CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

219122Orig1s000

CLINICAL PHARMACOLOGY REVIEW(S)

CLINICAL PHARMACOLOGY REVIEW

NDA:	NDA 219122 (SDN 1)
Submission Type:	505b2
Reference Listed Drug:	Januvia®
Brand Name:	Tradename pending
Drug Name:	Sitagliptin hydrochloride
Submission Date:	1/4/2024
PUDFA Goal Date:	11/4/2024
Priority:	Standard
Indications:	An adjunct to diet and exercise to improve glycemic control in adults with type 2 diabetes mellitus.
Dosage and Administration	100 mg once daily (4 mL), with or without food. Dosage adjustment is recommended for patients with eGFR < 45 mL/min/1.73m^2.
Dosage Form	Solution (25 mg per ml)
Route of Administration	Oral
Sponsor	Azurity Pharmaceuticals, Inc.
OCP Reviewers	Lin Zhou, Ph.D.
OCP Team Leaders	Edwin Chow, Ph.D.
OCP Division	Division of Cardiometabolic and Endocrine Pharmacology (DCEP)
OND Division	OND/OCHEN/DDLO

1. EXECUTIVE SUMMARY

In this 505b2 NDA, the applicant Azurity Pharmaceuticals Inc. is seeking approval for a ready-to-use Sitagliptin Hydrochloride Oral Solution. The listed product (LD) is Januvia®, sitagliptin hydrochloride oral tablets (NDA 021995).

This application relies on the Agency's previous finding of safety and effectiveness for the LD, Januvia® (sitagliptin) Tablets (NDA 021995, approved October 16, 2006), distributed by Merck Sharp & Dohme Corp., a subsidiary of Merck & Co., Inc. The Azurity's product differs from the LD of Januvia ® by dosage form (oral solution vs oral tablet) and salt form (sitagliptin hydrochloride vs sitagliptin phosphate). The Applicant claims that both formulations deliver an equivalent dose of sitagliptin when used according to the prescribing information.

To establish a scientific bridge between the proposed Sitagliptin Hydrochloride Oral Solution and the LD, and to evaluate the effect food on Sitagliptin Hydrochloride Oral Solution, the Applicant performed the following study:

- Study AZ17.001: A Randomized, Open-Label, Single-Dose, Three-Way Crossover, Bioavailability, Bioequivalence, and Food Effect Study of 100 mg Azurity Sitagliptin

Hydrochloride Oral Solution 25 mg/mL (4mL) and Januvia® Sitagliptin Phosphate) 100 mg Oral Tablets in Healthy Adults.

The PK study results of Study AZ17.001 showed that the 90% confidence intervals and the geometric mean ratio of proposed sitagliptin hydrochloride oral solution product to LD product are within the pre-specified limit of 80% to 125% for primary PK endpoints (C_{max}, AUC_{last}, and AUC_{inf}) for sitagliptin. Therefore, a scientific bridging between sitagliptin hydrochloride oral solution and Januvia® Tablet is established from a clinical pharmacology perspective.

In addition, food decreased ~28% of C_{max} and delayed T_{max} (~ 2 hours) for the Sitagliptin HCl Oral Solution product, but the findings are not considered clinically meaningful from a clinical pharmacology perspective.

1.1 Recommendations

The Office of Clinical Pharmacology/Division of Cardiometabolic and Endocrine Pharmacology has reviewed the information contained in NDA 219122/S0001 and finds the submitted PK data acceptable to support approval from a clinical pharmacology perspective.

Review Issue	Recommendations and Comments
Pivotal or supportive evidence of effectiveness	A relative bioavailability (BA) and Food Effect Study (Study AZ17.001) of 100 mg Azurity Sitagliptin Hydrochloride Oral Solution 25 mg/mL (4mL) and Januvia® Sitagliptin Phosphate) 100 mg Oral Tablets in Healthy Adults was conducted. The PK study results supported a finding of bioequivalence between the proposed sitagliptin hydrochloride oral solution product and Januvia® (sitagliptin) Tablets, which is the LD product. Therefore, a scientific PK bridging between sitagliptin hydrochloride oral solution and Januvia Tablet is established from a clinical pharmacology perspective.
General dosing instructions	100 mg once daily (4 mL), can be taken with or without food.
Dosing in patient subgroups (intrinsic and extrinsic factors)	<u>Food effect</u> Compared to fasting conditions, C_{max} was reduced by ~28% and Tmax was delayed by ~2 hrs under fed conditions for the Sitagliptin HCl Oral Solution. Food did not appear to impact AUCs. This is different than the information presented in Januvia® (LD) where there is no effect of food on the PK exposures (both C_{max} and AUC) of sitagliptin. But, based on the totality of evidence, we do not think the effect of food is clinically meaningful from a clinical pharmacology perspective. Dosing in other subgroups is the same as LD.

Labeling	Pending
OSIS inspection	Inspections were requested for the clinical and analytical site for Study AZ17.001. The Office of Study Integrity and Surveillance (OSIS) determined that inspections are not needed for the sites based on inspection results on the (b) (4) sites (b) (4) (DARRTS 4/29/2024; Reference ID: 5372133).

1.2 Post-Marketing Requirements and Commitments

None.

2. BACKGROUND

Azurity Pharmaceuticals Inc., (Azurity) has developed a ready-to-use Sitagliptin Hydrochloride Oral Solution containing 25 mg/mL sitagliptin, as a 505(b)(2) product.

This application relies on the Agency's previous finding of safety and effectiveness for the reference listed drug (LD), Januvia® (sitagliptin) Tablets (NDA 021995, approved October 16, 2006), distributed by Merck Sharp & Dohme Corp., a subsidiary of Merck & Co., Inc. The Azurity's product differs from the LD of Januvia® by dosage form (oral solution vs oral tablet) and salt form (sitagliptin hydrochloride vs sitagliptin phosphate). The Applicant claims that both formulations deliver an equivalent dose of sitagliptin when used according to the prescribing information.

Azurity also relies on previously approved data contained in the Januvia® NDA for the nonclinical safety data required for approval of Sitagliptin Hydrochloride Oral Solution.

To establish a scientific bridge between the proposed Sitagliptin Hydrochloride Oral Solution and the LD, and to evaluate the effect food on Sitagliptin Hydrochloride Oral Solution the Applicant performed the following comparative bioequivalence studies (BE):

- Study AZ17.001: A Randomized, Open-Label, Single-Dose, Three-Way Crossover, Bioavailability, Bioequivalence, and Food Effect Study of 100 mg Azurity Sitagliptin Hydrochloride Oral Solution 25 mg/mL (4mL) and Januvia® Sitagliptin Phosphate) 100 mg Oral Tablets in Healthy Adults.

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Summary of Study AZ17.001

Study Design:

A total of 30 eligible subjects (13 females and 17 males) participated in the study and 26 subjects completed the study. The primary objectives of the study were to determine the relative bioavailability (BA) between 100 mg Sitagliptin Hydrochloride Oral Solution 25 mg/mL (4 mL) and Januvia® (100 mg) oral tablets under fasted conditions. The secondary objectives were to assess the effect of food on the PK and relative BA of 100 mg dose of Sitagliptin Hydrochloride Oral Solution 25 mg/mL (4 mL) under fed (a high-fat, high calorie meal), versus fasted (for at least 10 hours pre-dose and 4 hours post-dose) conditions [both consistent with FDA's Food Effect Bioavailability and Fed Bioequivalence

Studies guidance (2002)] as well as to evaluate the safety and tolerability of a single dose (100 mg) of Sitagliptin Hydrochloride Oral Solution as compared to Januvia® (100 mg) oral tablets.

Each participant was randomized to receive a single 100 mg dose of each of the following 3 treatments on dosing Days 1, 6, and 11 separated by 2 five-day washouts between the start of each period:

- Treatment A: 4 mL of Sitagliptin Hydrochloride Oral Solution (25 mg/mL) administered as a single 100 mg dose, fasted.
- Treatment B: 4 mL of Sitagliptin Hydrochloride Oral Solution (25 mg/mL) administered as a single 100 mg dose, fed (FDA-standard high-fat, high-calorie meal).
- Treatment C: One (1) Januvia® (Sitagliptin Phosphate) 100 mg oral tablet administered as a single dose, fasted.

In each treatment period, Blood samples for plasma pharmacokinetic (PK) analysis of sitagliptin were obtained at 0 (pre-dose) and at 0.25, 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 10, 12, 15, 18-, 24-, 36-, and 48-hours post-dose in each dosing period for the determination of Sitagliptin plasma concentrations. The PK sampling timepoints are sufficient to cover approximately 4 half-lives of sitagliptin ($t_{1/2}$ ~12.4 hours). Safety was assessed throughout the study duration.

PK Data Analyses:

A validated LC-MS/MS method (See Appendix 4.2) was used to quantify the plasma concentration of sitagliptin in the PK samples collected in Study AZ17.001.

PK parameters were estimated from plasma sitagliptin concentration-time data using noncompartmental methods.

For the PK analysis, plasma PK concentrations below the limit of quantification (BLQ) up to the time of the first quantifiable concentration were treated as zero. Embedded (values between 2 quantifiable concentrations) and terminal BLQ concentrations were treated as "missing". Calculation of the PK characteristics was based on actual elapsed times [h] relative to dosing.

Comparison of the log-transformed parameters peak drug concentration (C_{max}), area under the concentration-time curve from time-zero to the time of the last quantifiable concentration (AUC_{last}), and area under the plasma concentration-time curve from time-zero extrapolated to infinity (AUC_{inf}) were compared by ANOVA, using MIXED procedure in SAS. The model contained terms for sequence, period, and treatment as fixed effects, and subject nested within sequence as a random effect. The geometric mean ratios (e.g., Solution/Tablet and Fed/Fasted) and the 90% confidence intervals were reported. In addition, a non-parametric method (e.g., Wilcoxon signed-rank test) was used to assess the difference in T_{max} between treatments.

The following comparisons were conducted:

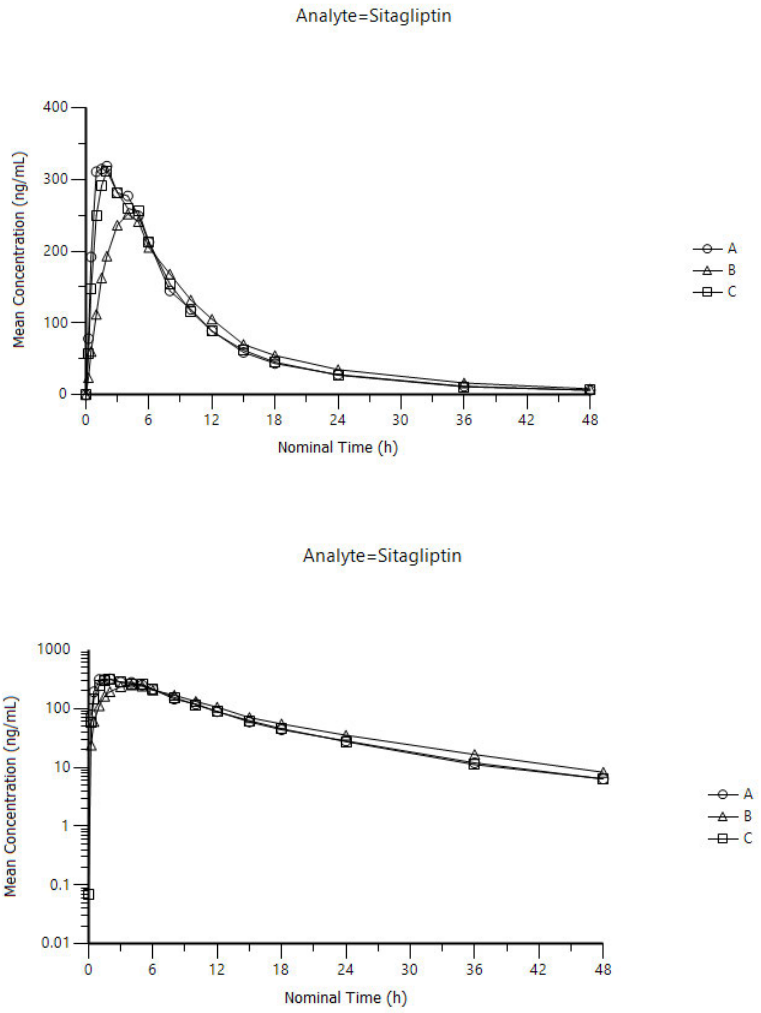
- Bioequivalence under Fasted Conditions
    - Treatment A (100 mg dose of Sitagliptin HCl Oral Solution 25 mg/mL (4 mL) under fasted conditions) vs. treatment C (Januvia® (sitagliptin phosphate) 100 mg tablet-fasted)
- Food Effect

- Treatment B (100 mg dose of Sitagliptin HCl Oral Solution 25 mg/mL (4 mL), under fed conditions) vs. Treatment A (100 mg dose of Sitagliptin HCl Oral Solution 25 mg/mL, under fasted conditions)

PK Results:

PK profiles and descriptive statistics for plasma PK parameters for sitagliptin shown in Figure 1 and Table 1.

Figure 1. Mean plasma concentration-time profiles of sitagliptin after sitagliptin hydrochloride oral solution, 100 mg, under fasted (Treatment A) and fed conditions (Treatment B) or Januvia® tablet, 100 mg under fasted conditions (Treatment C): PK analysis set



Treatment A: Azurity sitagliptin hydrochloride Oral Solution 4 mL (100 mg), fasted, n=27
Treatment B: Azurity sitagliptin hydrochloride Oral Solution 4 mL (100 mg), fed, n=27
Treatment C: Januvia® (sitagliptin phosphate) 100 mg Tablet, fasted, n=28
Source data: Figure 1 of Study AZ17.001

Table 1. Descriptive Statistics for Plasma Pharmacokinetic Parameters for Sitagliptin- PK analysis set

	Treatment A: 100 mg Sitagliptin HCl Oral Solution, Fasted				Treatment B: 100 mg Sitagliptin HCl Oral Solution, Fed				Treatment C*: 100 mg Januvia Tablet, Fasted			
Parameter	**n**	**Mean**	**SD**	**CV%**	**n**	**Mean**	**SD**	**CV%**	**n**	**Mean**	**SD**	**CV%**
[a]T_{max} (h)	27	1.50 (0.500-4.02)			27	4.00 (2.00-6.00)			28	2.00 (1.00-6.00)		
C_{max} (ng/mL)	27	379	106	27.9	27	264	43.9	16.6	28	370	111	29.9
AUC_{last} (h*ng/mL)	27	3340	557	16.7	27	3250	477	14.7	28	3280	506	15.4
AUC_{inf} (h*ng/mL)	27	3450	560	16.3	27	3400	516	15.2	27	3360	523	15.6
AUC_{Extrap} (%)	27	3.10	1.52	49.0	27	4.30	2.06	47.9	27	2.47	1.01	41.0
$T_{1/2}$ (h)	27	10.5	1.69	-	27	10.8	2.13	-	27	9.86	1.24	-
T_{last} (h)	27	47.6	2.32	-	27	47.1	4.62	-	28	48.0	0.0477	-
C_{last} (ng/mL)	27	6.73	2.41	-	27	9.69	6.60	-	28	6.36	4.29	-

[a]Tmax is presented as median (minimum-maximum); Mean = arithmetic mean
Treatment A: Azurity sitagliptin hydrochloride Oral Solution 4 mL (100 mg), fasted
Treatment B: Azurity sitagliptin hydrochloride Oral Solution 4 mL (100 mg), fed
Treatment C: Januvia® (sitagliptin phosphate) 100 mg Tablet, fasted
Source data: Table 8 of Study AZ17.001. Results verified by FDA reviewer.

Comparison of PK data between different arms are presented in Section 3 to answer the two key review questions.

3. KEY REVIEW TOPICS

3.1. Is the PK of Sitagliptin Oral Solution Bioequivalent to the PK of Januvia® (Sitagliptin tablet)?

Yes, the PK results of Study AZ17.001 supported no statistical differences in PK exposures between 100 mg Sitagliptin HCl Oral Solution and Januvia® tablets under fasting condition. The PK study results of Study AZ17.001 showed that the 90% confidence intervals and the geometric mean ratio of proposed sitagliptin hydrochloride oral solution product to LD product are within the pre-specified limit of 80% to 125% for primary PK endpoints (C_{max}, AUC_{last}, and AUC_{inf}) for sitagliptin as shown in Table 2. In addition, sitagliptin HCL Oral solution and Januvia® tablets has comparable T_{max} following dosing in the fasted state (Table 3).

Table 2. Statistical Analysis of Natural Log-Transformed Systemic Exposure of Sitagliptin Comparing Sitagliptin Hydrochloride Oral Solution, 100 mg (Treatment A, Test) vs. Januvia® Tablet, 100 mg (Treatment C, Reference) Under Fasted Conditions (Bioequivalence) – PK Analysis Set

Dependent Variable	GeoMean[a] Test	GeoMean[a] Ref	Ratio (%)[b] (T/R)	90% CI[c] Lower	90% CI[c] Upper	ANOVA CV%
C_{max}	361	356	101.46	93.37	110.25	17.78
AUC_{last}	3280	3260	100.62	97.98	103.32	5.59
AUC_{inf}	3390	3330	101.65	99.19	104.17	5.07

Treatment A (Test): Azurity Sitagliptin HCl Oral Solution 4 mL (100 mg), fasted
Treatment C (Reference): Januvia (sitagliptin phosphate) 100 mg Tablet, fasted
[a] Geometric Mean based on Least Squares Mean
[b] Ratio(%) = Geometric Mean (Test)/Geometric Mean (Ref)
[c] 90% Confidence Interval
Source: Table 8 of Study AZ17.001. Results verified by FDA reviewer.

Table 3. Wilcoxon Signed Rank Test Comparing Sitagliptin Hydrochloride Oral Solution, 100 mg (Treatment A, Test) vs. Januvia® Tablet, 100 mg (Treatment C, Reference) Under Fasted Conditions (Bioequivalence) – PK Analysis Set

Dependent Variable	Median Test (h)	Median Reference (h)	Range Test (h)	Range Reference (h)	p-value[a]	Median Difference[b]	90% CI[c] Lower	90% CI[c] Upper
T_{max}	1.50	2.00	0.50 - 4.02	1.00 - 6.00	0.1173	-0.50	-1.52	-0.29

Treatment A (Test): Azurity Sitagliptin HCl Oral Solution 4 mL (100 mg), fasted
Treatment C (Reference): Januvia (sitagliptin phosphate) 100 mg Tablet, fasted
[a] Significant difference defined *a priori* as $p < 0.05$
[b] Hodges-Lehmann Estimated Median Difference between Treatments
[c] Hodges-Lehmann Estimated 90% Confidence Interval for Median Difference between Treatments
Source: Table 10 of Study AZ17.001. Results verified by FDA reviewer.

Therefore, a scientific bridging between sitagliptin hydrochloride oral solution and Januvia® Tablet is established from a clinical pharmacology perspective.

3.2. Does Food have a Clinically Meaningful Impact on Clinical Efficacy/Safety of Proposed Sitagliptin Oral Solution?

In Study AZ17.001, compared to fasting conditions, C_{max} was reduced by ~28% and T_{max} was delayed by 2.5 hrs (from 1.5 hrs to 4 hrs) under fed conditions for the Sitagliptin HCl Oral Solution Table 4 and

Treatment A (Reference): Azurity Sitagliptin Hydrochloride Oral Solution 4 mL (100 mg), fasted
Treatment B (Test): Azurity Sitagliptin Hydrochloride Oral Solution 4 mL (100 mg), fed
[a] Geometric Mean based on Least Squares Mean
[b] Ratio (%) = Geometric Mean (Test)/Geometric Mean (Ref)
[c] 90% Confidence Interval

Source: Table 11 of Study AZ17.001. Results verified by FDA reviewer.

Table 5. Food did not appear to impact AUCs. This is different than the information presented in Januvia® where there is no effect of food on the PK exposures (both C_{max} and AUC) of sitagliptin.

Table 4. Statistical Analysis of Natural Log-Transformed Systemic Exposure of Sitagliptin Comparing Sitagliptin Hydrochloride Oral Solution, 100 mg under Fed (Treatment B, Test) or Fasted Conditions (Treatment A, Reference) (Food Effect) – PK Analysis Set

Dependent Variable	GeoMean[a] Test	GeoMean[a] Ref	Ratio (%)[b] (T/R)	90% CI[c] Lower	90% CI[c] Upper	ANOVA CV%
C_{max}	260	365	71.27	66.54	76.35	14.63
AUC_{last}	3220	3280	98.07	95.87	100.31	4.76
AUC_{inf}	3360	3390	99.38	97.21	101.60	4.65

Treatment A (Reference): Azurity Sitagliptin Hydrochloride Oral Solution 4 mL (100 mg), fasted
Treatment B (Test): Azurity Sitagliptin Hydrochloride Oral Solution 4 mL (100 mg), fed
[a] Geometric Mean based on Least Squares Mean
[b] Ratio (%) = Geometric Mean (Test)/Geometric Mean (Ref)
[c] 90% Confidence Interval
Source: Table 11 of Study AZ17.001. Results verified by FDA reviewer.

Table 5. Wilcoxon Signed Rank Test Comparing Sitagliptin Hydrochloride Oral Solution, 100 mg under Fed (Treatment B, Test) or Fasted Conditions (Treatment A, Reference) (Food Effect) – PK Analysis Set

Dependent Variable	Median Test (h)	Median Reference (h)	Range Test (h)	Range Reference (h)	p-value[a]	Median Difference[b]	90% CI[c] Lower	90% CI[c] Upper
T_{max}	4.00	1.50	2.00 - 6.00	0.50 - 4.02	0.0000	2.25	1.25	2.50

Treatment A (Reference): Azurity Sitagliptin Hydrochloride Oral Solution 4 mL (100 mg), fasted
Treatment B (Test): Azurity Sitagliptin Hydrochloride Oral Solution 4 mL (100 mg), fed
[a] Significant difference defined *a priori* as $p < 0.05$
[b] Hodges-Lehmann Estimated Median Difference between Treatments
[c] Hodges-Lehmann Estimated 90% Confidence Interval for Median Difference between Treatments
Source: Table 12 of Study AZ17.001. Results verified by FDA reviewer.

To evaluate whether the PK differences in C_{max} contributes to clinically meaningful differences in efficacy, we evaluated dose response data from NDA 021995 (Januvia®) and Applicant's generated PK simulations on steady-state PK exposure of sitagliptin. The conclusion is that we do not believe that the reduced C_{max} or delayed T_{max} will have a significant impact on clinical efficacy. The following data and justifications supported our conclusion.

- Under NDA 021995 (Januvia®), Study P014, a multinational, double-blind, randomized, placebo-controlled, parallel group, dose-range finding study evaluated HbA1 reductions with 4 doses of sitagliptin 25, 50, 100 mg q.d., 50 mg b.i.d. Compared to daily oral administration of 100 mg QD sitagliptin, plasma PK concentrations of sitagplitin at 1- and 2-hours post-dose in subjects who received 50 mg BID sitagliptin had were about 50% lower (Table 6).
  Note: In Study P014, PK samples were only collected at baseline, 1-, and 2-hours post-dose at Week 2, 4, and 6 visits. Given that the T_{max} of sitagliptin is between 1 to 4 hours in Januvia® USPI, plasma concentration at 1- and 2-hrs post-dose are good approximation of C_{max}.

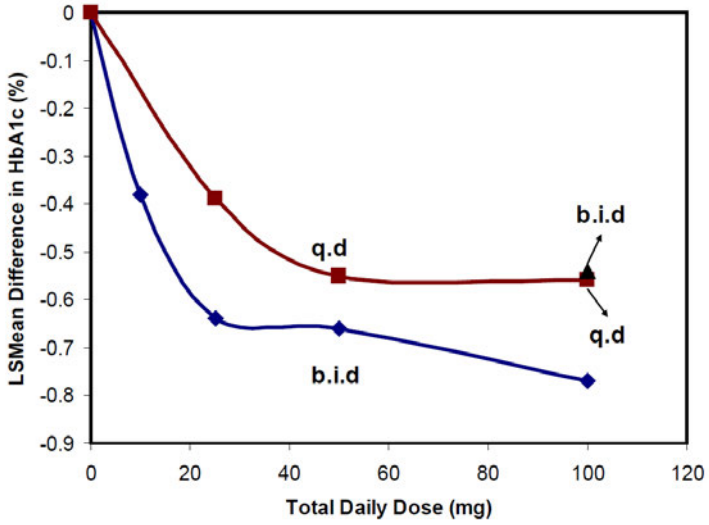
Table 6. Summary Statistics for Plasma Concentration (nM) for Study 014 at Week 6

Treatment	N	Mean (nM)	SD
1-hour postdose			
MK-0431 100 mg q.d.	33	994.5	718.6
MK-0431 50 mg b.i.d.	31	485.5	288.5
2-hour post dose			
MK-0431 100 mg q.d.	32	983.4	460.4
MK-0431 50 mg b.i.d.	31	501.9	232.8

Adapted based on table in PK report of Study P014 from NDA 021995 (Januvia®). 1nM is ~0.41 ng/mL.

- The 50% difference in plasma Cmax concentration of sitagplitin (Table 6) did not appear to impact efficacy (Figure below). Based on dose-response analysis from Januvia® (NDA 021995), there was no clinically meaningful difference in the primary endpoint (mean

difference in HbA1c) when sitagliptin tablet was administered as 50 mg b.i.d or 100 mg q.d as shown by the right line at 100 mg total daily dose in the figure below.



Redline: Study P014 (25, 50, 100 mg q.d., 50 mg b.i.d.)
Blueline: Study P010 (5, 12.5, 25 or 50 mg b.i.d.)
Source: Clinical pharmacology Review for NDA 021995 (Januvia®) by Dr. Xiaoxiong Wei dated 08/31/2006 in DARRTS.

- Compared to PK data a single oral dose of sitagliptin oral solution, food had a similar effect on steady-state PK (simulated) (Table 7). Food decreased steady state C_{max} by ~26% and reduced T_{max} by 2 hours. In addition, these simulations predict no difference in C_{avg} at steady state between fasted and fed conditions. [136.6 (Fasted) vs. 141.9 (Fed) ng/ml)].

Table 7. Predicted Mean ± SD (%CV) Pharmacokinetic Parameter Values – Steady State Sitagliptin Under Fasted and Fed Conditions Based on Superposition of AZ17.001 Single Dose Sitagliptin Hydrochloride Oral Solution Data

Parameter	Fasted	Fed
Dose (mg)	100	100
C^{SS}_{max} (ng/mL)	403 ± 103 (25.5)	299 ± 45.9 (15.3)
C^{SS}_{min} (ng/mL)	33.2 ± 12.3 (37.2)	43.1 ± 16.2 (37.5)
Tmax (hr)	1.88 ± 0.92 (49.1)	3.89 ± 1.07 (27.4)
AUC^{SS}_{tau} (ng*hr/mL)	3415 ± 570 (16.7)	3357 ± 519 (15.5)
Accumulation Ratio	1.15 ± 0.06 (5.1)	1.21 ± 0.08 (6.6)
Fluctuation %	259 ± 47.7 (18.4)	185 ± 28.2 (15.3)
Swing	13.7 ± 10.6 (77.3)	10.3 ± 20.3 (197)

Source: Table 1 of Response to Information Request dated 05/22/24

In addition, based on PK-PD relationship of sitagliptin and inhibition of plasma dipeptidyl peptidase-4 (DPP4) activity from NDA 021995 (Januvia®) and simulated steady-state PK, delayed T_{max} is not expected to influence efficacy.

- In a study of multiple oral doses of 25 mg to 600 mg sitagliptin in healthy subjects, there is a dose and concentration-related increase in inhibition of plasma DPP-IV enzyme activity (Clinical pharmacology review of Januvia®).

- When the relationship between plasma sitaglipin concentrations and inhibition of plasma DPP4 activity was explored, no significant hysteresis was observed. This indicates that inhibition of plasma DPP4 activity was described by plasma sitagliptin concentration alone and was independent of time (Clinical pharmacology review of Januvia®). Using an Emax model, the plasma EC50 was 25.7 nM and the EC80 was approximately 100 nM, which is equivalent to 40.65 ng/mL. (Clinical pharmacology review for Januvia® NDA 021995) reported "The lower limit of quantitation (LLOQ) for the plasma assay is 0.500 ng/mL (1.23 nM) and the linear calibration range is 0.500 to 1000 ng/mL (1.23 to 2455 nM)." This means 100 nM (100nmol/L) = 40.65 ng/ml.).

- Based on the simulated steady state PK, $C_{ss,avg}$ sitagliptin concentration remained above 40.65 ng/mL during the 24-hour dosing period. [mean $C_{ss,avg}$ (ng/ml): 136.6 (Fasted) vs. 141.9 (Fed)]. In addition, $C_{ss,min}$ is slightly higher under fed condition. [mean $C_{ss,min}$ (ng/m;): 43.1 (fed) vs. 33.2 (fasted)]

Based on the totality of data, we do not believe that the decrease of C_{max} (~28%) and delayed T_{max} by 2 hours with food may have significant impact on efficacy from a clinical pharmacology perspective.

4. APPENDICES

4.1. OSIS Inspections

Inspections were requested for the clinical and analytical site for Study AZ17.001. The Office of Study Integrity and Surveillance (OSIS) determined that inspections are not needed for the sites based on inspection results on the (b) (4) sites (b) (4). More details can be found in the memo by Felecia Hagood dated 04/29/2024 in DARRTS.

4.2. Bioanalytical Assay

The LC-MS/MS method for the quantification of sitagliptin in human plasma has been developed and validated for calibration curve ranging from 1 ng/mL to 500 ng/mL based on the analysis of 0.100 mL of plasma (DCN 1004227). Sitagliptin and Sitagliptin-D4 (IS) were extracted from human plasma by liquid-liquid extraction method. A portion of the organic layer was isolated, then dried and reconstituted. An aliquot was then injected onto a LC-MS-MS equipped with an HPLC column. The method was developed at (b) (4)

The method for sitagliptin in human K2-EDTA plasma (Method#: ATM-1632) was initially validated in 2010 in October 2010. Additional experiments were conducted to bring the method up to current

standard operating procedures as the following: Amendment 1 (January 2011), Amendment 2 (March 2012), Amendment 3 (April 2012), Amendment 4 (October 2012), Amendment 5 (November 2012), Amendment 6 (December 2023). In Amendment 6, a partial validation was conducted to bring the method ATM-1632 up to current standard operating procedures and the method number was updated to ATM-2729.

Validation parameters and performance of the methods used for the determination of sitagliptin in human plasma are found to be acceptable and summarized in Table 8.

Table 9. Bioanalytical Method Summary for Study A17.001

Method Description	The method was validated for a range of 1.00 to 500 ng/mL based on the analysis of 0.100 mL of plasma. Human plasma containing Sitagliptin and the internal standard, sitagliptin-D4, was extracted using a liquid-liquid extraction method. A portion of the organic layer was isolated, then dried and reconstituted. An aliquot was then injected onto a LC-MS-MS equipped with an HPLC column. The peak area of the Sitagliptin product ion was measured against the peak area of the sitagliptin-D4 internal standard product ion. Quantitation was performed using a weighted (1/x2) linear least squares regression analysis generated from calibration standards prepared immediately prior to each run.
Biological Matrix	Human Plasma
Anticoagulant	K_2-EDTA
Analyte	Sitagliptin
Internal Standard (ISTD)	Sitagliptin-D4
Range of quantitation	1.00 to 500 ng/mL
Limit of quantitation	1.00 ng/mL
Dilution Integrity	2500 ng/mL concentration diluted 10X CV 1.2% & Bias
QC concentrations	LLOQ 1.00 ng/mL, QC Low 3.00 ng/mL, QC Mid-Low 35 ng/mL, QC Medium 250.0 ng/mL, QC High 400 ng/mL and QC Very High 2500 ng/mL
QC intraday precision range (%)	1.8% to 125.8% Including Dixon Outlier* 1.8% to 8.4% Excluding Dixon Outlier
QC intraday accuracy range (%)	-6.5% to 133.7% Including Dixon Outlier -6.5% to 13.7% Excluding Dixon Outlier
QC interday precision range (%)	3.8% to 106. 2% Including Dixon Outlier 3.8% to 6.9% Excluding Dixon Outlier
QC interday accuracy range (%)	-2.8% to 39.3% Including Dixon Outlier -2.8% to 9.0% Excluding Dixon Outlier
Stability	Bench-top: 24 hrs Stock: 62 days (4°C); 24 hours at room temperature (22°C) Extract stability: 86 hrs Freeze-thaw (-20°C to room temperature): 5 cycles

	Long-term storage: 645 days at 20 °C and -70 °C Between 08/24/2023 and 09/07/2023, samples were received and stored at ~20 °C. The established long-term stability covers the period of study sample storage.
Selectivity	4.0 to 9.0% @ 1.0 ng/mL
Average recovery of drug (%)	82.0% (LQC: 3.0 ng/mL) 79.1% (MQC: 250 ng/mL) 83.8% (HQC: 400 ng/mL)
Average recovery of ISTD (%)	77.9%
Incurred Sample Reanalysis for Study AZ17-001	120 of 126 samples (95.2%) met the pre-specified run acceptance criteria.

*As pre-specified, for linear curves, a Dixon Outlier test of normalized response (per unit/mL) may be used to exclude a calibration standard point with approval of the laboratory Director.
Source: Table 3 of Summary of Biopharmaceutical and Associated Analytical Methods and Bioanalytical Study Report for Study AZ17-001.

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/s/

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