

# Evaluation of the safety of daily administration of capromorelin in cats

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Capromorelin is a ghrelin receptor agonist that is FDA approved for appetite stimulation in dogs. The objective of this study was to evaluate the safety of daily oral administration of capromorelin to cats over a range of doses and for an extended period. Two randomized, controlled studies were conducted: in Study 1, cats ( $n = 6$  per group) received placebo or capromorelin at a dose of 9, 15, 30 or 60 mg/kg once daily for 14 days; and in Study 2, cats received capromorelin at 6 mg/kg ( $n = 8$ ) or placebo ( $n = 4$ ) once daily for 91 days. Cats were evaluated using clinical observations and clinical pathology test results for both studies, with the addition of postmortem examination in Study 1 and measurements of growth hormone and insulin-like growth factor 1 in Study 2. Abnormal clinical observations were limited to emesis, hypersalivation, lethargy/depression, head shaking and lip smacking, which occurred more frequently in the capromorelin-treated groups than in the placebo group. There were no clinically relevant differences in clinical pathology test results between the capromorelin and placebo groups in either study.

## 1 | INTRODUCTION

Capromorelin is a ghrelin receptor agonist in a class of drugs also known as growth hormone secretagogues (GHSs) that bind to GHS receptors (GHS-Rs) in the hypothalamus, pituitary and other organs. The endogenous ligand for the GHS-R is the peptide hormone ghrelin, which is produced in the stomach in response to fasting, is secreted into the circulation and binds to GHS-Rs in the hypothalamus and pituitary to stimulate appetite and enhance the release of growth hormone (GH) from the pituitary (Cowley et al., 2003; Cummings, Frayo, Marmonier, Aubert, & Chapelot, 2004; Hainerova & Lebl, 2010; Muller et al., 2015; Takaya et al., 2000; Wren et al., 2001). In turn, GH promotes the release of insulin-like growth factor 1 (IGF-1) from the liver (Mathews, Hammer, Brinster, & Palmiter, 1988; Mathews, Norstedt, & Palmiter, 1986). IGF-1 has been shown to enhance differentiation of muscle cells in vitro, and elevated GH and IGF-1 levels are associated with increased overall growth in mice and increased body weight and muscle mass in Beagle dogs (Molon-Noblot et al., 1998; Palmiter, Norstedt, Gelin, Hammer, & Brinster, 1983; Palmiter et al., 1982; Schmid, Steiner, & Froesch, 1983). IGF-1 also regulates GH levels through a negative feedback

mechanism, thereby preventing overstimulation of GH production (Clemmons, 2004; Yakar et al., 2001).

Growth hormone secretagogues, such as capromorelin, represent a class of drugs that mimic the action of ghrelin to increase appetite and stimulate release of GH. GHSs were originally developed for the treatment of muscle loss and weakness in elderly human patients as well as anorexia and cachexia in human patients with cancer (Carpino et al., 2003; Hersch & Merriam, 2008; Smith, 2005; Temel et al., 2016; White et al., 2009). Treatment with oral capromorelin was shown to increase IGF-1 levels and total lean body mass in elderly men and women (White et al., 2009). In addition, capromorelin has been shown to stimulate appetite, food intake, weight gain and GH and IGF-1 release in dogs (Zollers, Huebner, Armintrout, Rausch-Derra, & Rhodes, 2017; Zollers, Rhodes, & Heinen, 2017; Zollers, Rhodes, & Smith, 2017; Zollers, Wofford, Heinen, Huebner, & Rhodes, 2016).

Capromorelin is the only Food and Drug Administration (FDA)-approved ghrelin receptor agonist and is currently approved for use in dogs to stimulate appetite. This report describes two randomized, placebo-controlled studies that evaluated the safety of capromorelin in healthy laboratory cats when dosed daily over a large range of doses for 14 or 91 days.

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## 2 | MATERIALS AND METHODS

### 2.1 | Study 1: 14-day dose-ranging safety study

#### 2.1.1 | Animals

Seventeen intact male and 17 intact female domestic shorthair cats  $\geq 6$  months old were bought from Harlan Laboratories (Indianapolis, IN). Two males and two females were excluded prior to study initiation due to unsatisfactory physical examination results; 15 males and 15 females received study treatments and were included in the analyses. Cats were housed at the Midwest Research Institute (Kansas City, MO) and allowed to acclimate for 10 days prior to study initiation. All cats were provided with IAMS™ ProActive Health™ Adult Original dry cat food ad libitum, except during fasting periods (overnight and prior to sample collection on days 8 and 15), as well as fresh tap water ad libitum.

Animal care and housing were in accordance with the Guide for the Care and Use of Laboratory Animals, 8th edition (National Research Council, 2011). The Midwest Research Institute has been continuously accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International since 1972. The study was conducted in accordance with Good Laboratory Practice as set forth by the FDA 21 Code of Federal Regulations (CFR; Part 58). The study protocol was reviewed and approved by the facility's Institutional Animal Care and Use Committee.

#### 2.1.2 | Study design

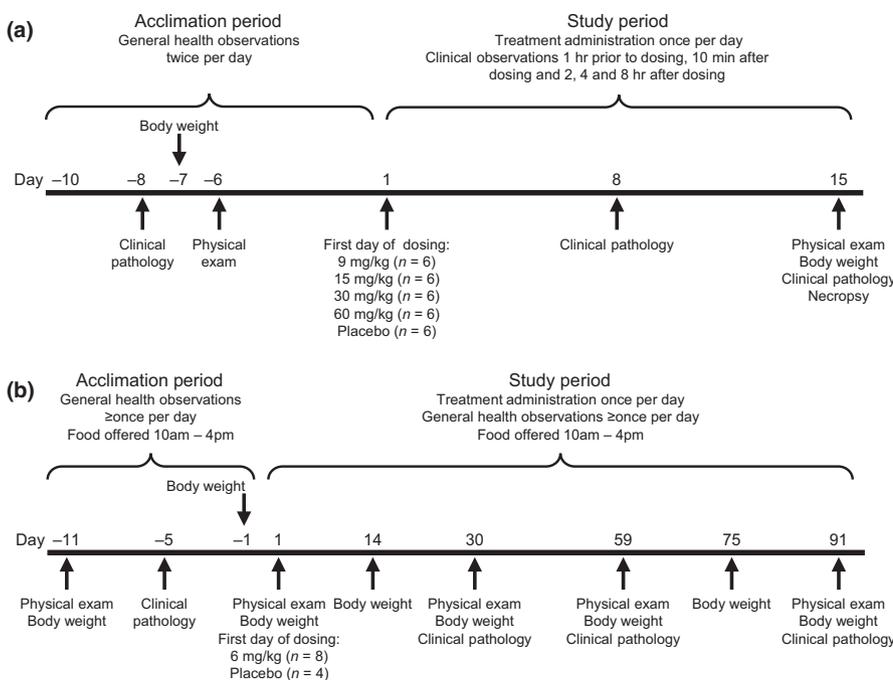
The study design is summarized in Figure 1a. Cats were randomized 1:1:1:1:1 into five treatment groups based on body weight measured 7 days prior to the first dosing, with six cats (three males and three females) per treatment group. Cats were administered capromorelin

at doses of 9, 15, 30 or 60 mg/kg or placebo once daily for 14 days approximately 1 hr prior to feeding. Placebo-treated cats received oral capsules containing microcrystalline cellulose alone, whereas capromorelin-treated cats received oral capsules containing the appropriate amount of capromorelin and backfilled with microcrystalline cellulose. Variables of interest were clinical observations for general health, clinical pathology evaluations (including serum chemistry, haematology and urinalysis) and postmortem examination, including histopathology.

#### 2.1.3 | Clinical and laboratory assessments

Cats were observed at least twice daily during acclimation, except on day -6, when they were observed once 6-7 hr after they were given a physical examination. During the dosing period (days 1-14), clinical observations were made prior to dosing,  $\leq 10$  min after treatment and 2, 4 and 8 hr after treatment. Body weights were recorded 7 days prior to dosing and at the time of euthanasia.

Haematology and serum chemistry were performed on blood samples collected on day -8 during acclimation and on days 8 and 15 after cats were fasted overnight. Blood samples were collected via jugular vein into BD Vacutainer® (Becton, Dickinson and Company) blood collection tubes containing ethylenediaminetetraacetic acid (EDTA) anticoagulant for haematology or no anticoagulant for serum chemistry. Haematology parameters included white blood cell (WBC) count, WBC differential, red blood cell (RBC) count, haemoglobin, platelet count, haematocrit, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration and reticulocytes. Serum chemistry parameters included albumin, globulin, creatine phosphokinase (CPK), alkaline phosphatase, glucose, alanine aminotransferase, blood urea nitrogen (BUN), phosphorus, calcium, potassium, chloride, sodium, total protein, creatinine, total bilirubin, direct bilirubin, amylase and aspartate



**FIGURE 1** Study designs for (a) Study 1, the 14-day dose-ranging study, and (b) Study 2, the 91-day study

aminotransferase (AST). Urinalysis was performed on samples collected on days -8, 8 and 15 by cystocentesis or free catch 28–48 hr after blood collection. Urinalysis parameters included urine specific gravity, pH, colour, blood, protein, bilirubin, glucose, ketones and microscopic examination of sediments.

On day 15, each cat was given a physical examination and then humanely euthanized for postmortem examination. Organ weights were measured, and cats were examined for any gross external or internal abnormalities upon necropsy; all gross lesions were examined histologically. Tissues from all major organs from cats that received placebo or capromorelin 60 mg/kg were evaluated by histopathology. Samples for histopathology were embedded in paraffin, sectioned to 5  $\mu$ m, stained with haematoxylin and eosin and examined by light microscopy.

#### 2.1.4 | Statistical analysis

Clinical observations and pathology results were summarized using frequency tables. Descriptive statistics (number of subjects, mean, standard deviation, standard error of the mean and minimum, median and maximum values) were presented for each parameter and time point for each dose level for body weight, clinical pathology test results and organ weights. Endpoints measured multiple times were analysed using a mixed-model repeated-measures analysis of covariance implemented by SAS/STAT Proc MIXED (SAS Institute Inc). The model included fixed effects of dose level, study day and sex and interaction terms for dose level by study day, dose level by sex, sex by study day and dose level by sex by study day. The baseline value was included in the model as a covariate. Variance component covariance structure was used. Pairwise comparisons were generated from the model using linear contrast statements. There were no adjustments for multiple comparisons. All tests of significance, except for the three-way interaction of dose level by sex by study day, were performed at  $\alpha = 0.10$ , two-sided. The interaction of dose level by sex by study day was declared statistically significant at  $\alpha = 0.05$ , two-sided. If the interaction of dose level by sex by study day was significant ( $p \leq .05$ ), then the results of the analysis were deemed inconclusive and only the qualitative analysis is presented. This occurred for potassium, platelet count, monocytes, observed reticulocytes and absolute reticulocytes. If the interaction of dose level by study day was significant ( $p \leq .10$ ), then the results for each dose level were compared to those of the control at each study day. The interaction of dose level by study day was statistically significant for CPK, AST, amylase, pH and urine specific gravity.

## 2.2 | Study 2: 91-day safety study

### 2.2.1 | Animals

Six neutered male and six intact female domestic shorthair cats (age range, 3.0–7.2 years) from the Sinclair Research Center (SRC; Auxvasse, MO) feline colony were included in the study. Cats were housed at the SRC and allowed to acclimate for 11 days prior to receiving the first study dose. All cats were offered 300 g of Purina® Cat Chow® for approximately 6 hr each day throughout the

acclimation and study drug exposure periods. Water was provided ad libitum.

Animal care and housing were in accordance with the Guide for the Care and Use of Laboratory Animals, 8th edition (National Research Council, 2011). The study was conducted in accordance with Good Laboratory Practice as set forth by the FDA 21 CFR (Part 58). The study protocol was reviewed and accepted by the facility's Institutional Animal Care and Use Committee.

### 2.2.2 | Study design

The study design is summarized in Figure 1b. Cats were randomized 2:1 into two treatment groups, with eight animals (four males and four females) assigned to receive capromorelin at a dose of 6 mg/kg (30 mg/ml solution) orally once per day and four animals (two males and two females) assigned to receive placebo (volume equivalent to a 6 mg/kg dose) orally once per day. The 6 mg/kg capromorelin dose was selected as it was likely to be several fold higher than the anticipated clinical dose, which has yet to be confirmed in cats. Cats were administered capromorelin or placebo via syringe on days 1 through 91. Initial dose was calculated using body weight measured on the day prior to first dosing, and dose was adjusted following body weight measurement on days 14, 30, 59 and 75.

Cats were offered food 1 hr after dosing, and food was removed after 6 hr. On days -11 through 90, food consumption was determined daily for all cats. During the acclimation period, baseline food consumption values were calculated using the average of days -3 through -1 for all animals. Variables of interest were clinical observations and physical examinations for general health, clinical pathology tests (including serum chemistry, haematology and urinalysis) and serum levels of GH and IGF-1.

### 2.2.3 | Clinical and laboratory assessments

General health observations were performed at least once daily throughout the study. Physical examinations were performed at the beginning of the acclimation period (day -11) and on days 1, 30, 59 and 91. Body weights were measured on days -11, -1, 1, 14, 30, 59, 75 and 91 prior to dose administration and feeding.

Haematology and serum chemistry were performed on samples collected on day -5 during acclimation and on days 30, 59 and 91 approximately 1 hr after dose administration. Animals were fasted for  $\geq 8$  hr prior to blood collection. Blood samples were collected via jugular vein and/or other appropriate vessel(s) into BD Vacutainer blood collection tubes containing K<sub>3</sub>EDTA anticoagulant for haematology or no anticoagulant for serum chemistry. Haematology parameters included WBC count, WBC differential, RBC count, WBC and RBC morphology, haemoglobin, platelet count, platelet morphology, haematocrit, mean corpuscular volume, mean corpuscular haemoglobin concentration, reticulocytes and blood smear. Serum chemistry parameters included albumin, globulin, albumin/globulin ratio, CPK, alkaline phosphatase, glucose, alanine aminotransferase, BUN, phosphorus, calcium, potassium, chloride, sodium, cholesterol, total

protein, creatinine and total bilirubin. In addition, serum samples were submitted to IDEXX Laboratories, Inc (West Sacramento, CA) for fructosamine analysis.

For the measurement of GH and IGF-1, additional blood samples were collected from each cat prior to dosing and 8 hr following treatment administration on days 1, 30, 59 and 91; hence, samples at the 0-hr time point on days 30, 59 and 91 were collected approximately 24 hr after the previous dose. Serum was processed for the measurement of GH and IGF-1 using a validated radioimmunoassay (Animal Health Diagnostic Center Endocrinology Laboratory, Cornell University College of Veterinary Medicine, Ithaca, NY).

Urinalyses were performed on samples collected on day -5 during acclimation and on days 30, 59 and 91 approximately 1 hr after dose administration. Urine samples were collected by cystocentesis or in pans/trays. Urinalysis parameters included pH, urine specific gravity, urobilinogen, colour, clarity, blood, protein, bilirubin, glucose, ketones and microscopic examination of the sediments for RBCs per high-power field, WBCs per high-power field, bacteria, epithelial cells, mucus, casts and crystals.

#### 2.2.4 | Statistical analysis

Statistical analyses were performed using SAS version 9.3 or higher, with the cat as the experimental unit. All tests of significance, except for the three-way interaction of dose level by sex by time, were performed at  $\alpha = 0.10$ , two-sided. The interaction of dose level by sex by time was declared statistically significant at  $\alpha = 0.05$ , two-sided. Assumptions of normality of residuals and homogeneity of variance were investigated for each continuous response measurement. If it was determined that the distribution could not be approximated by a normal curve, then values were ranked in ascending order, with tied values given a mean rank prior to running statistical models.

Descriptive statistics (number of subjects, mean, standard deviation, standard error of the mean and median, minimum and maximum values) were presented for each time point collected by treatment group. For continuous haematology, serum chemistry and urinalysis measurements, descriptive statistics were presented for each day by treatment group. For values collected at >1 time point, repeated-measures analysis of variance was used to test for differences between treatment groups. SAS/STAT Proc MIXED was implemented using a model containing terms for treatment group, study day and the interaction of treatment-by-study day, with the baseline value included in the model as a covariate. Possible differences between treatment groups on each study day were determined from this model. The covariance structure (variance components, compound symmetry, heterogeneous compound symmetry, first-order autoregressive or heterogeneous autoregressive structure) that provided the smallest Akaike information criterion value was used.

Analysis of variance was used to test for differences between treatment groups for parameters with one value per subject. SAS/STAT Proc MIXED was implemented using a model containing a term for treatment group, with the baseline value included in the model as a

covariate, if possible. For GH and IGF-1, differences within treatment groups were assessed by the Wilcoxon rank sum test.

For food consumption, the baseline value was defined as the mean of the values collected on Days -3 through -1 for each cat. The treatment period for each cat was the mean of the values collected each day from Days 1 through 91 during the dosing period. Note that Day 83 and Day 84 were not included in the analyses because the feeding was not consistent with the other days. Food was not removed from the cats on Day 83, and they were allowed to eat all that they wanted. As a result, they did not eat much on Day 84. The change was calculated between baseline average and treatment period average. For each animal, the rate of change was calculated as the slope of the best fit linear regression equation using SAS/STAT Proc REG with study day (values collected on Days -11 through 91) included as the independent variable.

### 3 | RESULTS

#### 3.1 | Study 1: 14-day dose-ranging safety study

##### 3.1.1 | Clinical observations

Animals in all groups gained weight during the study. There was no statistically significant treatment effect on body weight from day -7 to day 15 (Figure 2), and the pairwise comparisons were not statistically significant for any group. Abnormal clinical observations were limited to emesis, hypersalivation and lethargy/depression.

Cats that received capromorelin at a dose of 30 or 60 mg/kg experienced a greater frequency of abnormal clinical observations over 15 days compared with those that received placebo or capromorelin 9 or 15 mg/kg (Table 1). The incidence of emesis was comparable between the placebo group and the 9 mg/kg and 15 mg/kg groups, but higher in the 30 mg/kg and 60 mg/kg groups; emesis generally occurred within 2 hr after dosing. Hypersalivation was only observed in the 30 mg/kg and 60 mg/kg groups; hypersalivation primarily occurred during and immediately after dosing. Lethargy/depression, defined as reluctance to stand, was only observed for cats in the 30 mg/kg group (two cats on day 1) and the 60 mg/kg group (three cats on day 1 and one cat on day 2); lethargy/depression was most pronounced within 6 hr of dosing.

##### 3.1.2 | Haematology, serum chemistry and urinalysis

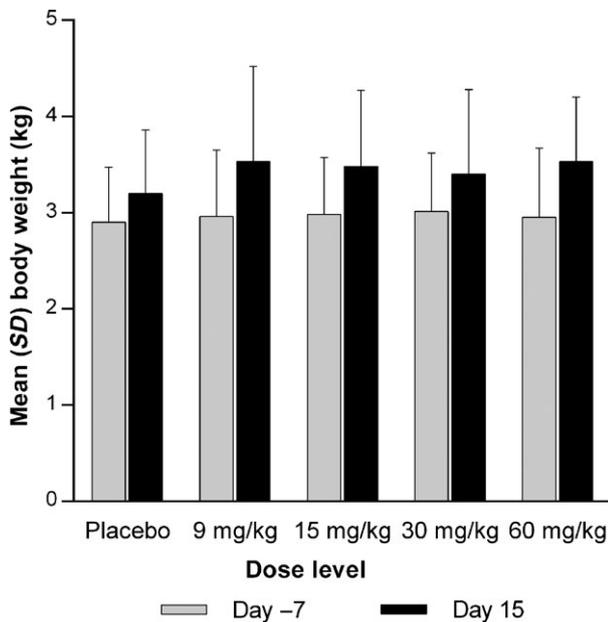
Haematology measurements were generally comparable between all capromorelin and placebo groups (Table S1). Reference ranges were not supplied by the laboratory for Study 1, so ranges from the Merck Veterinary Manual (MVM) are provided here (Aiello, 2016). However, the Study 2 ranges may be more relevant as they represent ranges in healthy laboratory cats. Occasional values outside the reference ranges for haematology parameters were reported in cats in all groups, including the placebo group, both at baseline and throughout the study. Some statistically significant differences in haematology measurements between cats that received capromorelin and

those that received placebo were observed. Additionally, the overall capromorelin treatment effect was statistically significant for elevated platelet count. However, most changes in haematology measurements remained within the corresponding reference ranges, and these changes were not considered clinically relevant. There was no significant treatment-by-study day interaction for any haematology measure. Some results were outside of the MVM reference ranges across all groups. For per cent lymphocytes and per cent eosinophils, all groups had mean values above the reference ranges at most timepoints; however, there was no treatment effect and these differences were likely due to factors unique to this population.

Serum chemistry measurements were generally comparable between all groups (Table S2). Some statistically significant differences in serum chemistry measurements were observed between cats that received capromorelin and those that received placebo; however, most changes remained within the corresponding reference ranges and were not considered clinically relevant. Based on the MVM reference ranges, results for most cats at most timepoints were above the listed ranges for ALP, CPK and phosphorus. The “elevated” ALP and phosphorus values were consistent with ranges reported for 4- to 6-month-old kittens (37-333 U/L for ALP and 6.0-10.4 mg/dl

for phosphorus) (von Dehn, 2014). The increased CPK values were likely due to struggling and traumatic venipuncture in this population of young laboratory cats. The overall capromorelin treatment effect was statistically significant for amylase, calcium, phosphorus and potassium. The treatment-by-study day interaction was statistically significant for amylase, CPK and AST. Although there was not a statistically significant treatment effect for glucose, elevated serum glucose (defined as >120 mg/dl) was reported in some of the capromorelin-treated cats: one female in the 15 mg/kg group on day 15 (190 mg/dl); one male in the 30 mg/kg group on days 8 and 15 (387 and 390 mg/dl, respectively); one female in the 60 mg/kg group on day 8 (141 mg/dl); and one male in the 60 mg/kg group on day 15 (431 mg/dl).

Urinalysis results were generally comparable between all capromorelin and placebo groups. The overall capromorelin treatment effect was statistically significant for urine specific gravity, and the treatment-by-study day interaction was statistically significant for urine specific gravity and urine pH (Table S3). Urine specific gravity was statistically significantly higher in all capromorelin-treated groups than in the placebo group on day 8, but the difference was statistically significant only for the 60 mg/kg group on day 15. Urine pH was statistically significantly lower in all capromorelin-treated groups than in the placebo group on day 8; however, only the 15 mg/kg group had a statistically significantly different urine pH compared with the placebo group (7.42 vs 6.58, respectively) on day 15. All cats had negative bilirubin and ketone tests throughout the study, with the exception of two males in the 15 mg/kg group and one male in the 60 mg/kg group that had trace ketone levels (5 mg/dl) on day 15. Some cats in all groups had transient elevations in pH and protein throughout the study, including pretreatment. Additionally, some cats in all groups had sporadic positive tests for occult blood, RBCs, WBCs, epithelial cells, bacteria and crystals throughout the study, including pretreatment. Glycosuria was reported in one male on day 8 and one male on days 8 and 15 in the 30 mg/kg group, which corresponded with elevated serum glucose in the second cat on both days. Glycosuria was also reported in two females on day 8 and one male on days 8 and 15 in the 60 mg/kg group, which corresponded with elevated serum glucose in the male on day 15 only.



**FIGURE 2** Mean body weight on days -7 and 15 in cats treated with capromorelin or placebo in Study 1

**TABLE 1** Abnormal clinical observations in cats treated with placebo or capromorelin 9, 15, 30 or 60 mg/kg once daily for 14 days in Study 1

Event, n of cats*	Capromorelin daily dose				
	Placebo n = 6	9 mg/kg n = 6	15 mg/kg n = 6	30 mg/kg n = 6	60 mg/kg n = 6
Emesis	1 (2)	2 (1-2)	0	5 (2-4)	6 (4-10)
Hypersalivation	0	0	0	6 (1-2)	4 (1-3)
Lethargy/depression	0	0	0	2 (1)	4 (1)

\*Data in parentheses are the numbers of days on which adverse events were observed.

### 3.1.3 | Necropsy and histology

The overall capromorelin treatment effect was statistically significant for absolute weight and relative weight of the liver (absolute,  $p = .048$ ; relative,  $p = .036$ ) and right adrenal gland (absolute,  $p = .064$ ; relative,

$p = .095$ ). For both the liver and right adrenal gland, the mean organ weight and mean organ weight relative to body weight were greater in each capromorelin-treated group than in the placebo group. Gross pathology findings were limited to one cat with a pale thymus (normal on histopathology) and one cat with a cryptorchid testis (degenerative on histopathology) in the placebo group and one cat with diffusely mottled lungs (congestion on histopathology) and an enlarged urinary bladder (normal on histopathology) in the 60 mg/kg capromorelin group. Histopathology findings were infrequent overall, generally of minimal or mild severity and similarly distributed between the capromorelin and placebo groups (summarized in Table S4).

## 3.2 | Study 2: 91-day safety study

### 3.2.1 | Clinical observations

All animals maintained or increased body weight during the study. Both the overall capromorelin treatment effect and the treatment-by-study day interaction for body weight were statistically significant ( $p < .0001$ ). The capromorelin-treated group had statistically significant increases in mean body weight from day 1 at day 14 ( $p = .003$ ), day 30 ( $p = .001$ ), day 59 ( $p < .0001$ ), day 75 ( $p < .0001$ ) and day 91 ( $p < .0001$ ) compared with the placebo-treated group, whose mean body weight was stable (Figure 3a). The change from baseline food consumption was larger for capromorelin-treated cats than for placebo-treated cats; however, neither the overall treatment effect nor the analysis of the rate of change was statistically significant ( $p = .091$  and  $p = .057$ , respectively; Figure 3b).

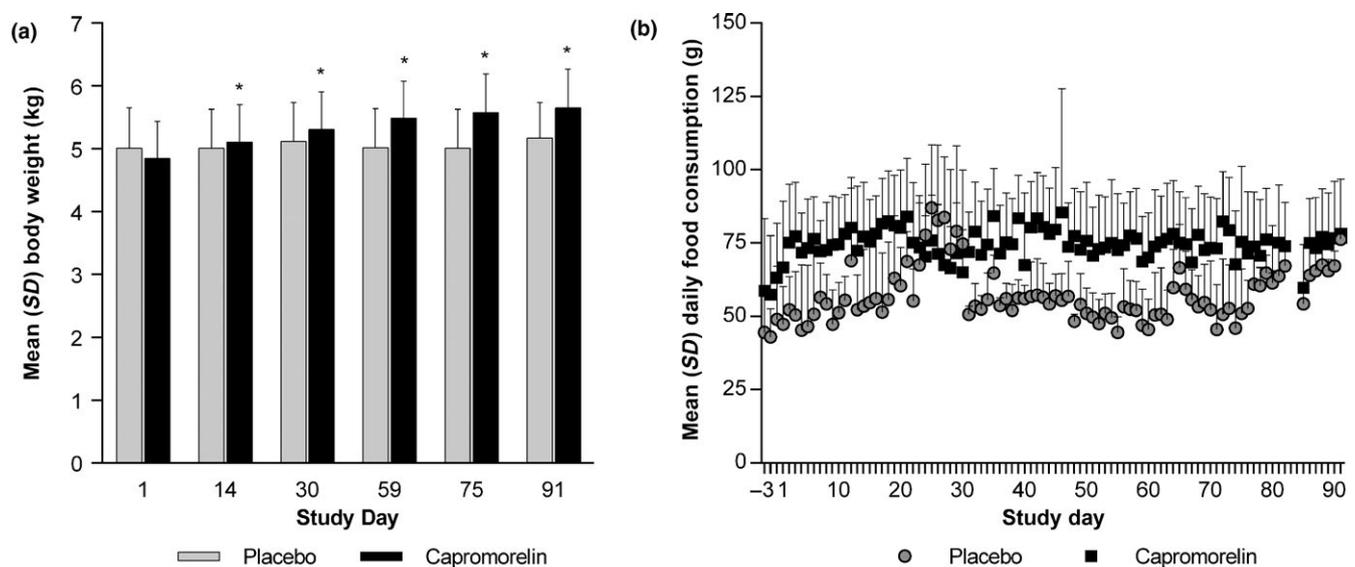
Abnormal clinical observations were limited to emesis, hypersalivation, head shaking and lip smacking, all of which occurred immediately after dosing and generally resolved within 5 min. Cats that received capromorelin 6 mg/kg experienced a greater frequency of

abnormal clinical observations compared with those that received placebo (Table 2). In particular, six cats (75%) in the capromorelin group experienced hypersalivation on a range of 1–89 days, whereas one cat (25%) in the placebo group experienced hypersalivation on 1 day. Similarly, eight cats (100%) in the capromorelin group displayed lip smacking on a range of 4–82 days, whereas three cats (75%) in the placebo group displayed lip smacking on a range of 1–2 days.

### 3.2.2 | Haematology, serum chemistry and urinalysis

Haematology measurements were generally comparable between the placebo and capromorelin groups (Table S5). The overall capromorelin treatment effect was statistically significant for per cent neutrophils ( $p = .002$ ), absolute lymphocytes ( $p = .002$ ) and per cent lymphocytes ( $p = .003$ ). Statistically significant treatment-by-study day interactions were observed for RBCs ( $p = .005$ ), haemoglobin ( $p = .003$ ), haematocrit ( $p = .005$ ), absolute eosinophils ( $p = .067$ ) and per cent eosinophils ( $p = .010$ ).

Serum chemistry measurements were also generally comparable between the groups (Table S6). The overall capromorelin treatment effect was statistically significant for potassium ( $p = .090$ ), glucose ( $p = .001$ ) and fructosamine ( $p = .034$ ). Although cats that received capromorelin had higher mean levels of glucose at all time points, individual glucose values did not exceed the reference range (64–170 mg/dl), with a maximum recorded value of 151 mg/dl in a capromorelin-treated cat on day 59. Similarly, the overall capromorelin treatment effect was statistically significant for fructosamine, with higher mean fructosamine levels observed in the placebo-treated group than in the capromorelin-treated group. Individual fructosamine values did not exceed the reference range (171–349  $\mu\text{mol/L}$ ), with a maximum recorded value of 264  $\mu\text{mol/L}$  in a capromorelin-treated cat and 269  $\mu\text{mol/L}$  in a placebo-treated cat, both observed on day 91.



**FIGURE 3** Mean (a) body weight ( $\pm$ SD) and (b) food consumption ( $\pm$ SD) over time in cats treated with capromorelin 6 mg/kg or placebo in Study 2. \*Change from baseline in body weight was significantly different ( $p < .05$ ) in the capromorelin group compared with that in the placebo group

Statistically significant treatment-by-study day interactions were observed for sodium ( $p = .042$ ), creatinine ( $p = .025$ ), albumin/globulin ratio ( $p = .015$ ) and cholesterol ( $p = .020$ ).

There were no statistically significant overall treatment effects or treatment-by-study day interactions observed for urine pH or urine specific gravity throughout the study (Table S7), and total urine protein measurements were consistent throughout. Urinalysis results for all cats were negative for WBCs, casts and ketones throughout the study, with the exception of one cat in the capromorelin group that had trace ketones on day 91. Most cats in both groups had few to moderate numbers of crystals throughout the study, including pretreatment. Some cats in both groups were transiently positive for low numbers of RBCs (1–3 cells per high-power field), rare squamous epithelial cells, low to moderate levels of bacteria and trace or low levels of bilirubin

**TABLE 2** Abnormal clinical observations in cats treated with placebo or capromorelin 6 mg/kg once daily for 91 days in Study 2

Event, n of cats*	Placebo n = 4	Capromorelin 6 mg/kg n = 8
Emesis	2 (1)	6 (1–2)
Hypersalivation	1 (1)	6 (1–89)
Head shaking	0	3 (1)
Lip smacking	3 (1–2)	8 (4–82)

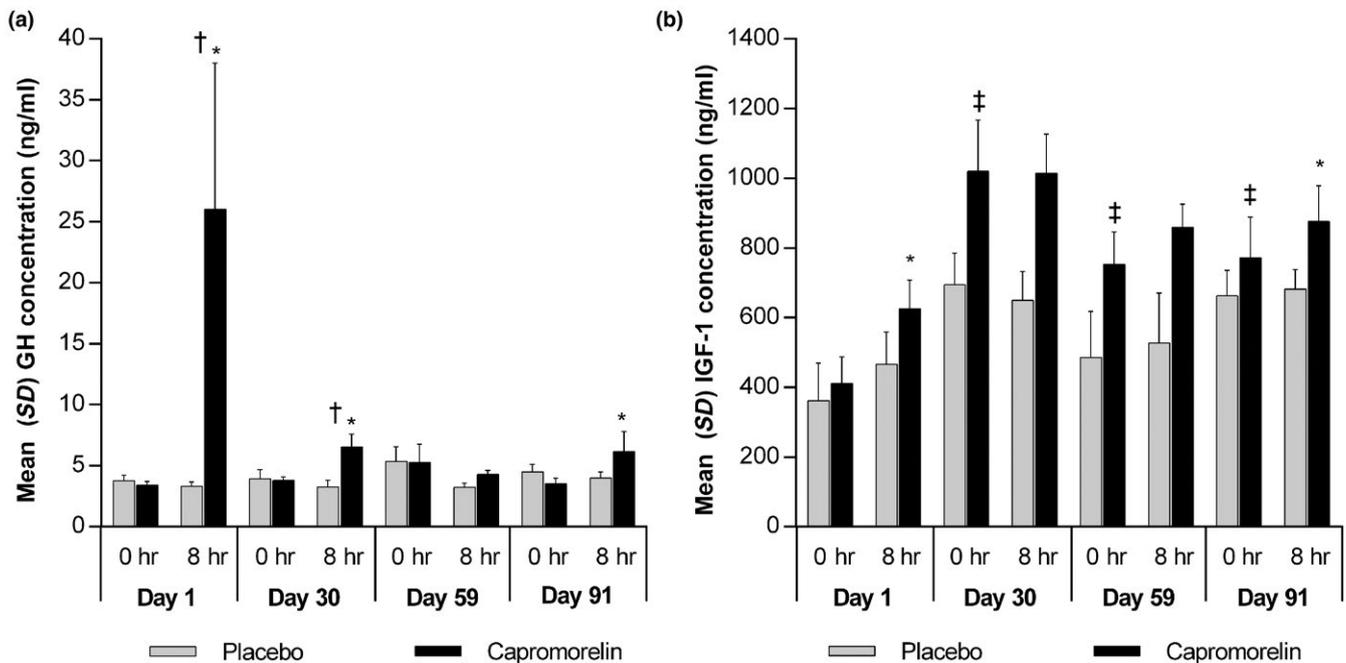
\*Data in parentheses are the numbers of days on which adverse events were observed.

and blood during the study, including pretreatment. Trace glycosuria was observed in one capromorelin-treated cat on days 30 and 91 and in one placebo-treated cat on day 30.

### 3.2.3 | Serum GH and IGF-1

Cats treated with capromorelin had statistically significant increases in mean serum GH from 0 hr to 8 hr on day 1 ( $p = .016$ ), day 30 ( $p = .039$ ) and day 91 ( $p = .023$ ); the greatest capromorelin-stimulated increase in GH was observed on day 1 (Figure 4a). No statistically significant differences in GH levels were found in the placebo group between the 0-hr and 8-hr time points on any day. When only 0-hr results for serum GH were analysed for the overall study, neither overall treatment effect nor treatment-by-study day interaction were statistically significant. Cats in the capromorelin group had significantly higher increases in mean GH from 0 hr to 8 hr on day 1 ( $p = .031$ ) and day 30 ( $p = .007$ ) compared with those in the placebo group.

Cats treated with capromorelin had statistically significant increases in mean serum IGF-1 from 0 hr to 8 hr on day 1 ( $p = .008$ ) and day 91 ( $p = .016$ ; Figure 4b). No statistically significant differences in IGF-1 levels were found in the placebo group between the 0-hr and 8-hr time points on any day. Cats in the capromorelin group, but not in the placebo group, had significantly higher mean serum IGF-1 levels at 0 hr on days 30, 59 and 91 ( $p = .008$  for all) than at 0 hr on day 1. When only 0-hr results were analysed for the overall study, the overall treatment-by-study day interaction was statistically significant ( $p = .022$ ). However, the differences in mean IGF-1 levels at 0 hr



**FIGURE 4** Mean serum (a) GH and (b) IGF-1 levels predosing (0 hr) and 8 hr after dosing in cats treated with capromorelin 6 mg/kg or placebo in Study 2. GH, growth hormone; IGF-1, insulin-like growth factor 1. \*Mean value was significantly greater at 8 hr ( $p < .05$ ) than at 0 hr on the same study day within the treatment group. †Change from 0 hr to 8 hr was significantly greater ( $p < .05$ ) in the capromorelin group compared with that in the placebo group on the same study day. ‡Mean value was significantly greater at 0 hr on a given study day ( $p < .05$ ) than at 0 hr on day 1 within the treatment group

between the placebo and capromorelin groups on any study day were not statistically significant.

## 4 | DISCUSSION

The results of these studies provided preliminary safety information on the daily administration of capromorelin over a range of doses and for an extended period in cats. Abnormal clinical observations were mild and included emesis, hypersalivation, lethargy/depression, head shaking and lip smacking. In Study 1, in which capromorelin was administered in an oral capsule formulation, abnormal clinical observations generally occurred immediately upon dosing to <6 hr after dosing and were primarily observed in cats that received 30 or 60 mg/kg of capromorelin. In Study 2, in which capromorelin was administered in a liquid formulation, abnormal clinical observations occurred immediately upon dosing and generally resolved within 5 min. Although there were some statistically significant differences in mean haematology and serum chemistry parameters between cats in treatment groups receiving capromorelin and those receiving placebo in both studies, most changes remained within the corresponding reference ranges and were not considered clinically significant; urinalysis results were comparable between the treatment groups.

Sporadic increases in serum and urine glucose were observed in capromorelin-treated cats during both studies. Based on the sporadic incidences of elevated glucose in Study 1, it was unclear whether the elevated glucose results were secondary to stress or represented persistent hyperglycaemia (Feldhahn, Rand, & Kinnaird, 1999; Kojima et al., 2000; Rand, Kinnaird, Baglioni, Blackshaw, & Priest, 2002). Findings from Study 1 prompted the inclusion of measurement of fructosamine as part of the serum chemistry panel in Study 2, as elevated serum fructosamine indicates prolonged hyperglycaemia. In Study 2, increased serum glucose was not accompanied by an increase in fructosamine, and fructosamine levels did not exceed the reference range. These data suggest that glucose elevations above the reference range in Study 1 may have been the result of transient hyperglycaemia associated with stress. Further study on the effects of capromorelin on serum glucose and fructosamine in a larger population of cats may aid in understanding the effect of capromorelin on glucose metabolism.

Increases in GH and IGF-1 are expected with capromorelin treatment (Hersch & Merriam, 2008; Zollers, Rhodes, & Smith, 2017). Chronically elevated GH and IGF-1 levels in cats are often a sign of hypersomatotropism (acromegaly) (Berg et al., 2007; Peterson et al., 1990); however, few studies have evaluated GH and IGF-1 levels in a large number of healthy cats (Tschour et al., 2012). Based on the overall results in the placebo-treated cats in Study 2, the observed ranges were 2.10–7.97 ng/ml for GH and 120–956 ng/ml for IGF-1 (reference ranges were not available from the laboratory). In capromorelin-treated cats, GH was markedly increased after the first dose, as expected. Following treatment, statistically significant differences in GH levels between the capromorelin and placebo groups were observed on day 1 and to a much lesser extent on day 30. Statistically

significant increases in IGF-1 8 hr after dosing were observed only on days 1 and 91 in the capromorelin group; however, statistically significant increases in IGF-1 were seen at 0 hr on days 30, 59 and 91 compared with 0 hr at day 1, indicative of chronic, consistent increases in IGF-1 levels. Presumably, negative feedback from these sustained elevated levels of IGF-1 attenuated capromorelin stimulation of GH release over time.

This situation is markedly different from that of cats with hypersomatotropism, in which the pituitary tumour persistently secretes GH, resulting in chronically elevated serum GH in addition to elevated IGF-1 levels (Berg et al., 2007; Peterson et al., 1990). An IGF-1 measurement >1000 ng/ml is generally used to aid in the diagnosis of hypersomatotropism (Niessen et al., 2007, 2015; Tschour et al., 2012). Some capromorelin-treated cats in Study 2 had IGF-1 levels >1000 ng/ml at some time points, with a maximum value of 1498 ng/ml recorded in this study (predosing on day 30). Interestingly, the maximum IGF-1 value observed in a placebo-treated cat (956 ng/ml) approached the 1000-ng/ml threshold. In hypersomatotropism, a tumour produces excess GH, which is resistant to negative feedback from IGF-1. Thus, the significance of constitutively increased IGF-1 in the context of down-regulated GH is unknown.

Postmortem evaluations in Study 1 did not reveal any toxic effects of capromorelin treatment. It is unknown whether the statistically significant increase in liver weight observed with capromorelin treatment is clinically significant. Histopathology showed hepatocellular vacuolation in six of six cats treated with placebo and five of six cats treated with capromorelin 60 mg/kg. Thus, no treatment-related changes were evident on histopathologic evaluation of the liver. The statistically significant increase in weight of the right adrenal gland observed with capromorelin treatment is unlikely to have any clinical significance.

One limitation of these studies is the lack of capromorelin pharmacokinetics in cats. However, biologically active levels of capromorelin were clearly achieved because the observed capromorelin-stimulated increases in serum GH and IGF-1 demonstrated the expected physiological activity of capromorelin.

These initial safety studies were performed in healthy laboratory cats under controlled conditions of food intake. Although it is encouraging that no serious adverse events were observed, clinical studies in client-owned cats will be required to demonstrate safety in the target population.

Capromorelin is FDA approved for appetite stimulation in dogs and has been shown to result in increased food consumption and body weight in laboratory Beagle dogs after as few as 4 days of treatment with capromorelin 3 mg/kg oral solution for dogs (Zollers, Rhodes, & Heinen, 2017). However, in Study 1, there was no statistically significant treatment effect of capromorelin on body weight, although the cats were  $\geq 6$  months of age at the start of the study and all cats gained weight from day -7 to day 15. Given the small group size, the study was likely underpowered to detect treatment-related differences in weight gain in growing kittens. In Study 2, treatment with capromorelin resulted in statistically increased body weight over 91 days. Although mean food consumption was greater

in capromorelin-treated cats than in placebo-treated cats in Study 2, there was no statistically significant treatment effect of capromorelin on food consumption. It is likely that due to the small group sizes, this study was underpowered to detect a statistically significant treatment effect on food consumption in the controlled conditions of the laboratory setting and the limited time (6 hr each day) during which food was available to the cats. It is also possible that, in contrast to dogs, healthy laboratory cats may more readily self-regulate food consumption.

Capromorelin was well-tolerated in healthy laboratory cats that received 9, 15, 30 or 60 mg/kg once daily for 14 consecutive days or 6 mg/kg once daily for 91 consecutive days, although gastrointestinal effects were observed more frequently in cats in the 30 mg/kg and 60 mg/kg groups. The doses used in these studies were  $\geq 2$  times the FDA-approved clinical dose of capromorelin 3 mg/kg oral solution (ENTYCE®, Aratana Therapeutics, Inc) used for stimulating appetite in dogs. The clinical dose of capromorelin in cats has yet to be confirmed. Given its mechanism of action, capromorelin has the potential to be clinically useful in a variety of medical conditions in cats in which inappetence and/or weight loss are factors. Further studies are planned to investigate the safety of capromorelin at elevated doses for a longer duration in laboratory cats and the safety and effectiveness of capromorelin in client-owned cats.

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## CONFLICT OF INTERESTS

All authors except Margie Bell are current or previous employees of Aratana Therapeutics, Inc. All authors except Margie Bell are stockholders in Aratana Therapeutics, Inc. Margie Bell has no conflicts of interest.

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## SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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