 0.06	—	0.8 ± 0.27	8 / 10000
	F	24	5	0.497 ± 0.08	—	0.9 ± 0.22	9 / 10000
SYR110322S 50 mg/kg	M	24	5	0.467 ± 0.04	-9	0.6 ± 0.42	6 / 10000
	F	24	5	0.478 ± 0.05	-4	0.5 ± 0.35	5 / 10000
100 mg/kg	M	24	5	0.457 ± 0.05	-11	0.3 ± 0.27	3 / 10000
	F	24	5	0.441 ± 0.03	-11	0.6 ± 0.22	6 / 10000
200 mg/kg	M	24	5	0.463 ± 0.05	-10	1.2 ± 0.57	12 / 10000
	F	24	5	0.461 ± 0.06	-7	0.5 ± 0.00	5 / 10000
Cyclophosphamide 50 mg/kg	M	24	5	0.314 ± 0.02	-39	18.0 ± 3.69	*180 / 10000
	F	24	5	0.326 ± 0.02	-34	17.1 ± 2.25	*171 / 10000
Corn oil	M	48	5	0.502 ± 0.06	—	0.6 ± 0.22	6 / 10000
	F	48	5	0.432 ± 0.05	—	0.5 ± 0.35	5 / 10000
SYR110322S 200 mg/kg	M	48	5	0.506 ± 0.06	1	0.8 ± 0.45	8 / 10000
	F	48	5	0.483 ± 0.02	12	0.4 ± 0.42	4 / 10000

[1]*Statistically significant, $p \leq 0.05$ (Kastenbaum-Bowman Tables)

2.6.6.5 Carcinogenicity

A 2-year oral carcinogenicity study of SYR-322 in CD-1 mice
50, 150, 300 mg/kg
*10, 42, 90 µg*h/ml*
NOAEL = 300 mg/kg (60X MRHD)

Key study findings: NOAEL = 300 mg/kg/day (non-neoplastic and neoplastic findings); 60X MRHD

The final study report of a GLP-compliant, standard two year oral (gavage) carcinogenicity in CD-1 mice was reviewed and results were discussed at a meeting of the Executive Carcinogenicity Assessment Committee (ECAC). The study was considered acceptable based on a 60X exposure multiple at the NOAEL (the high dose). The sponsor chose doses that were originally proposed to the ECAC but which the ECAC determined were likely to lead to excessive mortality. The high dose did not result in remarkable toxicity, however, exposure at the high dose provided 45-fold to 74-fold exposure margins (male and female, respectively) over the maximum recommended human dose of 25 mg (1.5 µg*h/ml).

Adequacy of the carcinogenicity study and appropriateness of the test model: Sponsor chose doses originally proposed to ECAC (0, 50, 150, 300), which the ECAC determined were likely to lead to excessive mortality in mice. Results showed low mortality in treatment groups compared to controls and confirmed the appropriateness of the model and doses. The high dose did not result in remarkable toxicity, however, exposure at the high dose provided 45-fold to 74-fold exposure margins (male and female, respectively) over the maximum recommended human dose of 25 mg (1.5 µg*h/ml).

Evaluation of tumor findings: Notable findings were limited to a 5% incidence of benign hepatocellular adenomas in 300 mg/kg/day females (74X MRHD). There were no similar tumors in any other group (up to 45X MRHD in males, 34X MRHD in females). The incidence was statistically significant according to the FDA statistical review, under the assumption that it was a "rare" tumor in female mice. The reviewing toxicologist did not consider hepatocellular adenoma to be a "rare" tumor in female mice, based on mean historical tumor incidence greater than 1% in both the conducting contract lab and the laboratory of the mouse supplier (b) (4) The incidence of hepatocellular adenoma was not statistically increased when considered a "common tumor". Based on an absence of malignant hepatocellular carcinomas in the high dose group and the fact that tumor incidence was within the historical range of some studies in CD-1 mice, the finding was not considered evidence of a treatment related carcinogenic effect. In addition, there were no increases in malignant hepatocellular carcinoma or additional histopathology correlates to suggest hyperplasia, pre-neoplastic lesions or extensive hepatotoxicity. The sponsor also concluded there were no statistically or biologically significant carcinogenic effects (i.e. the sponsor's analysis did not show statistical significance, presumably because it was not considered a rare tumor).

Mouse Histopathology – Neoplasm Summary									
Tissue	Finding	Male				Female			
		0 (n=60)	50 (n=60)	150 (n=60)	300 (n=60)	0 (n=60)	50 (n=60)	150 (n=60)	300 (n=60)
Liver	Hepatocellular adenoma (benign)	2	7	2	4	0	0	0	3
	Hepatocellular carcinoma (malignant)	2	1	2	0	0	0	1	0
	Adenocarcinoma	0	0	0	0	0	1	0	0

Mouse Human Exposure Summary †				
Dose (mg/kg)	AUC (µg*h/ml)		MRHD	
	Males	Females	Males	Females
50	10	9	7X	6X
150	33	51	22X	34X
300	68	111	45X	74X

† Mouse exposure at 18 months; human exposure multiples based on 25 mg MRHD (AUC_{0-24} = 1.5 µg*h/ml) in diabetic patients

A 2-year oral carcinogenicity study of SYR-322 in Sprague Dawley rats

75, 400, 800 mg/kg
*48, 425, 703 µg*h/ml*
NOAEL = 75 mg/kg (32X MRHD)

Key study findings: NOAEL = 75 mg/kg/day. There was an increased incidence of combined thyroid C-cell adenomas and carcinomas in males. MRHD at NOAEL = 32X and 288X at the tumor-inducing dose (LOAEL).

The final study report of a GLP-compliant, standard two year oral (gavage) carcinogenicity in Sprague-Dawley rat was reviewed and results were discussed at a meeting of the Executive Carcinogenicity Assessment Committee (ECAC). The study was considered acceptable. Doses were chosen based on the MTD (decrements in body weight gain) and ECAC concurred with doses proposed in the original protocol. Results confirmed body weight decreases in high dose groups and the exposure multiple of 32X at the NOAEL and 288X at the LOAEL provided adequate exposure margins.

Adequacy of the carcinogenicity study and appropriateness of the test model: Doses were based on MTD (decrements in body weight gain) with which the ECAC concurred. Body weight decreases in high dose males (-22%) and females (-18%) at scheduled termination confirmed dose selection based on high dose MTD predictions.

Evaluation of tumor findings: There was evidence for statistically significant increased incidence of combined thyroid C-cell adenomas and carcinomas in males rats. The incidence of thyroid C-cell adenomas were increased in female rats, however, the incidence of neither adenomas nor combined adenomas and carcinomas were statistically increased in female rats. No neoplastic findings in female rats were considered evidence of a drug-related effect by the reviewing toxicologist or by the ECAC. There was no non-neoplastic evidence that thyroid was a target organ and there was no drug-related increase in thyroid C-cell hyperplasia. At the doses in males which caused increased thyroid C-cell tumors, exposures were 288- and 533-fold higher than predicted maximum human exposure. Exposure at the male NOAEL was 32-fold higher than the MRHD. No treatment-related carcinogenic potential was identified in female rats exposed to more than 400-fold higher drug exposure than the MRHD.

SYR-322 poses minimal carcinogenic risk to humans based on high exposure multiples at the NOAEL for tumor incidence (32X) and very high exposure multiples (≥ 288X) at the doses of SYR-322 that caused tumors in a single sex in rats. In addition, no drug-related tumors were found in mice exposed to up to 60-times the MRHD.

Non-neoplastic findings: There was no treatment related increase in mortality. Data from premature decedents showed a small increase in treatment-related deaths from urinary and/or kidney related toxicity, which was consistent with treatment related deaths in the four week, high dose rat study. Decreased body weight gain in the high dose was consistent with predictions based on shorter term rat studies.

Gross pathology findings, inclusive of animals found dead and sampled at terminal necropsy (n=60 per group) included:

- Lung discoloration and focus/foci in mid and high dose animals (associated with increased lung histiocytosis seen histologically)
- Slight increased incidence of small **seminal vesicles** (1/60, 3/60, 2/60, 4/60, respectively) and marked increased small **testes** in MD (14/60) and HD (34/60) males compared to controls (5/60).
- A slight increase in **urinary bladder** calculus/calculi was seen in males (2/60, 1/60, 1/60, 5/60, respectively), up to 'severe' grade in 2/39 found dead/euthanized. There was no incidence reported in any female group.

Histological findings confirmed treatment-related testes toxicity seen sporadically in shorter duration rat studies. Evidence of male reproductive organ toxicity at mid and high doses included increased epididymal oligospermia and germ cell debris (0.75- to 2.5-fold), seminal vesicle depletion (2.5-fold), and testes bilateral seminiferous tubule degeneration/atrophy (up to 3-fold). The findings were consistent with marked increases

in gross testes size.

Liver (periportal vacuolation and focus of cellular alteration), lung (alveolar histiocytosis), urinary bladder (simple transitional cell hyperplasia), and kidney (transitional cell hyperplasia and increased chronic progressive nephropathy) were target organs in males and females.

The NOAEL was 75 mg/kg (32-times human exposure) for non-neoplastic effects based mainly on marked effects on male reproductive tissues and a variety of other modest effects.

Non-neoplastic findings from the IND review are summarized in tabular form below.

- **Male:**
  - ↑ **epididymides** oligospermia/gem cell debris, bilateral (15, 12, 26, 37, respectively); lower magnitude ↑ seen unilaterally (2, 3, 6, 4)
  - ↑ **eye cataracts**, minimal to moderate (0, 2, 3, 7 incidence, respectively)
  - ↑ **liver** periportal vacuolation, generally minimal to mild (8, 15, 25, 34, respectively)
  - ↑ **lung** alveolar histiocytosis, with increased severity with increased dose (32, 35, 46, 55, respectively); generally minimal to mild severity, with moderate severity limited to 2/60 HD animals
  - slight ↑ **seminal vesicle** depletion (moderate to severe in 2, 3, 2, 5, respectively)
  - ↑ **testes** degeneration/atrophy, seminiferous tubules, bilateral (16, 12, 28, 48, respectively); slight ↑ seen unilaterally (2, 5, 4, 5)
  - ↑ **urinary bladder** hyperplasia (simple transitional cell), minimal to mild (2, 6, 10, 14, respectively), largely limited to animals found dead/euthanized

- **Female:**
  - ↑ **kidney** (a) ***hyperplasia*** (transitional cell), generally minimal to mild (19, 26, 27, 28, respectively, with 2/28 moderate in HD); (b) ***chronic progressive nephropathy*** (25, 27, 45, 48, respectively), with severity generally higher in animals found dead/euthanized
  - ↑ **liver** (a) basophilic ***focus of cellular alteration***, predominantly minimal to mild (15, 15, 19, 28, respectively); (b) periportal ***vacuolation***, predominantly minimal to mild (15, 18, 28, 26, respectively), with severity generally higher in animals found dead/euthanized
  - ↑ **lung** alveolar histiocytosis, minimal to mild (37, 31, 44, 53, respectively)
  - ↑ **urinary bladder** hyperplasia (simple transitional cell), minimal to moderate and limited to animals found dead/euthanized (1, 1, 1, 4, respectively)

Rat Human Exposure Summary †				
Dose (mg/kg)	AUC (µg*h/ml)		MRHD	
	Males	Females	Males	Females
75	45	51	30X	34X
400	432	418	288X	279X
800	800	606	533X	404X

† Rat exposure at 18 months; human exposure multiples based on 25 mg MRHD (AUC_{0-24} = 1.5 µg*h/ml) in diabetic patients

2.6.6.6 Reproductive and developmental toxicology

Fertility and early embryonic development

Fertility and early embryonic development to implantation in rats administered SYR110322S

100, 500, 1000 mg/kg
*TK estimate (13-week rat) – 100 µg*h/ml (♂) and 250 µg*h/ml (♀)*
NOAEL = 500 mg/kg (67X (♂) to 167X (♀) MRHD)

Key study findings[11]:

- Toxicity in high dose males (1000 mg/kg) is indicated by a 22% decrement in weight gain and reduced food consumption in males. In high dose females, reduced weight gain is seen early in gestation, but final body weight is not effected. A previous 13 week toxicity study showed increased liver weight, minimal nephropathy, and minimal thymic atrophy in some animals at 1000 mg/kg.

- Fertility indices were not changed by treatment (e.g., # pregnant females, time to pregnancy, # males impregnating females).

- In males, cauda epididymal weight increased 10% at all doses corresponding with increased sperm production and increased percentage of abnormal sperm. Male fertility was apparently not compromised, however.

- In high dose females, estrus cycle length increased by ~1 day; however, the time to impregnation did not change (~3 days).

- One high dose female (#479) showed only 1 implant site and zero embryos. This individual reduced the high dose group mean for the number of implantation sites, number of viable embryos, and increased post-implantation loss. The high dose group is more comparable to control if this individual is excluded from analysis.

- ***NOAEL Determination:*** Increased sperm production and percent abnormal sperm at 1000 mg/kg suggests a NOAEL of 500mg/kg. This NOAEL is still associated with a non-significant but present increase in the percent abnormal sperm.
    - The total resorption in high dose female #479 may reflect surpassing a threshold of toxicity in this individual at 1000 mg/kg. No similar finding was observed in the 3 other groups with a collective 'n' of 67 females. A NOAEL of 500mg/kg is suggested.

[11] Key study findings from IND review

Embryofetal development

Study for effects of SYR110322S on embryo-fetal development in rats

250, 500, 1000 mg/kg;
*143, 323, 800 μg*h/ml*
NOAEL = 500 mg/kg (215X MRHD)

Key study findings[12]:
Dams

- Body weight and BW gain were reduced 5% and 12%, respectively, at 1000 mg/kg. Reduced BW gain was most notable at GD6-9 and GD15-18 intervals. A smaller reduction in BW and BW gain was seen at 500mg/kg (3% and 7% respectively).

- Food consumption was similarly reduced at 1000 mg/kg over most of the gestational period. Food intake was reduced most notably at GD6-9, the initiation of treatment, and generally followed the pattern of BW changes.

- TK data confirmed that SYR110322S and its active metabolite SYR110324 cross the placenta. Plasma concentrations of parent and metabolite were similar in the dam and fetuses at 500 and 1000mg/kg, but ~30% lower in fetuses in the 250mg/kg group.

- No other significant toxicological finding was observed in the dams.

Fetuses

- Pregnancy rate, pre- and post-implantation variables, and fetal viability were comparable across groups.

- Gravid uterine weight was unaffected; effects on the placenta were not presented.

- Fetal body weight, male and female, was reduced 8% at 1000 mg/kg compared to the control group; this difference is statistically significant.

- No drug-related effect was seen for malformations and visceral variations.

- The number of fetuses at 1000 mg/kg with skeletal variations increased 2-fold compared to control. Skeletal variations consisted of incompletely ossified sternebrae and skull bones, suggesting delayed fetal development at 1000 mg/kg.

NOAEL Determination: Toxicity in the dams is indicated by reduced food intake, BW, and BW gain at 1000 mg/kg; a smaller reduction in BW is seen at 500mg/kg. Reduced fetal body weight and increased skeletal variations at 1000 mg/kg suggests delayed fetal development, possibly related to the BW effects on the dams. Fetal findings at 250 and

[12] Key study findings from IND review

500 mg/kg are comparable to findings in the control group. A NOAEL of 500 mg/kg is established, providing a 215x AUC multiple at the MRHD of 25 mg.

Toxicokinetic data were summarized in the IND as follows:

> *"Dams:* Accumulation of exposure to the parent drug is seen at 500mg/kg (36%) and at 1000mg/kg (224%) from GD6 to GD17. No accumulation is seen at 250mg/kg. The active metabolite SYR110324 accounted for ~10% of exposure on the first day of dosing, but exposure decreased by ~50% in the mid and high dose groups on GD17. This pattern of accumulating parent and declining metabolite is good evidence for reduced metabolism at high drug concentrations.
>
> *Fetuses:* Parent and metabolite were found in fetal blood on GD20. Fetal and maternal plasma concentrations were identical in the mid and high dose groups, but fetal exposure was about 30% lower in the low dose group."

A summary table from the IND review shows the absence of drug-related fetal malformations and visceral variations and skeletal variations limited to the high dose.

Summary of fetal malformations and variations

	Control	250 mg/kg	500 mg/kg	1000 mg/kg
# fetuses w/ malformations	2/274 cleft palate; misshapen tympanic ring	1/291 absent eye lens	0/255	1/283 filamentous tail
# fetuses w/ visceral variations	1/139	1/146	1/125	0/142
# fetuses w/ skeletal variations	33/135 (16/23 litters)	44/145 (20/23 litters)	38/130 (19/22 litters)	63/141 (21/23 litters)

Study for effects of SYR110322S on embryo-fetal development in rabbits

100, 200, 500, 700 mg/kg;
*120, 310, 760, 1050 μg*h/ml*
NOAEL = 200 mg/kg for dams and fetuses (207X MRHD)

Key study findings[13]:

Dams

- Significant mortality at 700 mg/kg prompted termination of this dose group. Causes of most deaths were not identified, but some animals had ulcerated and perforated stomachs, and red-discolored thymuses.

- Mortality at 500 mg/kg was observed in 8 of 23 pregnant dams. Survivors of this group were continued to the scheduled necropsies.

- Additional signs of toxicity in dams at 500 mg/kg include decreased activity and breathing difficulty, a 75% decrement in weight gain, and a clinical chemistry that suggests renal dysfunction and a humoral immune response.

- Some mild signs of toxicity were also observed at 200 mg/kg, including breathing difficulty and decreased activity (low incidence), and milder clinical chemistry findings. Body weight and weight gain was comparable to control dams.

- In addition to 8 deaths of pregnant dams, the 500 mg/kg group had 2 dams give early delivery, and 1 dam with total implant resorption. This left 12 litters for analysis compared to ≥ 21 litters in other dose groups.

Fetuses:

- Excluding the 1 dam with total resorption at 500 mg/kg, there was no significant difference in pre- or post-implantation loss variables.

- Fetal body weight, males and females, was decreased 15% in the 500 mg/kg group.

- The incidence of an unossified hyoid body variation was significantly increased in the 500 mg/kg group relative to concurrent and historical controls.

- No other malformation or variation was specifically ascribed to drug treatment; all dose groups were comparable.

NOAEL Determination

The 500 mg/kg group showed mortality, reduced weight gain and food intake, early delivery, and total resorption in at least one dam. This group also produced litters

[13] Key study findings from IND review

with 15% decreased fetal weight and increased incidence of unossified hyoid body, suggesting a delay in fetal development.

The 200 mg/kg groups showed mild toxicity in the dams, including some clinical signs and changes in clinical chemistry, but the resultant litters were comparable to litters in the control group. Thus, a NOAEL of 200 mg/kg is established for dams and fetuses by this study.

Summary tables from the original IND review are shown below. Findings in dams were limited to doses that were acutely lethal. Modest hematology findings at the mid-high and high doses suggested generalized stress at the toxic doses, which are easily monitored in humans.

RBC parameters on GD18			
Dose, mg/kg	Abs. reticulocytes (10^3/ul)	% reticulocytes	Erythrocytes (10^6/ul)
0	365	5.9	6.2
100	398	6.2	6.4
200	442	6.5	6.8
500	366	5.8	6.4
700	**180**	**2.8**	6.6

Bold indicates statistical significance at $p \leq 0.05$

Clotting Parameters

APTT, PTT, and platelet count were not significantly changed by treatment.

WBC parameters on GD18			
Dose, mg/kg	Neutrophils (10^3/ul)	Lymphocytes (10^3/ul)	Eosinophils (10^3/ul)
0	1.2	4.1	0.19
100	1.1	3.1	0.12
200	1.7	**2.5**	0.08
500	2.2	**2.8**	**0.07**
700	**2.4**	**2.7**	0.11

Bold indicates statistical significance at $p \leq 0.05$

Clinical biomarkers and electrolyte levels suggested kidney toxicity at the lethal doses.

KIDNEY MARKERS ON GD18		
Dose, mg/kg	BUN (mg/dL)	Creatinine (mg/dL)
0	19	1.2
100	19	1.3
200	15	1.2
500	15	1.4
700	23	**1.5**

Bold indicates statistical significance at $p \leq 0.05$

Electrolytes at GD18				
Dose, mg/kg	Sodium (mEq/L)	Potassium (mEq/L)	Chloride (mEq/L)	Calcium (mg/dL)
0	139	4.7	102	13
100	139	4.4	101	13
200	141	**4.1**	99	13
500	140	**3.6**	**95**	**12**
700	137	**3.7**	**96**	**12**

Bold indicates statistical significance at $p \leq 0.05$

Pregnancy findings were limited to early delivery and total resorption in three dams at the maternally toxic dose. Treatment-related fetal findings were limited to unossified hyoid body, with no external or visceral malformations above historical control levels.

Disposition of Dams during Gestation				
	Control	100 mg/kg	200 mg/kg	500 mg/kg
# on study	23	23	23	23
# pregnant	21	22	23	23
# died pregnant	0	1	0	8
# early delivery	0	0	0	2
# with all resorptions	0	0	0	1
# with viable fetuses at GD29	**21**	**21**	**23**	**12**

Uterine Evaluation				
	Control n=21	100 mg/kg n=21	200 mg/kg n=23	500 mg/kg n=12
Post-implantation loss	8	4	6	14
Early resorptions	0.3	0.1	0.3	1.3
Gravid uterine weight, kg	0.51	0.51	0.51	0.48

Skeletal malformations and variations				
	Control n=174 fetus n=21 litters	100 mg/kg n=181 fetus n=21 litters	200 mg/kg n=195 fetus n=23 litters	500 mg/kg n=112 fetus n=12 litters
Malformations # litters / # fetuses	2 / 4	2 / 2	4 / 5	3 / 4
Variations # litters / # fetuses	20 / 76	20 / 94	22 / 85	12 / 61
unossified hyoid body variation # litters / # fetuses	0 / 0	3 / 10	0 / 0	**4 / 7**

Bold indicates statistical significance

Prenatal and postnatal development

Study for toxic effects of SYR110322S on pre- and postnatal development, including maternal function in rats

250, 500, 1000 mg/kg
No plasma TK (NOAEL = 250 mg/kg; ~95X MRHD)
Drug secretion in milk confirmed nursing F_1 pup exposure

Key study findings: NOAEL = 250 mg/kg/day for maternal and developmental toxicity; 95X MRHD (estimated from 143 µg*h/ml maternal exposure at 250 mg/kg in embryo-fetal development (Seg. II) study and 1.5 µg*h/ml human exposure at 25 mg dose).

There were decreased body weight gain and decreased food consumption in 500 and 1000 mg/kg/day F_0 dams, which resulted in decreased F_1 pup body weights which persisted throughout weaning and into adulthood. There were no gross abnormalities in F_1 pups. Delivery findings in F_0 included: dose-dependent ↑ females with stillborn pups and ↑ stillborn index; dose-dependent ↓ live pups/litter; dose-dependent ↓ pup viability index (i.e. ↓ pup survival). There were also slight developmental, sexual maturation, and behavioral abnormalities noted in high dose F_1 animals. There were no remarkable findings in F_1 mating, fertility, or pregnancy parameters. Modest delivery trends in high dose F_1 females included slightly lower implantation sites and viable embryos, and increased postimplantation loss, but results were confounded by lower maternal body weights and not considered remarkable.

Summary of F_1 study findings

F_1 physical development: No treatment-related gross observations in pups (F_1) pre- or post-weaning. F_1 pup body weights were decreased in 500 and 1000 mg/kg/day groups (7-23%), which persisted throughout the study period, including post-weaning.

Observations/necropsy: Gross abnormalities in F_1 pups were generally unremarkable. No gross abnormalities noted in stillborn F_1 pups. No dose-related gross abnormalities in F_1 pups that died on study; dose-independent findings of moderate to severe kidney renal pelvis dilation (2/28) and moderate distended ureters (2/28) in low dose.

F_1 behavioral evaluation: Slight developmental, sexual maturation, and behavioral abnormalities noted in high dose F_1 animals. Clinical signs were unremarkable in males and females prior to, during, and post-mating.

Developmental: Slight, statistically significant effects in 1000 mg/kg/day pups: ↑ time to pinna detachment; ↑ time to eye opening; ↑ time to air drop righting reflex; trend of ↓ auditory response (not statistically significant). Only eye opening and auditory response were outside the historical control ranges.

Sexual maturation: Slight delay in vaginal opening at 1000 mg/kg/day, which was outside the historical control range. Body weight remained lower in ≥ 500 mg/kg/day females.
Motor activity: Results at 1000 mg/kg/day typically out of the historical control range included: ↑ horizontal activity, ↑ stereotypy activity, ↑ total distance in males; and, early ↑ horizontal activity and stereotypy activity and total distance in females.
Learning/memory: Increased non-passive behavior at 1000 mg/kg/day male (28% vs. 0% control); number of trials to learn was increased in treatment group males but considered unremarkable because most animals learned within the standard assay length.

F_1 reproduction: Male and female body weights remained lower in ≥ 500 mg/kg/day groups throughout the study and through gestation of F_2 generation. There were no apparent treatment-related effect on fertility and reproduction.

2.6.6.7 Local tolerance

Local tolerance study of intravenously injected SYR-322 in rabbits

NOAEL = 2.5 mg/ml concentration of SYR-322 (as free base). Male Kbl:JW rabbits (n=6/group) were injected *iv* in V. auricularis posterior (ear vein) with vehicle or a 2.5 mg/ml SYR-322 solution for 3 min at 1 ml/min (3 ml total). Animals were necropsied on either day 2 or day 14 (n=3/group/time point). There were no macroscopic signs throughout the study and there were no macroscopic or histological lesions at scheduled necropsies. Results supported tolerability of up to 2.5 mg/ml SYR-322 for clinical *iv* use based on the absence of any findings in rabbits.

Local tolerance study of paravenously injected SYR-322 in rabbits

NOAEL = 2.5 mg/ml. Male Kbl:JW rabbits (n=6/group) were injected *sc* adjacent to the left V. auricularis posterior (ear vein) with vehicle or a 2.5 mg/ml SYR-322 solution (0.3 ml/site). Animals were necropsied on either day 2 or day 14 (n=3/group/time point; injection on day 0). Gross findings were limited to slight erythema in all animals starting on day 1, which persisted for 2 to 4 days (absent day 5). Histological analysis showed slight subcutis hemorrhage in 2/3 animals on day 2 but none on day 14. Results were considered modest and tolerable in the animals.

2.6.6.8 Special toxicology studies

Single-dosage phototoxicity study to determine the effects of oral (gavage) administration of SYR-322 on skin in hairless mice

Distribution studies showed retention and accumulation of SYR-322 in the eyes, and to a lesser extent skin, in pigmented Long-Evans rats but not in albino SD rats, indicating that SYR322 associates with melanin. Metabolism studies showed parent SYR-322 accounted for 74% and 67.5% of total administered radioactivity at 24 and 168 h post-dose, respectively. M-I metabolite was approximately 7.5% at 24 and 168 h post-dose, with limited M-II present. There were no histological changes or abnormal ophthalmic exams in dogs treated with SYR-322 for 9 months. The sponsor did not find an ocular safety signal in a retrospective analysis of adverse events in phase II studies. SYR-322 does not absorb light in the 300-700 nm range, but there is a small degree of absorbance at 290 nm (peak absorbance at 277 nm).

The sponsor investigated potential for SYR-322 mediated phototoxicity in the well-established SKH hairless mouse. The hairless mouse is an albino strain, nevertheless, the assay system was valid for identifying phototoxic agents. There was no evidence of skin reactions in a standard single oral dose phototoxicity study at SYR-322 doses up to 800 mg/kg. The expected response in positive controls (200 mg/kg lomefloxacin hydrochloride) confirmed the sensitivity of the assay system.

2.6.6.9 Discussion and Conclusions

See overall toxicology summary in Section 2.6.6.1 above.

2.6.6.10 Tables and Figures

None. Section intentionally left blank.

2.6.7 TOXICOLOGY TABULATED SUMMARY

Table 2.6.7.1 – FDA Review Summary of NOAELs and General Toxicity Findings

SPECIES/ STUDY	NOAEL	MULTIPLE OF MRHD *25 mg (1.5 µg*h/ml)*	BASIS
SD Rats Seg I Fertility 100, 500, 1000 mg/kg No TK	500 mg/kg 100 (♂), 250 (♀) µg*h/ml	(m): 67x (f): 167x	Males: ↑ sperm production and % abnormal sperm Females: Total resorption in high dose female #479 *Note- TK estimated from 13-wk study*
SD Rats Seg II Embryonic Development 250, 500, 1000 mg/kg 143, 323, 800µg*h/ml	Dams & Fetus: 500 mg/kg	215x	Dams: ↓ food intake, BW, & BW gain Fetus: ↓ BW, incomplete ossification
NZ Rabbits Seg II Embryonic Development 100, 200, 500, 700 mg/kg 120, 310, 760, 1050µg*h/ml	Dams & Fetus: 200 mg/kg	207x	Dams: ↓ BW gain, renal and immune toxicity, deaths at 500 mg/kg Fetus: ↓ BW, ↑ unossified hyoid body variation
SD Rat Seg III Prenatal and Postnatal Development 250, 500, 1000 mg/kg No TK	F_0 Dams, F_1 Develop.: 250 mg/kg F_1 Repro/F_2: 1000 mg/kg	F_0/F_1 Development: ~100x F_1 Repro./F_2: ~500x	Dams (F_0): ↓ BW gain, ↓ food consumption, sl. ↑ stillbirths, sl. ↓ pup survival (≥200x) Pups (F_1): ↓ BW (throughout lifespan), sl. developmental/behavior/maturation abnormalities (≥200x) Pup Repro (F_1): no Δ pregnancy/fertility, sl. ↓ implantation sites & viable embryos

SPECIES/ STUDY	NOAEL	MULTIPLE OF MRHD *25 mg (1.5 µg*h/ml)*	BASIS
Rat Single dose 500, 1000, 1500, 2000mpk	> 2000 mg/kg	no TK	No findings out to 14 days post-dose
5 Day 100, 300, 600, 1000mpk	> 1000mg/kg	no TK	No drug-related findings
28 day 30, 100, 300mpk 7.5, 29, 88 µg*h/ml	> 300 mg/kg	> 59x	No drug-related findings
28 day, high dose 1333, 1666, 2000mpk	< 1333 mg/kg (not established)	no TK	Mortality all doses, renal/urinary related ↓ body weight gain Kidney tubular degen./mineralization Bladder erosion/ulcer, hemorrhage Liver ↑ weight, hypertrophy
13 week 100, 400, 1000mpk m: 32, 89, 549 µg*h/ml f: 58, 240, 661 µg*h/ml	400mg/kg	59x-160x	↓ body weight gain ↑ liver weight (≤ 50%); hypertrophy Chronic progressive nephropathy
26 week 100, 400, 900mpk 44, 259, 664µg*h/ml	400mg/kg	173x	↓ body weight (≤ 13%) ↑ liver weight (≤ 15%); hypertrophy ↑ incidence of lung histiocytosis and renal nephropathy of uncertain relation to drug
2 year 75, 400, 800 mg/kg 48, 425, 703 µg*h/ml	75 mg/kg	32x	Carci: ↑ Thyroid C-cell tumors (male) ↓ body weight (max. 22%) ↑ testes degeneration/atrophy and ↑ oligospermia (epididymides) ↑ liver periportal vacuolation ↑ lung histiocytosis Kidney and bladder cell hyperplasia ↑ ♀ chronic progress. nephropathy (late onset, ↑ over background (~50% bkgd)

SPECIES/ STUDY	NOAEL	MULTIPLE OF MRHD *25 mg (1.5 µg*h/ml)*	BASIS
Mouse 28 Day 30, 100, 300, 400mpk 6, 22, 66, 60µg*h/ml	300mg/kg	44x	Unexplained mortality, 8% animals ↓ body weight gain in males Histology unremarkable
13 week 200, 300, 400, 600mpk 71, 96, 117, 180 µg*h/ml	200mg/kg	47x	Unexplained but drug-related mortality starting at 300mg/kg Histology unremarkable
2 year (CD-1) 50, 150, 300 mg/kg 10, 42, 90 µg*h/ml	300 mg/kg	60x	No treatment related tumors Toxicity unremarkable 5% incidence benign hepatocellular adenoma (high dose ♀ only) not considered drug-related

SPECIES/ STUDY	NOAEL	MULTIPLE OF MRHD *25 mg (1.5 µg*h/ml)*	BASIS
Dog Single dose 125, 300, 500mpk	125mg/kg	no TK	Transient and mild facial reddening at 125mg/kg, more severe and prolonged at 300 and 500mg/kg BW loss, ↓ activity, emesis in female at 500mg/kg
7 Day 30, 100, 300mpk	30mg/kg	no TK	*100mpk*: Facial/body reddening *300mpk* Facial/body reddening and swelling ↓ Activity ↓ Food intake and BW loss in female
28 day 7.5, 25, 75mpk 5, 31, 117 µg*h/ml	> 75 mg/kg	> 78x	Transient facial flushing and reddening of skin at 75mg/kg
13 week 10, 30, 90mpk Alo.+M-I: 13, 48, 149 µg*h/ml	male: 90mg/kg female: 30mg/kg	m: 99x f: 32x	Facial flushing, swelling, and reddening of skin Reduced body weight and food consumption in females at 90mg/kg
9 months 30, 100, 200mpk 40, 200, 400µg*h/ml	> 200 mg/kg	>267x	Facial flushing, swelling, and reddening of skin occurs at 100 and 200mg/kg Reduced BW and food intake in females at 200mg/kg (findings not considered toxicologically significant)

SPECIES/ STUDY	NOAEL	MULTIPLE OF MRHD *25 mg (1.5 µg*h/ml)*	BASIS
Monkey Single escalating doses 0.3, 1, 10, 30 mg/kg 0.3, 1, 14, 40 µg*h/ml	≥30 mg/kg	≥27x	Sporadic ↑ AST, LDH, CK *(not toxicologically significant)* *No histopathology analysis*
4 week 1, 10, 30 mg/kg 1, 12, 31 µg*h/ml	≥30 mg/kg	≥21x	No skin lesions up to C_{max} 22 µM (7300 ng/ml) ♂ enlarged prostate (↑81%) ♀ enlarged ovary (↑70%), pituitary glands (↑50%) *(not toxicologically significant)*
13 week 3, 10, 30 mg/kg 4, 13, 47 µg*h/ml	≥30 mg/kg	≥31x	No skin lesions up to C_{max} 10 µM (~3300 ng/ml) Unremarkable toxicity

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/s/

David Carlson
8/21/2008 11:22:50 AM
PHARMACOLOGIST
Recommend Approval

Todd Bourcier
8/27/2008 10:50:37 AM
PHARMACOLOGIST
Concur with reviewer. Please refer to supervisory memo.

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR A NEW NDA/BLA

NDA Number: 22-271 **Applicant:** Sanofi-Aventis **Stamp Date:** 12/27/07

Drug Name: Nesina (alogpliptin) Tablets **NDA/BLA Type:** 505(b)(1) (NME)

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		
2	Is the pharmacology/toxicology section indexed and paginated in a manner allowing substantive review to begin?	X		
3	Is the pharmacology/toxicology section legible so that substantive review can begin?	X		
4	Are all required (*) and requested IND studies (in accord with 505 b1 and b2 including referenced literature) completed and submitted (carcinogenicity, mutagenicity, teratogenicity, effects on fertility, juvenile studies, acute and repeat dose adult animal studies, animal ADME studies, safety pharmacology, etc)?	X		All studies completed and submitted. Stats team has been notified of mouse and rat carcinogenicity studies needing statistical review (files submitted in .xpt format).
5	If the formulation to be marketed is different from the formulation used in the toxicology studies, have studies by the appropriate route been conducted with appropriate formulations? (For other than the oral route, some studies may be by routes different from the clinical route intentionally and by desire of the FDA).	X		No difference in drug substance formulation. Changes in tablet formulation should be OK based on excipients used. Different salt formulations were used in early nonclinical development but shouldn't be an issue because dosing and analyses were based on free base concentrations of active moiety.
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		Statement included in 'Nonclinical Overview' (section 2.4) and statements/signatures included in individual study reports.
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?	X		Studies previously requested, including enantiomer characterization, phototoxicity/photosensitivity and subchronic monkey skin lesion studies completed.

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		Nonclinical section included in label. Sponsor's multiples based on exposure. After a cursory review of the nonclinical data it looks like there may need to be some revision of pharm/tox info. included.
10	Have any impurity – etc. issues been addressed? (New toxicity studies may not be needed.)			PharmTox will request CMC provide a copy of the impurity profile review to make sure there are no outstanding PharmTox issues.
11	Has the applicant addressed any abuse potential issues in the submission?	X		Addressed indirectly based on receptor binding assays *in vitro*. No notable abuse liability issues were identified in IND reviews.
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.

No pharm/tox comments.

Reviewing Pharmacologist Date

Team Leader/Supervisor Date

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

/s/

David Carlson
2/12/2008 12:34:39 PM
PHARMACOLOGIST

Todd Bourcier
2/12/2008 12:38:48 PM
PHARMACOLOGIST