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APPLICATION NUMBER:

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NON-CLINICAL REVIEW(S)

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

PHARMACOLOGY/TOXICOLOGY NDA/BLA REVIEW AND EVALUATION

Application number:	NDA 210134
Supporting document/s:	SDN 1
Applicant's letter date:	6/28/2018
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Product:	BAQSIMI™ (Glucagon Nasal Powder)
Indication:	Emergency Treatment of Severe Hypoglycemia
Applicant:	Eli Lilly and Company
Review Division:	Division of Metabolism and Endocrinology Products (DMEP)
Reviewer:	Dongyu Guo, Ph.D.
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1 Executive Summary

1.1 Introduction

BAQSIMI™ is a novel, nasally-administered glucagon powder formulation for the treatment of severe hypoglycemia. The glucagon powder formulation contains 3 mg synthetic glucagon, (b) (4) mg dodecylphosphocholine (DPC) (b) (4) mg beta-cyclodextrin (β-CD) (b) (4).

1.2 Brief Discussion of Nonclinical Findings

The pharmacological and toxicological properties of glucagon, an endogenous peptide hormone, are well known. The physiological effect of acute use of glucagon is to raise the concentration of blood glucose. At hyperphysiological levels, glucagon can induce reversible hyperglycemia. Glucagon is currently marketed as two injectable glucagon products (Glucagon for Injection: NDA 020928; Glucagon Hypo Kit: NDA 020918). Therefore, the nonclinical program for Nasal Glucagon was designed to evaluate the local effects of glucagon in the nasal cavity and to determine the local and systemic effects related to the two excipients (β-CD and DPC), which were not previously qualified for administration by the intranasal route.

Nasal Glucagon was shown to have a pharmacodynamic effect on blood glucose in dogs similar to that of other subcutaneously administered human glucagon products. Administration by the intranasal route to dogs also led to local drug product exposures to the nasal passages, nasopharynx, stomach, esophagus, and tongue of dogs, as indicated by a blue powder tracer dye. There was no evidence of dye in the larynx or trachea of the dogs, indicating nasal glucagon is unlikely to be inspired when administered as indicated.

Rats and dogs were administered the formulation by the nasal route during 28-day repeat-dose toxicity studies. These evaluations were meant to identify hazards that may not be observed in the small number of patients administered the product in short duration pharmacokinetic studies. There were no test article-related adverse effects on body weight and/or food consumption, ophthalmology, electrocardiography, hematology, coagulation parameters, clinical chemistry, urinalysis, or organ weights, and no macroscopic findings at necropsy in these studies. Reversible lesions (mild to moderate, unilateral or bilateral erosion/ulceration) were observed in the dorsal turbinates of the nasal cavity (especially the olfactory epithelium of the lamina propria) in the nasal cavity after 28 days of once-daily exposure with nasal glucagon. In the rat study, The NOAEL was 0.1 mg glucagon/day, based on findings in the turbinates. The safety margin at the NOAEL in rats for the clinical dose of 3 mg nasal glucagon was 45-times the human exposure, based on AUC. In the dog study, the NOAEL was not established based on the histopathology findings of mild to moderate atrophy and degeneration of the olfactory epithelium in the nasal cavity of all treated dogs. The

lowest dose represented 398-times the human exposure, based on AUC. All lesions observed in animals were reversible and are unlikely to occur in humans because Nasal Glucagon is intended for a single emergency use for patients with severe hypoglycemia.

The β-CD and DPC excipients were administered to vehicle control groups in the two 28-day repeat-dose toxicity studies by intranasal administration to rats (b) (4) mg/day β-CD, equivalent to the clinical exposure based on nasal surface area) and 0.3 mg/day DPC (less than the clinical exposure based on AUC), and to dogs ((b) (4) mg/day β-CD, equivalent to the clinical exposure based on nasal surface area) and (b) (4) mg/day DPC (7-times the clinical exposure based on AUC). No significant excipient-related toxicities were observed. Minimal local nasal irritation was noted in the vehicle control group in dogs. Rather, the primary nasal irritant in Nasal Glucagon is synthetic glucagon. β-CD and DPC were negative for genotoxicity and mutagenicity in standard genotoxicity tests.

Total impurities (b) (4) as well as specified degradation impurities (b) (4) were qualified in a 14-day GLP repeat-dose toxicity study in rats nasally instilled at (b) (4) mg glucagon/day (45-times the human exposure, based on AUC). No test item-related findings were observed.

In summary, the nonclinical data support approval of Nasal Glucagon. There are no safety concerns for Nasal Glucagon compared with marketed injectable glucagon products that should preclude its approval. Changes in Labeling for Sections 8.1 and 13.1 (compared to the Sponsor's proposed label) are recommended (see below).

1.3 Recommendations

1.3.1 Approvability

The nonclinical data support approval of BAQSIMI™.

1.3.2 Additional Nonclinical Recommendations

None

1.3.3 Labeling

Reviewer's recommended labeling:

Section 8.1 Pregnancy

Risk summary

Reproduction studies with nasal glucagon powder were not conducted in animals. In a rat reproduction study, with glucagon administered by injection during the period of organogenesis at doses representing up to 40 times the human dose of nasal glucagon powder, based on body surface area (mg/m^2), no embryofetal toxicity was observed.

Animal data

In pregnant rats given animal sourced glucagon twice-daily by injection at doses up to 2 mg/kg (up to 40 times the human dose based on body surface area extrapolation, mg/m^2) during the

period of organogenesis, there was no evidence of increased malformations or embryofetal lethality.

Section 13.1 Carcinogenesis, mutagenesis, impairment of fertility
Long term studies in animals to evaluate carcinogenic potential have not been performed. Recombinant glucagon was positive in the bacterial Ames assay. It was determined that an increase in colony counts was related to technical difficulties in running this assay with peptides. Studies in rats have shown that glucagon does not cause impaired fertility.

2 Drug Information

2.1 Drug

CAS Registry Number
16941-32-5

Generic Name
Glucagon

Code Name
LY900018, AMG504-1, glucagon nasal powder, nasal glucagon (NG), intranasal (IN) glucagon

Chemical Name
L-histidyl-L-seryl-L-glutaminyl-glycyl-L-threonyl-L-phenylalanyl-L-threonyl-L-seryl-L-alpha-aspartyl-L-tyrosyl-L-seryl-L-lysyl-L-tyrosyl-L-leucyl-L-alpha-aspartyl-L-seryl-L-arginyl-L-arginyl-L-alanyl-L-glutaminyl-L-alpha-aspartyl-L-phenylalanyl-L-valyl-L-glutaminyl-L-tryptophyl-L-leucyl-L-methionyl-L-asparagyl-L-threonine

Molecular Formula/Molecular Weight
$C_{153}H_{225}N_{43}O_{49}S$ / 3482.795 g/mol
Structure or Biochemical Description

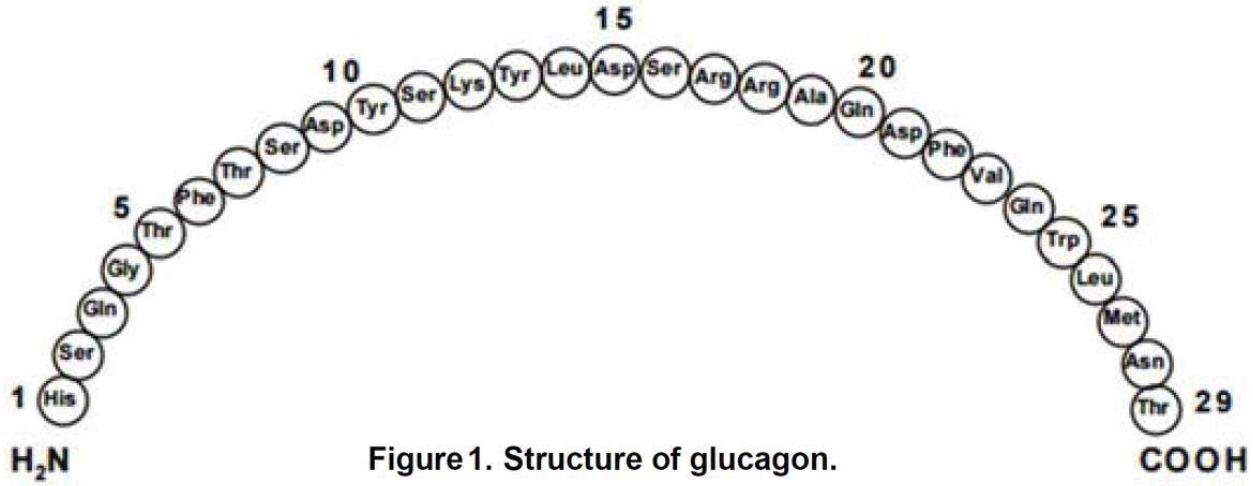


Figure 1. Structure of glucagon.

Pharmacologic Class
Antihypoglycemic agent

2.2 Relevant INDs, NDAs, BLAs and DMFs

NDA 020928 (Glucagon, Eli Lilly and Company); NDA 020918 (GlucaGen, Novo Nordisk); IND 110674; DMF (b) (4), glucagon drug substance manufacturer)

2.3 Drug Formulation

Nasal Glucagon (NG) is a combination product comprised of a (b) (4) powder (drug powder) administered to the nasal mucosa via a delivery device.

Table 1 Unit Formula for Nasal Glucagon Drug Powder

Ingredient	Quantity (mg/unit dose)	Function	Reference to Standards
Active Ingredient			
Glucagon (Synthetic)	3.0	Active ingredient	(b) (4)
Other Ingredients			
β-Cyclodextrin	(b) (4)		
Dodecylphosphocholine (DPC)			
(b) (4)			

Abbreviations: Ph. Eur. = European Pharmacopoeia; USP-NF = United States Pharmacopoeia and National Formulary.

(b) (4)

2.4 Comments on Novel Excipients

Beta-cyclodextrin (β-CD) and dodecylphosphocholine (DPC) are novel excipients in the drug product by the nasal route. The safety profile of DPC was evaluated by the sponsor in a series of pharmacology, genetic toxicity, and reproduction studies. Extensive reviews of toxicological studies with β-CD have been published (Stella et al,

2008)[1]. Therefore, no stand-alone toxicology studies were conducted with β-CD. B-CD together with DPC was tested in 28-day intranasal toxicity studies in rats and dogs. The studies were reviewed in appropriate sections of this review.

2.5 Comments on Impurities/Degradants of Concern

No impurities of concern were identified.

All degradation impurities were qualified in Sprague Dawley Rats given nasal glucagon (heat-stressed in a closed container with lid at 50°C for 82 days) at (b) (4) mg/day (45-fold the clinical dose, based on AUC) daily for 14 days by intranasal instillation. The impurities content included (b) (4) No test article-related toxicities were noted in the study.

CMC reviewer Dr. Muthukumar Ramaswamy asked the Pharmacology/Toxicology reviewer to comment on the sponsor's toxicology risk assessment of a potential leachable (b) (4) was performed in the review of Ventolin HFA (albuterol sulfate) Inhalation Aerosol (NDA 020983, approved in 2001). Human exposure up to (b) (4) µg/day is considered acceptable. The total amount of (b) (4) in Nasal Glucagon is (b) (4) µg. Furthermore, the sponsor reported "the ongoing stability results indicated that (b) (4) was not reported by GC-MS". The reviewer concluded that there was no safety concern of using (b) (4) in Nasal Glucagon.

2.6 Proposed Clinical Population and Dosing Regimen

BAQSIMI™ will be given as a single 3 mg intranasal dose in both adults and pediatric patients.

2.7 Regulatory Background

IND 110674 was submitted to FDA on April 03, 2012. FDA agreed that the proposed nonclinical assessments of both AMG504-1 (Nasal Glucagon) and DPC were reasonable at the End-of-Phase 2 meeting held on April 15, 2013. Pre-NDA meeting was held on May 07, 2015. FDA acknowledged the transfer of IND 110674 from Locemia to Eli Lilly on October 27, 2015.

[1] Stella VJ, He Q. Cyclodextrins. *Toxicol Pathol*. 2008; 36:30-42.

3 Studies Submitted

3.1 Studies Reviewed

- Dodecylphosphocholine (DPC): Single Dose Cardiovascular Function with Respiratory and Neurological Assessment in Conscious Beagle Dogs (Study No. 1012-2932)
- Pharmacokinetics of Dodecylphosphocholine in Sprague Dawley Rats Following a Single Intravenous or Single Intranasal Administration of Dodecylphosphocholine (Study No. 6901466)
- Pharmacokinetics of Dodecylphosphocholine in Male Beagle Dogs Following a Single Intravenous or Single Intranasal Administration of Dodecylphosphocholine (Study No. 6901467)
- Dodecylphosphocholine: Mammalian Erythrocyte Micronucleus Test in Rat Bone Marrow (Study No. 963848)
- Dodecylphosphocholine: An Intravenous Fertility Study in Male and Female Sprague-Dawley Rats (Study No. 1013-0531)
- Dodecylphosphocholine: An Intravenous Embryo-Fetal Development Toxicity in Sprague-Dawley Rats (Study No. 1013-0551)
- Dodecylphosphocholine: An Intravenous Embryo-Fetal Development Toxicity Study in New Zealand white Rabbits (Study No. 1013-0574)
- Dodecylphosphocholine: An Intravenous Range-Finding Embryo-Fetal Developmental Toxicity Study in Sprague-Dawley Rats (Study No. 2013-0541)
- Dodecylphosphocholine: An Intravenous Range-Finding Embryo-Fetal Developmental Toxicity Study in New Zealand White rabbits (Study No. 2013-0564)
- Dodecylphosphocholine: A 4-Day Intravenous Toxicity Study in Sprague-Dawley rats (Study No. 3012-2851)
- Dodecylphosphocholine: A 4-Day Intravenous Toxicity Study in New Zealand White rabbits (Study No. 3012-2864)
- Dodecylphosphocholine: An Intravenous Toxicity Study in New Zealand White rabbits (Study No. 8013-1654)
- Dodecylphosphocholine: An Intravenous Toxicity Study in New Zealand White rabbits (Study No. 8013-1774)
- Dodecylphosphocholine: A Pre and Post-Natal Intravenous Study in Female Sprague-Dawley Rats (Study No. 1013-0581)
- A Drug Product Impurity Qualification Study in Sprague Dawley Rats Given LY900018 Daily for 14 Days by Intranasal Instillation (Study No. 7300547)
- A Single Ocular Tolerance Study in New Zealand White Rabbits (Study No. 1013-0594)

3.2 Studies Not Reviewed

Studies previously reviewed under IND 110674 are not reviewed.

3.3 Previous Reviews Referenced

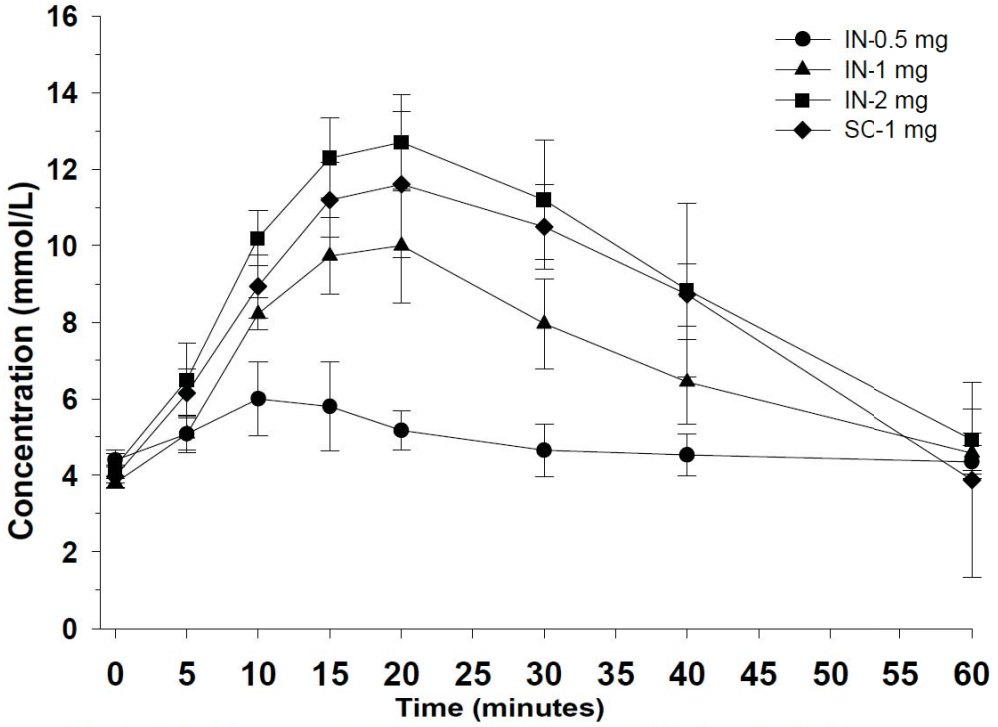
IND 110674 Pharmacology/Toxicology review dated May 01, 2012 by Dr. Parvaneh Espandiari is referenced.

4 Pharmacology

4.1 Primary Pharmacology

The physiological effect of glucagon, an endogenous peptide hormone produced by the pancreas, is to raise the concentration of blood glucose. Glucagon stimulates glycogen breakdown and release of glucose from the liver by activating hepatic glucagon receptors. Nasal Glucagon is a novel, nasally-administered glucagon powder formulation for the treatment of severe hypoglycemia. Nasal Glucagon was shown to have a glucose pharmacodynamic effect in dogs similar to that of SC administered human glucagon.

Figure 2 **Mean (± 95% Confidence Intervals) blood glucose concentrations (mmol/L) in Beagle dogs following IN administration of 0.5, 1, or 2 mg of synthetic glucagon as nasal glucagon and SC administration of 1 mg of glucagon.**



Number (n) of dogs per treatment group: IN, 0.5 mg (n=6); IN, 1 mg (n=5); IN, 2 mg (n=4); SC, 1 mg (n=5). Error bars show the 95% confidence intervals.

4.2 Secondary Pharmacology

No secondary pharmacology studies were conducted.

4.3 Safety Pharmacology

The potential effects of nasal glucagon and its excipients on cardiovascular function were evaluated as part of the 28-day repeat-dose toxicity study in dogs. Additionally,

DPC was evaluated for its potential effects on cardiovascular, respiratory, and central nervous system functioning following single intravenous (IV) or intranasal (IN) doses (Table 2). No effects on HR or ECG waveforms were observed in beagle dogs following daily IN dosing for 28 days with nasal glucagon containing up to 4 mg glucagon + 4 mg DPC + 32 mg β-CD /day or Placebo Control Powder containing 6 mg DPC + 34 mg β-CD. No neurological adverse effects were noted following single IN administration of DPC at 30 mg/dog, mild increase in respiratory parameters (respiratory rate and minute volume) was observed when compared to animals dosed with the reference item (β-CD). The single IV administration of DPC at a dose level of 5 mg/kg was associated with a non-adverse, mild, transient, and reversible increase in ABP without any effect on the ECG.

Table 2. Summary of Safety Pharmacology Studies

Organ Systems Evaluated	Species, Strain	Route	Test Article	Doses[a]	Sex #/Group	Noteworthy Findings
Cardiovascular	Dog, beagle	IV	DPC	5 mg/kg	4 males	NOEL: not defined ↓ HR (22.4% avg) 0.5–3 hr postdose; ↑ SABP (≤20.8%) 0.5–3 hr postdose; ↑ DABP (≤32.7%) 0.5–3 hr postdose; ↑ MABP (≤26.2%) 0.5–3 hr postdose
Central Nervous System	Dog, beagle	IN	DPC	30 mg	8 males	NOEL: not defined Salivation, sneezing, and clear nasal discharge 2–8 min postdose
Respiratory Function	Dog, beagle	IN	DPC	30 mg	8 males	NOEL: not defined ↑ Respiratory rate; ↑minute volume; salivation 0–8 min postdose; sneezing 0–8 min postdose; clear nasal discharge 0–8 min postdose
Cardiovascular	Dog, beagle	IN	Nasal Glucagon	0, 0, 2.0, 4.0 mg/day	3-5/sex/Group	None; NOEL 4.0 mg/day

Abbreviations: # = number; ↑ = increased; ↓ = decreased; avg = average; DABP = diastolic arterial blood pressure; DPC = dodecylphosphocholine; hr = hours; SABP = systolic arterial blood pressure; HR = heart rate; IN = intranasal; IV = intravenous; MABP = mean arterial blood pressure; min = minute; NOEL = no-observed-effect level.

[a] Single doses unless specified otherwise.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

The PK and TK of glucagon were evaluated after single-dose administration in dogs and repeat-dose administration in rats and dogs (Table 3). In addition, the PK of DPC was determined in rats and dogs.

Table 3. Summary of Nasal Glucagon PK studies

Pharmacokinetics of Glucagon in Male Dogs Following Single Subcutaneous or Intranasal Administration of Nasal Glucagon (Number of animals: 3/group)

Parameter	Geometric Mean (%CV)							
	Glucagon 0.5 mg IN		Glucagon 1 mg IN		Glucagon 2 mg IN		Glucagon 1 mg SC	
$AUC_{0\text{-}inf}$ (pg•min/mL)	15854	(145.2)	106461	(48.8)	185071	(45)	691560	(29.6)
$AUC_{0\text{-}t}$ (pg•min/mL)	6483	(119.7)	89251	(44.5)	169997	(43.4)	561342	(23.9)
CL/F (mL/min)	31538	(145.2)	9393	(48.8)	10807	(45)	1446	(29.6)
C_{max} (pg/mL)	563	(249.1)	3581	(50.4)	7050	(38.6)	18041	(36.9)

28-Day Study with a 14-Day Recovery in Sprague-Dawley Rats (Number of animals: 3/sex/group)

	Mean ± SE Toxicokinetics			
Dose (mg/day):	0.1 (Intranasal)		0.2 (Intranasal)	
Sex	M	F	M	F
Toxicokinetics (Day 1)				
C_{max} (pg/mL)	710 ± 425	192 ± 192	3956 ± 2499	15966 ± 12854
$AUC_{0\text{-}90min}$ (pg•min/mL)	9003 ± 5379	7187 ± 4720	82340 ± 31745	263447 ± 144044
T_{max} (min)	10	60	10	10
Toxicokinetics (Day 28)				
C_{max} (pg/mL)	970 ± 767	1742 ± 1732	3452 ± 2983	2346 ± 1230
$AUC_{0\text{-}90min}$ (pg•min/mL)	23328 ± 7901	62743 ± 42207	59472 ± 38190	60675 ± 11014
T_{max} (min)	10	60	20	10

28-Day Study with a 14-Day Recovery in Beagle Dogs (Number of animals: 3/sex/group)

	Mean ± SE Toxicokinetics			
Dose (mg/day):	2.0 (Intranasal)		4.0 (Intranasal)	
Sex	M	F	M	F
Toxicokinetics (Day 1)				
C_{max} (pg/mL)	7161 ± 1450	7236 ± 828	8693 ± 2346	7037 ± 2481
$AUC_{0\text{-}90min}$ (pg•min/mL)	223543 ± 22495	272208 ± 51413	226674 ± 38132	178888 ± 61957
T_{max} (min)	16.7 ± 3.33	10.0 ± 0.00	11.7 ± 4.41	10.0 ± 0.00
Toxicokinetics (Day 28)				
C_{max} (pg/mL)	9545 ± 2045	8252 ± 1630	17534 ± 5381	10036 ± 2368
$AUC_{0\text{-}90min}$ (pg•min/mL)	299633 ± 90168	273256 ± 43152	510712 ± 162997	287139 ± 78119
T_{max} (min)	8.33 ± 1.67	10.0 ± 0.00	11.7 ± 4.41	11.7 ± 4.41

No accumulation of glucagon was observed following once daily IN dosing for 28 days in rats or dogs. There were no apparent sex differences in glucagon exposure between male and female animals. Systemic glucagon exposure in dogs increased with increase in dose at a less-than-dose-proportional manner from 2 mg to 4 mg.

Table 4. Summary of DPC PK studies

Pharmacokinetic Parameters of DPC in Male Sprague Dawley Rats Following a Single Administration of DPC in Placebo Nasal Powder (3 animals/group/time point)

Route	Intravenous		Intranasal	
Parameter	**1 mg/kg**	**3 mg/kg**	**0.22 mg/kg**	**0.43 mg/kg**
C_{max} (ng/mL)	2740	7830	76.3	178
T_{max} (hr)	0.25	0.25	1	0.5
$AUC_{0\text{-}inf}$ (ng•hr/mL)	5840	28900	662	1080
CL (mL/hr/kg)	171	104	NA	NA
CL/F (mL/hr/kg)	NA	NA	332	398
$T_{1/2}$ (hr)	8.2	11.9	4.4	3.6

Pharmacokinetic Parameters (± SD) of DPC in Male Beagle Dogs Following a Single Administration of DPC in Placebo Nasal Powder (3 animals/group/time point)

Route	Intravenous		Intranasal	
Parameter	**1 mg/kg**	**3 mg/kg**	**0.32 mg/kg**	**0.65 mg/kg**
C_{max} (ng/mL)	5340 ± 349	16500 ± 954	372 ± 149	753 ± 262
T_{max} (hr)	0.25	0.25	4.8 ± 1.6	6.0 ± 0.0
$AUC_{0\text{-}inf}$ (ng•hr/mL)	23800 ± 2420	79300 ± 5220	5450 ± 2180	13300 ± 5930
CL (mL/hr/kg)	42.3 ± 4.42	38.0 ± 2.53	NA	NA
CL/F (mL/hr/kg)	NA	NA	75.8 ± 54.2	61.4 ± 38.3
$T_{1/2}$ (hr)	17.4 ± 1.43	26.9 ± 4.56	16.6 ± 5.92	21.5 ± 3.24

Plasma concentrations of DPC were detected following IV and IN administration in rats and dogs. The bioavailability for IN administration of DPC was determined to be approximately 38% in rats and 59% in dogs. The half-life of elimination following intranasal administration of DPC was approximately 4 hours and 19 hours in the rat and dog, respectively.

6 General Toxicology

Nasal Glucagon is a single-use device that delivers synthetic glucagon intranasally. The glucagon is absorbed in the nasal passages and is not intended for inhalation into the lungs. The pharmacological and toxicological properties of glucagon have been well characterized for the marketed injectable glucagon products. Therefore, the nasal glucagon toxicology program was limited to assessment of the local effects of this glucagon drug product and qualification of the two excipients in the drug formulation, DPC and β-CD.

6.1 Single-Dose Toxicity

Single-dose nasal glucagon toxicity studies were not conducted.

6.2 Repeat-Dose Toxicity

Two repeat-dose (28-day) pivotal toxicity studies in rats and dogs were submitted and reviewed by Dr. Espandiari under IND 110674. The key study findings are summarized as follows:

Study Title: AMG504-1: A 28-Day Intranasal Toxicity Study Followed by a 14-Day Recovery Period in Sprague-Dawley Rats (Study No. AMG015G)

- In rats administered 0.2 mg glucagon/day, unilateral or bilateral erosion/ulceration (mild or moderate) was observed in the dorsal turbinates of the nasal cavity (especially the olfactory epithelium of the lamina propria) in 2/10 males and 3/10 females (Table 5). These treatment-related lesions were not noted after a 14-day recovery period.
- All other histopathology findings in other tissues, including those in vehicle control (DPC + β-CD) and low dose groups were of no toxicological significance compared to a saline control.
- There were no adverse effects of nasal glucagon, DPC, or β-CD on survival, clinical signs, body weights, food consumption, ophthalmoscopy, hematology, clinical chemistry, urinalysis, or clinical pathology.
- The NOAEL was 0.1 mg glucagon/day, based on histopathology findings in the nasal cavity of the treated animals after 28-days of repeated once-daily dosing.

Table 5. Incidence of Microscopic Findings in the Nasal Cavity of Rats Exposured to AMG504-1

Observations: Neo-Plastic and Non Neo-Plastic	MALES				FEMALES			
Removal Reason: Terminal	Group 1 Placebo	Group 2 Control	Group 3 Low	Group 4 High	Group 1 Placebo	Group 2 Control	Group 3 Low	Group 4 High
Number of Animals on Study :	9	10	10	10	10	10	10	10
Number of Animals Completed:	(9)	(10)	(10)	(10)	(10)	(10)	(10)	(10)
NASAL CAVITY #1;								
Examined	(9)	(10)	(10)	(10)	(10)	(10)	(10)	(10)
Within Normal Limits	9	10	10	10	10	10	10	10
NASAL CAVITY #2;								
Examined	(9)	(10)	(10)	(10)	(10)	(10)	(10)	(10)
Within Normal Limits	9	10	10	8	10	10	10	7
Erosion/ulceration, olfactory epithelium	(0)	(0)	(0)	(2)	(0)	(0)	(0)	(3)
mild	0	0	0	1	0	0	0	1
moderate	0	0	0	1	0	0	0	2
Inflammation, lamina propia, olfactory epithelium	(0)	(0)	(0)	(2)	(0)	(0)	(0)	(2)
minimal	0	0	0	1	0	0	0	0
mild	0	0	0	1	0	0	0	2
NASAL CAVITY #3;								
Examined	(9)	(10)	(10)	(10)	(10)	(10)	(10)	(10)
Within Normal Limits	9	10	10	10	10	10	10	10
NASAL CAVITY #4;								
Examined	(9)	(10)	(10)	(10)	(10)	(10)	(10)	(10)
Within Normal Limits	9	10	10	10	10	10	10	10

GROUP 1: Placebo Control GROUP 2: Saline GROUP 3 and 4: AMG504-1

Table 6. Safety Margin for Intranasal Administration of Nasal Glucagon in Rats.

	Dose (mg/day)	Safety Margin based on a Single Dose
Glucagon	0.1	44.7X[a]
DPC	0.13	0.56X[a]
B-CD	1.7	1.1X[b]

[a] Calculation based on AUC data determined in Study 18R-MC-IGBA and Study 6901466.
[b] Calculation based on nasal surface area.

Study Title: AMG504-1: A 28-Day Intranasal Toxicity Study Followed by a 14-Day Recovery Period in Dogs (Study No. AMG014G)

- In dogs dosed at 2.0 or 4.0 mg glucagon/day, mild to moderate atrophy and degeneration of the olfactory epithelium were observed in the nasal cavity of all treated animals (Table 7). These treatment-related lesions were reversible after a 14-day recovery period.
- The primary nasal irritant in nasal glucagon drug product is the synthetic glucagon.
- There were no adverse effects of nasal glucagon, DPC, or β-CD on survival, clinical signs, body weights, food consumption, ophthalmoscopy, hematology, clinical chemistry, urinalysis, or clinical pathology.
- The NOAEL was not established based on the histopathology findings in the nasal cavity of the treated animals.

Table 7. Incidence of Microscopic Findings in the Nasal Cavity of Dogs Exposured to AMG504-1

Observations: Neo-Plastic and Non Neo-Plastic	MALES				FEMALES			
Removal Reason: Terminal	Group 1 Placebo	Group 2 Saline	Group 3 Low	Group 4 High	Group 1 Placebo	Group 2 Saline	Group 3 Low	Group 4 High
Number of Animals on Study :	3	3	3	3	3	3	3	3
Number of Animals Completed:	(3)	(3)	(3)	(3)	(3)	(3)	(3)	(3)
NASAL CAVITY #1;								
Examined	(3)	(3)	(3)	(3)	(3)	(3)	(3)	(3)
Within Normal Limits	3	3	3	3	3	3	3	3
NASAL CAVITY #2;								
Examined	(3)	(3)	(3)	(3)	(3)	(3)	(3)	(3)
Within Normal Limits	3	3	3	3	3	3	3	3
NASAL CAVITY #3;								
Examined	(3)	(3)	(3)	(3)	(3)	(3)	(3)	(3)
Within Normal Limits	3	3	3	2	3	3	3	3
Atrophy/degeneration, epithelium	(0)	(0)	(0)	(1)	(0)	(0)	(0)	(0)
minimal	0	0	0	1	0	0	0	0
NASAL CAVITY #4;								
Examined	(3)	(3)	(3)	(3)	(3)	(3)	(3)	(3)
Within Normal Limits	2	3	0	0	2	3	0	0
Atrophy/degeneration, epithelium	(1)	(0)	(3)	(3)	(1)	(0)	(3)	(3)
minimal	1	0	2	3	1	0	3	1
mild	0	0	1	0	0	0	0	0
moderate	0	0	0	0	0	0	0	2

GROUP 1: Placebo Control Powder GROUP 2: Saline Control GROUP 3 and 4: AMG504-1

Table 8. Safety Margin for Intranasal Administration of Nasal Glucagon in Dogs.

	Dose (mg/day)	Safety Margin based on a Single Dose
Glucagon	-	-
DPC	6.5	6.86X[a]
B-CD	34	0.93X[b]

[a] Calculation based on AUC data determined in Study 18R-MC-IGBA and Study 6901467.
[b] Calculation based on nasal surface area.

7 Genetic Toxicology

Nasal Glucagon was not tested for genotoxicity. β-CD was negative for genotoxicity and mutagenicity in standard genotoxicity tests (Stella et al, 2008)[2]. DPC did not show any

[2] Stella VJ, He Q. Cyclodextrins. *Toxicol Pathol*. 2008; 36:30-42.

evidence of genotoxic activity in two in vitro assays (reverse mutation assay, Ames; mammalian chromosome aberration) reviewed under IND 110674.

7.1 *In Vivo* Clastogenicity Assay in Rodent (Micronucleus Assay)

Study title: Dodecylphosphocholine Mammalian Erythrocyte Micronucleus Test in Rat Bone Marrow

Study no:	Test Facility Study No. 963848; Sponsor Reference No. AMG 019G
Study report location:	SDN 1, 06/28/2018
Conducting laboratory and location:	(b) (4)
Date of study initiation:	12/02/2013
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Dodecylphosphocholine (DPC), batch (lot) No.: 120CP-50, and 90.1%

Key Study Findings

- No increases in the number of micronucleated immature erythrocytes (MIE) in animals treated with DPC.
- No increases in the incidence of micronucleated mature erythrocytes (MME) in animals treated with DPC.
- No decreases in the proportion of immature erythrocytes in animals treated with DPC.

Methods

Doses in definitive study:	0, 10, 20, 40 mg/kg
Frequency of dosing:	Once
Route of administration:	Intravenous injection
Dose volume:	10 mL/kg
Formulation/Vehicle:	0.9% Sodium Chloride
Species/Strain:	Sprague-Dawley Hsd:SD
Number/Sex/Group:	5/Sex/Group
Satellite groups:	None
Basis of dose selection:	Estimated MTD, i.e. 40 mg/kg
Negative control:	0.9% Sodium Chloride
Positive control:	Cyclophosphamide (monohydrate) (CP) in ultra-pure water, 20 mg/kg

Study Validity

The data for concurrent vehicle control were within the ranges determined from laboratory historical data. The positive control induced clear, unequivocal increases in micronuclei. Therefore, the study was valid.

Results

DPC did not show evidence of induction of chromosome damage in rat immature erythrocytes, when tested with doses up to 40 mg/kg administered intravenously in both sexes in this in vivo test.

8 Carcinogenicity

Carcinogenicity studies were not warranted because Nasal Glucagon is a single-use product.

9 Reproductive and Developmental Toxicology

9.1 Fertility and Early Embryonic Development

Study title: Dodecylphosphocholine: An Intravenous Fertility Study in Male and Female Sprague-Dawley Rats

Study no.:	Test Facility Study No. 1013-0531; Sponsor Reference No. AMG 022G
Study report location:	SDN 1, 06/28/2018
Conducting laboratory and location:	(b) (4)
Date of study initiation:	September 3, 2013
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Dodecylphosphocholine, 120CP-49 and 98.2%

Key Study Findings

- There were no DPC-related adverse effects on male or female reproduction and early embryonic development.
- The NOAEL for parental toxicity was 1 mg/kg/day, based on reduction of food consumption and body weight.
- The NOAEL for reproductive performance of both sexes and early embryonic development was ≥3 mg/kg/day, based on no adverse effects observed in the study.

Methods

Doses:	0, 1, 3 mg/kg/day
Frequency of dosing:	Once daily
Dose volume:	0.5 mL/kg
Route of administration:	Intravenous injection
Formulation/Vehicle:	0.9% Sodium Chloride
Species/Strain:	Sprague-Dawley rats
Number/Sex/Group:	24/sex/group
Satellite groups:	None
Study design:	The test and reference items were administered daily to males for approximately 28 days prior to placement for mating, during mating and until necropsy and to females for 14 days prior to mating, during mating and up to GD 7. Estrous cycle was determined by daily vaginal smear for 7 days before start of dosing, during mating and until positive identification of mating.
Deviation from study protocol:	No significant deviations

Observations and Results

Mortality

There were no mortalities in the study.

Clinical Signs

DPC-related clinical signs were observed at the dosing sites (tail), including dark area/discoloration, scab, wound, dry and/or swelling in male and/or female rats treated with DPC at ≥1 mg/kg/day.

Body Weight

Body weights for males dosed at 3 mg/kg/day statistically significantly ($p \leq 0.05$ or $p \leq 0.01$) decreased (↓≤10.2%) on Days 11 to 39 of treatment (except for Days 29 to 32).

Feed Consumption

DPC-related reduction of food consumption (↓≤14.4%) was noted from Day 8 to 56 for males dosed at 3 mg/kg/day compared to controls.

Toxicokinetics

Not evaluated.

Dosing Solution Analysis

The accuracy of the label claimed concentration of the DPC dosing formulations were between 86.4% and 97.7%.

Necropsy

There were no DPC-related effects on the testes, epididymides, or prostate. The estrous cycles, mating, fertility index, numbers of corpora lutea, implantations, dead/live fetuses, sex ratio, or mean fetal and placental weights were not affected by treatment with DPC.

9.2 Embryonic Fetal Development

Study title: Dodecylphosphocholine: An Intravenous Embryo-Fetal Developmental Toxicity Study in Sprague-Dawley Rats

Study no.:	Test Facility Study No. 1013-0551; Sponsor Reference No. AMG 024G
Study report location:	SDN 1, 06/28/2018
Conducting laboratory and location:	(b) (4)
Date of study initiation:	July 3, 2013
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Dodecylphosphocholine, G120CP-11 and 99.7%

Key Study Findings

There were no DPC-related adverse maternal effects on body weights and food consumption and no evidence of embryo lethality, fetotoxicity or teratogenicity at doses up to 2.5 mg/kg/day.

Methods

Doses:	0, 1, 2.5 mg/kg/day
Frequency of dosing:	Once daily
Dose volume:	0.5 mL/kg
Route of administration:	Intravenous injection
Formulation/Vehicle:	0.9% Sodium Chloride
Species/Strain:	Female Sprague-Dawley rats
Number/Sex/Group:	24/group
Satellite groups:	None
Study design:	Pregnant Sprague-Dawley rats were administered DPC or vehicle from GD 6 to 17. The animals were euthanized on Day 21. The reproductive tracts were then dissected out, the

	ovaries removed and corpora lutea counted.
Deviation from study protocol:	No significant deviations

Observations and Results

Mortality

No animals died before the scheduled termination.

Clinical Signs

Animals were observed twice daily for clinical signs. Discoloration of skin surrounding dose sites were noted on a majority (20 of 22) of animals dosed at 1 mg/kg/day.

Body Weight

There were no DPC-related effects on body weights or body weight changes.

Feed Consumption

There were no DPC-related effects on food consumption.

Toxicokinetics

Not evaluated.

Dosing Solution Analysis

The accuracy of the label claimed concentration of the DPC dosing formulations were between 85.8% and 92.5%.

Necropsy

There were no DPC-related macroscopic findings.

Cesarean Section Data (Implantation Sites, Pre- and Post-Implantation Loss, etc.)

There were no DPC-related effects on gravid uterus weights, the number of corpora lutea, implantation sites, live or dead fetuses, sex ratio, resorptions or pre- and post-implantation losses.

Offspring (Malformations, Variations, etc.)

There was no significant effect on male, female, and total (sex combined) fetal weights at doses up to 2.5 mg/kg/day. There were no DPC-related major malformations, minor external, visceral or skeletal anomalies, or skeletal variants for any of the fetuses.

Study title: Dodecylphosphocholine: An Intravenous Embryo-Fetal Developmental Toxicity Study in New Zealand White Rabbits

Study no.:	Test Facility Study No. 1013-0574; Sponsor Reference No. AMG 026G
Study report location:	SDN 1, 06/28/2018
Conducting laboratory and location:	(b) (4)
Date of study initiation:	October 28, 2013
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Dodecylphosphocholine, 120CP-49 and 98.2%

Key Study Findings

There were no DPC-related adverse maternal effects on body weights and food consumption and no evidence of embryo lethality, fetotoxicity or teratogenicity at doses up to 1 mg/kg/day.

Methods

Doses:	0, 0.5, 1 mg/kg/day
Frequency of dosing:	Once daily
Dose volume:	1 mL/kg
Route of administration:	Intravenous injection
Formulation/Vehicle:	0.9% Sodium Chloride
Species/Strain:	Female New Zealand White rabbits (*Orytolagus cuniculus)*
Number/Sex/Group:	22/group
Satellite groups:	None
Study design:	Pregnant New Zealand rabbits were administered DPC or vehicle from GD 7 to 19. The animals were euthanized on GD 29. The reproductive tracts were then dissected out, the ovaries removed and corpora lutea counted.
Deviation from study protocol:	No significant deviations

Observations and Results

Mortality

No animals died before the scheduled termination.

Clinical Signs

Animals were observed twice daily for clinical signs. Discoloration of skin surrounding dose sites were noted on a majority (20 of 22) of animals dosed at 1 mg/kg/day.

Body Weight

There were no DPC-related effects on body weights or body weight changes.

Feed Consumption

There were no DPC-related effects on food consumption.

Toxicokinetics

Not evaluated.

Dosing Solution Analysis

The accuracy of the label claimed concentration of the DPC dosing formulations were between 95.4% and 99.3%.

Necropsy

There were no DPC-related macroscopic findings.

Cesarean Section Data (Implantation Sites, Pre- and Post-Implantation Loss, etc.)

There were no DPC-related effects on gravid uterus weights, the number of corpora lutea, implantation sites, live or dead fetuses, sex ratio, resorptions or pre- and post-implantation losses.

Offspring (Malformations, Variations, etc.)

There was no significant effect on male, female, and total (sex combined) fetal weights at any dose levels up to 1 mg/kg/day. There were no DPC-related major malformations, minor external, visceral or skeletal anomalies, or skeletal variants for any of the fetuses.

9.3 Prenatal and Postnatal Development

Study title: Dodecylphosphocholine: A Pre and Post-Natal Intravenous Study in Female Sprague-Dawley Rats

Study no.:	Test Facility Study No. 1013-0581; Sponsor Reference No. AMG 027G
Study report location:	SDN 1 06/28/2018
Conducting laboratory and location:	(b) (4)
Date of study initiation:	October 2, 2013
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Dodecylphosphocholine, 120CP-49 and 98.2%

Key Study Findings

- Administration of DPC up to 1 mg/kg/day in rats had no adverse effects on reproductive and development performance.
- The NOAEL for maternal toxicity, F_1 growth, behavior, and reproduction was 1 mg/kg/day.

Methods

Doses:	0, 0.5, 1 mg/kg/day
Frequency of dosing:	Once daily
Dose volume:	1 mL/kg
Route of administration:	Intravenous injection
Formulation/Vehicle:	0.9% Sodium Chloride
Species/Strain:	Female Sprague-Dawley rats
Number/Sex/Group:	22/group
Satellite groups:	None
Study design:	F_0 females were administered dodecylphosphocholine from gestation (GD 6) to *post-partum* day 20 (PPD20).)
Deviation from study protocol:	No significant deviations

Observations and Results

F_0 Dams

Survival:	There were no unscheduled mortalities during the study. One female from Group 2 (0.5 mg/kg/day) and one female from Group 3 (1 mg/kg/day) were euthanized on PPD 2 due to cannibalization of the litter and on PPD 3 due to inadequate milk production, respectively. There were no macroscopic findings at necropsy.
Clinical signs:	No DPC-related clinical signs on F_0 females in the study.
Body weight:	There was a statistically significant ($p \leq 0.05$ or $p \leq 0.01$) increase in body weight change during gestation period (Days 15 to 18) for F_0 females dosed at 0.5 and 1 mg/kg/day (↑25.7% and ↑17.1%, respectively) from Days 15 to 18 in comparison to the controls.
Feed consumption:	There were no DPC-related effects on food consumption during the gestation or lactation periods.
Uterine content:	There were no DPC-related effects on pregnancy rate, gestation index, gestation length, the live birth index and litter size.
Necropsy observation:	No DPC-related findings.
Toxicokinetics:	Not evaluated.
Dosing Solution Analysis	Concentrations ranged from 93.2 to 106.7% of the nominal concentrations.
Other:	None.

F_1 Generation

Survival:	No DPC-related effects.
Clinical signs:	No DPC-related effects.
Body weight:	There were no DPC-related effects on body weight and body weight changes on F_1 pups pre or post culling and surviving females during the gestation or lactation periods.
Feed consumption:	No DPC-related effects.
Physical development:	No DPC-related effects.
Neurological assessment:	No DPC-related effects.
Reproduction:	No DPC-related effects.
Other:	None.

F_2 Generation

Survival:	No DPC-related effects.
Body weight:	No DPC-related effects.
External evaluation:	No DPC-related effects.
Male/Female ratio:	There were no DPC-related effects on pup viability. There was a statistically significant ($p \leq 0.01$) decrease for sex ratio for the group 2 pups (↓21.3%) in comparison to pups from control group dams.
Other:	None.

10 Special Toxicology Studies

To qualify degradation impurities (including total impurities, (b) (4) found in the commercial drug product over the 18-month shelf-life, a 14-day GLP repeat-dose drug product impurity qualification study in rats was conducted.

Study title: A Drug Product Impurity Qualification Study in Sprague Dawley Rats Given LY900018 Daily for 14 Days by Intranasal Instillation

Study no.:	Test Facility Study No. 3012-2851; Sponsor Reference No. AMG 019
Study report location:	SDN 1, 06/28/2018
Conducting laboratory and location:	(b) (4)
Date of study initiation:	May 16, 2017
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	LY900018, RA8-H81439-037* and 75.3%**

* Heat-stressed in a closed container with lid at 50°C for 82 days to increase the level of impurities.
** Impurities content: glucagon-related (b) (4)

Key Study Findings

- There were no test article-related changes in body weight, food consumption or clinical pathology parameters (hematology, coagulation, clinical chemistry or urinalysis).
- No test article-related gross findings, organ weight changes, or microscopic findings were noted.

- Minimal to moderate degeneration/regeneration of the respiratory epithelium was observed in the nasal cavity of rats administered placebo control and LY900018 with a similar incidence and severity in both groups.

Methods

Doses:	0[a], 0[b], 0.1 mg/day
Frequency of dosing:	Once daily
Route of administration:	Intranasal instillation
Dose volume:	14 µL/nostril/day (2 X 14 µL/animal/day)
Formulation/Vehicle:	100 mM acetic acid
Species/Strain:	Crl:CD(SD) Sprague-Dawley rats
Number/Sex/Group:	10/sex/group
Age:	10 weeks
Weight:	Males: 281-333 g; Females: 197-235 g
Satellite groups:	None
Unique study design:	None
Deviation from study protocol:	No deviations affected the study results and conclusions.

[a] 0.9% Saline. [b] 88.9% β-CD + 11.1% DPC in 100 mM acetic acid

Mortality

There were no unscheduled mortalities over the course of the study.

Clinical Signs

There were no LY900018-related clinical signs over the course of the study.

Body Weights

There were no LY900018-related changes in body weights or body weight gains over the course of the study.

Table 9. Summar of Bod Wei hts

Group 1 - Sterile Saline Control Group 2 - Placebo Control Group 3 - LY900018 0.1 mg/day

Group / Sex		Day -10	-8	-1	7	14
1M	Mean	261.4	271.3	313.1	350.8	383.8
	SD	6.7	9.2	14.8	19.0	24.1
	N	10	10	10	10	10
2M	Mean	261.7	271.5	315.7	353.5	382.6
	SD	6.7	6.2	6.9	8.5	8.7
	N	10	10	10	10	10
3M	Mean	261.3	271.9	311.2	345.8	376.4
	SD	7.3	8.0	12.9	18.2	22.4
	N	10	10	10	10	10
1F	Mean	192.5	201.7	218.1	235.6	247.8
	SD	7.4	6.6	9.7	11.1	10.3
	N	10	10	10	10	10
2F	Mean	192.4	200.1	219.4	240.8	254.2
	SD	8.3	9.7	10.5	14.7	17.6
	N	10	10	10	10	10
3F	Mean	192.5	195.1	211.9	230.1	246.1
	SD	6.4	7.5	9.0	10.4	14.5
	N	10	10	10	10	10

Feed Consumption

There were no LY900018-related changes in food consumption over the course of the study.

Ophthalmoscopy

There were no LY900018-related ophthalmic changes over the course of the study.

Hematology

There were no LY900018-related differences in hematology or coagulation parameters compared to sterile saline and placebo control groups.

Clinical Chemistry

There were no LY900018-related differences in clinical chemistry parameters compared to sterile saline and placebo control groups.

Urinalysis

There were no LY900018-related differences in urinalysis parameters compared to sterile saline and placebo control groups.

Gross Pathology

No LY900018-related gross findings were observed.

Organ Weights

No LY900018-related organ weight changes were noted.

Histopathology

Adequate Battery
Yes

Peer Review
Yes

Histological Findings
Minimal to moderate degeneration/regeneration of the respiratory epithelium was observed in the nasal cavity of rats administered placebo control (Group 2) and LY900018 (Group 3) with a similar incidence and severity in both groups. No other microscopic findings were noted.

Table 10. Summary of Microscopic findings

Removal Reason: TERMINAL EUTHANASIA	Male			Female		
	0 mg/day Group 1	0 mg/day Group 2	0.1 mg/day Group 3	0 mg/day Group 1	0 mg/day Group 2	0.1 mg/day Group 3
Number of Animals:	10	10	10	10	10	10
BODY CAVITY, NASAL						
Examined	10	10	10	10	10	10
No Visible Lesions	9	1	0	10	0	0
Hyperplasia; cartilage	0	1	0	0	0	0
.... moderate	0	1	0	0	0	0
Degeneration/regeneration; olfactory epithelium	1	2	3	0	0	0
.... minimal	1	2	3	0	0	0
Degeneration/regeneration; respiratory epithelium	0	9	9	0	10	10
.... minimal	0	4	3	0	3	4
.... mild	0	5	5	0	6	6
.... moderate	0	0	1	0	1	0

Special Evaluation

None.

Dosing Solution Analysis

Formulations are considered acceptable if the mean results are within ±10% of the theoretical concentration and the mean relative standard deviation (RSD) is equal to or

less than 6.0%. The mean concentration of the LY900018 formulation samples (original and back-up samples) analyzed was 116% of their theoretical concentration, which was above the acceptance criteria of ±(b) (4)% of their theoretical concentration. This is deemed to have no impact on study integrity since the formulation results were only slightly higher than target and the study assessed the toxicity based on the target (lower concentration value of 3.61 mg/mL) value as more conservative. The relative standard deviation (RSD) of the LY900018 formulation samples (original and back-up samples) analyzed ranged from (b) (4)%, which were within the acceptance criteria.

11 Integrated Summary and Safety Evaluation

Nasal Glucagon is a novel, nasally-administered glucagon powder formulation for the treatment of severe hypoglycemia. The glucagon powder formulation contains 3 mg synthetic glucagon (b) (4)mg dodecylphosphocholine (DPC) (b) (4) mg beta-cyclodextrin (β-CD) (b) (4) .

The pharmacological and toxicological properties of glucagon are well known, based on the marketed injectable glucagon products (Glucagon for Injection: NDA 020928; Glucagon Hypo Kit: NDA 020918), the nonclinical program for nasal glucagon was limited to the local effects of this glucagon product and the systemic effects following nasal administration and the qualification of the two excipients (β-CD and DPC).

Glucagon

A single intranasal administration of 0.5, 1, or 2 mg nasal glucagon resulted in a glucose PD effect in dogs similar to that of SC administered human glucagon. The peak glucose response was similar after administration of nasal glucagon at 1 mg, 2 mg, or 1 mg SC comparator human glucagon. Maximal glucose response (median T_{max}) was observed at 20 minutes post dose for each treatment group, except for the 0.5 mg IN dose, where response peaked at 12.5 minutes post dose.

A single 20-mg dose of nasal glucagon drug product delivered by the intended clinical single-use nasal dosing device was disturbed in the nasal passages, the nasopharynx, stomach, esophagus, and on the tongue of dogs as indicated by a blue powder tracer dye. There was no evidence of dye in the larynx or trachea indicating nasal glucagon did not distribute to the lung.

Two repeat-dose (28-day) pivotal toxicity studies (AMG015G and AMG014G) in rats and dogs were conducted to evaluate the local tolerance of nasal glucagon. There were no test article-related adverse effects on body weight and/or food consumption, ophthalmology, electrocardiography, hematology, coagulation parameters, clinical chemistry, urinalysis, or organ weights, and no macroscopic findings at necropsy in the studies. In 2/10 males and 3/10 females intranasally administered 0.2 mg glucagon/day in the rat study, unilateral or bilateral erosion/ulceration (mild or moderate) was observed in the dorsal turbinates of the nasal cavity (especially the olfactory epithelium of the lamina propria). These treatment-related lesions were not noted after a 14-day recovery period. No other toxicologically significant histopathology findings in other

tissues were noted. The NOAL was 0.1 mg glucagon/day, based on histopathology findings in the nasal cavity of the treated animals. The safety margin at NOAEL to clinical exposure of 3 mg nasal glucagon was 60X, based on AUC. In dogs dosed at 2.0 or 4.0 mg glucagon/day, mild to moderate atrophy and degeneration of the olfactory epithelium were observed in the nasal cavity of all treated animals. These treatment-related lesions were reversible after a 14-day recovery period. The NOAEL was not established based on the histopathology findings in the nasal cavity of the treated dogs.

β-CD and DPC

β-CD + DPC as placebo controls in the two repeat-dose (28-day) pivotal toxicity studies (AMG015G and AMG014G) were administered daily by intranasal administration for 28 consecutive days in rats (1.7 mg β-CD (1.1X clinical exposure based on nasal surface area) + 0.3 mg DPC (0.56X clinical exposure based on AUC) /rat/day) or dogs (34 mg β-CD (0.93X clinical exposure based on nasal surface area) + 6 mg DPC (6.9X clinical exposure based on AUC) /day/day). No significant excipient-related toxicities were observed. Minimal irritation was noted in the placebo control group in dogs. The primary nasal irritant in nasal glucagon is synthetic glucagon.

No stand-alone toxicology studies with β-CD were submitted in this NDA. B-CD was designated generally recognized as safe (GRRAS) by the FDA (FDA, 2001)[3].The safety profile of β-CD has been reviewed by the Joint Food and Agriculture Organization/World Health Organization's (FAO/WHO) Expert Committee on Food Additives (JECFA) and by European Medicines Agency's (EMA) Committee for Human Medicinal Products (CHMP). JECFA allocated an ADI of 0-5 mg/kg for β-CD (JECFA, 1995)[4]; CHMP recommended an Oral Permitted Daily Exposure of 10 mg/kg for β-CD (EMA/CHMP, 2014)[5]. β-CD was negative for genotoxicity and mutagenicity in standard genotoxicity tests (Stella and He, 2008)[6]. Dietary administration of β-CD to rats at doses of 654, 1313, or 2655 mg/kg/day for males and 864, 1743 or 3614 mg/kg/day for females and to dogs at doses of 229, 456 or 1831 mg/kg/day for males and 224, 476 or 1967 mg/kg/day for females for 52 weeks revealed no effects on mortality, body weight gains, food consumption, ophthalmoscopic findings, hematology, organ weights and macroscopic pathology (Bellringer, et al. 1995)[7]. There was no evidence of systemic toxicity in the dogs, whereas the liver and kidney were the target organs for toxicity (single cell necrosis and minimal inflammatory response in the liver, increased incidence of minimal/trace amounts of pigment in the epithelium of the renal cortical tubules, respectively) of β-CD in rats in the high dose groups (≥1313 mg/kg/day for males; ≥1743 mg/kg/day for females).

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[3] https://www.accessdata.fda.gov/scripts/fdcc/?set=GRASNotices&id=74

[4] JECFA, 1995. Evaluation of Certain Food Additives and Comtaminants. WHO Technical Report Series No. 859.

[5] EMA/CHMP, 2014. Background review for cyclodextrins used as excipients.

[6] Stella VJ, He Q. Cyclodextrins. *Toxicol Pathol*. 2008; 36:30-42.

[7] Bellringer ME, Smith TG, Read R, Gopinath C and Olivier Ph. B-Cyclodextrin: 52-Week Toxicity Studies in the Rat and Dog. *Fd Chem. Toxic*. Vol. 33, No. 5, p367-376, 1995.

DPC was not genotoxic based on the results from the bacterial reverse mutation assay, the *in vitro* mammalian chromosome aberration assay, and the mammalian erythrocyte micronucleus assay. In embryo-fetal development studies, no adverse fetal effects were observed in rats dosed up to 2.5 mg/kg/day IV or in rabbits dosed up to 1 mg/kg/day IV. DPC did not affect male or female reproduction or early embryonic development through to the F_2 generation in a pre- and post-natal study in rats dosed up to 1 mg/kg/day IV DPC.

In summary, the nonclinical data support approval of Nasal Glucagon. There are no safety concerns for Nasal Glucagon compared with marketed injectable glucagon products. Reversible lesions in the nasal cavity after 28 days exposure with nasal glucagon in dogs or rats would not be expected to occur in humans because nasal glucagon is intended for emergency use for a single dose.

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/s/

DONGYU GUO
03/29/2019 08:17:43 AM

CALVIN L ELMORE
03/29/2019 08:28:19 AM
I concur.

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

PHARMACOLOGY/TOXICOLOGY IND REVIEW AND EVALUATION

Application number:	IND 110,674
Review number:	1
FDA SDN, **Sponsor SN (Vol #),** **Sponsor letter date,** **CDER stamp date:**	3 10 volume April 5, 2012 April 9, 2012
Product:	AMG504-1; Glucagon Intranasal Powder [redacted (b) (4)] actuation; [redacted (b) (4)]
Indication:	Emergency Treatment of Severe Hypoglycemia
Sponsor:	AMG Medical Inc.
Review Division:	DMEP
Reviewer:	Parvaneh Espandiari, Ph.D.
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1 Executive Summary

1.1 Introduction

AMG504-1 is a novel, intranasal (b) (4) powder formulation of glucagon being developed by AMG Medical INC. for treatment of severe hypoglycemia. The active ingredient is glucagon which is currently available as an injectable formulation. The Sponsor's rationale to develop AMG504-1 is as a simple portable nasal dosing product for the treatment of severe hypoglycemia in cases of emergency.

1.2 Brief Discussion of Nonclinical Findings (Internal Comments)

AMG504-1 has three ingredients: glucagon (an approved product), Betadex (beta-cyclodextran novel as administrated intranasal) and dodecylphosphocholine (DPC, a novel excipient). Nonclinical studies were conducted *in vitro* and *in vivo* to support proposed clinical studies.
Results of two *in vitro* genotoxicity studies suggested no evidence of genotoxicity activity of DPC.
Repeat-dose (28-day) pivotal toxicity studies were conducted in rats and dogs with AMG504-1; both studies evaluated respiratory and GI tissues only and represent assessments of local tolerance only. Findings of these studies suggested reversible treatment-related lesions in the nasal cavity of both rats and dogs after 28 days exposure to AMG504-1. In rats, at HD, unilateral or bilateral erosion/ulceration (mild to moderate) was observed in the dorsal turbinates of the nasal cavity (especially the olfactory epithelium of the lamina propria). NOAEL was established at LD with the safety margin to the MRHD of 89 fold (based on AUC pg.min/mL). In dogs, mild to moderate atrophy/degeneration of the olfactory epithelium was reported as following: Saline (0/6); Placebo (2/6); LD= 2 mg/day (6/6); HD= 4 mg/day (6/6). The NOAEL was not established for this study.
Repeat-dose toxicity studies were designed with over dose levels of AMG504-1 compared to the intended emergency use of AMG504-1 in humans (AUC at LD of 267261pg.min/mL in the dog study to the AUC of the MRHD of 479pg.min/mL represents greater than 557 fold). In addition, in both studies, histopathology findings in the nasal cavity were reversible (not reported at the end of the recovery period) and occurred after 28 days of IN treatment. AMG504-1 is intended for emergency use for a single dose; therefore, reversible lesions in the nasal cavity after 28 days exposure with overdose of AMG504-1 would not be expected to observe in humans.
Since the intranasal formulation of AMG504-1 contains a novel (b) (4) DPC; therefore, establishment of its systemic safety profile is needed or demonstration that systemic exposure of DPC does not occur.

1.3 Recommendations to the sponsor

Pharm/Tox recommends that clinical studies are reasonably safe to proceed for the proposed clinical studies based on prior clinical experience and 28-day repeat-dose rat toxicity study. However, because of potential effects of the AMG504-1 in the nasal cavity identified in dogs, subjects should be monitored for any clinical signs related to the nasal cavity and respiratory tract.

Characterization of the novel (b) (4) dodecylphosphocholine (DPC) is needed.
A complete gentox battery (i.e. in vivo micronucleus) is needed with DPC. Establishment of DPC's systemic safety profile is needed or demonstration that systemic exposure of DPC does

not occur is necessary. Study AMG011 which is a non-GLP study designed to address distribution of AMG504-1 in dog by the addition of powdered dye to the drug formulation is considered an inadequate assessment of systemic distribution as there is no apparent association between the admixed dye and the drug product or any of its components. Histopathological assessment of tissues which were collected but not assessed in the 28-day local tolerance studies may allow for an evaluation of systemic toxicity of the AMG504-1 and indirectly its components.

Sponsor's questions:

Regulatory

Glucagon is the treatment of choice outside of the hospital setting for severe hypoglycemia, a serious emergency in which the patient, typically an insulin-using diabetic, is unable or unwilling to take carbohydrate orally and requires third-party assistance to correct the hypoglycemia.

Glucagon has a long history of safe use, and two glucagon products for injection have been approved by the Agency – Glucagon for Injection (Eli Lilly; NDA 020928) and Glucagen Hypo Kit (Novo Nordisk; NDA 020918).

Glucagon is *currently available only as a powder that must be mixed with a diluent immediately prior to administration by injection*, a procedure which can be daunting and error-prone for the caregivers, family members, and friends who are frequently those who must deal with the emergency situation. AMG504-1 is a novel, nasally-administered glucagon powder formulation. The innovation that AMG504-1 brings is ease-of-use through needle-free intranasal administration therefore making treatment of severe hypoglycemia something that can be easily accomplished.

1. **Does the Agency agree that a Section 505(b)(2) application is appropriate for approval of this novel formulation and route of administration for glucagon?**

FDA Response:
Yes, however comparative bridging tox studies with an approved glucagon may be needed to qualify any differences in impurities/degradant profiles for the NDA.

IV. Questions Grouped by Discipline

Nonclinical

The Sponsor has conducted a nonclinical toxicology program that has focused primarily on local safety of the drug product at the site of administration while observing for evidence of potential systemic adverse effects. In addition, the Sponsor has conducted two of the three tests in the standard battery of genotoxicity studies for DPC and commits to conducting the mammalian micronucleus assay. The results of these studies indicate AMG504-1 has a clean toxicology profile and that DPC shows no evidence of genotoxicity.

In addition to these data and a mammalian erythrocyte micronucleus assay to complete the standard battery of genotoxicity studies on DPC, the Sponsor will provide additional support from publications and FDA's prior findings of safety for glucagon and beta-cyclodextrin.

1. **AMG504-1 is intended for infrequent administration associated with treatment of severe hypoglycemia. As such, patient exposure will be very small. Given the very long clinical history of glucagon in man, the extensive data already generated for glucagon and beta-cyclodextrin, the findings of toxicology studies with AMG504-1 and the absence of genotoxicity for DPC, does the FDA concur that additional toxicology studies should not be required to support a NDA?**

FDA Response: No, DMEP does not concur. Additional toxicology studies are needed to establish the safety profile of the novel (b) (4) DPC whether there is systemic exposure to DPC and its potential for in vivo genotoxicity during the clinical development. Comparative bridging tox studies with an approved glucagon may be needed to qualify any differences in impurities/degradant profiles for the NDA

2 Drug Information

2.1 Drug

CAS Registry Number
16941-32-5
Generic Name
AMG504-1
Proposed Trade Name
Glucagon (b) (4) nasal powder
Chemical Name
Glucagon
Molecular Formula/Molecular Weight
C153H225N43049S/3482.8 g/mol
Structure or Biochemical Description
H-His-Ser-Gln-Gly-Thr-Phe-Thr-Ser-Asp-Tyr-Ser-Lys-Tyr-Leu-Asp-Ser-Arg-Arg-Ala-Gln-Asp-Phe-Val-Gln-Trp-Leu-Met-Asn-Thr-OH
Pharmacologic Class
Glucagon
Planned Clinical Route of Administration
Intranasal (IN)

2.2 Relevant INDs, NDAs, and DMFs

NDA 020928; NDA 020918

2.3 Drug Formulation

See Table below from the Sponsor:

(1) Composition, i.e., list of all components of the dosage form, and their amounts on a per unit basis (including overages, if any):

Component and Quality Standard (and Grade, if applicable)	Function	Strength (label claim)					
		0.5 mg		1 mg		2 mg	
		Quantity per unit	%	Quantity per unit	%	Quantity per unit	%
Glucagon	Active	(b) (4)					
Dodecylphosphocholine							
Betadex USP/EP							
Total							

Reviewer: The dog repeat-dose toxicity study (GLP) was conducted with the proposed clinical formulation of AMG504-1 and the proposed intended nasal dosing device.

AMG504-1 contains (b) (4) mg of glucagon (USP monograph for injection) in a single-use IN powder delivery device (b) (4). The components of the device are USP grade for drug packaging materials and contain: the active ingredient of glucagon (b) (4) the inactive ingredient of Betadex (beta-cyclodextrin) as (b) (4); and the inactive ingredient of dodecylphosphocholine (DPC) as (b) (4).

2.4 Comments on Novel Excipients

- Betadex has been used as a pharmaceutical excipient and food additive; however, it is a novel intranasally (IN) excipient. There is extensive safety data available for Betadex including: genotoxicity, acute and chronic toxicity studies in rats and dogs, carcinogenicity studies in mice, and a 3-generation reproductive toxicity study in rats with a teratology phase. Betadex was designated as GRAS by the Agency (2001) with an acceptable daily intake of 0-5 mg/kg/day or 300 mg/ for a 60 kg person/ day.
- The DPC is a novel excipient in AMG504-1. The Sponsor conducted several toxicity studies to evaluate the safety profiles of DPC.

2.6 Proposed Clinical Protocol

Phase of Development:

The Phase I study was conducted in healthy volunteers for a cross-over PK/PD study of 3 doses (0.5, 1 and 2mg) of AMG504-1 (IN) and was compared to one dose (1mg) of an approved glucagon product (SC). Results of this study were reported as following: no considerable levels of glucagon were detected following of the 0.5 mg IN dose; the 1 mg SC treatment showed serum glucagon levels higher than the 1 and 2 mg IN doses; and the relative bioavailability of glucagon administered via the IN route at a dose of 2 mg was ~ 15% that of the SC route.

See Tables below from the Sponsor:

Table 5.1 Listing of Clinical Studies

Type of Study	Study Identifier	Location of Study Report	Objective(s) of the Study	Study Design and Type of Control	Test Product(s); Dosage Regimen; Route of Administration	Number of Subjects	Healthy Subjects or Diagnosis of Patients	Duration of Treatment	Study Status; Type of Report
Safety & tolerability	AMG101 (CRO Study No. (b) (4)-P1-557)	Vol. 8-9 Section 5.3.3.1	Safety and efficacy; PK PD	Cross-over, open, randomized Active control	0.5 mg IN powder 1 mg IN powder 2 mg IN powder 1 mg SC solution	16	Healthy subjects	Single dose	Complete; Full report
Safety & efficacy	AMG102 (CRO Study No. (b) (4)-P1-806)	Vol. 10 Section 5.3.32	Safety; efficacy in reversing insulin-induced hypoglycemia; PK PD	Cross-over; Active control	1 mg IN powder 2 mg IN powder 1 mg SC solution	12	Type 1 Diabetes	Single dose	Ongoing; No report

Table 2.20: Summary PK Parameters of baseline-adjusted Glucagon

PK Parameter	Serum Glucagon		
	AMG 504-1 (1 mg) IN Mean (CV%)	AMG 504-1 (2 mg) IN Mean (CV%)	Glucagon Solution (1 mg) SC Mean (CV%)
N	7	15	15
AUC_{0-t} (pg·h/mL)	124.9 (132.3)	478.8 (73.8)	2554 (53.9)
CL/F (mL/h)	2131835 (NC)[b]	2532711 (29.9)[c]	484874 (69.4)[d]
C_{max} (pg/mL)	508.4 (82.9)	1445 (69.6)	3818 (52.7)
$t_{1/2}$ (h)	0.540 (NC)[b]	0.157 (34.9)[c]	0.337 (44.0)[d]
t_{max}[a] (h)	0.25 (0.17, 0.28)	0.25 (0.17, 0.67)	0.33 (0.11, 0.67)

NC = Not calculated
[a] Median (Min, Max,) [b] N=1, [c] N=5, [d] N=14

Table 2.21: Summary PK Parameters of Glucose

Dose (mg)	Route		$AUEC_{0-4}$ (mmol.h/L)	C_{max} (mmol/L)	t_{max} (h)	$AUEC_{Within}$ (mmol.h/L)	T_{Above} (h)	T_{Below} (h)	T_{Within} (h)	$Duration_{Within}$ (h)
0.5	IN	N	15	15	15	15	6	15	6	15
		Mean	20.2	5.9	0.338	20.1	0.224	4.00	0.479	3.90
		CV%	7.1	8.9	35.6	7.0	22.9	0.0	34.6	4.3
1	IN	N	14	14	14	14	14	14	14	14
		Mean	20.8	7.1	0.397	20.4	0.209	4.00	0.613	3.551
		CV%	5.1	10.0	24.3	4.4	39.1	0.0	43.2	6.5
2	IN	N	16	16	16	16	16	16	16	16
		Mean	21.2	8.4	0.468	20.1	0.166	3.87	0.924	3.23
		CV%	6.4	11.9	19.8	4.9	23.7	13.6	26.5	8.5
1	SC	N	15	15	15	15	15	15	15	15
		Mean	21.3	8.5	0.419	19.7	0.157	3.42	1.04	2.96
		CV%	12.5	16.8	31.7	5.9	28.5	29.3	46.9	21.9

$AUEC_{0-4}$: area under the effect concentration time curve from time zero (pre-dose) up to 4 hours, C_{max}: maximum concentration, t_{max}: time to maximum concentration, $AUEC_{Within}$: area under the effect concentration-time curve within the normal range, T_{Above}: time to concentrations above normal range, T_{Below}: time to concentrations below normal range (after T_{Above}), T_{Within}: Time to concentrations within normal range, $Duration_{Within}$: duration within normal range. Note: Normal range for glucose was 3.8 to 6.0 mmol/L

Route of administration:
Intranasal (IN)

Study participants:
See Table below from the Sponsor for participants in proposed clinical studies:

Table 1: Summary overview of proposed clinical studies

Phase I				
Study #	**Subjects**	**N**	**Design**	**Endpoints**
AMG 101 (completed)	Healthy volunteers	16	Single center, randomized, four-period, four-way crossover study in fasted healthy volunteer adults evaluating 0.5 mg, 1 mg and 2 mg IN dose levels vs. 1 mg SC	Safety, tolerability, PK and PD.
AMG 104	Adult volunteers with head cold &/or SAR*	12	Single center, two-period, two-way crossover study evaluating PK & PD of IN glucagon in adult volunteers with SAR with and without symptoms of induced of allergic rhinitis OR in adults with and without head cold.	Safety, tolerability, PK and PD.
Phase II				
AMG 102	T1D adults	12	Single center, randomized, three-period, three-way crossover study evaluating 1 mg and 2 mg IN dose levels vs. 1 mg SC in T1D patients with insulin-induced hypoglycemia (glucose to ~ 3.5 mmol/L, 63 mg/dL).	Efficacy in reversing hypoglycemia. Safety, tolerability, PK and PD.
Phase III				
AMG 103	T1D children 15-30 kg	24-36	Single or multiple center, randomized, study evaluating 2 IN dose levels in fasted T1D children.	Safety, tolerability, PK and PD.
AMG 105	T1D and T2D adults	75	Multiple center, randomized, parallel study to evaluate the immunogenicity of repeated doses of glucagon following IN (N=50) and SC (N=25) administration in adult patients with T1D or T2D.	Immunogenicity. Safety and tolerability.
AMG 106	T1D and T2D adults	36	Confirmatory effectiveness study with the chosen IN dose rate. Multiple center, randomized, two-period, two-way crossover study evaluating chosen IN dose level vs. 1 mg SC in T1D or insulinized T2D patients with insulin-induced hypoglycemia (glucose to ~ 3.5 mmol/L, 63 mg/dL).	Effectiveness, safety and tolerability
Additional Studies				
AMG 107	Various	TBD	Simulated use testing covering potential users of the device as described in FDA Guidance on human factors testing.	Ease of use, comprehension of draft use instructions

* SAR: seasonal allergic rhinitis

Primary Endpoints:
Safety, tolerability, PK, PD and immunogenicity

Sponsor's Maximum Recommended Human Dose:
A single dose of 2 mg of glucagon during an emergency of severe hypoglycemia

2.8 Regulatory Background

On 11/26/2010 a PIND was requested. This request was denied. The Agency suggested to present questions with the IND application.

3 Studies Submitted

Primary Pharmacodynamic:

- AMG010/012 : Evaluation of the effects of intranasally administered glucagon powder on blood glucose and glucagon levels in beagle dogs

Toxicology
Single-Dose Toxicity

- Study AMG013G: AMG504-1: A Single Dose Intra-Tracheal Insufflation Toxicity Study in Sprague-Dawley Rats
- Study AMG011: Preliminary Evaluation of the Effect of Intranasally Administered DPC Powder on Nasopharyngeal Mucosa and the Distribution of Intranasally Administered Glucagon Powder Following Dosing Using a [redacted] (b) (4) Device in Beagle Dogs

Repeat-Dose Toxicity

- Study AMG015G: AMG504-1: A 28-Day Intranasal Toxicity Study Followed by a 14-Day Recovery Period in Sprague-Dawley Rats
- Study AMG014G: AMG504-1: A 28-Day Intranasal Toxicity Study Followed by a 14-Day Recovery Period in Beagle Dogs

Genotoxicity

- Study AMG016C: Dodecylphosphocholine: Bacterial Reverse Mutation Test in *Salmonella typhimurium* and *Escherichia coli*
- Study AMG017G: Dodecylphosphocholine: Chromosome Aberration Test

3.1 Studies Reviewed

All

3.2 Studies Not Reviewed

None

3.3 Previous Reviews Referenced

None

4 Pharmacology

4.1 Primary Pharmacology

The primary effect of glucagon, an endogenous hormone produced by the pancreas, is to raise blood glucose levels by binding to glucagon receptors in the liver causing liver cells to release glucose into circulation. Its effect is opposite to that of insulin which lowers blood glucose levels. Glucagon has been used for treatment of severe hypoglycemia and its pharmacology has been well-studied in animals and humans.

2.6.5.1 Pharmacokinetic Tabulated Summary

Type of Study	Test System	Method of Administration	Testing Facility	Study Number	Location	
					Vol.	Section
28-day subchronic toxicology study*	Rat	Intranasal	(b) (4)	AMG015G (b) (4) 75263)	5	4.2.2
Pharmacokinetics	Dog	Intranasal Subcutaneous		AMG010/012 (b) (4) 65113 (b) (4) PK 2010 11 18 34 1)	5	4.2.2
28-day subchronic toxicology study in beagle dogs*	Dog	Intranasal		AMG014G (b) (4) 65125)	6	4.2.2

*GLP-compliant study

Type of Study	Parameter measured	Species/ Strain	Method of Admin.	Doses (mg/kg)	Gender and No. per Group	Noteworthy Findings	GLP Compliance	Study Number (b) (4)
Primary pharmaco-dynamics	Serum glucose	Beagle dog	Intranasal Subcutaneous	0.5, 1 2 mg IN 1 mg SC	Male 6/group	1 or 2 mg IN glucagon results in similar glucose response as a 1 mg SC dose. Although a dose-response effect is seen with increasing doses of intranasal glucagon, there is evidence of a saturable response, in that the increase in blood glucose above baseline is not proportional to the increase in blood glucagon levels	No	65113 & PK 2010 11 18 34 1 (AMG010/12)

5 Pharmacokinetics/ADME/Toxicokinetics

5.2 Toxicokinetics

Effect of Intranasally Administered Powder Glucagon Formulation on Blood Glucose and Glucagon Levels in Beagle Dogs (#AMG010/012)

The effect of different doses of the AMG504-1 (IN) and the injected glucagon on blood glucose and blood glucagon levels was detected in dogs by using the intended device.

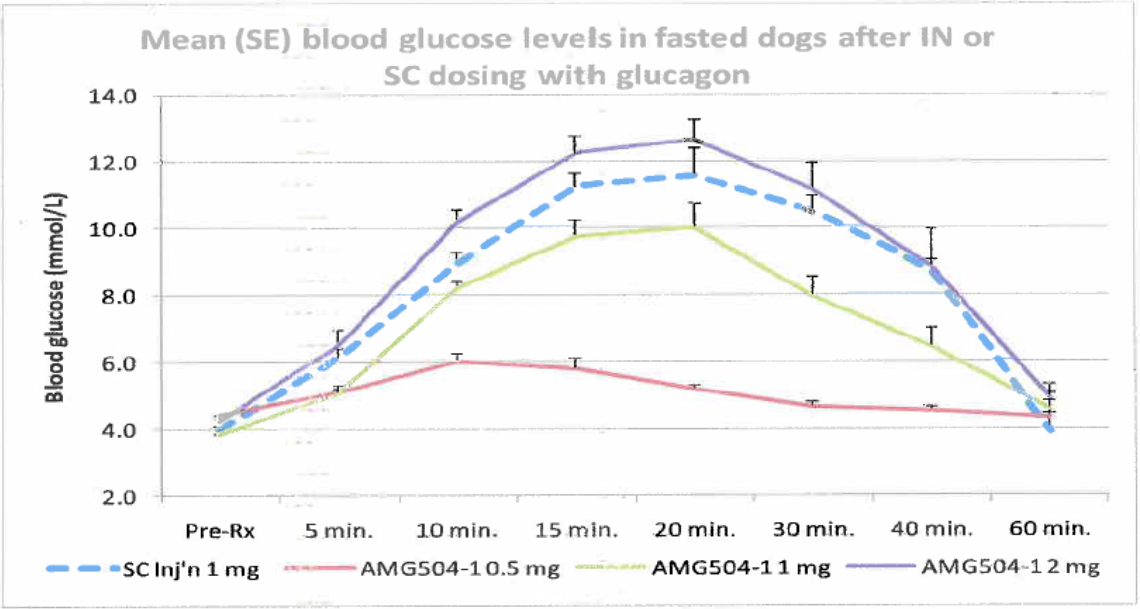
Results of this study were noted as following:

- Maximal glucose response was observed at 20 min after treatment for all doses except for the 0.5 mg IN dose which was at 12.5 min post dose
- Peak response was similar between the 1 and 2 mg IN administration and the 1 mg SC administration

- AUC values for glucagon were for: IN administration, 15854 to 185071 pg.min/mL (LD to HD respectively); and AUC value for SC dosing was 691560 pg.min/mL (bioavailability of glucagon dosed IN was ~ 15% of SC administration).

See Figure and Tables below from the Sponsor:

Figure 2.1: Mean blood glucose levels in fasted dogs after intranasal or subcutaneous dosing with glucagon



In the Figure above, the ratio of glucagon/AMG504-1 were as following: 0.5mg/10.5mg; 1mg/11mg; 2mg/12mg

Table 2.3: Glucagon pharmacokinetic parameters following intranasal and subcutaneous administration of glucagon in dogs

Parameter		Geometric Mean (%CV)							
		Glucagon 0.5 mg IN		Glucagon 1 mg IN		Glucagon 2 mg IN		Glucagon 1 mg SC	
$AUC_{0\text{-}inf}$	(pg.min/mL)	15854	(145.2)	106461	(48.8)	185071	(45)	691560	(29.6)
$AUC_{0\text{-}t}$	(pg.min/mL)	6483	(119.7)	89251	(44.5)	169997	(43.4)	561342	(23.9)
CL/F	(mL/min)	31538	(145.2)	9393	(48.8)	10807	(45)	1446	(29.6)
C_{max}	(pg/mL)	563	(249.1)	3581	(50.4)	7050	(38.6)	18041	(36.9)
$T_{1/2}$	(min)	57.2	(1564.4)	19.2	(13.8)	15.3	(14.3)	21.2	(28.0)
T_{max}	(min)	7.57	(134.1)	10	(0)	10	(0)	18.9	(12.9)
V_z	(mL)	2604374	(204)	260613	(59.4)	238036	(38.2)	44156	(15.6)

Findings of this study suggested that the maximal glucose response were almost similar after treatment for IN (1 and 2 mg) to SC (1 mg) bioavailability of glucagon dosed IN was ~ 15% of SC administration.

See Table below for the cross-species comparison of TK data from different studies:

Species	Rat		Dog				Dog		Human		
Type of Study	**28-day subchronic toxicology study**		**Exploratory single-dose dose-ranging PK/PD crossover**				**28-day subchronic toxicology study**		**Single dose PK/PD study in healthy subjects**		
Study number	AMG015G (b) (4)75263)		AMG010/012 (b) (4)65113 & (b) (4)PK 2010 11 18 34 1)				AMG014G (b) (4)65125)		AMG101		
Dose (mg/kg)	Low dose: 0.1 mg (0.4 mg/kg) glucagon daily High dose: 0.2 mg (0.8 mg/kg) glucagon daily 16µL in each nostril daily - placebo and control.		0.5 mg (0.05 mg/kg) IN 1 mg (0.1 mg/kg) IN 2 mg (0.2 mg/kg) IN 1 mg (0.1 mg/kg) SC				2 mg / day (0.2 mg.kg) 4 mg / day (0.4 mg/kg)		0.5 mg IN (0.007 mg/kg) 1 mg / IN (0.014 mg/kg) 2 mg IN (0.029 mg/kg) 1 mg SC (0.014 mg/kg)		
PK parameters[a]	**0.4 mg/kg**	**0.8 mg/kg**	**0.5 mg IN**	**1 mg IN**	**2 mg IN**	**1 mg SC**	**2 mg IN**	**4 mg IN**	**1 mg IN**	**2 mg IN**	**1 mg SC**
$AUC_{0\text{-}inf}$ (pg.min/mL)			15854 (145.2)	106461 (48.8)	185071 (45)	691560 (29.6)					
$AUC_{0\text{-}t}$ (pg*min/mL)	4757 (2466)	166298 (74716)	6483 (119.7)	89251 (44.5)	169997 (43.4)	561342 (23.9)	231274 (13.8)	202344 (42.1)	124.9 (132.3)	478.8 (73.8)	2554 (53.9)
$AUC_{0\text{-}90}$ (pg*min/mL)	6194 (2991)	166298 (74716)					248264 (26.4)	205081 (42.7)			
C_{max} (pg/mL)	341 (251)	9961 (6443)	563 (249.1)	3581 (50.4)	7050 (38.6)	18041 (36.9)	7302 (25.2)	8219 (50.8)	508.4 (82.9)	1445 (69.6)	3818 (52.7)
T_{max}			7.57	10	10	18.9	10.0	10.0	0.25	0.25	0.33
(min)	10.0	10.0	(134.1)	(0)	(0)	(12.90)	(10.0, 20.0)[b]	(10.0, 20.0)[b]	(0.17, 0.28)	(0.17, 0.67)	(0.11, 0.67)
$T_{1/2}$ (min)			57.2 (1564.4)	19.2 (13.8)	15.3 (14.3)	21.2 (28.0)			0.54 (NC)[c]	0.157 (34.9)	0.337 (44.0)
CL/F (mL/min)			31538 (145.2)	9393 (48.8)	10807 (45)	1446 (29.6)			2131835 (NC)	2532711 (29.9)	484874 (69.4)
V_z mL			2604374 (204)	260613 (59.4)	238036 (38.2)	44156 (15.6)					

a. Parameters are stated as mean ± % CV
b. Time stated as median (minimum, maximum)
c. Not calculated

6 General Toxicology

Non-GLP Studies:

Exploratory studies were conducted to find out preliminary safety and pharmacology data of IN administered of AMG504-1 or pure DPC.

See Tables below from the Sponsor for nonclinical studies that were conducted:

Table 2.14: Exploratory studies conducted with AMG504-1 and DPC

Study No.	Study Type	Species/N/ Gender	Dosage	Primary Findings
AMG011 (non-GLP)	DPC nasal toxicity	Dog/2/Male	4 or 10 mg pure DPC powder IN per dog per day X 5 days	No adverse clinical signs. No gross pathology or significant histological findings beyond minimal irritation.
	Powder dispersion after IN dosing	Dog/2/Male	20 mgs drug product plus dye given IN using intended device	Dogs dosed IN after last DPC dose (above). Necropsy 1 or 5 min after dosing. Drug product in nasal passage and nasopharynx. No powder in trachea or lungs
AMG 010/012 (non-GLP)	Dose range PK/PD cross-over	Dog/6/Male	0.5, 1, 2 mg IN; 1 mg SC	No adverse clinical signs other than transient snorting in a few dogs. IN AMG504-1 was well tolerated.

Table 2.15: Pivotal toxicology studies conducted with AMG504-1 and DPC

Study No.	Study Type	Species/N/Gender	Test Articles, Dosage, Route of Administration
AMG015G	28 day subchronic toxicology	Rat/70M/70Fe	Saline, placebo liquid, AMG504-1 ingredients in solution at 0.1 and 0.2 mg/rat/day for 28 days, intranasal
AMG014G	28 day subchronic toxicology	Dog/16M/16Fe	Saline, placebo powder, AMG504-1 at 2 and 4 mg/dog/day for 28 days, intranasal
AMG013G	Acute toxicology	Rat/16M/16Fe	Air placebo control, AMG504-1 at 0.5 mg intratracheally
AMG016C	Bacterial reverse mutation	*S. typhimurium* *E. coli* Pure DPC, up to 5000 µg/plate	
AMG017C	*In vitro* mammalian chromosome aberration	Human peripheral lymphocytes. Pure DPC, doses up 0.01M	

Single-Dose Toxicity Studies:

Non-GLP Study

Preliminary Evaluation of the Effect of Intranasally Administered DPC Powder on Nasopharyngeal Mucosa and the Distribution of Intranasally Administered Glucagon Powder Following Dosing Using a (b) (4) Device in Beagle Dogs (AMG011)

Species: Beagle Dogs

Doses and Administration: 4 and 10mg DPC (IN) and 20mg of glucagon powder

 # animals: 2

Maximal Non-Lethal Dose :

20mg

See Table below from the Sponsor for the experimental design:

	Study Days 1-5		Study Day 5	
	Formulation	Dose (mg/dog/day)	Formulation	Dose (mg powder/dog)
1001	DPC powder	4	Glucagon powder	20
1002		10		

The study was designed with the intended device and IN treatment to evaluate: the effect of high doses of pure DPC (4 and 10 mg for 5 days) and the distribution of AMG504-I (a single dose of 20mg with blue powdered dye).

Mortality:

none

Body Weight:

not reported

Clinical Signs: not remarkable

Pathology: not performed

Results:

- In the nasal cavity of both animals, minimal inflammation and mild accumulation of basophilic material was noted (there was no control animal)
- No differences were noted between the treated (left) and the untreated nostril (right)
- The distribution of powder in the respiratory and gastrointestinal tracts was similar in two animals that were euthanized 1 minute or 5 minutes post-dosing
- The blue powdered dye was present in different area as following: in the nasal passages, nasopharynx, stomach, esophagus, and on tongue; however, no evidence of dye was detected in the larynx or trachea (no powder in the lung).

Results suggested no clinical signs or gross pathology findings after the high dose exposure of DPC for 5 days as well as no distribution of AMG504-1 after IN treatment in the lung.

Non-GLP Study

Preliminary Evaluation of the Effect of Intranasally Administered DPC Powder on Nasopharyngeal Mucosa and the Distribution of Intranasally Administered Glucagon Powder Following Dosing Using a (b) (4) Device in Beagle Dogs (AMG012)	
Species: Beagle Dogs **Doses and Administration:** 0.5, 1, 2 mg **# animals:** 6 males/group **Follow-up:** none	**Maximal Non-Lethal Dose :** 2 mg
1: dogs were given glucagon (1mg, SC) and crossed-over to AMG504(0.5 mg IN) 2: AMG504-1 (1 &2mg IN)	**Mortality:** none **Body Weight:** not reported **Clinical Signs:** Transient snorting and salivation following IN dosing
Pathology: None	
Results suggested no adverse clinical signs	

GLP Study

AMG504-1: A Single Dose Intra-Tracheal Insufflation Toxicity Study in Sprague-Dawley Rats (AMG013G)

Species: SD rats

Doses and Administration: 2mg/kg glucagon (IT)

 # animals: 5/group/sex (16/sex)

Recovery: 3/sex

Followed up: 14 days

Maximal Non-Lethal Dose : 2mg/kg glucagon

Group No.	Group Designation	Powder Dose Amount (mg)	No. of Animals/Sex	
			Main	Recovery
1	Air Control	N/A	5	3
2	AMG504-1	0.5	5	3

Mortality:

None

Body Weight:

no changes

Clinical Signs:

not remarkable

Macroscopic Findings: In the lung, reversible dark area was observed as following: Air control: 5/10 (male: 4/5; female: 1/5); AMG504-1-treated 2/10 (male:1/5 and female: 1/5); not reported at the end of the recovery period.

Microscopic Findings: Histopathology evaluation was performed only on respiratory tract tissues. Results were reported as following: minimal alveolar histiocytosis and minimal alveolar hemorrhage in the lungs of both Air Control and AMG504-1-treated rats were reported as following: . These findings were also present at the end of the 14-day recovery period.

Microscopic Findings	Males N=5		Females N=5	
Lungs	Air control	AMG504-1	Air control	AMG504-1
Histiocytosis, alveolar (minimal)	5/5	5/5	5/5	5/5
Hemorrhage, alveolar (minimal)	4/5	1/5	2/5	1/5
Hyperplasia, neuroendocrine (present)	0/5	1/5	0/5	0/5
Metaplasia, osseous (present)	0/5	1/5	0/5	1/5

Results of this study suggested no treatment-related effects of a single IT treatment of AMG504-1 in the respiratory tract tissues. The Sponsor noted that a dose of 0.5 mg AMG504-1 in a 250g rat represents a 35 fold increase over the potential lung exposure of a 70 kg human following a single dose of AMG504- 1 (based on a body equivalent).

Repeat-Dose Toxicity Studies:
Pivotal Studies (GLP)

AMG504-1: A 28-Day Intranasal Toxicity Study Followed by a 14-Day Recovery Period in Sprague-Dawley Rats	
Study #	AMG015G ((b) (4) 75263)
Study report location	(b) (4)
CRO/Laboratory name	
CRO/Laboratory address	
Date of study initiation	03/22/2011
GLP compliance statement	yes
GLP issues identified	no
QA statement	yes
Drug, lot #, and % purity	AMG504-1, B110308-001, 92.8%

Key Study Findings

The NOAEL was established at LD (0.1 mg glucagon/day) based on histopathology findings in the nasal cavity (mild to moderate erosion/ulceration of the olfactory epithelium with inflammation of the lamina propria) in 2/10 males and 3/10 females at HD (0.2 mg/glucagon/day). These lesions were not reported at the end of the recovery period.

The Sponsor's NOEL was established at LD and reported that "Assuming the final human dose is 2 mg of glucagon in AMG504-1, 0.2 and 0.1 mg doses in rats (average body weight of 0.25 kg) would represent body weight-based multiples of 28X and 14X respectively on a single dose basis. Based on comparative nasal surface areas (NSA), these dose levels represent 1.8 and 0.9X multiples compared to a 2 mg dose in man. Over the course of 28 days, assuming a person would be dosed once, the multiples increase substantially to 784X and 392X based on body weight and 50X and 25X based on NSA".

28-Days Rat Study	**NOAEL Rat at LD (AUC pg.min/mL)**	**Human Single dose at MRHD (AUC pg.min/mL)**	**Multiple of MRHD**
Adverse Effect: In the nasal cavity: unilateral or bilateral erosion/ulceration of the olfactory epithelium	AUC at LD of 0.1mg or 0.4mg/kg =43036	AUC at MRHD of 2mg or 0.029 mg/kg=478	89 fold

MRHD of 2 mg has AUCpg.min/mL of 478

Methods	
Doses	0.1 and 0.2 mg glucagon /rat/day or 0.4 and 0.8 mg/kg
Frequency of dosing	Daily for 28 days
Route of administration	Intranasal (IN)
Dose volume	Low dose: 8µL/nostril/day High dose: 16µL/nostril/day
Formulation/Vehicle	Saline: 16µL/nostril/day of PBS Placebo liquid: 16µL/nostril/day of DPC (15% w/w) and Betadex (85% w/w) solubilized in 100 mM acetic acid without glucagon.
Species/Strain	Sprague-Dawley rats
Number/Sex/Group	10/sex/group (70 male and 70 female); 5/sex/group of recovery
Age	9-10 Weeks
Weight	281 to 393g for males and 210 to 286g for females at onset of treatment
Satellite groups	6/sex/group except for the saline group (3/sex/group)
Unique study design	AMG504-1 was solubilized in 100 mM acetic acid immediately prior to IN instillation; the dose was divided equally into each nostril.
Deviation from study protocol	Not remarkable
Recovery	5/sex; two groups of the Placebo and the HD; for 14 days

See Table below from the Sponsor for the study design:

Table 2.16: Study outline for subchronic toxicology rat study AMG015G

Group Number	Group Designation	Dose Level of Glucagon (mg/rat/day)	Main		Recovery		TK		Volume administered (µL)
			Male	Female	Male	Female	Male	Female	
1	Placebo Control	0	10	10	5	5	6	6	16/nostril
2	Saline	0	10	10	-	-	3	3	16/nostril
3	Low Dose	0.1	10	10	-	-	6	6	8/nostril
4	High Dose	0.2	10	10	5	5	6	6	16/nostril

Animals in the placebo liquid control group received 16 µL/nostril/day (i.e. 32 µL/rat/day) of

Placebo liquid contained DPC (15% w/w) and Betadex (85% w/w) without glucagon in 100 mM acetic acid (exposed to same levels of excipients as HD group).

Observations and Results

Mortality:

Mortality checks were performed once/day. Animals that died prematurely were examined for internal necropsy.
Two animals in the Placebo control group died prematurely; one cause of death was reported as physical trauma based on the histopathology findings.

Clinical Signs:

Clinical signs were recorded once/day.
Not remarkable

Body Weights

Body weights were recorded once prior to group assignment; one week prior to initiation of treatment; 1 day prior to dosing; once weekly during the treatment; once prior to necropsy (fasted); and at the end of the recovery study.
Results showed no treatment-related changes.

Feed Consumption

Feed consumption for each animal was recorded for all main and recovery animals during the last week of the pre-treatment period and throughout the treatment and recovery periods.
Results were not remarkable.

Ophthalmoscopy

Funduscopic (indirect ophthalmoscopy) and biomicroscopic (slit lamp) examinations were performed once for all animals during the pre-treatment period and once on Week 4 for all animals.
Results were not remarkable

Hematology and Coagulation

Samples for hematology and coagulation were performed for all animals at termination. Blood samples were collected from the abdominal aorta or by cardiac puncture at termination. See Tables below from the Sponsor for parameters that were selected to measure.

The following parameters were measured on blood samples (nominal 1 mL) collected into EDTA anticoagulant:

Red blood cell count	Mean Corpuscular Hemoglobin (calculated)
Hematocrit (calculated)	Mean Corpuscular Volume
Hemoglobin	Morphology of cells
White blood cell count	Platelet count
WBC differential (absolute)	Reticulocyte (absolute and percentage)
Mean Corpuscular Hemoglobin Concentration (calculated)	

The following parameters were measured on blood samples (nominal 1.3 mL) collected into citrate anticoagulant:

Activated partial thromboplastin time
Prothrombin time

In females, at HD, the number of monocytes decreased (ss) compared to the Saline control (not to the Placebo and not reported at the end of the recovery period).
See Tables below from the Sponsor on Day 29 and 43; the number of monocytes was compared to two Controls:

Day: 29 Relative to Start Date Sex: Female		MONO (10⁹ /L) GEN AN (AUTO)
Group 1 Placebo Control	Mean	0.11 L²
	SD	0.060
	N	10
Group 2 Control Saline	Mean	0.16
	SD	0.070
	N	10
Group 3 Low Dose	Mean	0.10
	SD	0.027
	N	9
Group 4 High Dose	Mean	0.09
	SD	0.039
	N	9

¹[I - Automatic Transformation Selected: Identity
²[L - Automatic Transformation Selected: Log]
³[R - Automatic Transformation Selected: Rank]

		MONO (10⁹ /L) GEN AN (AUTO)
Group 2 Control Saline	Mean	0.16 L²
	SD	0.070
	N	10
Group 3 Low Dose	Mean	0.10
	SD	0.027
	N	9
Group 4 High Dose	Mean	0.09 *³
	SD	0.039
	N	9

¹[I - Automatic Transformation Selected: Identity (
²[L - Automatic Transformation Selected: Log]
³[* - Statistical Test: Dunnett 2 Sided p < 0.05]
⁴ [R - Automatic Transformation Selected: Rank]

Day: 43 Relative to Start Date Sex: Female		MONO (10⁹ /L) GEN AN (AUTO)
Group 1 Placebo Control	Mean	0.14
	SD	0.045
	N	5
Group 4 High Dose	Mean	0.11
	SD	0.034
	N	5

Coagulation
Not treatment-related changes were reported.

Clinical Chemistry

Samples for clinical chemistry were performed on all main and recovery animals at termination.
See Table below from the Sponsor for parameters that were measured:

The following parameters were measured on blood samples (nominal 1.1 mL) collected into tubes containing a clotting activator:

A/G ratio (calculated)	Creatinine
Alanine aminotransferase	Globulin (calculated)
Albumin	Glucose
Alkaline phosphatase	Phosphorus (inorganic)
Aspartate aminotransferase	Potassium
Bilirubin (total)	Sodium
Calcium	Total protein
Chloride	Triglycerides
Cholesterol (total)	Urea

In the main study, in males, the serum levels of creatinine slightly increased (up to 18%) at both LD and HD. This change was present at the end of the recovery period in HD treated males (nss) as well as in HD treated females (ss). The weights of kidneys were increased slightly (nss). Histopathology did not evaluate for kidney tissues. Although this change appears in a dose-related, it was very small (up to 18%) and the Sponsor noted that it was in the range of normal serum creatinine in SD rats. See Table below for changes in the serum creatinine:

Kidney Markers

Dose, mg/kg	Creatinine (mmol/L)			
	Main Study (28 Days)		Recovery Study (43 Days)	
	Males	Females	Males	Females
Placebo (N=9)	22±3.3	28±2.5	23±4.3	25±5.7
Saline (N=10)	21±3.0	28±.5.1	---	---
LD(N=10)	25±2.9*	28±5.5	----	----
HD (N=10)	26±2.0*	29±3.0	31±11.6**	32±2.5*

*Dunnett 2 Sided <0.05 Statistical Test
** Biological variation was large between animals

Urinalysis

Samples for urinalysis were performed on all main and recovery animals at termination. Animals were deprived of food during these collections.
See Table below from the Sponsor for parameters that were measured:

The following parameters were measured on urine samples:

Bilirubin
Blood
Color and appearance
Glucose
Ketones
pH
Protein
Sediment microscopy
Specific gravity
Urobilinogen
Volume

Not treatment related changes were noted.

Gross Pathology

Animals were euthanized upon completion of the treatment and were examined for any gross changes.
Changes were reported only for one found dead animal in the Placebo control group (1006B) as following: soft dark red material surrounding the heart; moderate multifocal pulmonary intra-alveolar hemorrhage and many dark red areas in all lung lobes. There were no gross findings for other found dead animal (1518D).

Organ Weights

The selected organs were examined and their weighs were recorded.

No-treatment-related effects in AMG504-1 treated animals.
In females, the Placebo control animals compared to the Saline animals, a slight increase in organ weights relative to body weights was reported in several organs such as adrenal, brain, heart, kidney, lung/trachea, ovaries and thyroid/parathyroid.

Histopathology

Battery Considered Adequate? Only respiratory and GI organs were assessed.
Peer Review Performed? Yes
Tissues for microscopy evaluation were fixed with neutral buffered 10% formalin for fixation and preservation. The histopathology examination was performed mostly on the respiratory tissues.
See Table below from the Sponsor for tissues that were examined.

ORGANS/TISSUES	Retained (•)	Weighed (√)	Examined (€)	ORGANS/TISSUES	Retained (•)	Weighed (√)	Examined (€)
Adrenals	•	√		Duodenum	•		€
Animal identification	•			Jejunum	•		€
Aorta (thoracic)	•			Ileum	•		€
Blood	•			SC, cervical	•		
Bone marrow smears (3)	•			Spleen	•	√	
Brain	•	√		Sternum & marrow	•		
Cecum	•			Stomach	•		€
Colon	•			Testes	•d	√	
Epididymides	•d			Thymus	•	√	
Esophagus	•		€	Thyroid gland/parathyroids	•	√	
Eyes	•a			Tongue	•		
Femur & marrow	•			Trachea	•c		€
Heart	•	√		Urinary bladder	•		
Kidneys	•	√		Uterus	•	√	
Liver (2 lobes)	•	√		Vagina	•		
Lungs (2 lobes)	•bc	√	€				
LN, mandibular	•			Abnormal findings	•		€
LN, mesenteric	•						
Mammary gland (inguinal)	•						
Optic nerves	•a			**Additional Tissues presented below**			
Ovaries	•	√					
Pancreas	•			Nasopharynx	•		€
Pituitary	•	√		Nasal Cavity	•		€
Prostate	•	√					
Rectum	•						
SG, mandibular	•						
Sciatic nerve	•						
Seminal vesicles	•						
Skeletal muscle	•						
Skin & subcutis (inguinal)	•						

a	Davidson's fluid (euthanized animal only)
b	Lungs were infused with 10% neutral buffered formalin (euthanized animal only)
c	Lungs were weighed with trachea and recorded in Provantis as lungs in organ weight record
d	Bouin's fluid (euthanized animal only)
LN	Lymph node
SG	Salivary gland
SC	Spinal cord
€	Examined microscopically

See Tables below for all changes that were reported during the main and the recovery studies:

Main Study								
Findings	**Males**				**Females**			
	Placebo	**Saline**	**LD**	**HD**	**Placebo**	**Saline**	**LD**	**HD**
Nasal Cavity #2 Erosion/ulceration, olfactory epithelium	**0/9**	**0/10**	**0/10**	**2/10 1mild 1moderate**	**0/10**	**0/10**	**0/10**	**3/10 1mild 2moderate**

Recovery Study					
Findings		**Males**		**Females**	
		Placebo	**HD**	**Placebo**	**HD**
Lungs	**Hemorrhage, intra-alveolar**	**0/5**	**3/5 minimal**	**0/5**	**0/5**

In Summary, at HD, mild to moderate, unilateral or bilateral erosion/ulceration of the olfactory epithelium (with minimal to mild acute to subacute inflammation of the lamina propria) was reported at the dorsal turbinates of nasal cavity (2/10 males and 3/10 females); this change was not noted at the end of the recovery study. In the recovery animals, 3/5 in HD treated males was reported with minimal hemorrhage (intra-alveolar), which was not reported during the main study.

See Tables below from the Sponsor for the histopathological evaluation:

Observations: Neo-Plastic and Non Neo-Plastic	MALES				FEMALES			
Removal Reason: Terminal	Group 1 Placebo	Group 2 Control	Group 3 Low	Group 4 High	Group 1 Placebo	Group 2 Control	Group 3 Low	Group 4 High
Number of Animals on Study :	9	10	10	10	10	10	10	10
Number of Animals Completed:	(9)	(10)	(10)	(10)	(10)	(10)	(10)	(10)
EPIDIDYMIDES;								
Examined	(0)	(0)	(0)	(1)	(-)	(-)	(-)	(-)
Within Normal Limits	0	0	0	0	-	-	-	-
Hypoplasia, immaturity	(0)	(0)	(0)	(1)	(-)	(-)	(-)	(-)
moderate	0	0	0	1	-	-	-	-
ESOPHAGUS;								
Examined	(9)	(0)	(0)	(10)	(10)	(0)	(0)	(10)
Within Normal Limits	9	0	0	10	10	0	0	10
HEART;								
Examined	(1)	(0)	(0)	(0)	(0)	(0)	(0)	(0)
Within Normal Limits	1	0	0	0	0	0	0	0
KIDNEYS;								
Examined	(0)	(2)	(0)	(1)	(0)	(1)	(0)	(0)
Within Normal Limits	0	1	0	1	0	1	0	0
Cyst	(0)	(1)	(0)	(0)	(0)	(0)	(0)	(0)
mild	0	1	0	0	0	0	0	0
LIVER;								
Examined	(0)	(0)	(1)	(0)	(0)	(0)	(0)	(0)
Within Normal Limits	0	0	0	0	0	0	0	0
Not Examined: Not Present On The Section	0	0	0	0	0	0	1	0
Inflammation, portal	(0)	(0)	(1)	(0)	(0)	(0)	(0)	(0)
mild	0	0	1	0	0	0	0	0
Leukocytosis, sinusoidal	(0)	(0)	(1)	(0)	(0)	(0)	(0)	(0)
mild	0	0	1	0	0	0	0	0
LUNGS;								
Examined	(9)	(2)	(2)	(10)	(10)	(0)	(1)	(10)
Within Normal Limits	8	0	0	8	10	0	1	10
Hemorrhage, intra-alveolar	(1)	(2)	(2)	(2)	(0)	(0)	(0)	(0)
minimal	1	2	1	2	0	0	0	0
mild	0	0	1	0	0	0	0	0

Observations: Neo-Plastic and Non Neo-Plastic	MALES				FEMALES			
Removal Reason: Terminal	Group 1 Placebo	Group 2 Control	Group 3 Low	Group 4 High	Group 1 Placebo	Group 2 Control	Group 3 Low	Group 4 High
Number of Animals on Study :	9	10	10	10	10	10	10	10
Number of Animals Completed:	(9)	(10)	(10)	(10)	(10)	(10)	(10)	(10)
LN, BRONCHIAL;								
Examined	(0)	(1)	(0)	(0)	(0)	(1)	(0)	(0)
Within Normal Limits	0	1	0	0	0	0	0	0
Hyperplasia, lymphoid	(0)	(0)	(0)	(0)	(0)	(1)	(0)	(0)
minimal	0	0	0	0	0	1	0	0
LN, ILIAC;								
Examined	(0)	(1)	(1)	(1)	(1)	(1)	(0)	(0)
Within Normal Limits	0	0	1	1	1	0	0	0
Hyperplasia, lymphoid	(0)	(1)	(0)	(0)	(0)	(1)	(0)	(0)
minimal	0	0	0	0	0	1	0	0
mild	0	1	0	0	0	0	0	0
LN, MANDIBULAR;								
Examined	(0)	(0)	(1)	(1)	(0)	(2)	(0)	(1)
Within Normal Limits	0	0	0	0	0	0	0	0
Congestion/hemorrhage, sinusal	(0)	(0)	(1)	(1)	(0)	(2)	(0)	(1)
minimal	0	0	1	1	0	2	0	1
LN, MEDIASTINAL;								
Examined	(0)	(1)	(1)	(0)	(0)	(1)	(1)	(0)
Within Normal Limits	0	0	0	0	0	0	0	0
Hyperplasia, lymphoid	(0)	(0)	(0)	(0)	(0)	(0)	(1)	(0)
mild	0	0	0	0	0	0	1	0
Congestion/hemorrhage, sinusal	(0)	(1)	(1)	(0)	(0)	(1)	(0)	(0)
minimal	0	0	1	0	0	1	0	0
mild	0	1	0	0	0	0	0	0
NASAL CAVITY #1;								
Examined	(9)	(10)	(10)	(10)	(10)	(10)	(10)	(10)
Within Normal Limits	9	10	10	10	10	10	10	10
NASAL CAVITY #2;								
Examined	(9)	(10)	(10)	(10)	(10)	(10)	(10)	(10)
Within Normal Limits	9	10	10	8	10	10	10	7

Observations: Neo-Plastic and Non Neo-Plastic	MALES				FEMALES			
Removal Reason: Terminal	Group 1 Placebo	Group 2 Control	Group 3 Low	Group 4 High	Group 1 Placebo	Group 2 Control	Group 3 Low	Group 4 High
Number of Animals on Study :	9	10	10	10	10	10	10	10
Number of Animals Completed:	(9)	(10)	(10)	(10)	(10)	(10)	(10)	(10)
NASAL CAVITY #2; (continued)								
Erosion/ulceration, olfactory epithelium	(0)	(0)	(0)	(2)	(0)	(0)	(0)	(3)
mild	0	0	0	1	0	0	0	1
moderate	0	0	0	1	0	0	0	2
Inflammation, lamina propia, olfactory epithelium	(0)	(0)	(0)	(2)	(0)	(0)	(0)	(2)
minimal	0	0	0	1	0	0	0	0
mild	0	0	0	1	0	0	0	2
NASAL CAVITY #3;								
Examined	(9)	(10)	(10)	(10)	(10)	(10)	(10)	(10)
Within Normal Limits	9	10	10	10	10	10	10	10
NASAL CAVITY #4;								
Examined	(9)	(10)	(10)	(10)	(10)	(10)	(10)	(10)
Within Normal Limits	9	10	10	10	10	10	10	10
NASOPHARYNX;								
Examined	(9)	(10)	(10)	(10)	(10)	(10)	(10)	(10)
Within Normal Limits	9	10	10	10	10	10	10	10
PANCREAS;								
Examined	(1)	(0)	(0)	(0)	(0)	(0)	(0)	(0)
Within Normal Limits	0	0	0	0	0	0	0	0
Inflammation, subacute	(1)	(0)	(0)	(0)	(0)	(0)	(0)	(0)
mild	1	0	0	0	0	0	0	0
PEYERS PATCH;								
Examined	(0)	(2)	(0)	(0)	(0)	(0)	(0)	(0)
Within Normal Limits	0	0	0	0	0	0	0	0
Hyperplasia, lymphoid	(0)	(2)	(0)	(0)	(0)	(0)	(0)	(0)
mild	0	2	0	0	0	0	0	0
SEMINAL VESICLES;								
Examined	(1)	(0)	(0)	(0)	(-)	(-)	(-)	(-)
Within Normal Limits	0	0	0	0	-	-	-	-

Observations: Neo-Plastic and Non Neo-Plastic	MALES				FEMALES			
Removal Reason: Terminal	Group 1 Placebo	Group 2 Control	Group 3 Low	Group 4 High	Group 1 Placebo	Group 2 Control	Group 3 Low	Group 4 High
Number of Animals on Study :	9	10	10	10	10	10	10	10
Number of Animals Completed:	(9)	(10)	(10)	(10)	(10)	(10)	(10)	(10)
TESTES;								
Examined	(0)	(0)	(0)	(1)	(-)	(-)	(-)	(-)
Within Normal Limits	0	0	0	0	-	-	-	-
Hypoplasia, immaturity	(0)	(0)	(0)	(1)	(-)	(-)	(-)	(-)
moderate	0	0	0	1	-	-	-	-
THYMUS;								
Examined	(2)	(0)	(2)	(1)	(1)	(1)	(2)	(1)
Within Normal Limits	0	0	0	0	0	0	0	0
Hemorrhage	(2)	(0)	(2)	(1)	(1)	(1)	(2)	(1)
minimal	2	0	2	1	1	1	2	1
THYROID GLAND;								
Examined	(0)	(0)	(1)	(0)	(2)	(0)	(0)	(0)
Within Normal Limits	0	0	1	0	2	0	0	0
TRACHEA;								
Examined	(9)	(0)	(0)	(10)	(10)	(0)	(0)	(10)
Within Normal Limits	9	0	0	10	10	0	0	10

See Tables below from the Sponsor for the Recovery Group:

Observations: Neo-Plastic and Non Neo-Plastic	------- MALES -------		------ FEMALES ---	
Removal Reason: Recovery	Group 1 Placebo	Group 4 High	Group 1 Placebo	Group 4 High
Number of Animals on Study :	5	5	5	5
Number of Animals Completed:	(5)	(5)	(5)	(5)
ESOPHAGUS;				
Examined	(5)	(5)	(5)	(5)
Within Normal Limits	5	5	5	5
KIDNEYS;				
Examined	(0)	(2)	(1)	(0)
Within Normal Limits	0	1	0	0
Inflammation, pelvic	(0)	(0)	(1)	(0)
mild	0	0	1	0
Infarct, healed	(0)	(1)	(0)	(0)
mild	0	1	0	0
LUNGS;				
Examined	(5)	(5)	(5)	(5)
Within Normal Limits	5	2	5	5
Hemorrhage, intra-alveolar	(0)	(3)	(0)	(0)
minimal	0	3	0	0
LN, BRONCHIAL;				
Examined	(0)	(1)	(0)	(0)
Within Normal Limits	0	1	0	0
LN, MANDIBULAR;				
Examined	(0)	(0)	(1)	(0)
Within Normal Limits	0	0	0	0
Congestion/hemorrhage, sinusal	(0)	(0)	(1)	(0)
minimal	0	0	1	0
LN, PANCREATIC;				
Examined	(0)	(2)	(0)	(0)
Within Normal Limits	0	2	0	0
NASAL CAVITY #1;				
Examined	(5)	(5)	(5)	(5)
Within Normal Limits	5	5	5	5

Observations: Neo-Plastic and Non Neo-Plastic	------- MALES -------		------ FEMALES -----	
Removal Reason: Recovery	Group 1 Placebo	Group 4 High	Group 1 Placebo	Group 4 High
Number of Animals on Study :	5	5	5	5
Number of Animals Completed:	(5)	(5)	(5)	(5)
NASAL CAVITY #2;				
Examined	(5)	(5)	(5)	(5)
Within Normal Limits	5	5	5	5
NASAL CAVITY #3;				
Examined	(5)	(5)	(5)	(5)
Within Normal Limits	5	5	5	5
NASAL CAVITY #4;				
Examined	(5)	(5)	(5)	(5)
Within Normal Limits	5	5	5	5
NASOPHARYNX;				
Examined	(5)	(5)	(5)	(5)
Within Normal Limits	5	5	5	5
PEYERS PATCH;				
Examined	(1)	(0)	(0)	(0)
Within Normal Limits	0	0	0	0
Lymphomatoid change	(1)	(0)	(0)	(0)
severe	1	0	0	0
DUODENUM;				
Examined	(5)	(5)	(5)	(5)
Within Normal Limits	5	5	5	5
ILEUM;				
Examined	(5)	(5)	(5)	(5)
Within Normal Limits	5	5	5	5
JEJUNUM;				
Examined	(5)	(5)	(5)	(5)
Within Normal Limits	5	5	5	5

Observations: Neo-Plastic and Non Neo-Plastic	------- MALES -------		------ FEMALES ------	
Removal Reason: Recovery	Group 1 Placebo	Group 4 High	Group 1 Placebo	Group 4 High
Number of Animals on Study :	5	5	5	5
Number of Animals Completed:	(5)	(5)	(5)	(5)
STOMACH;				
Examined	(5)	(5)	(5)	(5)
Within Normal Limits	5	5	4	5
Congestion	(0)	(0)	(1)	(0)
minimal	0	0	1	0
THYMUS;				
Examined	(0)	(0)	(0)	(1)
Within Normal Limits	0	0	0	0
Hemorrhage	(0)	(0)	(0)	(1)
minimal	0	0	0	1
TRACHEA;				
Examined	(5)	(5)	(5)	(5)
Within Normal Limits	5	5	5	5

See Tables below from the Sponsor for two found dead animals:

Observations: Neo-Plastic and Non Neo-Plastic	-- MALES -
Removal Reason: Found Dead	Group 1 Placebo
Number of Animals on Study :	1
Number of Animals Completed:	(1)
ESOPHAGUS;	
Examined	(1)
Within Normal Limits	1
LUNGS;	
Examined	(1)
Within Normal Limits	0
Hemorrhage, intra-alveolar	(1)
moderate	1
LN, MANDIBULAR;	
Examined	(1)
Within Normal Limits	0
Congestion/hemorrhage, sinusal	(1)
mild	1
NASAL CAVITY #1;	
Examined	(1)
Within Normal Limits	1
NASAL CAVITY #2;	
Examined	(1)
Within Normal Limits	1
NASAL CAVITY #3;	
Examined	(1)
Within Normal Limits	1
NASAL CAVITY #4;	
Examined	(1)
Within Normal Limits	1
NASOPHARYNX;	
Examined	(1)
Within Normal Limits	1

Observations: Neo-Plastic and Non Neo-Plastic	-- MALES -
Removal Reason: Found Dead	Group 1 Placebo
Number of Animals on Study :	1
Number of Animals Completed:	(1)
DUODENUM;	
Examined	(1)
Within Normal Limits	1
ILEUM;	
Examined	(1)
Within Normal Limits	1
JEJUNUM;	
Examined	(1)
Within Normal Limits	1
STOMACH;	
Examined	(1)
Within Normal Limits	1
THYMUS;	
Examined	(1)
Within Normal Limits	0
Hemorrhage	(1)
mild	1
TRACHEA;	
Examined	(1)
Within Normal Limits	1

Toxicokinetics

The glucagon assay was performed with collected blood samples on Day 1 and 28 from the TK animals. Results of TK data were reported as following:

- Day 1, AUC0-t to glucagon increased with dosing (8095 vs. 172893 pg*min/mL, for the LD and HD, respectively). Mean peak glucagon was observed 10 min after dosing and increased with dose (390 vs. 9961 pg/mL, for the LD and HD, respectively)
- Day 28, AUC0-t and Cmax values were 43036 pg*min/mL and 988 pg/mL, respectively for the LD and 60073 pg*min/mL and 2109 pg/mL, respectively for the HD
- At HD, AUC0-t and Cmax to glucagon appeared to be higher on Day 1 as compared to Day 28 (indicating no accumulation)
- No statistical differences in either Cmax or AUC0-t were found between sexes

See table below from the Sponsor:

Table 4.1 Summary TK Parameters of Glucagon in Rats (Gender Combined)

Day	Group	Dose Level of Glucagon (mg/day)	Mean (± SE)				
			AUC_{0-t} (pg*min/mL)	AUC_{0-90} (pg*min/mL)	C_{max} (pg/mL)	t_{max} (min)	R_A
1	Low Dose	0.1	8095 ± 3266	8095 ± 3266	390 ± 240	10.0	NC
	High Dose	0.2	172893 ± 72774	172893 ± 72774	9961 ± 6443	10.0	NC
28	Low Dose	0.1	43036 ± 21047	43036 ± 21047	988 ± 851	60.0	5.31
	High Dose	0.2	60073 ± 20639	60073 ± 20639	2109 ± 1464	20.0	0.347

NC = Not calculated

Dosing Formulation Analysis

The Sponsor reported that the glucagon content in the test article formulation for Day 1 and for Day 28 was 5.7 and 5.8 mg/mL respectively (within at least 2% of the desired value of 5.7 mg of glucagon/mL). The absence of glucagon was also noted in both control groups on both Days 1 and 28.

AMG504-1: A 28-Day Intranasal Toxicity Study Followed by a 14-Day Recovery Period in Beagle Dogs	
Study #	AMG014G (b) (4) 65125)
Study report location	(b) (4)
CRO/Laboratory name	
CRO/Laboratory address	
Date of study initiation	03/28/2011
GLP compliance statement	yes
GLP issues identified	no
QA statement	yes
Drug, lot #, and % purity	AMG405-1, F110315-001, 92.8% (2 mg glucagon/device or 20 mg total powder). For Placebo control powder: DPC (15%) and Betadex (85%)

Key Study Findings

The NOAEL was not established based on histopathology findings on mild to moderate atrophy/degeneration of the olfactory epithelium in the nasal cavity treated animals as following: Placebo powder control (2/6); Saline control (0/6); LD (6/6) and HD (6/6). These lesions were not reported after a 14-day recovery period.

The Sponsor reported NOEL at LD (0.1 mg glucagon/day) and noted "Assuming the final human dose is 20 mg of AMG504-1 (i.e., 2 mg of glucagon), 20 and 40 mg doses in dogs (body weight average of 10 kg) would represent body weight-based multiples of 7X and 14X respectively on a daily basis. Based on comparative nasal surface areas (NSA), these dose levels represent 0.8 and 1.6X multiples compared to a 20 mg dose in man. Over the course of 28 days, assuming a person would be dosed once, the multiples increase substantially to 23X and 46X based on NSA and 196X and 392X based on body weight".

Methods	
Doses	2 and 4 mg/day
Frequency of dosing	Daily for 28 days
Route of administration	Intranasal (IN)
Dose volume	LD: 8µL/nostril/day; HD: 16µL/nostril/day
Formulation/Vehicle	Saline and Placebo Powder
Species/Strain	Beagle dogs
Number/Sex/Group	3/sex/group
Age	6-7 month
Weight	6.5 to 8.9 kg for males and 6.7 to 7.9 kg for females
Satellite groups	None
Deviation from study protocol	Not remarkable
Recovery	2/sex/group (Placebo and the HD); for 14 days

Reviewer: the intended clinical nasal dosing device and the clinical powder drug product formulation was used for the dog study.

AMG504-1 was dissolved in 100 mM acetic acid immediately prior to IN instillation and were given to animals as following for: the LD, the entire dose was administered in one nostril (single device/dog; received 20 mg of powder/day with a total of 2 mg of glucagon); the HD, one device was discharged into each nostril/day (20 mg of powder/day/nostril with a total of 4 mg of glucagon); the placebo control powder, the dose consisted of one device/nostril/dog/day with each dog (receiving 40 mg of placebo powder per day); and the saline control group, a micropipette was used to deliver 10 µL of saline/nostril/dog/day.

See Table below form the Sponsor for the study design:

Group Number	Group Designation	Targeted Dose of Glucagon (mg/day)	Toxicology Animals			
			Main		Recovery	
			Male	Female	Male	Female
1	Placebo Control Powder	0	3	3	2	2
2	Saline Control	0	3	3	-	-
3	Low Dose	2.0	3	3	-	-
4	High Dose	4.0	3	3	2	2

See Table below from the Sponsor for the average powder dose (mg/dog) that was used for each group.

Group Number	Group Designation	Targeted Powder Dose (mg/dog)	Achieved Average Powder Dose (mg/dog)	Achieved Average Glucagon Dose (mg/dog)
1	Placebo Control	40.0	33.0*	0
			37.1**	0
2	Saline Control	0	0	0
3	Low Dose	20.0	18.9*	1.9
			19.9**	2.0
4	High Dose	40.0	36.8*	3.7
			39.6**	4.0

* Calculated using all values from the entire study including discharge weights obtained before implementation of a device wiping procedure to correct the adverse effect of device-related electrostatic charge on the analytical balance.

** Calculated using values beginning April 8, 2011 (equivalent to study day 10, 11 or 12 depending on the dog) after instituting a device wiping procedure to remove electrostatic charge and thus resulting in accurate and expected discharge weights.

Observations and Results

Mortality:
Mortality checks were performed once/day during all phases of the study.
No mortality was reported

Clinical Signs:
Clinical signs were noted once/daily during the acclimation period, once/daily during the treatment and recovery study.
Most dogs had transient salivation and some sneezing after IN dosing.

Body Weights:
Body weights were recorded for all animals as following: once prior to group assignment, one week prior to initiation of treatment, at once weekly during the treatment and at the end of the recovery study. No treatment-related changes were reported.
See Tables below from the Sponsor:

GROUP 1: Placebo Control Powder
GROUP 2: Saline Control
GROUP 3 and 4: AMG504-1

65125 - BODY WEIGHTS
SUMMARY OF MEANS
PRETREATMENT AND TREATMENT PERIODS

Bodyweight (kg)

Sex: Male		Day(s) Relative to Start Date				
		-1	7	14	21	28
Group 1	Mean	7.9 [1]	7.7 [1]	7.8 [1]	8.0 [1]	8.2 [1]
Placebo	SD	0.62	0.65	0.64	0.56	0.70
Control	N	5	5	5	5	5
Group 2	Mean	7.6	7.5	7.6	7.8	7.9
Saline	SD	0.50	0.55	0.56	0.76	0.70
Control	N	3	3	3	3	3
Group 3	Mean	7.6	7.7	7.8	7.9	8.0
Low	SD	0.61	0.45	0.50	0.40	0.36
Dose	N	3	3	3	3	3
Group 4	Mean	7.7	7.5	7.6	7.7	7.6
High	SD	0.96	1.00	1.00	0.93	1.11
Dose	N	5	5	5	5	5

Statistical Test: Generalised Anova/Ancova Test Transformation: Automatic
[1][I - Automatic Transformation Selected: Identity (No Transformation)]

Sex: Female		Day(s) Relative to Start Date				
		-1	7	14	21	28
Group 1	Mean	7.3 [1]	7.1 [1]	7.1 [1]	7.2 [1]	7.2 [2]
Placebo	SD	0.37	0.38	0.50	0.53	0.73
Control	N	5	5	5	5	5
Group 2	Mean	6.9	7.0	7.0	7.3	7.5
Saline	SD	0.21	0.38	0.32	0.45	0.53
Control	N	3	3	3	3	3
Group 3	Mean	7.4	7.3	7.4	7.4	7.3
Low	SD	0.46	0.38	0.32	0.21	0.26
Dose	N	3	3	3	3	3
Group 4	Mean	7.1	7.0	7.0	7.0	7.0
High	SD	0.29	0.41	0.48	0.48	0.64
Dose	N	5	5	5	5	5

Statistical Test: Generalised Anova/Ancova Test Transformation: Automatic
[1][I - Automatic Transformation Selected: Identity (No Transformation)]
[2][R - Automatic Transformation Selected: Rank]

Sex: Male		Day(s) Relative to Start Date	
		35	42
Group 1	Mean	8.0	8.2
Placebo	SD	0.35	0.07
Control	N	2	2
Group 4	Mean	7.8	8.2
High	SD	1.06	0.99
Dose	N	2	2

Sex: Female		Day(s) Relative to Start Date	
		35	42
Group 1	Mean	7.3	7.6
Placebo	SD	0.85	0.85
Control	N	2	2
Group 4	Mean	7.0	7.1
High	SD	0.07	0.28
Dose	N	2	2

Feed Consumption

Food intake was recorded for each animal as following: daily during the last week of the pre-treatment period and daily throughout the treatment and recovery periods.
Not treatment-related changes were noted

Ophthalmoscopy

Funduscopic (indirect ophthalmoscopy) and biomicroscopic (slit lamp) examinations were conducted for all animals once during the pre-treatment period and once during Week 4 of the treatment period.
No treatment-related findings were noted

ECG

Electrocardiograms (limb leads I, II and III, and augmented leads aVR, aVL and aVF) were obtained for each dog once during the pre-treatment period and once on Week 4 of the treatment period.
No treatment-related findings were noted for heart rate, PR interval, QRS duration, QT and QTc intervals values.

Blood/Urine Sampling:
Samples for hematology, coagulation, clinical chemistry and urinalysis were performed on all animals prior to start of treatment, on Day 28 and at the end of the recovery period.

Hematology and Coagulation
See Tables below from the Sponsor for parameters that were measured:
The following parameters were measured on blood samples (nominal 1 mL) collected into EDTA anticoagulant:

- Red blood cell count
- Hematocrit (calculated)
- Hemoglobin
- White blood cell count
- WBC differential (absolute)
- Mean Corpuscular Hemoglobin Concentration (calculated)
- Mean Corpuscular Hemoglobin (calculated)
- Mean Corpuscular Volume
- Morphology of cells
- Platelet count
- Reticulocyte (absolute and percentage)
- Activated partial thromboplastin time
- Prothrombin time

No treatment-related changes were reported.

Clinical Chemistry

See Table below from the Sponsor for parameters that were measured:

The following parameters were measured on blood samples (nominal 1.1 mL) collected into tubes containing clotting activator:

A/G ratio (calculated)	Creatinine
Alanine aminotransferase	Globulin (calculated)
Albumin	Glucose
Alkaline phosphatase	Phosphorus (inorganic)
Aspartate aminotransferase	Potassium
Bilirubin (total)	Sodium
Calcium	Total protein
Chloride	Triglycerides
Cholesterol (total)	Urea

In both females and males, serum levels of urea decreased in all AMG504-1 treated animals compared to both control groups; this change was independent of doses, not associate to any other related-changes in livers (weight and gross pathology), and not reported at the end of the recovery period.

See Table below for serum levels of urea:

Main Study								
	Males (N=3)				Females (N=3)			
	Placebo	Saline	LD	HD	Placebo	Saline	LD	HD
Urea (mmol/L)	7.7± 1.62	8.0± 1.30	4.5± 1.25*	5.7± 1.88	8.3± 1.27	7.6± 0.96	5.9± 1.81*	5.3± 0.80**

* - Statistical Test: Dunnett 2 Sided p < 0.05]
** - Statistical Test: Dunnett 2 Sided p < 0.01]

See Tables below from the Sponsor for clinical chemistry:

Day: 28 Relative to Start Date

Sex: Male

		Clinical Chemistry								
		ALT (U/L) GEN AN (AUTO)	AST (U/L) GEN AN (AUTO)	ALP (U/L) GEN AN (AUTO)	T-BIL (µmol/L)	CHOL (mmol/L) GEN AN (AUTO)	TRIG (mmol/L) GEN AN (AUTO)	GLUC (mmol/L) GEN AN (AUTO)	TP (g/L) GEN AN (AUTO)	ALB (g/L) GEN AN (AUTO)
Group 1	Mean	35 [1]	58 [1]	117 [2]	-	4.80 [3]	0.28 [3]	4.7 [1]	53 [1]	32 [1]
Placebo	SD	11.0	20.5	47.0	-	0.544	0.074	0.70	2.1	2.0
Control	N	5	5	5	0	5	5	5	5	5
Group 2	Mean	36	59	129	-	4.46	0.26	4.9	54	33
Saline	SD	7.5	8.9	106.8	-	0.146	0.085	0.06	1.0	2.0
Control	N	3	3	3	0	3	3	3	3	3
Group 3	Mean	35	41	110	-	5.16	0.27	4.6	53	32
Low	SD	2.3	4.6	21.7	-	0.179	0.074	0.38	4.0	3.8
Dose	N	3	3	3	0	3	3	3	3	3
Group 4	Mean	43	46	105	1.9	5.17	0.28	5.2	55	34
High	SD	22.9	6.8	42.0	-	0.836	0.064	0.32	4.7	2.5
Dose	N	5	5	5	1	5	5	5	5	5

[1][I - Automatic Transformation Selected: Identity (No Transformation)]
[2][L - Automatic Transformation Selected: Log]
[3][R - Automatic Transformation Selected: Rank]

Sex: Male

		Clinical Chemistry								
		GLOB (g/L) GEN AN (AUTO)	A/G GEN AN (AUTO)	UREA (mmol/L) GEN AN (AUTO)	CRE (µmol/L) GEN AN (AUTO)	CA (mmol/L) GEN AN (AUTO)	PHOS (mmol/L) GEN AN (AUTO)	NA (mmol/L) GEN AN (AUTO)	K (mmol/L) GEN AN (AUTO)	CL (mmol/L) GEN AN (AUTO)
Group 1	Mean	21 R[1]	1.6 I[2]	7.7 L[3]	50 I[2]	2.70 R[1]	1.86 I[2]	154 I[2]	4.7 I[2]	116 R[1]
Placebo	SD	1.3	0.16	1.62	5.5	0.098	0.183	4.3	0.15	5.2
Control	N	5	5	5	5	5	5	5	5	5
Group 2	Mean	21	1.6	8.0	51	2.77	1.69	157	4.7	118
Saline	SD	1.7	0.23	1.30	13.7	0.040	0.108	0.6	0.21	1.5
Control	N	3	3	3	3	3	3	3	3	3
Group 3	Mean	22	1.5	4.5 *[4]	47	2.70	1.61	151	4.5	114
Low	SD	2.1	0.23	1.25	9.8	0.104	0.136	2.0	0.45	2.1
Dose	N	3	3	3	3	3	3	3	3	3
Group 4	Mean	21	1.7	5.7	48	2.73	1.71	153	4.8	115
High	SD	3.1	0.25	1.88	9.0	0.070	0.134	3.6	0.32	2.3
Dose	N	5	5	5	5	5	5	5	5	5

[1][R - Automatic Transformation Selected: Rank]
[2][I - Automatic Transformation Selected: Identity (No Transformation)]
[3][L - Automatic Transformation Selected: Log]
[4] [* - Statistical Test: Dunnett 2 Sided p < 0.05]

Sex: Female

		Clinical Chemistry								
		ALT (U/L) GEN AN (AUTO)	AST (U/L) GEN AN (AUTO)	ALP (U/L) GEN AN (AUTO)	T-BIL (µmol/L)	CHOL (mmol/L) GEN AN (AUTO)	TRIG (mmol/L) GEN AN (AUTO)	GLUC (mmol/L) GEN AN (AUTO)	TP (g/L) GEN AN (AUTO)	ALB (g/L) GEN AN (AUTO)
Group 1	Mean	35 I[1]	65 L[2]	93 I[1]	-	4.37 I[1]	0.45 L[2]	4.6 I[1]	56 R[3]	35 I[1]
Placebo	SD	6.9	40.6	13.0	-	0.471	0.282	0.79	2.9	2.1
Control	N	5	5	5	0	5	5	5	5	5
Group 2	Mean	27	86	88	-	4.54	0.37	4.6	52	33
Saline	SD	10.8	71.7	17.8	-	0.707	0.085	0.36	1.5	1.5
Control	N	3	3	3	0	3	3	3	3	3
Group 3	Mean	39	63	61	-	4.14	0.25	4.7	55	35
Low	SD	3.8	6.2	9.6	-	0.376	0.127	0.25	1.5	0.6
Dose	N	3	3	3	0	3	3	3	3	3
Group 4	Mean	32	48	83	-	4.45	0.25	5.2	56	36
High	SD	12.3	11.9	17.7	-	0.831	0.033	0.40	1.3	1.2
Dose	N	5	5	5	0	5	5	5	5	5

[1][I - Automatic Transformation Selected: Identity (No Transformation)]
[2][L - Automatic Transformation Selected: Log]
[3][R - Automatic Transformation Selected: Rank]

GROUP 1: Placebo Control Powder
GROUP 2: Saline Control
GROUP 3 and 4: AMG504-1

65125 - CLINICAL CHEMISTRY
SUMMARY OF MEANS

Day: 28 Relative to Start Date

Sex: Female

		Clinical Chemistry								
		GLOB (g/L) GEN AN (AUTO)	A/G GEN AN (AUTO)	UREA (mmol/L) GEN AN (AUTO)	CRE (µmol/L) GEN AN (AUTO)	CA (mmol/L) GEN AN (AUTO)	PHOS (mmol/L) GEN AN (AUTO)	NA (mmol/L) GEN AN (AUTO)	K (mmol/L) GEN AN (AUTO)	CL (mmol/L) GEN AN (AUTO)
Group 1	Mean	21 [1]	1.7 [1]	8.3 [1]	55 [4]	2.76 [1]	1.74 [1]	159 [5]	4.7 [1]	119 [1]
Placebo	SD	1.9	0.18	1.27	8.6	0.212	0.225	7.7	0.44	4.8
Control	N	5	5	5	5	5	5	5	5	5
Group 2	Mean	20	1.7	7.6	49	2.76	1.81	157	5.0	119
Saline	SD	1.5	0.18	0.96	4.0	0.061	0.201	2.1	0.29	3.5
Control	N	3	3	3	3	3	3	3	3	3
Group 3	Mean	21	1.7	5.9 [*2]	46	2.69	1.45	155	4.5	116
Low	SD	1.2	0.08	1.81	4.4	0.046	0.200	3.5	0.40	2.5
Dose	N	3	3	3	3	3	3	3	3	3
Group 4	Mean	20	1.8	5.3 [**3]	52	2.74	1.56	155	4.3	116
High	SD	1.6	0.19	0.80	6.6	0.059	0.056	1.0	0.32	1.6
Dose	N	5	5	5	5	5	5	5	5	5

[1][I - Automatic Transformation Selected: Identity (No Transformation)]
[2][* - Statistical Test: Dunnett 2 Sided p < 0.05]
[3][** - Statistical Test: Dunnett 2 Sided p < 0.01]
[4] [L - Automatic Transformation Selected: Log]
[5] [R - Automatic Transformation Selected: Rank]

Sex: Male

		Clinical Chemistry								
		ALT (U/L) GEN AN (AUTO)	AST (U/L) GEN AN (AUTO)	ALP (U/L) GEN AN (AUTO)	T-BIL (µmol/L)	CHOL (mmol/L) GEN AN (AUTO)	TRIG (mmol/L) GEN AN (AUTO)	GLUC (mmol/L) GEN AN (AUTO)	TP (g/L) GEN AN (AUTO)	ALB (g/L) GEN AN (AUTO)
Group 2	Mean	30 [1]	32 [1]	226 [R2]	1.9	3.97 [1]	0.35 [1]	5.6 [1]	52 [1]	33 [R2]
Saline	SD	6.2	6.0	278.6	-	0.404	0.044	0.25	1.0	0.6
Control	N	3	3	3	1	3	3	3	3	3
Group 3	Mean	30	41	244	-	4.27	0.34	4.4 [3]	50	30
Low	SD	4.0	5.5	126.5	-	0.183	0.085	0.21	2.5	1.5
Dose	N	3	3	3	0	3	3	3	3	3
Group 4	Mean	28	53	107	2.0	4.16	0.34	5.1 [*4]	52	34
High	SD	4.3	16.2	44.7	-	0.777	0.061	0.16	6.4	3.8
Dose	N	5	5	5	1	5	5	5	5	5

[1][I - Automatic Transformation Selected: Identity (No Transformation)]
[2][R - Automatic Transformation Selected: Rank]
[3][*** - Statistical Test: Dunnett 2 Sided p < 0.001]
[4] [* - Statistical Test: Dunnett 2 Sided p < 0.05]

Sex: Male

		Clinical Chemistry								
		GLOB (g/L)	A/G	UREA (mmol/L)	CRE (µmol/L)	CA (mmol/L)	PHOS (mmol/L)	NA (mmol/L)	K (mmol/L)	CL (mmol/L)
Group 1	Mean	20	1.7	7.8	54	2.75	1.90	151	4.7	113
Placebo	SD	0.7	0.06	2.55	0.7	0.021	0.028	0.7	0.21	0.0
Control	N	2	2	2	2	2	2	2	2	2
Group 4	Mean	23	1.4	5.8	55	2.69	1.91	150	4.7	113
High	SD	0.7	0.05	0.35	6.4	0.049	0.191	1.4	0.00	2.1
Dose	N	2	2	2	2	2	2	2	2	2

Day: 42 Relative to Start Date

Sex: Female

		Clinical Chemistry								
		ALT (U/L)	AST (U/L)	ALP (U/L)	T-BIL (µmol/L)	CHOL (mmol/L)	TRIG (mmol/L)	GLUC (mmol/L)	TP (g/L)	ALB (g/L)
Group 1	Mean	38	37	103	-	3.85	0.39	4.7	56	36
Placebo	SD	4.9	7.8	18.4	-	0.721	0.148	0.14	2.1	2.1
Control	N	2	2	2	0	2	2	2	2	2
Group 4	Mean	20	35	90	1.8	5.16	0.28	5.1	55	36
High	SD	7.8	2.1	15.6	-	0.587	0.028	0.21	0.7	0.7
Dose	N	2	2	2	1	2	2	2	2	2

Sex: Male

		Clinical Chemistry								
		ALT (U/L)	AST (U/L)	ALP (U/L)	T-BIL (µmol/L)	CHOL (mmol/L)	TRIG (mmol/L)	GLUC (mmol/L)	TP (g/L)	ALB (g/L)
Group 1	Mean	33	36	91	-	4.75	0.31	4.7	54	34
Placebo	SD	11.3	2.1	0.7	-	0.820	0.028	0.42	0.7	0.0
Control	N	2	2	2	0	2	2	2	2	2
Group 4	Mean	54	40	80	-	4.91	0.30	4.8	55	33
High	SD	12.7	2.8	29.7	-	1.039	0.014	0.35	2.8	2.1
Dose	N	2	2	2	0	2	2	2	2	2

Day: 42 Relative to Start Date

Sex: Female		Clinical Chemistry								
		GLOB (g/L)	A/G	UREA (mmol/L)	CRE (µmol/L)	CA (mmol/L)	PHOS (mmol/L)	NA (mmol/L)	K (mmol/L)	CL (mmol/L)
Group 1	Mean	20	1.8	8.3	53	2.68	1.86	152	4.8	115
Placebo	SD	0.0	0.11	4.10	7.1	0.092	0.163	4.2	0.07	2.1
Control	N	2	2	2	2	2	2	2	2	2
Group 4	Mean	19	1.9	6.8	54	2.74	1.64	154	4.7	116
High	SD	0.0	0.04	0.85	4.2	0.035	0.354	0.0	0.21	0.0
Dose	N	2	2	2	2	2	2	2	2	2

Urinalysis

See Table below from the Sponsor for parameters that were measured:

The following parameters were measured on urine samples:

Bilirubin	Protein
Blood	Sediment microscopy
Color and appearance	Specific gravity
Glucose	Urobilinogen
Ketones	Volume
pH	

No treatment-related changes were reported.

Gross Pathology

Animals were pre-anesthetized and then euthanized by an intravenous overdose of sodium pentobarbital followed by exsanguination (transsection of major blood vessels). The gross examination was performed for all animals.

Gross pathology changes were summarized in the Table below.

Gross Pathology								
	Males				Females			
	Placebo	Saline	LD	HD	Placebo	Saline	LD	HD
	Main Study (N=3)							
Kidney	0/3	0/3	0/3	0/3	1/3 cyst	0/3	0/3	1/3 Pale Discoloration
Thyroid Gland	0/3	0/3	0/3	0/3	0/3	0/3	0/3	1/3 Cyst
Lungs	0/3	1/3 Dark area Pale Discoloration	0/3	1/3 Pale Discoloration	0/3	0/3	0/3	0/3
	Recovery Study (N=2)							
Lungs	1/2 Adhesion	ND	ND	1/2 Dark area	1/2 Adhesion	ND	ND	0/2

Organ Weights

After the gross examination, weights of selected organs were recorded.
No treatment-related changes were reported

Histopathology

Battery Considered Adequate? Only respiratory and GI organs were assessed.
Peer Review Performed? Yes
Tissues were prepared for microscopic examination by embedding in paraffin wax, sectioning and staining with hematoxylin and eosin. Histological processing was conducted for all animals but not for all tissues. The nasal cavity was evaluated anatomically at different levels of 1-4.
See Table below from the Sponsor for tissues that were examined.

ORGANS/TISSUES	Retained (•)	Weighed (√)	Examined (€)	ORGANS/TISSUES	Retained (•)	Weighed (√)	Examined (€)
Adrenals	•	√		Sciatic nerve	•		
Animal identification	•			Skeletal muscle	•		
Aorta (thoracic)	•			Skin & subcutis (inguinal)	•		
Blood				Duodenum	•		€
Bone marrow smears (3)	•			Jejunum	•		€
Brain	•	√		Ileum	•		€
Cecum	•			SC, cervical	•		
Colon	•			Spleen	•	√	
Epididymides	•d			Sternum & marrow	•		
Esophagus	•		€	Stomach	•		€
Eyes	•a			Testes	•d	√	
Femur & marrow	•			Thymus	•	√	
Gallbladder	•			Thyroid gland/parathyroids	•	√	
Heart	•	√		Tongue	•		
Kidneys	•	√		Trachea	•c		€
Liver (2 lobes)	•	√		Urinary bladder	•		
Lungs (all lobes)	•b	√c	€	Uterus	•	√	
LN, mandibular	•			Vagina	•		
LN, mesenteric	•						
Mammary gland (inguinal)	•			Abnormal findings	•		€
Optic nerves	•a						
Ovaries	•	√					
Pancreas	•						
Pituitary	•	√		**Additional Tissues presented below**			
Prostate	•	√		Nasal Cavity (all 4 levels)	•		€
Rectum	•			Nasopharynx	•		€
SG, mandibular	•			Carina	•		€

a	Davidson's fluid
b	Lungs were infused with 10% neutral buffered formalin
c	Lungs were weighed with trachea
d	Bouin's fluid
LN	Lymph node
SG	Salivary gland
SC	Spinal cord
€	Examined microscopically

Paired organs weighed together

Results of microscopic examination were reported as following:

- Minimal atrophy/degeneration of the olfactory epithelium in the nasal cavity (level 3) was reported in 1/3 HD treated males (not reported at the end of the recovery period).
- Minimal to moderate subacute bronchioloalveolar inflammation was observed in the lungs as following: Placebo control 3/6 (2/3 males and 1/3 females); Saline control 4/6 (3/3 males and 1/3 females); LD, 4/6 (2/3 males and 2/3 females); and HD 4/6 (2/3 males and 2/3 females). This change correlated with the macroscopic finding of dark and/or pale discoloration in one animal in the Saline group and one animal in the HD group.
- Minimal to mild focal hyperplasia or squamous metaplasia of the respiratory epithelium was reported in the carina as following: Placebo control, 3/6 (2/3 males and 1/3 females); Saline, 3/6 (2/3 males and 1/3 females); LD, 2/6 (0/3 males and 2/3 females); and HD, 4/6 (2/3 males and 2/3 females). This change was not reported at the end of the recovery period.
- Minimal to moderate atrophy/degeneration of the olfactory epithelium (with/without subacute inflammation) was reported in the nasal cavity (level 4) as following: Placebo control, 2/6; Saline, 0/6; LD 6/6; and HD 6/6.

See Table below for all histopathological changes on Day 29:

Main Study									
Findings		**Males**				**Females**			
		Doses Glucagon (mg/day)				**Doses Glucagon (mg/day)**			
		Placebo	**Saline**	**2.0**	**4.0**	**Placebo**	**Saline**	**2.0**	**4.0**
Carina		**(2/3)**	**(2/3)**	**(0/3)**	**(2/3)**	**(1/3)**	**(1/3)**	**(2/3)**	**(2/3)**
	Metaplasia, squamous	**1 min**	**1 min**			**1 min**	**1 min**	**1 min 1 mild**	**1 min**
	Hyperplasia, respiratory epithelium	**1 min**	**1min**		**2 min**				**1 min**
Kidney		**ND**	**ND**	**ND**	**ND**	**(1/1)**	**ND**	**ND**	**(1/1)**
	Fibrosis, interstitial								**1 min**
	Cyst, cortical					**present**			
lungs		**(2/3)**	**(3/3)**	**(3/3)**	**(3/3)**	**(1/3)**	**(1/3)**	**(3/3)**	**(2/3)**
	Microgranuloma	**2 min**	**1 min**	**0**	**2 min**		**1 min**	**1 min**	
	Inflammation, subacute	**2 min**	**2 min 1 mild**	**2 min 1 mild**	**1 min 1 mild**	**1 min**	**1 min**	**3 min**	**2 min**
Nasal Cavity Level 3		**(0/3)**	**(0/3)**	**(0/3)**	**(1/3)**	**(0/3)**	**(0/3)**	**(0/3)**	**(1/3)**
	Atrophy/degeneration olfactory Epithelium				**1 min**				
Nasal Cavity Level 4		**(1/3)**	**(0/3)**	**(3/3)**	**(3/3)**	**(1/3)**	**(0/3)**	**(3/3)**	**(3/3)**
	Atrophy/degeneration olfactory Epithelium	**1 min**		**2 min 1 mild**	**3 min**	**1 min**		**3 min**	**1 min 2 mode**
Trachea		**(0/3)**	**(0/3)**	**(0/3)**	**(0/3)**	**(0/3)**	**(1/3)**	**(0/3)**	**(2/3)**
	Cell infiltrate, mononuclear						**1 min**		**2min**

See Table below for all histopathological changes at the end of the recovery period:

Recovery Study					
		Males		Females	
Findings		Glucagon (mg/day) N=2		Glucagon (mg/day) N=2	
		Placebo	4.0	Placebo	4.0
Carina		(1/2)	(0/2)	(0/2)	(2/2)
	Metaplasia, Squamous	1 min			1 min 1 mild
lungs		(1/2)	(1/2)	(0/2)	(1/2)
	Fusion, Lobal	present		present	
	Hemorrhage, Focal		1 min		
	Inflammation, Subacute			1 min	1 min
Trachea		(0/2)	(1/2)	(0/2)	(0/2)
	Cell Infiltrate, Neutrophilic		1 min		

See Tables below from the Sponsor:

Table 1 Incidence of Microscopic Findings in the Nasal Cavity (Level 4) of Terminal (Main) Dogs Exposed to AMG504-1

Sex		Male				Female			
Targeted Dose Level of Glucagon (mg/day)		0	0	2.0	4.0	0	0	2.0	4.0
Number of animals examined		3	3	3	3	3	3	3	3
Finding	Severity								
Atrophy/degeneration olfactory epithelium		1	0	3	3	1	0	3	3
	minimal	1	0	2	3	1	0	3	1
	mild	0	0	1	0	0	0	0	0
	moderate	0	0	0	0	0	0	0	2

APPENDIX 11

GROUP 1: Placebo Control Powder
GROUP 2: Saline Control
GROUP 3 and 4: AMG504-1

65125 - INCIDENCE OF HISTOPATHOLOGY

Date: 23/06/11 16:12

Observations: Neo-Plastic and Non Neo-Plastic	MALES				FEMALES			
Removal Reason: Terminal	Group 1 Placebo	Group 2 Saline	Group 3 Low	Group 4 High	Group 1 Placebo	Group 2 Saline	Group 3 Low	Group 4 High
Number of Animals on Study :	3	3	3	3	3	3	3	3
Number of Animals Completed:	(3)	(3)	(3)	(3)	(3)	(3)	(3)	(3)
CARINA;								
Examined	(3)	(3)	(3)	(3)	(3)	(3)	(3)	(3)
Within Normal Limits	1	1	3	1	2	2	1	1
Metaplasia, squamous	(1)	(1)	(0)	(0)	(1)	(1)	(2)	(1)
minimal	1	1	0	0	1	1	1	1
mild	0	0	0	0	0	0	1	0
Hyperplasia, respiratory epithelium	(1)	(1)	(0)	(2)	(0)	(0)	(0)	(1)
minimal	1	1	0	2	0	0	0	1
ESOPHAGUS;								
Examined	(3)	(3)	(3)	(3)	(3)	(3)	(3)	(3)
Within Normal Limits	3	3	3	3	3	2	2	3
Cell infiltrate, mononuclear	(0)	(0)	(0)	(0)	(0)	(1)	(1)	(0)
minimal	0	0	0	0	0	1	1	0
KIDNEYS;								
Examined	(0)	(0)	(0)	(0)	(1)	(0)	(0)	(1)
Within Normal Limits	0	0	0	0	0	0	0	0
Cyst, cortical	(0)	(0)	(0)	(0)	(1)	(0)	(0)	(0)
present	0	0	0	0	1	0	0	0
Fibrosis, interstitial	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(1)
minimal	0	0	0	0	0	0	0	1
LIVER;								
Examined	(0)	(0)	(0)	(0)	(1)	(0)	(0)	(0)
Within Normal Limits	0	0	0	0	0	0	0	0
Fibrosis, capsular	(0)	(0)	(0)	(0)	(1)	(0)	(0)	(0)
mild	0	0	0	0	1	0	0	0
LUNGS;								
Examined	(3)	(3)	(3)	(3)	(3)	(3)	(3)	(3)
Within Normal Limits	1	0	0	0	2	2	0	1
Microgranuloma	(2)	(1)	(0)	(2)	(0)	(1)	(1)	(0)
minimal	2	1	0	2	0	1	1	0
Inflammation, subacute	(2)	(3)	(3)	(2)	(1)	(1)	(3)	(2)

GROUP 1: Placebo Control Powder
GROUP 2: Saline Control
GROUP 3 and 4: AMG504-1

65125 - INCIDENCE OF HISTOPATHOLOGY

Date: 23/06/11 16:12

Observations: Neo-Plastic and Non Neo-Plastic	MALES				FEMALES			
Removal Reason: Terminal	Group 1 Placebo	Group 2 Saline	Group 3 Low	Group 4 High	Group 1 Placebo	Group 2 Saline	Group 3 Low	Group 4 High
Number of Animals on Study :	3	3	3	3	3	3	3	3
Number of Animals Completed:	(3)	(3)	(3)	(3)	(3)	(3)	(3)	(3)
LUNGS; (continued)								
minimal	2	2	2	1	1	1	3	2
mild	0	1	1	1	0	0	0	0
NASAL CAVITY #1;								
Examined	(3)	(3)	(3)	(3)	(3)	(3)	(3)	(3)
Within Normal Limits	3	3	3	3	3	3	3	3
NASAL CAVITY #2;								
Examined	(3)	(3)	(3)	(3)	(3)	(3)	(3)	(3)
Within Normal Limits	3	3	3	3	3	3	3	3
NASAL CAVITY #3;								
Examined	(3)	(3)	(3)	(3)	(3)	(3)	(3)	(3)
Within Normal Limits	3	3	3	2	3	3	3	3
Atrophy/degeneration, epithelium	(0)	(0)	(0)	(1)	(0)	(0)	(0)	(0)
minimal	0	0	0	1	0	0	0	0
NASAL CAVITY #4;								
Examined	(3)	(3)	(3)	(3)	(3)	(3)	(3)	(3)
Within Normal Limits	2	3	0	0	2	3	0	0
Atrophy/degeneration, epithelium	(1)	(0)	(3)	(3)	(1)	(0)	(3)	(3)
minimal	1	0	2	3	1	0	3	1
mild	0	0	1	0	0	0	0	0
moderate	0	0	0	0	0	0	0	2
NASOPHARYNX;								
Examined	(3)	(3)	(3)	(3)	(3)	(3)	(3)	(3)
Within Normal Limits	3	3	3	3	3	3	3	3
PITUITARY;								
Examined	(1)	(0)	(0)	(0)	(0)	(0)	(0)	(1)
Within Normal Limits	0	0	0	0	0	0	0	0
Cyst	1	0	0	0	0	0	0	1

GROUP 1: Placebo Control Powder
GROUP 2: Saline Control
GROUP 3 and 4: AMG504-1

65125 - INCIDENCE OF HISTOPATHOLOGY

Date: 23/06/11 16:12

Observations: Neo-Plastic and Non Neo-Plastic	MALES				FEMALES			
Removal Reason: Terminal	Group 1 Placebo	Group 2 Saline	Group 3 Low	Group 4 High	Group 1 Placebo	Group 2 Saline	Group 3 Low	Group 4 High
Number of Animals on Study :	3	3	3	3	3	3	3	3
Number of Animals Completed:	(3)	(3)	(3)	(3)	(3)	(3)	(3)	(3)
DUODENUM;								
Examined	(3)	(3)	(3)	(3)	(3)	(3)	(3)	(3)
Within Normal Limits	3	3	3	3	3	3	3	3
ILEUM;								
Examined	(3)	(3)	(3)	(3)	(3)	(3)	(3)	(3)
Within Normal Limits	3	3	2	3	3	3	3	3
Necrosis, focal	(0)	(0)	(1)	(0)	(0)	(0)	(0)	(0)
minimal	0	0	1	0	0	0	0	0
JEJUNUM;								
Examined	(3)	(3)	(3)	(3)	(3)	(3)	(3)	(3)
Within Normal Limits	3	3	3	3	3	3	3	3
SPLEEN;								
Examined	(1)	(1)	(0)	(0)	(0)	(0)	(0)	(0)
Within Normal Limits	1	0	0	0	0	0	0	0
Fibrosis, capsular	(0)	(1)	(0)	(0)	(0)	(0)	(0)	(0)
moderate	0	1	0	0	0	0	0	0
STOMACH;								
Examined	(3)	(3)	(3)	(3)	(3)	(3)	(3)	(3)
Within Normal Limits	3	3	3	3	3	3	3	3
THYROID GLAND;								
Examined	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(1)
Within Normal Limits	0	0	0	0	0	0	0	0
Cyst	0	0	0	0	0	0	0	1
TRACHEA;								
Examined	(3)	(3)	(3)	(3)	(3)	(3)	(3)	(3)
Within Normal Limits	3	3	3	3	3	2	3	1
Cell infiltrate, mononuclear	(0)	(0)	(0)	(0)	(0)	(1)	(0)	(2)
minimal	0	0	0	0	0	1	0	2

GROUP 1: Placebo Control Powder
GROUP 2: Saline Control
GROUP 3 and 4: AMG504-1

65125 - INCIDENCE OF HISTOPATHOLOGY

Date: 23/06/11 16:11

Observations: Neo-Plastic and Non Neo-Plastic	MALES		FEMALES	
Removal Reason: Recovery	Group 1 Placebo	Group 4 High	Group 1 Placebo	Group 4 High
Number of Animals on Study :	2	2	2	2
Number of Animals Completed:	(2)	(2)	(2)	(2)
CARINA;				
Examined	(2)	(2)	(2)	(2)
Within Normal Limits	1	2	2	0
Metaplasia, squamous	(1)	(0)	(0)	(2)
minimal	1	0	0	1
mild	0	0	0	1
ESOPHAGUS;				
Examined	(2)	(2)	(2)	(2)
Within Normal Limits	2	2	2	2
LUNGS;				
Examined	(2)	(2)	(2)	(2)
Within Normal Limits	1	1	0	1
Hemorrhage, focal	(0)	(1)	(0)	(0)
mild	0	1	0	0
Fusion, lobal	(1)	(0)	(1)	(0)
present	1	0	1	0
Inflammation, subacute	(0)	(0)	(1)	(1)
minimal	0	0	1	1
LN, MEDIASTINAL;				
Examined	(1)	(1)	(0)	(0)
Within Normal Limits	0	0	0	0
Hemorrhage/erythrophagocytosis	(1)	(1)	(0)	(0)
moderate	1	1	0	0
NASAL CAVITY #1;				
Examined	(2)	(2)	(2)	(2)
Within Normal Limits	2	2	2	2
NASAL CAVITY #2;				
Examined	(2)	(2)	(2)	(2)
Within Normal Limits	2	2	2	2

GROUP 1: Placebo Control Powder
GROUP 2: Saline Control
GROUP 3 and 4: AMG504-1

65125 - INCIDENCE OF HISTOPATHOLOGY Date: 23/06/11 16:11

Observations: Neo-Plastic and Non Neo-Plastic	------- MALES -------		------ FEMALES ------	
Removal Reason: Recovery	Group 1 Placebo	Group 4 High	Group 1 Placebo	Group 4 High
Number of Animals on Study :	2	2	2	2
Number of Animals Completed:	(2)	(2)	(2)	(2)
NASAL CAVITY #3;				
Examined	(2)	(2)	(2)	(2)
Within Normal Limits	2	2	2	2
NASAL CAVITY #4;				
Examined	(2)	(2)	(2)	(2)
Within Normal Limits	2	2	2	2
NASOPHARYNX;				
Examined	(2)	(2)	(2)	(2)
Within Normal Limits	2	2	2	2
DUODENUM;				
Examined	(2)	(2)	(2)	(2)
Within Normal Limits	2	2	2	2
ILEUM;				
Examined	(2)	(2)	(2)	(2)
Within Normal Limits	2	2	2	2
JEJUNUM;				
Examined	(2)	(2)	(2)	(2)
Within Normal Limits	2	2	2	2
STOMACH;				
Examined	(2)	(2)	(2)	(2)
Within Normal Limits	2	2	2	2
TRACHEA;				
Examined	(2)	(2)	(2)	(2)
Within Normal Limits	2	1	2	2
Cell infiltrate, neutrophilic	(0)	(1)	(0)	(0)
minimal	0	1	0	0

Toxicokinetics

Blood samples were collected from animals of the main study on Day 1 and Day 28 of the treatment period.
Results of TK data were reported as following:

- Day 1, the AUC0-t to glucagon were 213385 and 148561 pg*min/mL for the LD and HD, respectively. Peak glucagon concentration was observed 10 minutes after dosing and increased with dose (LD, 6984 vs. HD, 7251 pg/mL).
- Day 28, the AUC0-t (pg*min/mL) were 267261 for LD and 343597 for HD; Cmax (pg/mL) values were: 8684 for LD and 13171 for HD; therefore, both values of AUC and Cmax were increased with doses.
- Based on the short half-life of glucagon in serum, minimal accumulation was observed following multiple AMG504-1
- No gender-related differences in glucagon exposure parameters were reported

See Table below from The Sponsor for the TK data:

Table 4.2 Baseline-Corrected TK Parameters of Glucagon in Dogs

Parameters	Mean (%CV)			
	Day 1		Day 28	
	AMG504-1 2 mg/day	AMG504-1 4 mg/day	AMG504-1 2 mg/day	AMG504-1 4 mg/day
N	6	6	6	6
AUC_{0-90} (pg.min/mL)	229465 (27.9)	151619 (52.1)	267261 (41.6)	345747 (61.4)
AUC_{0-t} (pg.min/mL)	213385 (15.8)	148561 (52.7)	267261 (41.6)	343597 (62.4)
C_{max} (pg/mL)	6984 (26.0)	7251 (50.6)	8684 (34.1)	13171 (56.4)
T_{max}[a] (min)	10.0 (10.0, 20.0)	10.0 (5.00, 20.0)	10.0 (5.00, 10.0)	10.0 (5.00, 20.0)
$T_{1/2}$ (min)	NC (NC)[b]	11.4 (NC)[c]	21.1 (18.4)[d]	7.79 (NC)[e]

[a] Median (Min, Max), [b] n = 0, [c] n = 2, [d] n = 4, [e] n = 1, NC = Not calculated

Dosing Formulation Analysis

The mean glucagon content in the test article devices for both Days 1 and 28 was 2.1 mg (within 10% of the desired value of 2 mg per device). The amount of glucagon for Days 1 and 28 was consistent. There was no glucagon present in any control article device from Day 1 or Day 28. See Table below from the Sponsor for the efficiency of devices:

Achieved powder concentrations of the devices were as follows:

Group Number	Group Designation	Targeted Powder Dose (mg)	Achieved Average Powder Dose (mg)	% Efficiency
1	Placebo Control	40.0	33.0*	82.5
			37.1**	92.8
3	Low Dose	20.0	18.9*	94.5
			19.9**	99.5
4	High Dose	40.0	36.8*	92.0
			39.6**	99.0

* Calculated using all values from the entire study including discharge weights obtained before implementation of a device wiping procedure to correct the adverse effect of device-related electrostatic charge on the analytical balance.

** Calculated using values beginning April 8, 2011(equivalent to study Day 10, 11 or 12 depending on the dog) after instituting a device wiping procedure to remove electrostatic charge and thus resulting in accurate and expected discharge weights.

7 Genetic Toxicology

7.1 In Vitro Reverse Mutation Assay in Bacterial Cells (Ames)

Dodecylphosphocholine: Bacterial Reverse Mutation Test in Salmonella typhimurium and Escherichia coli	
Study #	AMG016C
Study report location	(b) (4)
CRO/Laboratory name	
CRO/Laboratory address	
Date of study initiation	03/15/2011
GLP compliance statement	yes
GLP issues identified	no
QA statement	yes
Drug, lot #, and % purity	Dodecylphosphocholine (DPC),120CP-47, 100%

Key Study Findings

- DPC did not induce in revertants relative to the solvent control in any of the test strains
- DPC was not considered mutagenic under the conditions of this AMES Assay

Methods	
Strains	Salmonella typhimurium strains (TAI535, TA1537, TA98, TA100) and Escherichia coli strain (WP2 uvrA)
Concentrations in definitive study	0, 5.0, 15.8, 158, 500, 1581 and 5000 ug/plate
Basis of concentration selection	Based on general rule of the assay, which was the 5 highest levels below the toxic level or , if non-toxic, at five levels up to the standard limit of 5000 µg/plate".
Negative control	DMSO, sodium chloride
Positive control	Sodium azide; 9-Aminoacridine; 2-nitrofluorine; 4-nitroquinoline N-oxide; 2-aminoanthracene; Benzo (a) pyrene
Formulation/Vehicle	For DPC: Saline For positive controls: Water (USP) or DMSO
Incubation & sampling time	Plate with ±S9 for 60 to 72h at 37°C

Study Validity

The expected number of revertants (± S9) was noted in assays with positive controls.

Results

- DPC was toxic to the bacteria at the highest level tested with: TAI00 (± S9); with TA1535 and TAI00 (+S9); and at ≥ 1581 µg/plate with TA1537 and TAI00 (-S).
- DPC did not show any increase in the number of revertant colony counts with any strain (± S9)

See Tables below from the Sponsor:

Test Article: Number of revertant colonies (mean ± SD)

Metabolic Activation	Assay	Strain	Concentration (µg per plate)							
			0	5.0	15.8	50	158	500	1581	5000
No	Plate Incorporation	TA1535				18 ± 2	17 ± 5	21± 7	22 ±3	18 ± 4
		TA1537	14 ± 3			19 ± 4	16 ±1	19 ± 3	13 ± 2	14 ± 5
		TA98	27 ± 10			36 ± 3	33 ± 3	38 ± 9	37 ± 10	25 ± 6
		TA100	187 ± 3		163 ± 14	166 ± 26	176 ± 12	154 ± 37	121 ± 4	Toxic
		WP2 *uvrA*	53 ± 3			35 ± 8	60 ± 12	57 ± 4	58 ± 5	47 ± 6
No	Pre-incubation	TA1535	16 ±1			25 ± 3	21 ± 6	21 ± 6	11 ± 3	13 ± 2
		TA1537	14 ± 3	11 ± 2	12 ± 4	12 ± 4	9 ± 2	9 ± 2	Toxic	Toxic
		TA98				27 ± 10	40 ± 5	33 ± 3	28 ± 2	34 ± 2
		TA100	187 ± 3	154 ±11	157 ± 30	157 ±8	117 ± 13	111 ± 13	Toxic	Toxic
		WP2 *uvrA*	53 ± 3			44 ± 4	52 ± 8	51 ± 2	48 ± 14	34 ± 10
Yes	Plate Incorporation	TA1535	27 ± 7			20 ± 4	23 ± 2	18 ± 7	23 ± 7	
		TA1537	18 ± 2			17 ± 4	18 ± 2	17 ± 4	17 ± 3	22 ± 5
		TA98	30 ± 6			51 ± 8	49 ± 9	48 ± 7	51 ± 6	27 ± 5
		TA100	145 ± 5		132 ± 6	119 ± 21	200 ± 18	199 ± 8	150 ± 16	49 ± 11 (Toxic)
		WP2 *uvrA*	61 ± 7		41 ± 10	60 ± 5	61 ± 1	69 ± 9	51 ± 19	
Yes	Pre-incubation	TA1535	27 ± 7		21 ± 11	19 ± 2	16 ± 4	20 ± 6	18 ± 5	14 ± 5 (Toxic)
		TA1537	18 ± 2			15 ± 7	22 ± 5	21 ± 2	23 ± 5	21 ± 2
		TA98	39 ± 6			46 ± 5	44 ± 4	45 ±10	46 ± 7	27 ± 8
		TA100	145 ± 5		180 ± 3	148 ± 27	183 ± 11	139 ± 6	112 ± 11	31 ± 6 (Toxic)
		WP2 *uvrA*	61 ± 7			64 ± 5	68 ±1	54 ± 5	66 ±5	47 ± 15

Positive controls

Metabolic Activation	Assay	Strain	Test Article	Concentration (µg per plate)	Number of revertants (mean ± SD)	Fold response [a]
No	Plate incorporation	TA1535	NaAz	0.5	302 ± 9	18
		TA1537	9AC	50	256 ± 55	18
		TA98	2NF	1	210 ± 11	7.8
		TA100	NaAz	0.5	538 ± 14	2.9
		WP2 *uvrA*	NQO	0.5	171 ± 9	3.2
No	Pre-incubation	TA1535	NaAz	0.5	302 ± 9	18
		TA1537	9AC	1.5	451 ± 58	32
		TA98	2NF	1	218 ± 218	8.1
		TA100	NaAz	0.5	563 ± 563	3.0
		WP2 *uvrA*	NQO	0.5	1236 ± 61	23
Yes	Plate incorporation	TA1535	2AA	5	368 ± 7	14
		TA1537	BaP	5	92 ± 6	5.0
		TA98	BaP	5	351 ± 14	9.0
		TA100	BaP	5	1065 ± 26	7.3
		WP2 *uvrA*	2AA	15	256 ± 26	4.2
Yes	Pre-incubation	TA1535	2AA	5	377± 17	14
		TA1537	BaP	5	89 ± 13	4.9
		TA98	BaP	5	249 ± 13	6.4
		TA100	BaP	5	998 ± 21	6.9
		WP2 *uvrA*	2AA	15	326 ± 24	5.4

a. Fold response in mean revertants compared to concurrent vehicle control.

7.2 In Vitro Assays in Mammalian Chromosome Aberration

Dodecylphosphocholine: In Vitro Mammalian Chromosome Aberration Test in Human Peripheral Blood Lymphocytes	
Study #	AMG017C
Study report location	(b) (4)
CRO/Laboratory name	
CRO/Laboratory address	
Date of study initiation	03/15/2011
GLP compliance statement	yes
GLP issues identified	no
QA statement	yes
Drug, lot #, and % purity	Dodecylphosphocholine (DPC),120CP-47, 100%

Key Study Findings

Cultures treated with DPC at levels up to the limit of toxicity (±S9) did not show any increases in the incidence of aberrant metaphases.

Methods	
Cell line	Human peripheral blood lymphocytes
Concentrations in definitive study	6.25 to 3515 µg/mL
Basis of concentration selection	A range of concentrations were selected (only assessed at the highest levels below the toxic level).
Negative control	Solvent: water
Positive control	Mitomycin C (MMC); Cyclophosphamide monohydrate
Formulation/Vehicle	For DPC: Saline For Positive Controls: Sterile Water USP
Incubation & sampling time	4 hours (±S9) and for 21 hours (-S9)

Study Validity

The sensitivity of the system was confirmed by induced significant increases in aberrations with the positive controls.

Results:

DPC at levels up to the limit of toxicity did not show any increases (ss) in the incidence of aberrant metaphases.

The Sponsor noted that "the highest dose level initially chosen for examination for chromosome aberrations caused a substantial reduction in the absolute number of analyzable metaphases (as well as the RMI) as a result of general cell toxicity. Consequently, a representative number of analyzable metaphases could not be examined and no results are presented in this report for these dose levels).

See Tables below from the Sponsor:

Text Table 1 Study Design

Dose number	Material	Formulation conc. (µg/mL)	Final conc. (µg/mL)	Number of cultures		
				4 Hours (0S9)	4 Hours (+S9)	21 Hours (0S9)
0	Vehicle	-	-	2	2	2
1	DPC	125	6.25	2	2	2
2		250	12.5	2	2	2
3		500	25.0	2	2	2
4		1000	50.0	2	2	2
5		2000	100	2	2	2
6		4000	200	2	2	2
7		8000	400	2	2	2
8		16000	800	2	2	2
9		35147	1757	2	2	2
10		70294	3515†	2	2	2
1	MMC	5	0.05	2		2
2		10	0.10	2		2
3		20	0.20	2		2
1	CP	800	8.0		2	
2		1200	12		2	
3		1600	16		2	

† The highest dose tested was the standard limit of 0.01M as recommended by the OECD.

Table 1 Results and Statistical Analysis

Treatment	Conc. (µg/mL)	MI	RMI (%)	Number cells examined	% Aberrant	Number of aberrations					Incidental observations †			
						b	e	B	E	other	(g	G	P	C)
4 hours treatment in the absence of S9 (0S9)														
Saline	-	7.9	100	200	0.5	1	0	0	0	0	1	0	0	0
DPC	100	7.1	90	200	0.5	1	0	0	0	0	0	0	0	0
	200	6.1	77	200	2.0	4	0	0	0	0	4	0	0	0
	400	4.5	57	200	1.5	3	0	0	0	0	3	0	0	1
	800	0.0	0	0					N/A					
MMC	0.10	7.1	90	200	12.0**	23	5	1	0	0	5	0	0	0
4 hours treatment in the presence of S9 (+S9)														
Saline	-	8.0	100	200	0.5	1	0	0	0	0	1	0	0	0
DPC	100	10.2	128	200	0.0	0	0	0	0	0	2	0	0	0
	200	8.5	106	200	1.0	2	0	0	0	0	1	0	0	0
	400	4.6	57	200	1.0	3	0	0	0	0	8	0	1	1
	800	0.0	0	0					N/A					
CP	8.0	3.6	45	200	28.0**	64	18	1	0	1	9	2	0	0
21 hours treatment in the absence of S9 (0S9)														
Saline	-	5.6	100	200	0.5	1	0	0	0	0	3	1	0	1
DPC	50.0	7.4	131	200	1.5	3	0	0	0	0	7	0	0	0
	100	5.3	93	200	1.0	2	0	0	0	0	6	0	0	1
	200	3.2	57	200	1.5	4	0	0	0	0	5	0	0	0
	400	0.1	2	0					N/A					
MMC	0.05	4.8	86	200	11.5**	23	4	0	0	0	5	2	0	0

MI, RMI Mitotic Index, Relative Mitotic Index (vehicle = 100%)
b, e, g Chromatid break, exchange, gap
B, E, G Chromosome break, exchange, gap
other Includes pulverized chromosomes and cells with > 8 aberrations
P Polyploidy and endoreduplication
C Centromeric disruption
† g, G, P and C are excluded from the calculation of % aberrant cells
N/A Not assessable (insufficient cells available due to toxicity)

Results of statistical analysis using one-tailed Fisher's exact test
*p ≤ 0.01 (significant)
**p ≤ 0.001 (highly significant)
Otherwise, p > 0.01 (not significant)

Cytotoxic Effects: Cytotoxic effects were seen at the following concentrations:

Test Condition	Minimum cytotoxic concentration
4 hour incubation without S9	800 µg / mL
4 hour incubation with S9	800 µg / mL
21 hour incubation without S9	400 µg / mL

Test Article: % Aberrant Cells

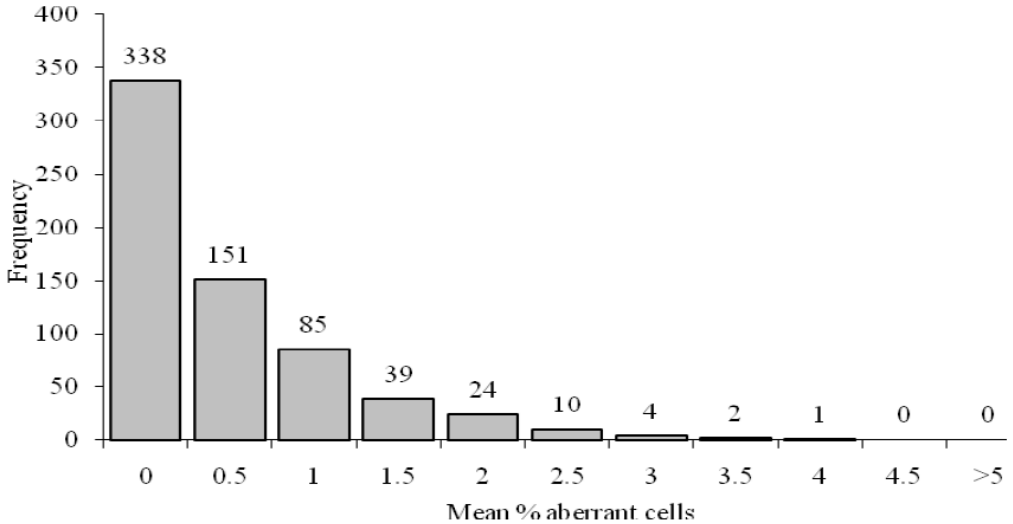
Metabolic activation	Incubation Period (hr)	Test Article	Concentration (µg / mL)					
			0	50	100	200	400	800
Without activation	4	DPC	0.5		0.5	2.0	1.5	N/A
Without activation	21	DPC	0.5	1.5	1.0	1.5		
With activation	4	DPC	0.5		0.0	1.0	1.0	N/A

N/A Not assessable (insufficient cells available due to toxicity)

Positive Controls: % Aberrant Cells

Metabolic activation	Incubation Period (hr)	Test Article	Concentration (µg / mL)	% Aberrant Cells
Without activation	4	MMC	0.10	12.0
Without activation	21	MMC	0.05	11.5
With activation	4	CP	8.0	28.0

Figure 1 Historical Control Values



The laboratory historical mean incidence of aberrant metaphase cells for negative/vehicle control cultures for the human lymphocyte chromosome aberration test is 0.48% (SD 0.67) for 654 treatments. These QA audited results were collected from GLP compliant studies performed from 05 February 2003 to18 March 2010.

The historical positive control values (for QA-audited and GLP compliant studies) are listed below:

Mitomycin C (4 hour 0S9):	mean 10.3%, SD 4.8, 174 treatments
Mitomycin C (21 hour 0S9):	mean 12.0 %, SD 4.9, 180 treatments
Cyclophosphamide (4 hour +S9):	mean 23.1 %, SD 8.7, 177 treatments

11 Integrated Summary and Safety Evaluation

Clinical Safety Margins

Toxicity	Species	Sex	NOAEL (Dose)	Human Safety Margin (Based on AUC*pg.min/mL)
At the nasal cavity, erosion/ulceration of the olfactory epithelium at HD	28-day Rats	F/M	LD (0.4mg/kg)	AUC at LD (0.4mg/kg)= 43036 AUC at MRHD=479 43036/479=89X
At the nasal cavity, atrophy/degeneration of the olfactory epithelium at LD and HD	28-day Dogs	F/M	NOAEL not established	------

*Human AUC at MRHD=478.8 pg·min/mL

AMG504-1, a novel nasally-administered glucagon powder formulation, is under development for severe hypoglycemia for patients that typically use insulin and, in emergency circumstances unable or unwilling to take carbohydrates orally and require third-party assistance to correct the hypoglycemia. It is intended for frequently a single administration of 1 mg of glucagon and a second dose might be given if the patient fails to respond within15 to 20 minutes. Phase I studies have been conducted with AMG504-1 (IN up to 2mg) and was compared to approved glucagon (SC, Img) in healthy volunteers and results of one completed study suggested that AMG504-1 was well tolerated. The Sponsor has submitted nonclinical studies to support proposed future clinical studies of AMG504-1 in Phase II and Phase III.

AMG504-1 has 3 ingredients as following:
Glucagon for the treatment of hypoglycemia has a long history of safe use and currently there are two approved glucagon products for injection (Glucagon for Injection; NDA 020928; and Glucagon Hypo Kit; NDA 020918). These products are available as a powder that must be mixed with a diluent immediately prior to administration by injection.
Betadex has been used as an excipient; however, it is a novel excipient as given IN. The submitted repeat dose toxicity studies address the local tolerance of beta cyclodextran. AMG504-1 at MRHD (2mg) contains (b) (4) mg/unit of Betadex which is much less than the acceptable daily intake of 0-5 mg/kg/day or 300 mg/person/day for a 60 kg person. In addition, there is extensive data for the safety profiles of the Betadex and it was designated as GRAS by the Agency (2001). Betadex has been used as a 1% topical gel and in oral tablets up to 133 mg in other marketed products as an excipient.
DPC has not been used in previously marketed products. it is contains a choline group, a phosphate group and a saturated aliphatic chain which is 12-carbons in length (all present in phospholipids of cell membranes of humans). DPC is used in the formulation (b) (4) %) to increase (b) (4) . For safety profiles of DPC, the Sponsor conducted several nonclinical studies and submitted published studies with positive safety profiles regarding dosing glucagon (IN) with a closely related phospholipid (didecanoyl-phosphatidyl-choline).
Results of two submitted *in vitro* genotoxicity assays (reverse mutation assay, Ames; mammalian chromosome aberration) suggested no genotoxicity activity of DPC. In exploratory studies in male dogs, safety of DPC on nasopharyngeal was evaluated with high doses of pure DPC (IN, at 4 and 10 mg) for 5 days in 2 dogs. Results of this study suggested no adverse clinical or gross necropsy at necropsy. Minimal inflammation and mild accumulation of basophilic material were reported in the nasal cavity of both animals. The safety profiles of DPC

also were tested in two 28-day repeat-dose toxicity studies in rats and dogs with AMG504-1 and the placebo (both excipients). Results of these studies were explained under the repeat-dose toxicity studies.
DPC as a novel excipient for short term use should be evaluated: 1)-in the standard battery of genetic toxicology studies before Phase II studies; AMG504-1 was not evaluated for *in vivo* genotoxicity study; 2)- in one month repeat-dose toxicology studies; AMG504-1 was evaluated in two repeat-dose toxicity studies; however, histopathology evaluation was performed only for the respiratory tract tissues; and 3)- in the reproductive toxicology study; AMG504-1 was not evaluated in any reproductive study.
However, considering the chemical structure of DPC that resembles the phospholipid cell membrane in humans, further nonclinical studies may not be required if the Sponsor submitted sufficient data that systemic exposure of DPC dose not occur.

Nonclinical studies:
PK/PD studies:
In dogs, the levels of blood glucose and glucagon by different doses of IN AMG504-1 (with the device) were compared to the injected glucagon. Results of these studies suggested that despite the lower bioavailability of IN glucagon (~15%) to SC glucagon, the time to peak glucose response and the magnitude of the post-treatment glycemic excursion were similar for both IN and SC administration.

Single-Toxicity Studies:
To evaluate inadvertent pulmonary exposure of AMG504-1 into the lungs, a single dose of intra-tracheal (IT) insufflation toxicity study (GLP) was conducted with 2mg/kg in rats. Results of this study suggested no treatment-related effects of a single dose of 2mg/kg AMG504-1 in the respiratory tract of rats (after treatment and after 14 day recovery period). Based on the body surface area, a single dose of 2mg/kg AMG504-1 in rats compared to a single MHRD (2mg) dose has 10X of safety margin.
The distribution of glucagon powder (20 mg/dog; IN) in the respiratory tract with dye was evaluated, which showed evidence of dye in the nasal passages, nasopharynx, stomach, esophagus, and on tongue; however, no evidence of dye was reported in the larynx, trachea and lung.

Repeat-Dose Toxicity Studies:
Two 28-day repeat-dose toxicity studies in rats and dogs were conducted to evaluate the local tolerance of AMG504-1; therefore, the histopathology evaluation was conducted only for the respiratory tract tissues in both studies. In the rat study, the intended device was too large for animals; therefore, rats were treated with solubilized formulation (0.1 and 0.2 mg glucagon/rat/day) in 100 mM acetic acid for 28 days. In dogs, the intended clinical nasal dosing device and the clinical powder drug product formulation (2.0 and 4.0 mg glucagon/dog/day) was used for 28 days. Both studies showed reversal treatment-related lesions in the nasal cavity as following:
In the rat study, at HD, unilateral or bilateral erosion/ulceration (mild to moderate) was observed in the dorsal turbinates of the nasal cavity (especially the olfactory epithelium of the lamina propria) in 2/10 males and 3/10 females. These treatment-related lesions were not reported after the recovery period. The NOAEL was established at LD (0.1 mg glucagon/day). Based on the AUC pg.min.mL, the safety margin at NOAEL to MRHD was 89X (AUC of LD in rats of 43036/AUC of MRHD of 479) with limited histopathological assessment of respiratory and GI organs only.
In the dog study, histopathology evaluation of respiratory tract was reported as mild to moderate atrophy and degeneration of the olfactory epithelium in the nasal cavity of all treated

animals except for the Saline control group as following: Saline (0/6); Placebo (2/6); LD (6/6); HD (6/6). These treated-related lesions were not noted after the recovery period. The NOAEL was not established for this study. The LD represent <2X based on NSA to the human therapeutic dose 20 mg AMG504-1 or 2 mg glucagon.

In summary, there are two safety concerns for AMG504-1: 1)- Reversible treatment-related lesions findings in the nasal cavity in the dog study with no established NOAEL at clinically relevant exposures based on NSA. However, AMG504-1 is intended for emergency use for a single dose; therefore, reversible lesions in the nasal cavity after 28 days exposure with overdose of AMG504-1 would not be expected to observe in humans. 2)- DPC as a novel excipient has not been evaluated for all the required nonclinical studies; however, these studies may not be required if the Sponsor submits sufficient data that systemic exposure of DPC dose not occur.

12 Appendix/Attachments

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/s/

PARVANEH ESPANDIARI
05/07/2012

KAREN L DAVIS BRUNO
05/07/2012