2.6.3 NONCLINICAL PHARMACOLOGY TABULATED SUMMARY

Lixisenatide (AVE0010)

Date: [redacted]-[redacted]-20[redacted] **Document Number:** -

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1 PHARMACOLOGY OVERVIEW

TS 2.6.3.1.1 - Pharmacology tabulated summary - pharmacology overview

Test Article: Lixisenatide

Type of Study	Test System	Method of Administration	Testing Facility	Report Number	Location
Primary Pharmacodynamics *Pharmacological Model*					
Binding to the GLP-1 receptor	CHO-K1 cells	in vitro	[redacted] Taiwan [redacted]	[MVT0010]	4.2.1.1-1
Glucose-stimulated insulin secretion in vitro	Isolated perfused pancreas from rat	Ex vivo	Sanofi-Aventis Deutschland GmbH, Frankfurt, Germany	[MVT0012]	4.2.1.1-3
Oral glucose tolerance test	Mouse	Intraperitoneal	[redacted] Denmark	[MVV0002]	4.2.1.1-4
Duration of effect in oral glucose tolerance test	Mouse	Intraperitoneal	[redacted] Denmark	[MVV0013]	4.2.1.1-5
Gastric emptying	Mouse	Intraperitoneal	[redacted] Denmark	[MVV0005]	4.2.1.1-6
Oral glucose tolerance test	Rat	Subcutaneous	Sanofi-Aventis Deutschland GmbH, Frankfurt, Germany	[MVV0008]	4.2.1.1-7

TS 2.6.3.1.1 - Pharmacology tabulated summary - pharmacology overview (continued)

Test Article: Lixisenatide

Type of Study	Test System	Method of Administration	Testing Facility	Report Number	Location
Primary Pharmacodynamics *Pharmacological Model (continued)*					
Oral glucose tolerance test	Dog	Subcutaneous	Sanofi-Aventis Deutschland GmbH, Frankfurt, Germany	[MVV0009]	4.2.1.1-8
Effects of single subcutaneous injection of lixisenatide and liraglutide on oral glucose tolerance in male dogs	Dog	Subcutaneous	Sanofi-Aventis Deutschland GmbH, Frankfurt, Germany	[DIVV0006]	4.2.1.1-9
Chronic effects on blood glucose levels, oral glucose tolerance and HbA1c	Mouse	Intraperitoneal	[redacted] Denmark	[MVV0007]	4.2.1.1-10
Stereological determination of insulin positive vs. total pancreas area	Mouse	Intraperitoneal	[redacted] Denmark	[MVV0006]	4.2.1.1-11
Chronic effects on fasting blood glucose levels, oral glucose tolerance, HbA1c and pancreatic insulin mRNA	Mouse	Intraperitoneal	[redacted] Denmark	[MVV0003]	4.2.1.1-12
Chronic effects on oral glucose tolerance, basal blood glucose and plasma insulin levels and HbA1c	Rat	continuous subcutaneous infusion	Sanofi-Aventis Deutschland GmbH, Frankfurt, Germany	[MVV0010]	4.2.1.1-13
Glucose-stimulated insulin secretion ex vivo	Isolated perfused pancreas from chronically treated rat	Subcutaneous, ex vivo perfusion of pancreas	Sanofi-Aventis Deutschland GmbH, Frankfurt, Germany	[MVT0013]	4.2.1.1-14

TS 2.6.3.1.1 - Pharmacology tabulated summary - pharmacology overview (continued)

Test Article: Lixisenatide

Type of Study	Test System	Method of Administration	Testing Facility	Report Number	Location
Secondary Pharmacodynamics					
Cardioprotective effects	Ischemia/reperfusion in isolated perfused heart from rat	Ex vivo	Sanofi-Aventis Deutschland GmbH, Frankfurt, Germany	[DIVT0002]	4.2.1.2-1
Protection from atherosclerotic plaque formation	Mouse (ApoE knockout)	continuous subcutaneous infusion	Sanofi-Aventis Deutschland GmbH, Frankfurt, Germany	[DIVV0007]	4.2.1.2-2
Receptor binding profile	91 different (mainly human) receptors	in vitro	[redacted] France	[MVT0011]	4.2.1.2-3
Inhibition of native N-type calcium channels	Cultured neurons of rat dorsal root ganglia (DRG)	in vitro in extracellular medium	Sanofi-Aventis France, Bagneux, France	[NVT0222]	4.2.1.2-7
Safety Pharmacology					
Central nervous system	Rat (Wistar)	Intravenous	[redacted] England	1927/003 – D6146 [DSE 20[redacted]-1177]	4.2.1.3-1
	Mouse (CD-1)	Subcutaneous	Aventis Pharma, Alfortville, France	[DSE 20[redacted]-1224]	4.2.1.3-2
Cardiovascular system In vitro	CHO cells (human channel: hERG)	In vitro	Aventis, Bridgewater, US	[DSE 20[redacted]-1521]	4.2.1.3-3
	Rabbit (New Zealand)	In vitro	Sanofi-Aventis, Alfortville, France	[DSE 20xx-0098]	4.2.1.3-4
Cardiovascular system In vivo	Rat (Wistar and Sprague-Dawley)	Intravenous	[redacted] Denmark	[redacted]-104, [redacted]-127 [DIV1154]	4.2.1.3-5

TS 2.6.3.1.1 - Pharmacology tabulated summary - pharmacology overview (continued)

Test Article: Lixisenatide

Type of Study	Test System	Method of Administration	Testing Facility	Report Number	Location
Cardiovascular and respiratory system In vivo	Dog (Beagle)	Intravenous	[redacted] England	1927/010 – D6146 [DSE 20[redacted]-1182]	4.2.1.3-6
Cardiovascular system In vivo	Dog (Beagle)	Intravenous	Sanofi-Aventis, Alfortville, France	[CVR0345]	4.2.1.3-7
Pharmacodynamic Drug Interactions					
No data available					

2 PRIMARY PHARMACODYNAMICS

TS 2.6.3.2.1 - Primary Pharmacodynamics overview - noteworthy findings

Test Article: Lixisenatide

Type of Study	Test System	Method of Administration	Dose or Concentration	No. per Group and Gender	Noteworthy Findings	Report Number	Location
Binding to the GLP-1 receptor	CHO-K1 cells	In vitro	0.1 nmol/L – 10 µmol/L	n = 2 / NA	AVE0010 had a strong binding affinity for the human GLP-1 receptor with an IC_{50} of 1.43 ± 0.239 nmol/L (K_i = 1.33 ± 0.222 nmol/L) that is ~4-times greater than that of human GLP-1-	[MVT0010]	4.2.1.1-1
Glucose-stimulated insulin secretion in vitro	Isolated perfused pancreas from rat	In vitro	10 nmol/L	n = 6-8 / M	At hyperglycemic glucose concentrations, AVE0010 significantly increases insulin secretion of the isolated perfused pancreas in male Wistar rats, compared to control.	[MVT0012]	4.2.1.1-3
Oral glucose tolerance test	Mouse	Intraperitoneal	0.17, 1.7, 17 and 170 nmol/kg (= 0.826, 8.26, 82.6 and 826 µg/kg	n = 7-8 / M	AVE0010 effectively and dose-dependently improved oral glucose tolerance in diabetic animals with an ED_{50} of 0.256 [0.0425; 1.55] nmol/kg IP	[MVV0002]	4.2.1.1-4

TS 2.6.3.2.1 - Primary pharmacodynamics overview – noteworthy findings (continued)

Test Article: Lixisenatide

Type of Study	Test System	Method of Administration	Dose or Concentration	No. per Group and Gender	Noteworthy Findings	Report Number	Location
Duration of effect in oral glucose tolerance test	Mouse	Intraperitoneal	100 nmol/kg (= 486 µg/kg)	n = 7-9 / M	The anti-diabetic effect of a maximal dose of AVE0010 was strong and statistically significant for 12 hours in db/db mice. It is concluded that a long-lasting antidiabetic effect can be achieved by AVE0010.	[MVV0013]	4.2.1.1-5
Gastric emptying	Mouse (NMRI)	Intraperitoneal	AVE0010: 0.0005 – 1000 nmol/kg (= 0.0024-4859 µg/kg), exendin-4: 0.1 – 10000 nmol/kg (= 0.4187-41866 µg/kg)	n = 5-15 / M	ED_{50} of AVE0010 on gastric emptying was estimated to be 6.39 [3.38; 12.1] nmol/kg (Slope factor = 0.60). ED_{50} of e exendin-4(1-39)-NH_2 was estimated to be 12.9 [5.72; 29.1] nmol/kg (Slope factor = 0.40). Both AVE0010 and exendin-4(1-39)-NH_2 decreased the rate of gastric emptying in NMRI mice with similar ED50 values and widely overlapping confidence intervals when administered IP. There was no difference between both compounds with respect to inhibition of gastric emptying in NMRI mice.	[MVV0005]	4.2.1.1-6
Oral glucose tolerance test	Rat (ZDF)	Subcutaneous	1, 5 and 10 µg/kg	n = 8 / M	Single subcutaneous injections of 5 and 10 µg/kg AVE0010 30 minutes prior to an oral glucose load significantly and dose-dependently improved oral glucose tolerance in obese, diabetic ZDF rats.	[MVV0008]	4.2.1.1-7

TS 2.6.3.2.1 - Primary pharmacodynamics overview – noteworthy findings (continued)

Test Article: Lixisenatide

Type of Study	Test System	Method of Administration	Dose or Concentration	No. per Group and Gender	Noteworthy Findings	Report Number	Location
Oral glucose tolerance test	Dog (Beagle)	Subcutaneous	AVE0010: 0.03, 0.1, 0.3 and 1.0 nmol/kg (= 0.146, 0.486, 1.46, 4.86 µg/kg), exendin-4: 0.03, 0.1, 0.3 and 1.0 nmol/kg (= 0.126, 0.487, 1.256 and 4.187 µg/kg)	n = 6-9 / M	Single SC injections of 0.03 to 1.0 nmol/kg AVE0010 30 minutes prior to an oral glucose load significantly improved oral glucose tolerance in healthy, normoglycemic Beagle dogs similar to single SC injections of equal doses of exendin-4. In parallel to the abolished blood glucose excursion plasma insulin levels following an oral glucose challenge were also significantly reduced by both AVE0010 and exendin-4. With respect to plasma insulin both, AVE0010 and exendin-4, showed a delayed return of insulin levels back to baseline as compared to control animals. Similar observations were made with plasma c-peptide levels	[MVV0009]	4.2.1.1-8
Acute effects of lixisenatide and liraglutide on oral glucose tolerance	Dog (Beagle)	Subcutaneous	Lixisenatide: 1 µg/kg (= 0.206 nmol/kg), Liraglutide: 50 and 100 µg/kg (= 15.162 and 30.324 nmol/kg)	n = 6/ M	Acute effect on oral glucose tolerance by lixisenatide was superior to that of liraglutide although 50 and 100 times higher doses of liraglutide were used	[DIVV0006]	4.2.1.1-9

TS 2.6.3.2.1 - Primary pharmacodynamics overview – noteworthy findings (continued)

Test Article: Lixisenatide

Type of Study	Test System	Method of Administration	Dose or Concentration	No. per Group and Gender	Noteworthy Findings	Report Number	Location
Chronic effects on blood glucose levels, oral glucose tolerance and HbA1c	Mouse (db/db)	Intraperitoneal	1, 10 and 100 nmol/kg BID (= 4.86, 48.6 and 486 µg/kg BID)	n = 15 / M	Twice daily treatment with 100 nmol/kg AVE0010 prevented the progressive development of diabetes in diabetic db/db mice during the six weeks study period. This was demonstrated by a highly significant improvement of oral glucose tolerance, decreased water intake, decreased fasting blood glucose and HbA1c. The therapeutic effect of AVE0010 had an immediate onset after the first dose and was preserved throughout the six weeks of study. No signs for tolerance to treatment or tachyphylaxis were observed. These results indicate that long-term treatment with AVE0010 is a highly efficacious therapy in diabetic db/db mice.	[MVV0007]	4.2.1.1-10
Stereological determination of insulin positive vs. total pancreas area	Mouse (db/db)	Intraperitoneal	1, 10 and 100 nmol/kg BID (= 4.86, 48.6 and 486 µg/kg BID)	n = 5 out of a total of n = 15 / M	In order to visualise the β-cells histologic sections were stained for insulin. The β-cells were intensively stained and the exocrine cells were all devoid of staining. Stereology analysis of the β-cell volume showed a tendency towards a dose-dependent increase of the β-cell volume after administration of AVE0010. On a descriptive level, AVE0010 tends to cause a dose-dependent increase of the β-cell volume.	[MVV0006]	4.2.1.1-11

TS 2.6.3.2.1 - Primary pharmacodynamics overview – noteworthy findings (continued)

Test Article: Lixisenatide

Type of Study	Test System	Method of Administration	Dose or Concentration	No. per Group and Gender	Noteworthy Findings	Report Number	Location
Chronic effects on fasting blood glucose levels, oral glucose tolerance, HbA1c and pancreatic insulin mRNA	Mouse (db/db)	Intraperitoneal	100 nmol/kg QD (= 486 µg/kg QD)	n = 9-11 / M	Once daily AVE0010, 100 nmol/kg i.p. prevented the progressive development of diabetes in db/db mice. Thus, 90 days of AVE0010 treatment significantly increased glucose tolerance, decreased FBG level and decreased water intake. On a descriptive level HbA1C was also decreased and the expression of insulin mRNA in pancreatic β-cells was increased relative to vehicle-treated control mice. Interestingly, in mice treated with AVE0010 only during the first 50 days of the study period, AVE0010 treatment produced a sustained improvement in glucose tolerance, a decreased FBG, lower water intake and an elevated expression of insulin mRNA compared to vehicle-treated animals. These results demonstrated that once daily administration of AVE0010 effectively prevented the progression of diabetes in db/db mice. No signs for tolerance to treatment or tachyphylaxis were observed. The sustained effect on glucose metabolism and pancreatic expression of insulin in the group shifted from AVE0010 treatment to vehicle indicates that AVE0010 preserved insulin production and β-cell function in diabetic db/db mice.	[MVV0003]	4.2.1.1-12

TS 2.6.3.2.1 - Primary pharmacodynamics overview – noteworthy findings (continued)

Test Article: Lixisenatide

Type of Study	Test System	Method of Administration	Dose or Concentration	No. per Group and Gender	Noteworthy Findings	Report Number	Location
Chronic effects on oral glucose tolerance, basal blood glucose, plasma insulin levels and HbA1c	Rat (ZDF)	Continuous subcutaneous infusion	AVE0010: 0.1, 1 and 10 nmol/kg/day (= 0.486, 4.86 and 48.59 µg/kg/day), exendin-4: 1 nmol/kg/day (= 4.187 µg/kg/day)	n = 8 / M	In obese diabetic ZDF rats 10 nmol/kg*day AVE0010 significantly improved oral glucose tolerance, hyperglycemia and HbA1c and preserved the pancreatic production and glucose responsiveness while the same dose did not induce hypoglycemia or insulin release in lean ZDF rats. Reduction in food intake was observed in both lean and obese ZDF rats but did only result in weight reduction in the lean cohort while in the obese any weight effect was probably counteracted by improvements in metabolic control which result in less glucosuria and thereby less calorie loss as compared to diabetic controls.	[MVV0010]	4.2.1.1-13
Glucose-stimulated insulin secretion ex vivo	Isolated perfused pancreas from chronically treated rat (ZDF)	Continuous subcutaneous infusion, ex vivo perfusion of the isolated pancreas	50 µg/kg/day (= 10.3 nmol/kg/day)	n = 6-7 / M	AVE0010 treatment over 6 weeks preserved 1st phase insulin release and Glucose-stimulated insulin secretion in obese, male ZDF rats.	[MVT0013]	4.2.1.1-14

TS 2.6.3.2.2A - Primary pharmacodynamics - in vitro

Test Article: Lixisenatide

Type of Study	Test System	Method of Administration (Vehicle)	Dose or Concentration, Duration of Treatment*	No. per Group and Gender	Noteworthy Findings	GLP status	Location Report Number
Binding to the GLP-1 receptor	CHO-K1 cells transfected with human GLP-1 receptor	In vitro (0.4% DMSO)	0.1 nmol/L – 10 µmol/L, 90 min	n = 2 / NA	IC_{50} (K_i): AVE0010: 1.43 ± 0.239 nmol/L (1.33 ± 0.222 nmol/L). Human GLP-1: 5.48 ± 1.28 nmol/L (5.09 ± 1.19 nmol/L). Conclusion: AVE0010 has a strong binding affinity to the human GLP-1 receptor that is ~4-times greater than that of human GLP-1.	NR	4.2.1.1-1 [MVT0010]

Batch of AVE0010 used: [redacted]
NA: not applicable; NR: not required

TS 2.6.3.2.2B - Primary pharmacodynamics - in vitro

Test Article: Lixisenatide

Type of Study	Test System	Method of Administration (Vehicle)	Dose or Concentration, Duration of Treatment	No. per Group and Gender	Noteworthy Findings	GLP status	Location Report Number
Glucose-stimulated insulin secretion in vitro	Isolated perfused pancreas from rat (Wistar)	In vitro (Krebs-Henseleit-Buffer)	10 nmol/L, 60 min	n = 6-8 / M	Insulin secretion AUC(10-60min) (min*µg/L) Median [25 / 75% quartiles] p-value CTRL 775.5 [234.5/863.5] AVE0010 3784 [2888 / 5034] p=0,0002 GLP-1 2391.5 [1296 / 2817 p=0,0188 Conclusion: AVE0010 significantly increases insulin secretion of the isolated perfused pancreas in male Wistar rats compared to control levels.	NR	4.2.1.1-3 [MVT0012]

Batch of AVE0010 used: [redacted]
NR: not required

TS 2.6.3.2.3A - Primary pharmacodynamics - in vivo

Test Article: Lixisenatide

Type of Study	Test System	Method of Administration (Vehicle)	Dose or Concentration, Duration of Treatment	No. per Group and Gender	Noteworthy Findings	GLP status	Location Report Number
Oral glucose tolerance test	Mouse (db/db)	Intraperitoneal	0.17, 1.7, 17 and 170 nmol/kg (= 0.826, 8.26, 82.6 and 826 µg/kg), single administration	n = 7-8 / M	AVE0010 when given 15 minutes prior to an oral glucose load is active and possesses a powerful anti-hyperglycaemic effect in db/db mice. The effect illustrated as the calculated AUC within the first 120 minutes observation period (AUC_{0-120}) revealed a strong effect for all doses of AVE0010 compared to vehicle ($p = 0.0008$ for 0.17 nmol/kg and $p \leq 0.0001$ for larger doses). The ED_{50} was found to be 0.256 [0.0425; 1.55] nmol/kg IP. Conclusion: The present study showed that AVE0010 effectively and dose-dependently improved oral glucose tolerance in diabetic animals.	NR	4.2.1.1-4 [MVV0002]

Batch of AVE0010 used: [redacted]
NR: not required

TS 2.6.3.2.3B - Primary pharmacodynamics - in vivo

Test Article: Lixisenatide

Type of Study	Test System	Method of Administration (Vehicle)	Dose or Concentration, Duration of Treatment	No. per Group and Gender	Noteworthy Findings	GLP status	Location Report Number
Duration of effect in oral glucose tolerance test	Mouse (db/db)	Intraperitoneal (Phosphate buffered saline with 0.1 % albumin)	100 nmol/kg (= 486 µg/kg), single administration	n = 7-9 / M	The duration of action of AVE0010 was evaluated in oral glucose tolerance tests in db/db mice. AVE0010 was given in a maximal dose of 100 nmol/kg IP at 20 different time points prior to the oral glucose tolerance test. AVE0010 was effective in reducing glucose AUC after OGTT for 12 hrs. Even after 18 hours a considerable reduction of the $AUC_{0\text{-}240\ min}$ for the AVE0010 treated group compared to the vehicle is observed but being not statistically significant. Conclusion: The anti-diabetic effect of a maximal dose of AVE0010 was strong and statistically significant for 12 hours in db/db mice. It is concluded that a long-lasting antidiabetic effect can be achieved by AVE0010.	NR	4.2.1.1-5 [MVV0013]

Batch of AVE0010 used: [redacted]
NR: not required

TS 2.6.3.2.3C - Primary pharmacodynamics - in vivo

Test Article: Lixisenatide

Type of Study	Test System	Method of Administration (Vehicle)	Dose or Concentration, Duration of Treatment	No. per Group and Gender	Noteworthy Findings	GLP status	Location Report Number
Gastric emptying	Mouse (NMRI)	Intraperitoneal (phosphate buffered saline)	AVE0010: 0.0005 – 1000 nmol/kg (= 0.0024-4859 µg/kg), exendin-4: 0.1 – 10000 nmol/kg (= 0.4187-41866 µg/kg), single administration	n = 5-15 / M	ED_{50} of AVE0010 on gastric emptying was estimated to be 6.39 [3.38; 12.1] nmol/kg (Slope factor = 0.60). ED_{50} of e exendin-4(1-39)-NH_2 was estimated to be 12.9 [5.72; 29.1] nmol/kg (Slope factor = 0.40). Conclusion: Both AVE0010 and exendin-4(1-39)-NH_2 decreased the rate of gastric emptying with similar ED_{50} values and widely overlapping confidence intervals. Therefore it was concluded that there is no difference between both compounds with respect to inhibition of gastric emptying.	NR	4.2.1.1-6 [MVV0005]

Batch of AVE0010 used: [redacted]
NR: not required

TS 2.6.3.2.3D - Primary pharmacodynamics - in vivo

Test Article: Lixisenatide

Type of Study	Test System	Method of Administration (Vehicle)	Dose or Concentration, Duration of Treatment	No. per Group and Gender	Noteworthy Findings	GLP status	Location Report Number
Oral glucose tolerance test	Rat (ZDF lean and obese)	Subcutaneous (phosphate-buffered saline)	1, 5 and 10 µg/kg, single administration	n = 8 / M	Blood glucose excursion in response to an oral glucose load was determined in drug-treated animals in comparison to placebo-treated obese and lean control animals. Following the oral glucose challenge the mean blood glucose rose as the followings (mmol/L): Lean controls: 1.32 ± 0.23 Obese controls: 4.30 ± 0.32 Obese AVE0010 1 µg/kg: 2.84 ± 0.65 Obese AVE0010 5 µg/kg: 1.81 ± 0.44 ($p<0.01$) Obese AVE0010 10 µg/kg: 1.27 ± 0.41 ($p<0.01$) Conclusion: Single subcutaneous injections of 5 and 10 µg/kg AVE0010 30 minutes prior to an oral glucose load significantly and dose-dependently improved oral glucose tolerance in obese, diabetic ZDF rats.	NR	4.2.1.1-7 [MVV0008]

Batch of AVE0010 used: [redacted]
NR: not required

TS 2.6.3.2.3E - Primary pharmacodynamics - in vivo

Test Article: Lixisenatide

Type of Study	Test System	Method of Administration (Vehicle)	Dose or Concentration, Duration of Treatment	No. per Group and Gender	Noteworthy Findings	GLP status	Location Report Number
Oral glucose tolerance test	Dog (Beagle)	Subcutaneous (compound-free HOE901 placebo solution)	AVE0010: 0.03, 0.1, 0.3 and 1.0 nmol/kg (= 0.146, 0.486, 1.46, 4.86 µg/kg), exendin-4: 0.03, 0.1, 0.3 and 1.0 nmol/kg (= 0.126, 0.487, 1.256 and 4.187 µg/kg), single administration	n = 6-9 / M	Following the oral glucose challenge the blood glucose excursion and plasma insulin levels in dogs treated with 0.03, 0.1, 0.3 and 1.0 nmol/kg sc of both, AVE0010 and exendin-4, 30 min prior to challenge, were significantly different from the control group. Similar observations were made with plasma c-peptide levels on a descriptive level. Conclusion: Single subcutaneous injections of 0.03 to 1.0 nmol/kg AVE0010 30 minutes prior to an oral glucose load significantly improved oral glucose tolerance in healthy, normoglycemic Beagle dogs similar to those of equal doses of exendin-4	NR	4.2.1.1-8 [MVV0009]

Batch of AVE0010 used: [redacted] and [redacted]
NR: not required

TS 2.6.3.2.3F - Primary pharmacodynamics - in vivo

Test Article: Lixisenatide

Type of Study	Test System	Method of Administration (Vehicle)	Dose or Concentration, Duration of Treatment	No. per Group and Gender	Noteworthy Findings	GLP status	Location Report Number
Acute effects of lixisenatide and liraglutide on oral glucose tolerance	Dog (Beagle)	Subcutaneous (solution for injection, placebo: NaCl 0.9%)	Lixisenatide: 1 µg/kg (= 0.206 nmol/kg), Liraglutide: 50 and 100 µg/kg (= 15.162 and 30.324 nmol/kg) single administration	n = 6 / M	Following the oral glucose challenge the blood glucose concentrations of both liraglutide groups were lower between 0.75h and 2h after treatment as compared to blood glucose levels in the control group being statistically significant only for 100 µg/kg SC liraglutide at time 1h (p=0.0109). However, the blood glucose concentration of lixisenatide was lower between 0.75h and 2.5h as compared to both liraglutide groups being statistically significant at time 1h (p<0.0320). The excursion of serum glucagon as exhibited in the placebo group was almost completely inhibited by 1 µg/kg SC lixisenatide and 50 µg/kg and 100 µg/kg liraglutide and serum glucagon levels remained below baseline level for all three groups. There was no major difference of any treatment compared to control on both serum insulin and c-peptide levels although both lixisenatide and liraglutide. Conclusion: Acute effect on oral glucose tolerance by lixisenatide was superior to that of liraglutide although 50 and 100 times higher doses of liraglutide were used	NR	4.2.1.1-9 [DIVV0006]

Batches used: Lixisenatide ([redacted]), Liraglutide ([redacted])
NR: not required

TS 2.6.3.2.3G - Primary pharmacodynamics - in vivo

Test Article: Lixisenatide

Type of Study	Test System	Method of Administration (Vehicle)	Dose or Concentration, Duration of Treatment	No. per Group and Gender	Noteworthy Findings	GLP status	Location Report Number
Chronic effects on blood glucose levels, oral glucose tolerance and HbA_{1c}	Mouse (db/db)	Intraperitoneal (phosphate buffer with 0.1 % BSA)	1, 10 and 100 nmol/kg BID (= 4.86, 48.6 and 486 µg/kg BID), 42 days	n = 15 / M	Twice daily treatment with 100 nmol/kg AVE0010 prevented the progressive development of diabetes in diabetic db/db mice during the six weeks of study period. Conclusion: Treatment with AVE0010 was shown to prevent the progressive development of diabetes in db/db mouse	NR	4.2.1.1-10 [MVV0007]

Batch of AVE0010 used: [redacted]
NR: not required

TS 2.6.3.2.3H - Primary pharmacodynamics - in vivo

Test Article: Lixisenatide

Type of Study	Test System	Method of Administration (Vehicle)	Dose or Concentration	No. per Group and Gender	Noteworthy Findings	GLP status	Location Report Number
Stereological determination of insulin positive vs. total pancreas area	Mouse (db/db)	Intraperitoneal (phosphate buffer with 0.1 % BSA)	1, 10 and 100 nmol/kg BID (= 4.86, 48.6 and 486 µg/kg BID), 42 days	n = 5 out of a total of n = 15 / M	This report contains histological evaluation of SPRFU-MVV0007. In order to visualise the β-cells histologic sections were stained for insulin. The β-cells were intensively stained and the exocrine cells were all devoid of staining. Stereology analysis of the β-cell volume showed a tendency towards a dose-dependent increase of the β-cell volume after administration of AVE0010. Conclusion: The present study demonstrated that long-term administration of AVE0010 tends to cause a dose-dependent increase of the β-cell volume.	NR	4.2.1.1-11 [MVV0006]

Batch of AVE0010 used: [redacted]
NR: not required

TS 2.6.3.2.3I - Primary pharmacodynamics - in vivo

Test Article: Lixisenatide

Type of Study	Test System	Method of Administration (Vehicle)	Dose or Concentration	No. per Group and Gender	Noteworthy Findings	GLP status	Location Report Number
Chronic effects on fasting blood glucose levels, oral glucose tolerance, HbA1c and pancreatic insulin mRNA	Mouse (db/db)	Intraperitoneal (phosphate buffer with 0.1 % BSA)	100 nmol/kg QD (= 486 µg/kg QD), AVE0010 90 days, AVE0010 1-50 days + vehicle 51-90 days, Vehicle 1-50 days + AVE0010 50-90 days	n = 9-11 / M	Once daily AVE0010, 100 nmol/kg i.p. prevented the progressive development of diabetes in db/db mice. Thus, 90 days of AVE0010 treatment significantly increased glucose tolerance, decreased FBG level and decreased water intake. On a descriptive level HbA1C was also decreased and the expression of insulin mRNA in pancreatic β-cells was increased relative to vehicle-treated control mice. Interestingly, in mice treated with AVE0010 only during the first 50 days of the study period, AVE0010 treatment produced a sustained improvement in glucose tolerance, a decreased FBG, lower water intake and an elevated expression of insulin mRNA compared to animals that were treated with vehicle throughout the 90 days study period. These results demonstrated that once daily administration of AVE0010 effectively prevented the progression of diabetes in db/db mice. No signs for tolerance to treatment or tachyphylaxis were observed. Conclusion: The sustained effect on glucose metabolism and pancreatic expression of insulin in the group shifted from AVE0010 treatment to vehicle indicates that AVE0010 preserved insulin production and β-cell function in diabetic db/db mice.	NR	4.2.1.1-12 [MVV0003]

Batch of AVE0010 used: [redacted], NR: not required; NA: not applicable

TS 2.6.3.2.3J - Primary pharmacodynamics - in vivo

Test Article: Lixisenatide

Type of Study	Test System	Method of Administration (Vehicle)	Dose or Concentration, Duration of Treatment	No. per Group and Gender	Noteworthy Findings	GLP status	Location Report Number
Chronic effects on oral glucose tolerance, basal blood glucose, plasma insulin levels and HbA1c	Rat (ZDF and lean)	Continuous subcutaneous infusion (water)	0.1, 1 and 10 nmol/kg/day (= 0.486, 4.86 and 48.6 µg/kg/day), 12 weeks	n = 8 / M	Introduction of HFD to obese ZDF rats resulted in hyperglycemia, hyperinsulinemia, decreased oral glucose tolerance and increased HbA1c as compared to lean ZDF rats. In obese ZDF rats 10 nmol/kg/day AVE0010 significantly decreased basal blood glucose during the diabetic phase and HbA1c at the end of the study. 10 nmol/kg*day AVE0010 significantly improved oral glucose tolerance at the time point of highest blood glucose excursion (1h) in a 2nd OGTT that was repeated after 5.5 weeks on HFD, when the obese ZDF cohort was overtly diabetic. At study end plasma insulin levels in obese ZDF rats with 10 nmol/kg/day AVE0010 was significantly increased compared to control. Conclusion: In obese diabetic ZDF rats 10 nmol/kg*day AVE0010 significantly improved oral glucose tolerance, hyperglycemia and HbA1c and preserved the pancreatic production and glucose responsiveness while the same dose did not induce hypoglycemia or insulin release in lean ZDF rats. Reduction in food intake was observed in both lean and obese ZDF rats but did only result in weight reduction in the lean cohort while in the obese any weight effect was probably counteracted by improvements in metabolic control which result in less glucosuria and thereby less calorie loss as compared to diabetic controls.	NR	4.2.1.1-13 [MVV0010]

Batch of AVE0010 used: [redacted]; NR: not required

TS 2.6.3.2.3K - Primary pharmacodynamics - in vivo

Test Article: Lixisenatide

Type of Study	Test System	Method of Administration (Vehicle)	Dose or Concentration, Duration of Treatment	No. per Group and Gender	Noteworthy Findings	GLP status	Location Report Number
Glucose-stimulated insulin secretion ex vivo	Isolated perfused pancreas from rat (ZDF and lean)	Subcutaneous (phosphate-buffered saline)	50 µg/kg(= 10.3 nmol/kg), 6 wks in vivo, 60 min in vitro	n = 6-7 / M	Treatment with AVE0010 preserved glucose stimulated insulin secretion in obese ZDF rats compared with vehicle treated obese rats but did not significantly alter GSIS in lean ZDF rats. Insulin secretion AUC(10-60min) (min*µg/L) mean / SEM Lean CTRL 896 294 Lean, AVE0010 645 123 Obese, CTRL 507 137 Obese, AVE 2026 390 Conclusion: AVE0010 treatment over 6 weeks preserved 1st phase insulin release and glucose-stimulated insulin secretion.	NR	4.2.1.1-14 [MVT0013]

Batch of AVE0010 used: [redacted]
NR: not required

3 SECONDARY PHARMACODYNAMICS

TS 2.6.3.3.1 - Secondary pharmacodynamics overview - noteworthy findings

Test Article: Lixisenatide

Type of Study	Test System	Method of Administration	Dose or Concentration	No. per Group and Gender	Noteworthy Findings	Report Number	Location
Cardioprotective effects of lixisenatide on ischemia/ reperfusion-induced injury in the isolated rat heart	Isolated perfused heart from Sprague Dawley rat	Ex vivo perfusion	Lixisenatide: 0.3 nmol/L GLP-1: 0.3 nmol/L Liraglutide: 0.3 nmol/L	n = 10-11 / M	Lixisenatide administered at 0.3 nmol/L starting 35 minutes after occlusion of the left ventricular artery (LAD) which was 10 minutes prior to reperfusion and during the 120 minutes reperfusion phase significantly reduced the development of myocardial infarction induced by transient LAD occlusion and reperfusion. Similar effects were observed with same concentrations of GLP-1 and liraglutide. Thus, it could be demonstrated that lixisenatide protects against myocardial ischemia-reperfusion injury in isolated rat hearts.	[DIVT0002]	4.2.1.2-1
Effects of chronic subcutaneous infusion of lixisenatide on atherosclerotic plaque formation in male ApoE knockout mouse	ApoE knockout mouse (B.129P2-apoe ^{tm1Unc}/J)	continuous subcutaneous infusion	Continuous subcutaneous infusion of 3.6-5.04 µg/day lixisenatide for 16 weeks	n = 17-18 / M	Total serum cholesterol was reduced by lixisenatide. After 16 weeks of treatment with lixisenatide, a significant reduction of atherosclerotic plaque formation by about ~30% was shown compared to placebo in all three methods. Lixisenatide significantly reduced atherosclerotic lesions of the total inner surface of the aorta by 27% (oil red staining) and the aortic root semilunar valve region by 29% (Movat-Pentachrome staining) or 30% (USPIO-based MRI imaging), respectively. It is concluded from this study that lixisenatide showed beneficial effects on total serum cholesterol in association with a robust anti-atherosclerotic activity in ApoE KO mice.	[DIVV0007]	4.2.1.2-2

TS 2.6.3.3.1 - Secondary pharmacodynamics overview - noteworthy findings (continued)

Test Article: Lixisenatide

Type of Study	Test System	Method of Administration	Dose or Concentration	No. per Group and Gender	Noteworthy Findings	Report Number	Location
Receptor binding profile	91 different (mainly human) receptors	In vitro	100 nmol/L	n = 2 / NA	AVE0010 had low to very low affinity to a wide range of receptors. From >90 receptors tested at the high concentration of 100 nM only the Ca^{2+}-channel (N) showed inhibition >50%. AVE0010 appears to act as a very selective agonist at the GLP-1 receptor.	[MVT0011]	4.2.1.2-3
Inhibition of the N-type calcium channels: a patch clamp study	Rat cultured DRG neurons	In vitro	1.0 and 10 µmol/L	n = 3-9 / NA	The blocking effect of Lixisenatide at native N-type calcium channel was incomplete and considered as weak compared to that of the selective N-type Ca2+-channel blocker ω-conotoxin GVIA	[NVT0222]	4.2.1.2-7

TS 2.6.3.3.2A - Secondary pharmacodynamics - in vitro

Test Article: Lixisenatide

Type of Study	Test System	Method of Administration (Vehicle)	Dose or Concentration, Duration of Treatment	No. per Group and Gender	Noteworthy Findings	GLP status	Location Report Number
Receptor binding profile	91 different (mainly human) receptors	In vitro (water)	100 nmol/L, 15-240 min	n =2 / NA	AVE0010 did not inhibit (< 10 %) specific radioligand binding to the following receptors: A_1*(h)*, α_1(non-selective), α_2(non-selective), β_1*(h)*, β_2*(h)*, NE transporter *(h)*, AT_2*(h)*, ANP, BZD (central), BZD (peripheral), bombesin (non-selective), B_2*(h)*, CGRP*(h)*, CB_1*(h)*, CB_2*(h)*, CCK_A*(h)*, CCK_B*(h)*, D1*(h)*, D2*(h)*, D3*(h)*, D4.4*(h)*, D5*(h)*, DA transporter *(h)*, ET_B*(h)*, GABA (non-selective), GAL1*(h)*, PDGF, IL-8B*(h)*, TNF-α*(h)*, CCR1*(h)*, H_1(central), H_2, ML_1, M_2*(h)*, M_3*(h)*, M_4*(h)*, M_5*(h)*, NK_1*(h)*, NK_2*(h)*, Y_1*(h)*, Y_2*(h)*, NT_1*(h)*, δ*(h)*, κ*(h)*, μ*(h)*, ORL_1*(h)*, PACAP, PCP, PGH_2*(h)*, PGI_2*(h)*, P2Y, 5-HT_{1A}*(h)*, 5-HT_{1B}, 5-HT_{2C}*(h)*, 5-HT_3*(h)*, 5-HT_{5A}*(h)*, 5-HT_6*(h)*, 5-HT_7*(h)*, σ (non-selective), sst (non-selective), VIP_1*(h)*, Ca^{2+}-channel (L, DHP site), Ca^{2+}-channel (L, diltiazem site), Ca^{2+}-channel (L, verapamil site), K^+_{ATP}-channel, K^+_V-channel, SK^+_{Ca}-channel, Na^+-channel (site 1), Cl^- channel, nAChR subtype α7 (N neuronal α-BGTX-sensitive), at 100 nM, while AVE0010 at that concentration inhibited specific radioligand binding with 12 – 22% to the following receptors: A_{2A}*(h)*, A_3*(h)*, AT_1*(h)*, ET_A*(h)*, M_1*(h)*, NK_3*(h)*, P2X, 5-HT_{2A}*(h)*, 5-HT transporter (h), V_{1A}*(h)*, Na^+-channel (site 2), nAChR subtype α4β2 (N neuronal α-BGTX-insensitive), nAChR N muscle-type (h). AVE0010 inhibited specific radioligand binding to the Ca^{2+}-channel (N) with 71%.	NR	4.2.1.2-3 [MVT0011] and 4.2.1.2-4 [MVT0011 EXTa] and 4.2.1.2-5 [MVT0011 EXTb] and 4.2.1.2-6 [MVT0011 EXTc]

Batch of AVE0010 used: [redacted], [redacted]; NA: not applicable; NR: not required

TS 2.6.3.3.2A - Secondary pharmacodynamics - in vitro (continued)

Test Article: Lixisenatide

Type of Study	Test System	Method of Administration (Vehicle)	Dose or Concentration, Duration of Treatment	No. per Group and Gender	Noteworthy Findings	GLP status	Location Report Number
Receptor binding profile	91 different (mainly human) receptors	In vitro (water)	100 nmol/L, 15-240 min	n =2 / NA	Conclusion: In the present study AVE0010 is reported to have low to very low affinity to a wide range of receptors. From 91 receptors tested at the high concentration of 100 nmol/L only the Ca^{2+}-channel (N) showed inhibition >50%. Thus, AVE0010 appears to act as a selective agonist at the GLP-1 receptor.	NR	4.2.1.2-3 [MVT0011] (continued)

Batch of AVE0010 used: [redacted], [redacted]; NA: not applicable; NR: not required

TS 2.6.3.3.2B - Secondary pharmacodynamics - in vitro

Test Article: Lixisenatide

Type of Study	Test System	Method of Administration (Vehicle)	Dose or Concentration, Duration of Treatment	No. per Group and Gender	Noteworthy Findings	GLP status	Location Report Number
Inhibition of the N-type calcium channels: a patch clamp study	Cultured neurons of rat dorsal root ganglia (DRG)	in vitro in extracellular medium	1 µmol/L and 10 µmol/L, 10 min	n = 3-9 / M	At 1 and 10 µmol/L, AVE0010 exerted 20 and 52% of omega conotoxin GVIA effect, respectively, without affecting other calcium channel subtypes since its effects were not additive to those of omega conotoxin GVIA. Conclusion: The blocking effect of Lixisenatide at native N-type calcium channel was incomplete and considered as weak compared to that of the selective N-type Ca^{2+}-channel blocker ω-conotoxin GVIA	NR	4.2.1.2-7 [NVT0222]

Batch of AVE0010 used: [redacted]
NR: not required

TS 2.6.3.3.3A - Secondary pharmacodynamics - in vivo

Test Article: Lixisenatide

Type of Study	Test System	Method of Administration (Vehicle)	Dose or Concentration, Duration of Treatment	No. per Group and Gender	Noteworthy Findings	GLP status	Location Report Number
Cardioprotective effects of lixisenatide on ischemia/reperfusion-induced injury in the isolated rat heart	Isolated perfused heart from Sprague Dawley rat	Ex vivo perfusion	Lixisenatide: 0.3 nmol/L GLP-1: 0.3 nmol/L Liraglutide: 0.3 nmol/L	n = 10-11 / M	Lixisenatide administered at 0.3 nmol/L starting 35 minutes after occlusion of the left ventricular artery (LAD) which was 10 minutes prior to reperfusion and during the 120 minutes reperfusion phase significantly reduced the development of myocardial infarction induced by transient LAD occlusion and reperfusion. Similar effects were observed with same concentrations of GLP-1 and liraglutide. Thus, it could be demonstrated that lixisenatide, protects against myocardial ischemia-reperfusion injury in isolated rat hearts.	NR	4.2.1.2-1 [DIVT0002]

Batch of AVE0010 used: [redacted]
NR: not required

TS 2.6.3.3.3B - Secondary pharmacodynamics - in vivo

Test Article: Lixisenatide

Type of Study	Test System	Method of Administration (Vehicle)	Dose or Concentration, Duration of Treatment	No. per Group and Gender	Noteworthy Findings	GLP status	Location Report Number
Effects of chronic subcutaneous infusion of lixisenatide on atherosclerotic plaque formation in male ApoE knockout mouse	ApoE knockout mouse (B.129P2-apoe ^{tm1Unc}/J)	continuous subcutaneous infusion	lixisenatide 3.6-5.04 µg/day for 16 weeks	n = 17-18 / M	Total serum cholesterol was reduced by lixisenatide. The decrease in total serum cholesterol was related to a decrease in the atherogenic non-HDL fractions. Treatment with lixisenatide had no significant effect on relative liver weight, hepatic cholesterol, triglyceride or phospholipid concentrations at study end. At the end of the study the ApoE knockout mice but not the wildtype background strain clearly developed atherosclerotic lesions at the total inner surface of the aorta and at the aortic root semilunar valve region of the heart. In contrast, treatment with lixisenatide showed a significant reduction of atherosclerotic plaque formation by about ~30% compared to placebo in all three methods. Lixisenatide significantly reduced atherosclerotic lesions of the total inner surface of the aorta by 27% (oil red staining) and the aortic root semilunar valve region by 29% (Movat-Pentachrome staining) or 30% (USPIO-based MRI imaging), respectively. It is concluded from this study that lixisenatide showed beneficial effects on total serum cholesterol in association with a robust anti-atherosclerotic activity in ApoE KO mice.	NR	4.2.1.2-2 [DIVV0007]

Batch of lixisenatide: [redacted]
NR: not required

4 SAFETY PHARMACOLOGY

TS 2.6.3.4.1 - Safety pharmacology overview – noteworthy findings

Test Article: Lixisenatide

Organ System Evaluated	Test System	Method of Administration	Dose or Concentration	No. per Group and Gender (M/F)	Noteworthy Findings	GLP Compliance	Study Number	Location
Central Nervous System	Rat (Wistar)	Intravenous	0.1, 1, 10, 50, 150, 500 µg/kg	6 M	From 10 µg/kg: reversible and slight decrease in locomotor activity and body tone	Yes	1927/003 – D6146 [20[redacted]-1177]	4.2.1.3-1
	Mouse (CD-1)	Subcutaneous	0.02, 0.2, 2 mg/kg	8 M	No effect on general behaviour	Yes	[20[redacted]-1224]	4.2.1.3-2
Cardiovascular System (in vitro)	CHO cells expressing hERG channel	In vitro	10, 30 µg/mL	4 cells	Inhibition of peak tail currents by 12.5% (10 µg/mL) and 37.3% (30 µg/mL)	No	[20[redacted]-1521]	4.2.1.3-3
	Rabbit (New Zealand)	In vitro	0.57 µg/mL	6 Purkinje fibers	No effect in resting membrane potential and in action potential parameters	Yes	[20[redacted]-0098]	4.2.1.3-4

TS 2.6.3.4.1 - Safety pharmacology overview – noteworthy findings (continued)

Test Article: Lixisenatide

Organ System Evaluated	Test System	Method of Administration	Dose or Concentration	No. per Group and Gender (M/F)	Noteworthy Findings	GLP Compliance	Study Number	Location
Cardiovascular System (in vivo)	Rat (Wistar and Sprague-Dawley)	Intravenous	Part 1 : 50, 150, 500 µg/kg cumulatively every 30 min	7 male Wistar in total	Increases in mean arterial blood pressure and blood glucose at 50 µg/kg. No further increases at 150 and 500 µg/kg	No	[redacted]-104, [redacted]-127 [DIV1154]	4.2.1.3-5
			Part 2: 500 µg/kg	4 male Wistar and 4 male Sprague-Dawley in total	No effect in Wistar rats. Increases in mean arterial blood pressure and blood glucose in Sprague-Dawley rats			
Cardiovascular and Respiratory Systems (in vivo)	Dog (Beagle)	Intravenous	0.1, 1, 10 µg/kg cumulatively at intervals of at least 30 minutes	Vehicle: 2 M, 2 F ZS42-0010: 2 M, 2 F	No effect on systolic, diastolic, mean arterial blood pressures, heart rate, left ventricular systolic pressure and its derivative, femoral artery blood flow and corresponding vascular resistance, PR, QRS, QT, QTc_B , QTc_F intervals, respiratory rate, tidal volume, minute volume, peak inspiratory and expiratory flows	Yes	1927/010 – D6146 [20[redacted]-1182]	4.2.1.3-6

TS 2.6.3.4.1 - Safety pharmacology overview – noteworthy findings (continued)

Test Article: Lixisenatide

Organ System Evaluated	Test System	Method of Administration	Dose or Concentration	No. per Group and Gender (M/F)	Noteworthy Findings	GLP Compliance	Study Number	Location
Cardiovascular System (in vivo)	Dog (Beagle)	Intravenous	10 µg/kg AVE0010 alone and in combination with insulin glargin	8 M / group	AVE0010: moderate decrease in serum glucose concentration, slight increase in heart rate, marginal but significant decrease of PQ interval duration related to heart rate increase; Combination AVE0010/insulin glargine: effects observed with insulin glargine alone were not modified by AVE0010	Yes	[CVR0345]	4.2.1.3-7

TS 2.6.3.4.2 - Safety pharmacology - central nervous system – Irwin's test

Title: ZS42-0010: Effects on Genaral Activity and Behaviour in the Rat following Intravenous Administration

Species/Strain: Rat / Wistar
Number per Group/Gender: 6 M
Weight/Age: 163 – 237 g / 7 weeks
Test Article (Batch): Lixisenatide ([redacted])
Vehicle/Formulation: phosphate buffered saline
Method of Administration: intravenous route
Study No.: 1927/003 – D6146 / [20[redacted]-1177]
Location: 4.2.1.3-1
Start Date: [redacted] 20[redacted]
GLP Compliance: Yes
Observation Period/Times: 5, 15, 30, 60 and 120 minutes post-dose

Clinical Signs	ZS42-0010=Lixisenatide (µg/kg)						
	vehicle	0.1	1	10	50	150	500
General activity and behaviour	No behavioral or physiological changes	No noteworthy behavioral or physiological changes	Slight transient decrease in body tone at 5 minutes post dose in one animal	Slight apathy and decreases in locomotor activity and body tone in the majority of animals. Slight to moderate impairment of the righting reflex for 50% of the animals, fully recovered by 60 minutes post-dose. By 120 minutes post-dose, the remaining signs were slight apathy (2/6 animals) and a slight decrease in body tone (6/6 animals)	At 50, 150 and 500 µg/kg, signs were comparable in frequency and severity. Abnormal dispersion within the home cage was noted for the majority of animals. This sign was fully recovered by 30 minutes post-dose at 50 and 150 µg/kg and by 60 minutes at 500 µg/kg. The majority of animals displayed slight apathy and decreased body tone, these were the only signs that remained apparent at 120 minutes. A slight impairment of the righting reflex was seen in the majority of animals, with full recovery seen in all animals by 60 minutes. Slightly decreased spatial locomotion and decreased grip strength were observed in up to 50% of the animals and was not specifically dose-related. A minority of animals exhibited a slight decrease in pain response, with a full recovery by 60 minutes. Hypothermia was observed for one animal from each dose level at the 60-minute time-point. Slightly flatten posture (2/6 animals) and slightly decreased locomotor activity (6/6 animals) and transfer arousal (2/6 animals) were noted at 50 µg/kg only.		

Additional Information: one observation was made for each of the following: landing with splayed hind limbs (500 µg/kg, 5 minutes post-dose), landing on side (150 µg/kg, 5 and 15 minutes post-dose) and clonic convulsion (50 µg/kg, 5 minutes post-dose). By Day 2 all animals appeared normal and no further signs were apparent throughout the study.
Conclusion: In the Irwin's test carried out in male Wistar rats, the intravenous administration of ZS42-0010 produced slight and reversible decreases in locomotor activity and body tone from the dose of 10 µg/kg.

TS 2.6.3.4.3 - Safety pharmacology - central nervous system - modified Irwin

Title: AVE0010: subcutaneous general behavior study (Irwin profile) in male mice

Species/Strain: Mouse/ CD-1
Number per Group/Gender: 8 M
Weight/Age: 25.2-28.8g / 5-6 weeks

Test Article (Batch): Lixisenatide ([redacted])
Vehicle/Formulation: sodium citrate buffer stock solution was diluted with sterile isotonic saline
Method of Administration: subcutaneous route

Study No.: [20[redacted]-1224]
Location: 4.2.1.3-2
Start Date: [redacted]20[redacted]
GLP Compliance: Yes

Observation Period/Times: 0.5, 1, 2, 5 and 24 h post-dose

Clinical Signs	AVE0010 (mg/kg)			
	0	0.02	0.2	2
Clinical and behavioral observations (Irwin profile)	No autonomic symptoms, no changes in alertness, reactivity, motor activity, tone and coordination. No mortality	Same behavior as controls	Same behavior as controls	Same behavior as controls

Conclusion: there were no effects on general behavior up to 2 mg/kg

TS 2.6.3.4.4 - Safety pharmacology - cardiovascular in vitro - Patch Clamp (hERG channel)

Title: AVE0010: exploratory hERG channel affinity (IC50) assay

Test System: hERG Channel Stably Expressed in Chinese Hamster Ovary (CHO) Cells

Test Article (Batch): Lixisenatide (xxxxxxxxx)

Vehicle/Formulation: solution containing (mM): NaCl 130; KCl 5; sodium acetate 2.8; $MgCl_2$ 1; HEPES 10; glucose 10; $CaCl_2$ 1.

Study No.: [20[redacted]-1521]

Location: 4.2.1.3-3

Number of Cells: 4

Start Date: [redacted] 20[redacted]

Patch Clamp Protocol: hERG currents initiated by a positive voltage pulse (20mV) followed by a negative pulse (-40 mV). Peak amplitude of the steady-state hERG tail current measured at – 40 mV.

GLP Compliance: No

Parameter (Unit)	AVE0010[a] (µg/mL)		
	0	10	30
Tail I_{Kr} current (% control)	100	87.5 ± 5.0	62.7 ± 3.5

Conclusion: AVE0010 blocked hERG currents concentration-dependently (12.5% and 37.3% inhibition at 10 and 30 µg/mL, respectively)

[a] Successive cumulative concentrations.

TS 2.6.3.4.5 - Safety pharmacology - cardiovascular in vitro - Purkinje Fiber (action potential)

Title: AVE0010: electrophysiology assay on isolated male rabbit Purkinje fibers

Test System: Purkinje Fiber, Male albino Rabbit (New Zealand)
Test Article (Batch): Lixisenatide ([redacted])
Study No.: [20[redacted]0098]
Vehicle/Formulation: Krebs solution
Location: 4.2.1.3-4
Number of Preparations: 6
Pacing Rate: 1Hz, 0.2 Hz, then 3 Hz
Start Date: [redacted] 20[redacted]
Measured Parameters: RMP: Resting Membrane Potential; APA: Action Potential Amplitude; $APD_{50, 90}$: Action Potential Duration at 50 and 90% of repolarization; V_{max}: maximal rate of depolarization.
GLP Compliance: Yes

	AVE0010 at 0.57 µg/mL					
Parameter (Unit) (mean ± SEM)	1 Hz		0.2 Hz		3 Hz	
	Control (Vehicle)		Control (Vehicle)		Control (Vehicle)	
RMP (mV)	- 90 ± 1	- 91 ± 1	- 89 ± 1	- 90 ± 1	- 92 ± 1	- 92 ± 1
APA (mV)	127 ± 1	126 ± 1	125 ± 1	126 ± 1	127 ± 1	127 ± 1
APD_{50} (ms)	257 ± 13	260 ± 13	339 ± 25	341 ± 32	163 ± 8	166 ± 9
APD_{90} (ms)	323 ± 13	329 ± 14	446 ± 34	458 ± 41	219 ± 2	223 ± 3 *
V_{max} (V/s)	670 ± 16	677 ± 18	681 ± 19	694 ± 25	646 ± 19	658 ± 21

Additional Information: AVE0010 was tested at the nominal concentrations of 0.01, 0.1 and 1 µg/mL sequentially applied every 30 minutes. The analytical study of the bath solutions indicated that AVE0010 was probably adsorbed on the experimental set-up. As a consequence, only the highest concentration tested (nominal 1 µg/mL, actual 0.57 µg/mL) was validated.
Conclusion: At the highest actual concentration tested of 0.57 µg/mL and whatever the stimulation rate, AVE0010 did not induce any change in resting membrane potential and action potential parameters of Rabbit Purkinje fibers.

Statistical significance: * $p \leq 0.05$ vs control value (Newman Keul's test)

TS 2.6.3.4.6 - Safety pharmacology - cardiovascular in vivo - blood pressure and blood glucose (rat)

Title: Cardiovascular effects of ZS42-0010 in conscious rats

Species/Strain: Rat / Wistar and Sprague Dawley
Number per Group/Gender: 11 male Wistar and 4 male Sprague-Dawley used in total
Age: not mentioned. Body weight: 245 ± 5g

Test Article (Batch): Lixisenatide ([redacted])
Vehicle/Formulation: phosphate buffer saline
Method of Administration: intravenous

Study No.: [redacted]-104, [redacted]-127 ([DIV1154])
Location: 4.2.1.3-5
Start Date: [redacted] 20[redacted]
GLP Compliance: No

Measurement Times: Part 1: before then every 5 minutes for 90 minutes after administration. Part 2: before then every 5 minutes for 60 minutes after administration
Measured Parameters: MAP: Mean arterial Blood Pressure, BG: Blood Glucose concentration

Parameter (Unit, mean ± SEM)	ZS42-0010=AVE0010 (µg/kg, cumulative doses every 30 minutes)				
Part 1 (protocol [redacted]-104): Wistar rats (n = 7)	Time	Vehicle	50	150	500
MAP (mmHg) maximal change	5 minutes	+ 3 ± 4	+ 27 ± 4 *	No further increase	No further increase
BG (mM) maximal change	10 minutes	- 0.3 ± 0.3	+ 2.9 ± 0.5 *	No further increase	No further increase

Part 2 (protocol [redacted]-127): Wistar (n=4) and Sprague-Dawley (n=4) rats	Time	Vehicle	50	150	500
MAP maximal change (mmHg) Wistar rats	-	No effect	Not performed	Not performed	No effect
BG maximal change (mM) Wistar rats	-	No effect	Not performed	Not performed	No effect
MAP maximal change (mmHg) Sprague-Dawley rats	5 minutes	No effect	Not performed	Not performed	+ 10 ± 6 *
BG maximal change (mM) Sprague-Dawley rats	35 minutes	No effect	Not performed	Not performed	+ 2.0 ± 0.8 *

Conclusion: immediate increase in blood pressure and blood glucose levels at 50 µg/kg in Wistar rats. Immediate increase in blood pressure and blood glucose levels at 500 µg/kg in Sprague-Dawley rats.

Statistical significance: * $p < 0.05$ *vs* vehicle (two-way analysis of variance for repeated measures)

TS 2.6.3.4.7 - Safety pharmacology - cardiovascular and respiration in vivo (dog)

Title: ZS42-0010: cardiovascular and respiratory effects in the anesthetised dog following intravenous administration

Species/Strain: Dog/ Beagle
Test Article (Batch): Lixisenatide ([redacted])
Study No.: 1927/010-D6146 / ([20[redacted]-1182])
Number per Group/Gender: 2 M and 2 F per group
Vehicle/Formulation: phosphate buffer
Location: 4.2.1.3-6
2 groups: vehicle and tested article
Method of Administration: Intravenous route
Start Date: [redacted] 20[redacted]
Age: 6 to 8 months. **Body Weight:** 9.2 to 13.25 Kg
Anesthesia: intravenous administration of propofol
GLP Compliance: Yes

Measured or Calculated Parameters: Systolic, Diastolic, Mean arterial Blood Pressures (SBP, DBP, MBP), Heart Rate (HR), Left Ventricular Systolic Pressure (LVSP) and its derivative (dP/dt_{max}), Femoral Artery Blood Flow (FBF) and corresponding vascular resistance (FVR), PR, QRS, QT, QTc_B (Bazett), QTc_F (Fridericia) intervals, Respiratory Rate (RR), Tidal Volume (TV), Minute Volume (MV), Peak Inspiratory Flow (PIF), Peak Expiratory Flow (PEF), measured before and at 2, 10, 20, 30 minutes after the end of infusion.

Parameter (Unit)	Vehicle				ZS42-0010=Lixisenatide			
(Mean ± SEM)	Pre-treatment	Dosing 1	Dosing 2	Dosing 3	Pre-treatment	0.1 µg/kg	1 µg/kg	10 µg/kg
SBP (mmHg)	117 ± 5	125 ± 7	125 ± 7	131 ± 13	122 ± 2	130 ± 5	128 ± 3	118 ± 6
DBP (mmHg)	57 ± 2	61 ± 6	63 ± 6	76 ± 14	54 ± 2	56 ± 4	56 ± 4	58 ± 2
MBP (mmHg)	80 ± 3	85 ± 4	85 ± 5	97 ± 12	78 ± 2	81 ± 4	79 ± 3	81 ± 2
HR (beats/min)	93 ± 2	89 ± 1	91 ± 4	101 ± 7	79 ± 8	77 ± 8	84 ± 8	85 ± 9
dP/dt_{max} (mmHg/s)	3419 ± 150	3565 ± 98	3524 ± 144	3799 ± 314	3476 ± 56	3362 ± 9	3718 ± 208	3621 ± 138
FBF (mL/min)	112 ± 15	133 ± 23	126 ± 23	94 ± 15	85 ± 12	88 ± 10	91 ± 4	90 ± 8
FVR (mmHg.min/mL)	0.76 ± 0.11	0.69 ± 0.11	0.73 ± 0.12	1.08 ± 0.18	0.99 ± 0.18	0.96 ± 0.13	0.88 ± 0.06	0.88 ± 0.10
PR interval (ms)	134 ± 6	131 ± 6	130 ± 6	127 ± 8	145 ± 8	139 ± 6	134 ± 5	135 ± 4
QRS complex (ms)	48 ± 6	48 ± 6	46 ± 5	44 ± 4	57 ± 5	53 ± 6	56 ± 5	55 ± 5
QT interval (ms)	244 ± 4	241 ± 4	237 ± 4	232 ± 6	245 ± 13	249 ± 4	241 ± 5	241 ± 9
QTc_B interval (ms)	304 ± 3	297 ± 4	296 ± 1	298 ± 2	279 ± 3	282 ± 8	285 ± 5	286 ± 5
QTc_F interval (ms)	283 ± 3.3	278 ± 3.8	275 ± 2.3	274 ± 2.0	267 ± 6.1	270 ± 4.3	270 ± 2.4	270 ± 2.0
RR (breath per min)	34 ± 6	26 ± 1	27 ± 3	24 ± 3	28 ± 6	25 ± 5	26 ± 6	23 ± 6
TV (mL)	58 ± 7	62 ± 6	62 ± 7	68 ± 7	67 ± 8	72 ± 11	73 ± 12	83 ± 21
MV (mL)	1836 ± 263	1626 ± 111	1613 ± 115	1551 ± 166	1765± 349	1676 ± 243	1717 ± 226	1551 ± 265
PIF (mL/s)	171 ± 22	163 ± 21	155 ± 19	152 ± 21	162 ± 17	176 ± 16	180 ± 13	192 ± 21
PEF (mL/s)	215 ± 23	237 ± 28	230 ± 27	260 ± 38	227 ± 33	258 ± 50	264 ± 47	297 ± 79

Additional Information: Each dog per group received ascending doses of ZS42-0010 or the same number of doses of vehicle by infusion over 30 minutes at intervals of at least 30 minutes. Maximal or minimal value from pre-treatment are shown.

Conclusion: Intravenous administration of ZS42-0010 at doses of 0.1, 1 and 10 µg/kg had no biologically significant effects on the cardiovascular and respiratory systems of the anesthetised dog when compared with vehicle control group.

TS 2.6.3.4.8 - Safety pharmacology - cardiovascular in vivo (dog)

Title: Insulin glargine/AVE0010 combination - Effect of a single intravenous dose on cardiovascular function in anesthetized dogs

Species/Strain: Dog/ Beagle
Number per Group/Gender: 8 M
4 groups: vehicle, lixisenatide, insuline glargine, lixisenatide + insulin glargine
Age: 19.5 to 29.4 months. Body Weight: 7.2 to 10.9 Kg

Test Article (Batch): Lixisenatide ([redacted])
Vehicle/Formulation: ready-to-use solutions
Method of Administration: Intravenous route
Anesthesia: intravenous administration of thiopental, followed by isoflurane inhalation (2 to 3%)

Study No.: [CVR0345]
Location: 4.2.1.3-7
Start Date: [redacted] 20[redacted]
GLP Compliance: Yes

Measured or Calculated Parameters: Serum glucose, Serum potassium, Body temperature, Heart Rate (HR), PQ, QRS, QT, QTcF (Fridericia), QTcW (Van de Water's) intervals

- Before injection of the second treatment

Parameter (Unit) (Mean ± SEM)	Vehicle	10 µg/kg lixisenatide	
Glucose (mmol/L)	5.54 ± 0.154	4.11 ± 0.159**	
Potassium (mmol/L)	5.2 ± 0.08	4.9 ± 0.08*	
Body temperature (°C)	38.0 ± 0.02	38.0 ± 0.02	
HR (beats/min)	107 ± 1.1	112 ± 1.2**	
PQ interval (ms)	100 ± 0.6	99 ± 0.6*	
QRS complex (ms)	37 ± 0.2	38 ± 0.2	
QT interval (ms)	227 ± 1.1	226 ± 1.1	
QTcF interval (ms)	275 ± 1.2	278 ± 1.2*	
QTcW interval (ms)	265 ± 0.8	266 ± 0.8	

Additional Information: * p<0.05; ** p<0.001;

Conclusion: AVE0010 induced a moderate decrease in serum glucose concentration, reaching a maximum at the end of infusion, a slight increase in heart rate at mid infusion and at the end of infusion, and a marginal but significant decrease of PQ interval duration at mid infusion related to heart rate increase. The combination of AVE0010 with insulin glargine did not modify the effects observed with insulin glargine alone.

TS 2.6.3.4.8 - Safety pharmacology - cardiovascular in vivo (dog) (continued)

Title: Insulin glargine/AVE0010 combination - Effect of a single intravenous dose on cardiovascular function in anesthetized dogs

Species/Strain: Dog/ Beagle
Test Article (Batch): Lixisenatide ([redacted])
Study No.: [CVR0345]
Number per Group/Gender: 8 M
Vehicle/Formulation: ready-to-use solutions
Location: 4.2.1.3-7
4 groups: vehicle, lixisenatide, insuline glargine, lixisenatide + insulin glargine
Method of Administration: Intravenous route
Start Date: [redacted] [redacted] 20[redacted]
Age: 19.5 to 29.4 months. Body Weight: 7.2 to 10.9 Kg
Anesthesia: intravenous administration of thiopental, followed by isoflurane inhalation (2 to 3%)
GLP Compliance: Yes

Measured or Calculated Parameters: Serum glucose, Serum potassium, Body temperature, Heart Rate (HR), PQ, QRS, QT, QTcF (Fridericia), QTcW (Van de Water's) intervals

- During / end of infusion

Parameter (Unit)* (Mean ± SEM)	Vehicle	10 µg/kg lixisenatide	0.1 U/kg insuline glargine	10 µg/kg lixicsenatide + 0.1 U/kg insuline glargine	
Glucose (mmol/L)**	5.77 ± 0.388	4.23 ± 0.483	5.46 ± 0.363	3.85 ± 0.155	
Potassium (mmol/L)**	5.3 ± 0.13	5.0 ± 0.40	5.2 ± 0.16	4.8 ± 0.16	
Body temperature (°C)	38.0 ± 0.14	38.2 ± 0.12	38.0 ± 0.18	37.9 ± 0.08	
HR (beats/min)	109 ± 4.4	115 ± 2.9	107 ± 5.0	111 ± 3.2	
PQ interval (ms)	102 ± 4.8	97 ± 4.3	99 ± 5.5	99 ± 3.8	
QRS complex (ms)	37 ± 1.0	37 ± 0.7	37 ± 0.6	38 ± 0.6	
QT interval (ms)	223 ± 6.7	222 ± 8.0	229 ± 8.7	225 ± 6.3	
QTcF interval (ms)	271 ± 6.1	275 ± 8.3	276 ± 7.4	276 ± 7.2	
QTcW interval (ms)	261 ± 5.4	263 ± 7.1	266 ± 6.9	264 ± 5.9	

Additional Information: * end of 30-minute infusion; ** mean during infusion

Conclusion: AVE0010 induced a moderate decrease in serum glucose concentration, reaching a maximum at the end of infusion, a slight increase in heart rate at mid infusion and at the end of infusion, and a marginal but significant decrease of PQ interval duration at mid infusion related to heart rate increase. The combination of AVE0010 with insulin glargine did not modify the effects observed with insulin glargine alone.

TS 2.6.3.4.8 - Safety pharmacology - cardiovascular in vivo (dog) (continued)

Title: Insulin glargine/AVE0010 combination - Effect of a single intravenous dose on cardiovascular function in anesthetized dogs

Species/Strain: Dog/ Beagle
Test Article (Batch): Lixisenatide ([redacted])
Study No.: [CVR0345]
Number per Group/Gender: 8 M
Vehicle/Formulation: ready-to-use solutions
Location: 4.2.1.3-7
4 groups: vehicle, lixisenatide, insuline glargine, lixisenatide + insulin glargine
Method of Administration: Intravenous route
Start Date: [redacted] [redacted] 20[redacted]
Age: 19.5 to 29.4 months. Body Weight: 7.2 to 10.9 Kg
Anesthesia: intravenous administration of thiopental, followed by isoflurane inhalation (2 to 3%)
GLP Compliance: Yes

Measured or Calculated Parameters: Serum glucose, Serum potassium, Body temperature, Heart Rate (HR), PQ, QRS, QT, QTcF (Fridericia), QTcW (Van de Water's) intervals

- After infusion

Parameter (Unit)* (Mean ± SEM)	Vehicle	10 µg/kg lixisenatide	0.1 U/kg insuline glargine	10 µg/kg lixicsenatide + 0.1 U/kg insuline glargine	
Glucose (mmol/L)**	5.52 ± 0.325	4.33 ± 0.403	1.69 ± 0.143	1.52 ± 0.158	
Potassium (mmol/L)**	5.4 ± 0.10	5.2 ± 0.38	4.5 ± 0.09	4.3 ± 0.12	
Body temperature (°C)	38.0 ± 0.16	38.3 ± 0.13	38.1 ± 0.19	38.0 ± 0.07	
HR (beats/min)	112 ± 5.1	111 ± 2.5	124 ± 5.0	122 ± 3.4	
PQ interval (ms)	100 ± 5.4	97 ± 5.1	94 ± 4.5	98 ± 3.6	
QRS complex (ms)	37 ± 0.8	37 ± 0.6	38 ± 0.7	38 ± 0.8	
QT interval (ms)	219 ± 6.8	222 ± 7.7	227 ± 7.3	231 ± 6.5	
QTcF interval (ms)	268 ± 6.2	272 ± 8.4	289 ± 8.0	293 ± 8.4	
QTcW interval (ms)	258 ± 5.5	262 ± 7.1	272 ± 6.5	275 ± 6.5	

Additional Information: * 30 minutes after end of infusion; ** mean 2 to 30 minutes after infusion

Conclusion: AVE0010 induced a moderate decrease in serum glucose concentration, reaching a maximum at the end of infusion, a slight increase in heart rate at mid infusion and at the end of infusion, and a marginal but significant decrease of PQ interval duration at mid infusion related to heart rate increase. The combination of AVE0010 with insulin glargine did not modify the effects observed with insulin glargine alone.

5 PHARMACODYNAMIC DRUG INTERACTIONS

TS 2.6.3.5.1 - Pharmacodynamic drug interactions – noteworthy findings

Test Article: Lixisenatide

Not applicable, because no studies were performed.