A randomised, placebo controlled study to investigate the safety, tolerability, pharmacokinetics and pharmacodynamics of G3215 in adult subjects

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adm	administration
ADR	Adverse drug reaction
AE	Adverse event
ALK	Alkaline phosphatase
ALT	Alanine aminotransferase
API	Active pharmaceutical ingredient
aPTT	Activated partial thromboplastin time
AST	Aspartate aminotransferase
AUC	Area under the plasma concentration-time curve
BMI	Body mass index (kg/m^2)
CI	Confidence interval
C_{max}	Maximum plasma concentration
CRF	Case report form
CRP	C-reactive protein
CV	Coefficient of variation
ECG	Electrocardiogram
EDC	Electronic data capture
EMA	European Medicines Agency
EU	European Union
FDA	Food and Drug Administration
FIH	First in human
FT4	Free thyroxine
GCP	Good Clinical Practice
GGT	Gamma glutamyl transpeptidase
GLP	Good Laboratory Practice
GLP-1	Glucagon-like peptide-1
GMP	Good Manufacturing Practice
h	Hour
HBsAg	Hepatitis B surface antigen
HED	Human equivalent dose
hERG	Human ether-a-go-go related gene
HIV	Human immunodeficiency virus
IB	Investigator's brochure
ICF	Informed consent form
ICH	International Conference on Harmonization
IMP	Investigational medicinal product
IMPD	Investigational medicinal product dossier
IUD	Intrauterine device
IUS	Intrauterine system
LDH	Lactate dehydrogenase
MABEL	Minimum anticipated biological effect level
MAD	Multiple ascending dose

Max	Maximum
MHRA	Medicines and Healthcare products Regulatory Agency
Min	Minimum
MRSD	Minimum recommended starting dose
MTD	Maximum tolerated dose
NOAEL	No observed adverse effect level
OGTT	Oral glucose tolerance test
OXM	Oxyntomodulin
PD	Pharmacodynamic
PK	Pharmacokinetic
PP	Pancreatic polypeptide
PT	Prothrombin time
PTFE	Polytetrafluoroethylene
PYY	Peptide YY
QMS	Quality management system
QP	Qualified person
R_o	Extent of accumulation in plasma
SAD	Single ascending dose
SAE	Serious adverse event
SC	Subcutaneous
SD	Standard Deviation
SEM	Standard Error of the Mean
SGLT-2	Sodium-Glucose co-transporter 2
SI	Statutory Instrument
SUSAR	Suspected unexpected serious adverse reaction
$t_{½}$	Apparent terminal half life
t_{max}	Time to maximum plasma concentration
TSH	Thyroid stimulating hormone
UK	United Kingdom
US	United States of America
VAS	Visual analogue scale
WHO DD	World Health Organization Drug Dictionary
Y1r	Neuropeptide Y subtype 1 receptor
Y2r	Neuropeptide Y subtype 2 receptor
Y4r	Neuropeptide Y subtype 4 receptor
Y5r	Neuropeptide Y subtype 5 receptor
λ_z	Apparent terminal rate constant

1 INTRODUCTION

A powerful and complex physiological system exists in humans to ensure that adiposity and body weight remain constant despite variations in daily food intake and energy expenditure [1]. Daily energy intake is determined not only by the composition of the food being eaten but also by an individual's appetite, which can be considered as their drive to eat. The level of appetite is controlled predominantly by peptide hormones released in the gut. In particular, pancreatic polypeptide (PP), glucagon-like peptide-1 (GLP-1), oxyntomodulin (OXM) and peptide YY (PYY) increase satiety and therefore reduce food intake following a meal [2-7]. However, excessive appetite and the easy availability of highly calorific foods with enhanced hedonic properties can lead to an increase in body weight and the potential for the development of obesity.

Obesity is defined as a body mass index (BMI) of more than 30 kg/m^2. Obesity reduces life expectancy and increases the incidence of cardiovascular disease, diabetes, certain cancers and depression [8,9].

In 2008, about 24% of men and 25% of women in the United Kingdom were obese [10]. The prevalence of obesity appears to be increasing and a recent estimate indicated that obesity would affect up to 33% of the US adult population [11].

Advice on dietary and lifestyle changes has been ineffective in reducing the incidence of obesity. Currently, two general approaches to treatment are available: first, the use of oral medication (orlistat) to reduce fat absorption from the diet, and, second, the use of surgical interventions. However, both approaches are subject to potential problems regarding efficacy or safety. The use of orlistat is limited by side effects and its effect on body weight is relatively short-lived [12]. Other drugs, e.g. sibutramine and rimonabant, have recently been withdrawn from marketing in Europe due to side effects [13]. Surgical interventions e.g. gastric bypass and band surgery can be effective, but also carry a risk of death to the patient, and are expensive, being dependent on the availability of specialist surgeons and support facilities.

1.1 Background Information for G3215

G3125 is an analogue which combines the actions of two important hormones, glucagon, which increases energy expenditure, and GLP-1, which inhibits appetite and enhances insulin release, similar to the gut peptide oxyntomoduin.

G3215 was selected from 2,000 G analogues that were screened for human GLP-1 and glucagon receptor binding, and for receptor activation via cAMP activity. Bioactivity was measured via its anorectic and weight-reducing effect in mice and rats. The amino acid sequence of G3215 is based on human Oxyntomodulin, with the following features:

- Deletion of 2 residues;

- Substitution of 14 other residues within the 35-residue peptide, with the receptor active sequence of oxyntomodulin being unchanged;
- Composition wholly of natural L-amino acids without derivatisation or cross linking.

For further information please refer to the Investigator's Brochure (IB) [14].

1.2 Risk Assessment

The amino acid sequence of G3215 is similar to that of OXM. Therefore indications of potential safety issues related to the administration of G3215 can be predicted from review of the effects of OXM in humans.

Oxyntomodulin was infused intravenously into 13 human volunteers at a dose of 3.0 pmol/kg/min (6.6 ng/kg/min) for 90 minutes (total dose 594 ng/kg). At this dose, there was no nausea reported by subjects (4).

Oxyntomodulin was subsequently studied in healthy overweight and obese volunteers. Fourteen volunteers were treated with 400 nmol (1.77 mg, approximately 0.019 mg/kg) of oxyntomodulin, administered subcutaneously 3 times a day before meals, for 28 days. Transient mild nausea was reported with 3% of oxyntomodulin injections compared to 0.2% of placebo injections. Although a transient mild discomfort at the injection site was reported with 6.9% of oxyntomodulin injections, this was also reported in 6.6% of saline injections, and this adverse effect was not considered linked to the oxyntomodulin injection. No significant effects on cardiovascular parameters (blood pressure, heart rate) were detected when oxyntomodulin was given (22).

In a placebo-controlled cross-over study in 15 overweight/obese volunteers, the volunteers self-administered subcutaneously 400 nmol (1.77 mg or approximately 0.019 mg/kg) of oxyntomodulin three times a day before meals for 4 days. Only one subject reported nausea, as assessed by visual analogue scales, and this was associated with significantly higher plasma levels of oxyntomodulin, implying that nausea is related to drug exposure. No statistically significant effects on cardiovascular parameters (blood pressure, heart rate) were detected when oxyntomodulin was given (21).

In a study which examined the effects of the combination of peptide YY and oxyntomodulin when given as intravenous infusions for 110 minutes to 12 volunteers, oxyntomodulin alone was given in two arms of the study, one arm receiving a low dose (1.5 pmol/kg/min or 6.6 ng/kg/min, 726 ng/kg total dose) and another arm receiving a high dose (3.0 pmol/kg/min or 13.2 ng/kg/min, 1452 ng/kg total dose). Although some reduction in food intake was seen in these arms, this was not statistically significant from placebo. One subject receiving the high dose of oxyntomodulin reported nausea. The infusion was stopped and the nausea settled. No statistically significant effects on cardiovascular parameters (blood pressure, heart rate) were detected when oxyntomodulin was given (23).

The placebo-controlled Phase I clinical trial sponsored by Merck, Sharp and Dohme (Clinicaltrials.gov NCT01373450) reported that of the 12 participants receiving the oxytomodulin infusion (3.0 pmol/kg/min for 160 mins, 13.2 ng/kg/min, 1452 ng/kg total dose), 0 had serious adverse effects, and 6 had other adverse effects. None reported nausea. 1/12 participants had a catheter site adverse reaction (versus 2/12 for placebo), 2/12 reported fatigue (versus 1/12 for placebo), 1/12 reported peripheral oedema (versus 0/12 for placebo), 1/12 reported rhinitis (versus 0/12 for placebo), 2/12 reported headache (versus 3/12 for placebo).

In summary, most studies examining the effects of oxyntomodulin have investigated its effects over a relatively short timespan, the notable exception being (22). The most commonly reported adverse effect of oxyntomodulin is that of nausea. The nausea appears to be related to drug exposure (C_{max}), and transient. There appear to be no significant cardiovascular adverse effects noted with oxyntomodulin treatment.

In view of the testicular findings from the repeat dose toxicology study, all study volunteers will have LH, FSH and testosterone monitored pre and post dosing. In Part A, the post dose test will be done at the follow up visit. In Part B, the post dose test will be done at Day 15, 29 and at the final follow up visit.

As the SAD study (Part A) was a FIH study a sentinel dosing strategy was used for the first dosing group. Safety data up to and including 24 h postdose was reviewed by the Investigator prior to dosing the remaining subjects at that dose level 48 h later. Dose escalation only occurred if the previous dose level was deemed to be safe and well tolerated.

With the completion of the Part A (SAD) study, G3215 has now been tested in humans, and found to be well tolerated in single doses of up to 48 mg. See Section 12 for study report from the SAD study. As per Section 12, the MTD was not reached during the Part A study where a maximum dose of 48 mg was tested.

Administration of subsequent dose levels will be based on review of available safety, tolerability and PK data from previous doses. As with Part A, progression to the next higher dose will only occur if the previous dose level was deemed to be safe and well tolerated and the single doses have been shown to be safe and well tolerated in Part A. In Part B and Part C, dose titration for an individual subject may be necessary within a dosing regimen depending on the activity profile and tolerability. The highest doses in Part B and Part C will not exceed the maximum dose of 48 mg tested in the SAD study.

The study will be conducted in compliance with United Kingdom (UK) regulations and guidance, EU and ICH GCP, GMP, and current GLP. The study requires clinical trial authorisation and National Research Ethics Service approval.

The study will be conducted on an in-patient basis, with dosing in a Phase I unit with supplementary accreditation from the Medicines and Healthcare Products Regulatory

Agency (MHRA). The Principal Investigator and the investigating team will be experienced in the administration of novel molecules to man for the first time.

There is no known antidote for G3215. Full resuscitation facilities and emergency medication will be available at all times.

1.3 Urgent safety measures

In accordance with UK Law (Medicines for Human Use [Clinical Trials] as amended: Statutory Instrument [SI] 1031 Part 4 Section 30) the Sponsor and Investigator may take appropriate urgent safety measures in order to protect the subjects of a clinical trial against any immediate hazard to their health or safety. If such measures are taken the Sponsor shall immediately (no later than 3 days from the date the measures are taken) give written notice to the licensing authority and the relevant ethics committee of the measures taken and the circumstances giving rise to those measures.

STUDY OBJECTIVES

1.4 Primary Objective

- To investigate the safety and tolerability of single doses of G3215 in overweight/obese but otherwise healthy male subjects.
- To investigate the safety and tolerability of multiple doses of G3215 in overweight/obese male subjects with normal glucose tolerance, Type 2 diabetes or prediabetes via weekly subcutaneous injections.
- To investigate the safety and tolerability of sequentially incrementing doses of G3215 in overweight/obese male subjects with normal glucose tolerance, Type 2 diabetes or prediabetes via a three day subcutaneous infusion.

1.5 Secondary Objectives

- To assess the pharmacokinetic (PK) profile of single ascending doses of G3215 in overweight/obese but otherwise healthy male subjects via subcutaneous injection.
- To assess the PK profile of multiple ascending doses of G3215 in overweight/obese male subjects with normal glucose tolerance, Type 2 diabetes or prediabetes via weekly subcutaneous injections.
- To investigate the PK profile of a three day subcutaneous infusion of G3215 in overweight/obese male subjects with normal glucose tolerance, Type 2 diabetes or prediabetes.

1.6 Exploratory Objectives

- To investigate the effects of multiple doses of G3215 on food consumption, body weight, enteropancreatic hormone changes and glucose tolerance in overweight/obese male subjects with normal glucose tolerance, Type 2 diabetes or prediabetes.
- To assess the analytical performance of the Imperial College radioimmunoassay for G3215 compared with the LC-MS/MS assay for G3215.

2 INVESTIGATIONAL PLAN

2.1 Overall Study Design

This is a single centre, randomised, placebo controlled Phase I first time in human study of G3215 in 3 parts: Part A (single ascending dose – SC administration) and Part B (multiple ascending dose – weekly SC administration) and Part C (ascending doses – via continuous SC infusion).

2.1.1 Part A (Single Ascending Dose)

Part A was a double blinded, randomised, placebo controlled, single ascending dose study (SAD) and has been completed as of the time of writing of the version of this protocol. The following reflects the plan prior to execution of Part A. Approximately 28 eligible subjects will be enrolled in 5 sequential cohorts of overweight but otherwise healthy male subjects (BMI range of 25 to 38 kg/m^2). No subject will be a member of more than 1 cohort.

Cohort 1 will comprise 4 subjects (3 active and 1 placebo). In cohort 1, each volunteer will be dosed in three treatment periods (TPs) with three ascending dose levels (proposed doses 0.1 mg, 0.5 mg, 1.5 mg G3215). The subjects will be randomised to either placebo or active treatment at each of these visits, i.e. different subjects will receive placebo in each TP. There will be a minimum washout period of 1 week between TPs. If any subject in Cohort 1 drops out between doses, they will be replaced.

Cohort 2 onwards will comprise 6 subjects (5 active and 1 placebo) for each cohort. Each volunteer in Cohort 2 onwards will only be dosed once in one TP. Proposed doses for cohorts 2-5 are 4 mg, 10 mg, 20 mg and 40mg. All doses are subject to change according to safety and PK review.

Up to eight additional cohorts of up to 6 subjects may be enrolled if recommended by the Safety Committee (see Section 2.1.4) to increase scientific value of this study, e.g. to verify PK data or further characterise PD markers.

Proposed doses of 0.1 mg, 0.5 mg, 1.5 mg, 4.0 mg, 10 mg and 20 mg and 40mg G3215 will be investigated in ascending order. Randomisation for each dose level will be generated prior to study start.

For each dose level, subjects will be randomised to receive a single dose of G3215 or matching placebo by SC injection (with fractionation if needed). A sentinel dosing strategy will be used as outlined in Table 2.1.

Table 2.1 Dosing Schedule: Part A

Cohort	Treatment Periods	Dosing Session	Number of Subjects	
			G3215	Placebo
1	1	Sentinel subjects	1	1
		Non-sentinel subjects	2	0
1	2-3	Sentinel subjects	0	0
		Non-sentinel subjects	3	1
2 onwards	1	Sentinel subjects	0	0
		Non-sentinel subjects*	20	4

*May be increased if additional groups are dosed

In Cohort 1, TP1, the first two subjects will be randomised such that 1 subject receives G3215 and 1 subject receives placebo. This sentinel pair will be dosed first and will be observed for at least 48 h before study drug is administered to the remainder of the cohort. Safety (vital signs, ECGs, and clinical laboratory safety tests), and tolerability (adverse event profile) data up to and including 24 h postdose will be reviewed by Covance's clinical team prior to dosing the remaining subjects at that dose level. Dosing of the subsequent subjects in cohort 1 will be at the discretion of the Principal Investigator.

Treatments will be administered double blinded (except for Cohort 1, TP1 where it will be known that the 3rd and 4th subjects will receive the active dose); subjects will be blinded with regard to treatment and clinical staff will remain blinded with regard to treatment until the Safety Committee meeting.

An interval of at least 7 days will separate the dosing of the last subject in one cohort and dosing of the first subject in the next cohort to permit a timely review and evaluation of interim safety, tolerability and PK data. PK data will be reviewed up to 48hours post-dose prior to the dose escalation decision. If Tmax is significantly later than predicted, PK data to a later timepoint may be reviewed prior to the dose escalation decision. The data of up to at least 3 subjects receiving G3215 in cohort 1 and 4 subjects, (i.e. a minimum of 3 subjects receiving G3215) in cohort 2 onwards will be reviewed before dose escalation.

Following a 28-day screening period eligible subjects will be admitted to the clinical unit on Day -1 (1 day prior to dosing) for eligibility checks and baseline assessments. On Day 1 subjects will receive a single dose of G3215 or placebo by SC injection, and will remain in the clinical unit under medical supervision until 72 h postdose (Day 4) or until C_{max} is achieved, whichever is the later.

Safety monitoring will include assessment of adverse events (AEs), physical examination, 12-lead ECGs, vital signs (blood pressure pulse rate and body temperature) and clinical laboratory safety tests (serum biochemistry, haematology, coagulation, thyroid stimulating hormone [TSH], FT4, LH, FSH, testosterone and urinalysis).

Blood samples for biomarker (tryptase) will be collected immediately after any possible allergic or anaphylactic adverse reaction, and 12 hours later. These samples will only be analysed if relevant adverse reactions occur.

The PK evaluation will include measurement of plasma G3215 levels and associated PK parameters.

Exploratory PD evaluation will include evaluation of visual analogue scales (VAS) of nausea and satiety. Subjects in Cohort 3 onwards will undergo food intake studies on Day -1 and Day 1. The timing of the food intake study may be modified according to emerging PK data.

Blood samples for immunogenicity assays will be collected predose and at the final follow-up visit for the volunteers from Cohort 1 only (as these volunteers have received multiple doses of G3215).

All subjects will return for a follow up visit 14 days (+/- 1 day) after the final dose.

Following discharge of subjects and depending on emerging PK data, the subjects (except cohort 1, dose level 1 and 2) may return to the clinical unit for further outpatient visits . The number of additional visits per subject will not exceed six and will not extend beyond 8 weeks after each final dosing occasion. If additional outpatient visits are scheduled the final outpatient visit will be considered to be the final follow up visit where the follow-up visit assessments will be performed.

2.1.2 Part B (Multiple Ascending Dose)

Part B will be a double blind, randomised, placebo controlled, multiple ascending dose (MAD) study in sequential groups of male subjects (BMI range 25-38 kg/m^2). These subjects can either have normal glucose tolerance, prediabetes (impaired glucose tolerance or impaired fasting glucose), or Type 2 diabetes, defined according to WHO 2006 and 2011 diagnostic criteria. If the subject is diagnosed with diabetes or prediabetes, this condition should be stably controlled either with:

1. diet alone;
2. monotherapy with a sulphonylurea, metformin or SGLT-2 inhibitor;
3. dual therapy with any two of the following drug classes: sulphonylurea, metformin or SGLT-2 inhibitor;
4. or triple therapy with sulphonylurea/metformin/SGLT-2 inhibitor.

Patients treated with other anti-diabetic treatments are excluded. Where subjects have prediabetes or diabetes, their HbA1c at screening should be 6.0–8.5% (42–69 mmol/mol) and <±1.0% (±11 mmol/mol) from a previous HbA1c reading within the last 6 months, where available. Where a HbA1c reading within the last 6 months is not available, the subject should have HbA1c re-measured after at least 4 weeks to assure stability of

glycaemia before inclusion in the study. This remeasurement may take place any time up to and including check in on Day -2.

For other subjects without known diabetes or pre-diabetes, or in whom the glycaemic status is in doubt, they may attend a pre-screening visit no more than 10 weeks before Day 1, where a fasting glucose and HbA1c will be measured, and an oral glucose tolerance test performed to classify their diagnosis according to the WHO 2006 and 2011 criteria.

It is proposed to investigate 3 dose levels of G3215 in up to 3 cohorts of 8 subjects, an anticipated total of 24 subjects. In each cohort, 6 subjects will receive G3215 and 2 subjects will receive placebo. Where possible, subjects of a similar glycaemic status (i.e. diabetics, prediabetics or normal glucose tolerance) will be grouped in a cohort.

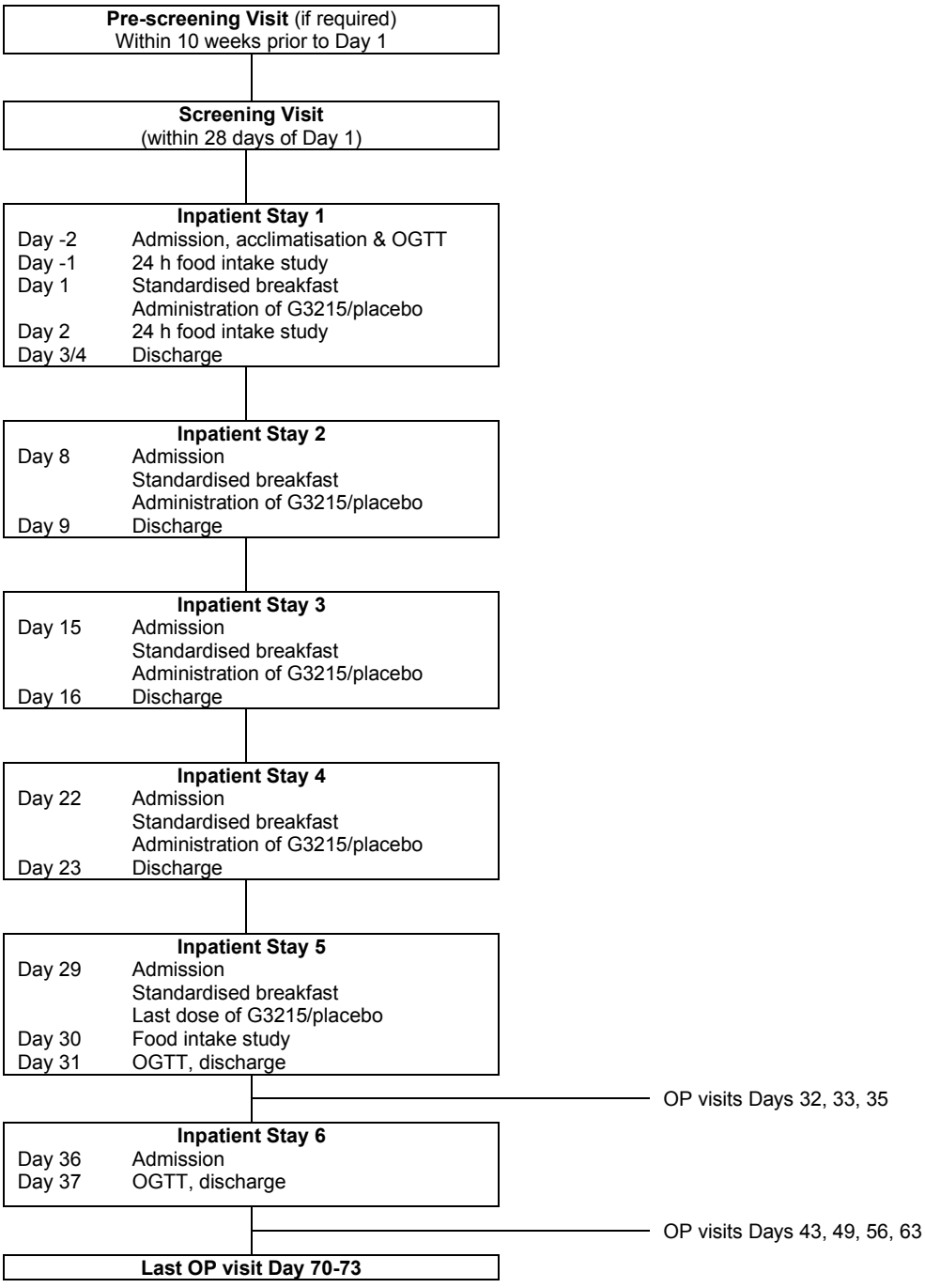
Up to 3 additional cohorts of 8 patients each, i.e. up to 24 subjects in addition, may be enrolled if recommended by the Safety Committee (see Section 2.1.4) to increase scientific value of this study, e.g. to assess additional doses of G3215 or further characterise PD markers.

Part B may commence prior to the completion of Part A (SAD) and the total daily dose will not exceed a single dose shown to be safe and well tolerated.

Subjects will receive 5 doses of G3215 or placebo by SC injection (with fractionation if needed), each separated by 7 days (i.e., on Days 1, 8, 15, 22 and 29). The starting dose level to be administered in Part B is 24 mg and the dose frequency weekly. Administration of subsequent doses will be based on review of available safety, tolerability and PK data from previous doses, and will not exceed a single dose which has been shown to be safe and well tolerated. Dose titration for an individual subject may be necessary within a dosing regimen depending on the activity profile and tolerability. The highest dose in Part B will not exceed the maximum dose of 48 mg tested in the SAD trial.

Subjects are proposed to have 6 inpatient stays in Part B as outlined in Figure 2.1.

Figure 2.1 Study Overview: Part B



Following a pre-screening visit within 10 weeks prior to Day 1 (if applicable), and a 28-day screening period, eligible subjects will be admitted to the clinical unit on the morning of Day -2 (2 days prior to first dosing) for the first inpatient stay. At Day -2, the volunteers

will undergo an OGTT, and therefore will need to attend the visit after an overnight fast. Subjects will receive a single dose of G3215 or placebo by SC injection(s) on Day 1. Subjects will remain in the clinical unit for at least 24 h postdose. It is the intention that subjects will be discharged on Day 4, but they may be discharged on Day 3 after the food intake study has been completed and if the subject is in good health. If volunteers are discharged on Day 3 they will return on Day 4 for collection of a pharmacokinetic sample.

For most of the subsequent inpatient stays (stays 2 to 4) subjects will be admitted to the clinical unit on the day of dosing and will remain in the clinical unit at least until 24 h postdose. In stay 5, the subjects will be admitted to the clinical unit on the day of dosing and will remain in the clinical unit until 72 h postdose. It is proposed that doses will be administered 7 days apart (i.e. on Days 1, 8, 15, 22 and 29).

The proposed duration of the inpatient stays may be altered based on emerging safety, tolerability and PK data from Part A (SAD) or previous cohorts in Part B.

After each inpatient stay, subjects will return to the clinical unit for outpatient visits as described in Figure 2.1.

Safety monitoring will include assessment of adverse events (AEs), physical examination, 12-lead ECGs, vital signs (blood pressure, pulse rate and body temperature) and clinical laboratory safety tests (serum biochemistry, haematology, coagulation, TSH, FT4, LH, FSH, testosterone and urinalysis).

Blood samples for biomarker (tryptase) will be collected immediately and 12 hours after any possible allergic or anaphylactic adverse reaction. These samples will only be analysed if there is a relevant adverse reaction.

The PK evaluation will include measurement of plasma G3215 levels and associated PK parameters.

Exploratory PD evaluation will include evaluation of the following: glucose tolerance (as assessed by a standard 75 g oral glucose tolerance test before and after treatment), energy intake at mealtimes, body weight, visual analogue scales (VAS) of nausea and satiety and enteropancreatic hormones.

Blood samples for immunogenicity assays will be collected predose and at the final follow-up visit.

Subjects will attend a final follow-up visit on Day 70-73.

2.1.3 Part C (Ascending Dose)

Part C will be a single blind, randomised, placebo controlled, ascending dose study in sequential groups of male subjects (BMI range from 25-38 kg/m^2). These subjects can either have normal glucose tolerance, prediabetes (impaired glucose tolerance or impaired

fasting glucose), or Type 2 diabetes, defined according to WHO 2006 and 2011 diagnostic criteria.

It is proposed to investigate 3 sequentially incrementing infusion doses of G3215 in a cohort of 4 subjects; and all 4 subjects will receive G3215 and placebo. Within this cohort, two subjects will receive placebo on Day 1 and two subjects will receive placebo on Day 4 (see Table 2.2). Cohort 1 will include normal glucose tolerance subjects only. Up to 3 additional cohorts of 4 subjects each (up to 12 subjects) may be enrolled if recommended by the Safety Committee to increase scientific value of this study (e.g. to assess additional doses of G3215 or further characterise PD markers). Where possible, subjects of a similar glycaemic status (i.e. diabetics, prediabetics or normal glucose tolerance) will be grouped in a cohort.

Part C may commence prior to the completion of Part B (MAD).

Subjects will receive 3 doses of G3215 by SC infusion, with a dose increment every 24 hours. The starting dose will be 0.8 mg/24 hr and if there is no safety and tolerability issues, the dose will be increased after 24 hours to 1.6 mg/24 hr and then 3.2 mg/24 hr. Dose titration for an individual subject may be necessary within a dosing regimen depending on the activity profile and tolerability. The highest total dose given in Part C (over the entire dosing interval) will not exceed the maximum dose of 48 mg tested in the SAD trial.

If applicable, subjects may undergo a pre-screening visit within 10 weeks prior to Day 1 (see Part B for criteria for pre-screening). After a 28-day screening period eligible subjects will be admitted to the clinical unit on Day -1 (1 day prior to the first dosing). This is to help them acclimatise to the unit and study procedures. On day 1, after a standardised breakfast, the subjects will receive an infusion of G3215 or placebo (0.9% saline) by SC infusion pump, at a set infusion dose and rate. The infusion and infusion set will be changed on a daily basis, and at this pump change the dose will be escalated. During the infusion, if the subject experiences nausea, the infusion rate will be reduced to 50% and the subject reviewed hourly by study personnel after that. If the nausea settles, the infusion rate will be increased to 75% of the original rate and then again back to the original rate after two hours if tolerated. If vomiting occurs, the infusion pump will be stopped and only restart an hour later after the nausea has settled. At this point when the infusion is restarted, the infusion rate will be 50% of the original rate, and then increased to 75% and then 100% of the original rate every two hours if the nausea remains settled.

If skin irritation/reaction occurs at the infusion site, the infusion set and infusion site will be changed.

Food intake will also be semi-quantitatively measured throughout the admission.

It is the intention that subjects will be discharged on Day 5 after the infusion has been discontinued. They should remain in the unit for three hours after the infusion pump has been disconnected for observations, to check that the subject is stable.

After the inpatient stay, subjects will return to the clinical unit for one final outpatient visit on day 7. Depending on emerging safety and PK data, the subjects may return to the clinical unit for further outpatient visits . The number of additional visits per subject will not exceed six and will not extend beyond 8 weeks after each final dosing occasion. If additional outpatient visits are scheduled the final outpatient visit will be considered to be the final follow up visit where the follow-up visit assessments will be performed.

Safety monitoring will include assessment of adverse events (AEs), physical examination, 12-lead electrocardiograms (ECGs), vital signs (blood pressure pulse rate and body temperature) and clinical laboratory safety tests (serum biochemistry, haematology, coagulation, TSH, FT4 and urinalysis).

Blood samples for biomarker (tryptase) will be collected immediately and 12 hours after any possible allergic or anaphylactic adverse reaction. These are to be analysed only if relevant adverse reactions occur.

The PK evaluation will include measurement of plasma G3215 levels and associated PK parameters.

Exploratory PD evaluation will include evaluation of the following: capillary blood glucose monitoring, semi-quantitative energy intake at mealtimes, body weight, visual analogue scales (VAS) of nausea and satiety. This assessment will be done during the inpatient stay.

Blood samples for immunogenicity assays will be collected predose and at the final follow-up visit.

In the cohort, each of the 4 healthy volunteers will receive three escalating doses of G3215/24 hr. Two volunteers will receive G3215 on day 1 to day 3 and the other two volunteers will receive saline/placebo on day 1 and then G3215 on day 2 to 4 (Table 2.2).

Table 2.2 Dosing Schedule: Part C

	N=2	N=2
Day 1	G3215 0.8 mg/24 hr	Saline/Placebo
Day 2	G3215 1.6 mg/24 hr	G3215 0.8 mg/24 hr
Day 3	G3215 3.2 mg/24 hr	G3215 1.6 mg/24 hr
Day 4	Saline/Placebo	G3215 3.2 mg/24 hr

Figure 2.2 Study Overview: Part C

Day -1	Participants check in to the research facility
Day 1	Standardised breakfast to start 2 hours before pump start

	Start G3215/Placebo infusion pump
Day 2	Standardised breakfast to start 2 hours before pump change Change G3215/Placebo infusion pump and increase infusion rate as per protocol
Day 3	As per day 2
Day 4	As per day 2 If the subject received G3215 on day 1 to 3, then on day 4, they will receive a placebo infusion. If the subject received Placebo on day 1, then on day 4, they will receive the next incremental G3215 infusion.
Day 5	Infusion completes.

2.1.4 Dose Escalation Criteria

After each dose level in Part A (SAD) and Part B (MAD) a Safety Committee will review and assess the safety, tolerability and PK data to determine the next dose level. This PK analysis will utilise data from the GLP-validated LC-MS/MS assay for G3215. For Part C, a review by the safety committee will take place to review the safety, tolerability and PK data 24 hours after the completion of the infusion within the cohort. The committee will at that stage decide if further cohorts infusing similar or different doses of G3215 should be done.

In Part A (SAD), an appropriate interval of at least 7 days will separate the dosing of the last subject in one cohort and dosing of the first subject in the next cohort to permit a timely review and evaluation of interim data. The committee will review interim safety (vital signs, ECGs, and clinical laboratory safety tests), tolerability (adverse event profile) up to 72 hours post dose, and PK data collected up to at least 48 hours post-dose in at least 3 subjects receiving G3215 in Cohort 1 and at least 4 subjects (i.e. a minimum of 3 subjects receiving G3215) in Cohort 2 onwards. PK data collected from previous dose levels may also be reviewed.

For Part B (MAD) the committee will review interim safety (vital signs, ECGs, and clinical laboratory safety tests), tolerability (adverse event profile), and PK data collected up to Day 31 from a minimum of 6 subjects, such that there will be at least 4 subjects receiving G3215 at each dose level.

Progression to the next higher dose level in Part A and Part B will only occur if the previous dose level was deemed to be safe and well tolerated by the Safety Committee. When it is not appropriate to escalate the dose to the next higher dose level, the Safety Committee may decide to administer the same dose (see dose fractionation criteria), a previous dose or an intermediate dose to the next cohort of subjects.

For Part C, three sequentially incrementing doses per 24 hour infusion of G3215 are planned. During the infusion, if the subject experiences nausea, the infusion rate will be reduced to 50% and the subject reviewed hourly by study personnel after that. If the nausea settles, the infusion rate will be increased to 75% of the original rate and then again back to the original rate after two hours if tolerated. If vomiting occurs, the infusion pump will be stopped and only restart an hour after the nausea has settled. At this point when the infusion is restarted, the infusion rate will be 50% of the original rate, and then increased to 75% and then 100% of the original rate every two hours if the nausea remains settled.

For Part A, Part B, and Part C additional optional cohorts may be enrolled if recommended by the Safety Committee to increase scientific value of this study (e.g., to verify PK data or further characterise PD markers). The Safety Committee may decide to administer the same dose, a previous dose, an intermediate dose or an escalated dose to the optional cohort. The optional cohorts may include up to 8 cohorts of 6 subjects each (up to 48 patients) in Part A, up to 3 cohorts of 8 subjects each (up to 24 subjects) in Part B and up to 3 cohorts of 4 subjects each (up to 12 subjects) in Part C.

If emerging safety, tolerability and PK data indicate it is appropriate, the Safety Committee may vary the dose level providing none of the stopping criteria are met.

Data will be reviewed unblinded for Part A, B and C as outlined in Section 3.9.

Safety Committee

The Safety Committee's membership will be composed of a minimum of the Sponsor team (Sponsor Senior Physician and Sponsor Senior Scientist or an appropriate delegate) and Principal Investigator (or an appropriate delegate). Additional members may be invited as needed (e.g. PK scientist, project manager).

All data reviewed at the dose escalation meeting (safety, tolerability and available PK data) will be subjected to a quality control review.

2.1.5 Stopping Rules

Dosing for any individual subject will be stopped if the subject experiences a possibly drug-related serious adverse event or a possibly drug-related significant non serious adverse event, which in the opinion of the study physician, Principal Investigator or Chief Investigator (Sponsor's medical representative), warrants discontinuation of the study for that subject's well being.

In Parts A, B, and C, following consultation with the Sponsor, dose escalation will stop if:

- Clinically relevant signs or symptoms of similar nature, occur in 2 or more subjects within a group/cohort, which in the opinion of the Investigator warrant stopping of dose escalation.
- A serious adverse event (SAE) in one or more subjects thought to be related to the study drug.

- Moderate nausea or vomiting that prevent subjects from eating a meal on 3 or more occasions on 2 successive days are seen in 2 or more subjects in a group.
- Severe diarrhoea, defined as 7 or more episodes, rated as ≥ 5 on the Bristol Stool Chart, in 1 day for 2 consecutive days are seen in 2 or more subjects in a group.
- The mean systemic exposure is predicted to exceed a Cmax of 46.2 ng/mL and/or $AUC_{0\text{-}72h}$ of 1582 ng·h/mL i.e. systemic exposure will be no greater than that the lowest exposure at the NOAEL for cynomolgus monkeys (0.1 mg/kg). This limit is chosen on the basis that non-human primates are expected to be the species that will most closely predict toxicity in humans.

2.1.6 Discussion of Study Design

This is a randomised, placebo-controlled study in three parts: part A, a single ascending dose study, part B, multiple ascending dose study (via a weekly SC injection over 28 days) and part C multiple ascending dose study via a three day continuous SC infusion. Part A and B will be conducted in a double blinded fashion and part C will be single blinded. Note, with the exception of Cohort 1 TP1 which is partially blinded, subjects will remain blinded. The time interval between each TP should be no less than 1 week. As G3215 is intended as an anti-obesity drug, overweight/obese subjects have been chosen to provide more clinically relevant PK and PD information. With regards to Part B and Part C, we propose to recruit overweight/obese subjects without diabetes, with Type 2 diabetes or pre-diabetes.

It is the intent of Part B to dose volunteers such that steady-state is achieved and maintained for several days. Based on the available pre-clinical data it is expected that this will be achieved following 28 days once weekly dosing, however, a full review of all the safety, tolerability and pharmacokinetic data from Part A will be performed to confirm the dose regimen for Part B. If the elimination half-life of G3215 is shorter than anticipated from the pre-clinical data, more frequent dosing (e.g. twice weekly) may be appropriate. If the elimination half-life of G3215 is longer than anticipated from the pre-clinical data, dosing every two weeks may be more appropriate (as steady-state will take longer to achieve). The dose frequency will be no more frequent than once a day and no less frequent than once every 14 days. The dosing duration will comprise no less than 28 consecutive days and will not exceed 35 consecutive days of dosing.

Details of the dosing regimen and duration used for Part B of the study will be documented in the SMF.

The sample size (approximately 28 subjects in Part A [SAD], 24 subjects in Part B [MAD], and 4 subjects in Part C) has been chosen to minimise the number of subjects exposed to G3215 whilst obtaining sufficient information to assess the safety, tolerability, PK and PD of G3215.

2.2 Study Population

Approximately 28 subjects will be enrolled in Part A (SAD), 24 subjects will be enrolled in Part B (MAD), and 4 subjects in Part C.

Overweight male volunteers will be entered into this study provided that they satisfy the following inclusion/exclusion criteria.

2.2.1 Subject Inclusion Criteria

1. Adult males aged 18 to 65 years inclusive with BMI between 25.0 and 38.0 kg/m^2 inclusive;
2. (PART A and PART C, Cohort 1) Subjects who have normal glucose tolerance;
3. (PART B and PART C, Cohort 2 onwards) Subjects who have normal glucose tolerance, Type 2 diabetes, impaired glucose tolerance or impaired fasting glucose according to WHO 2006 and 2011 criteria;
   a) In subjects who are identified as being prediabetic or diabetic, they should be stably treated either with:
      (1) diet only;
      (2) monotherapy with a sulphonylurea, metformin, or SGLT-2 inhibitor;
      (3) dual therapy with sulphonylurea/metformin, or sulphonylurea/SGLT-2 inhibitor;
      (4) or triple therapy with sulphonylurea/metformin/SGLT-2 inhibitor.
   b) Patients treated with other anti-diabetic treatments are excluded;
   c) In subjects who are identified as being prediabetic or diabetic, the HbA1c at screening should be 6.0–8.5% (42–69 mmol/mol) and <±1.0% (±11 mmol/mol) from a previous HbA1c reading within the last 6 months, where available. Where an HbA1c reading within the last 6 months is not available, the subject should have HbA1c re-measured after at least 4 weeks to assure stability of glycaemia before inclusion in the study. This remeasurement may take place any time up to and including check in on Day -2;
   d) To allow assessment of eligibility, subjects without known diabetes or prediabetes, or in whom the glycaemic status is in doubt, may undergo a pre-screening visit no more than 10 weeks before Day 1 for assessment of fasting glucose, HbA1c and glucose 2 hours after a 75 g oral glucose tolerance test;
4. Subjects who are otherwise healthy enough to participate, as determined by pre-study medical history, physical examination and 12-lead ECG;
5. Subjects whose clinical laboratory test results are either within the normal range or if outside this range the abnormalities are judged to be not clinically relevant and are acceptable to the Investigator;
6. Subjects who are negative for hepatitis B surface antigen (HBsAg), hepatitis C antibody and human immunodeficiency virus (HIV) I and II tests at screening;

7. Subjects who are negative for drugs of abuse and alcohol tests at screening and admissions;
8. Subjects who are non-smokers for at least 3 months preceding screening;
9. Subjects who agree to use medically acceptable methods of contraception for at least 3 months after study drug administration;
10. Subjects who agree not to donate sperm for at least 3 months after study drug administration;
11. Subjects who are able and willing to give written informed consent.
12. Subjects' medical history must be verified by either a personal physician or medical practitioner as appropriate.

2.2.2 Subject Exclusion Criteria

1. Subjects who do not conform to the above inclusion criteria;
2. Subjects who have a clinically relevant history or presence of gastrointestinal (especially associated with vomiting), respiratory, renal, hepatic, haematological, lymphatic, neurological (especially if associated with balance disorders or vomiting e.g. migraine or labyrinthitis), cardiovascular, psychiatric, musculoskeletal, genitourinary, immunological, dermatological, connective tissue diseases or disorders;
3. Subjects who have a clinically relevant surgical history;
4. Subjects who are currently taking any of the following classes of diabetes medications: thiazolidinediones, dipeptidyl peptidase IV inhibitors ('gliptins'), GLP-1 analogues, and insulin;
5. Subjects who have a history of relevant and severe atopy e.g. asthma, angioedema requiring emergency treatment, severe hayfever requiring regular treatment (i.e. taking antihistamines and/or glucorticoids more regularly than 3 times a week), severe eczema requiring regular treatment (i.e. taking antihistamines and/or glucocorticoids more regularly than 3 times a week);
6. Subjects who have a history of relevant drug hypersensitivity;
7. Subjects who have a history of alcohol abuse or alcohol dependence according to DSM-IV criteria within the last two years;
8. Subjects who have a history of drug or substance abuse according to DSM-IV criteria within the last 2 years;
9. Subjects who have a history of clinically significant migraine as judged by the Investigator. Subjects can be included if they have not had a migraine for the last 3 years;
10. Subjects with a history of pancreatitis or pancreatic cancer;
11. Subjects who consume more than 21 units of alcohol a week (unit = 1 glass of wine (125 mL) = 1 measure of spirits = ½ pint of beer);
12. Subjects who have a significant infection or known inflammatory process on screening;

13. Subjects who have acute gastrointestinal symptoms at the time of screening or admission (e.g. nausea, vomiting, diarrhoea, heartburn);
14. Subjects who have an acute infection such as influenza at the time of screening or admission;
15. Subjects who have used prescription drugs within 2 weeks of first dosing. For Part B, patients are allowed: monotherapy with sulphonylureas, metformin, or SGLT-2 inhibitors; dual therapy with any two of the following drug classes: sulphonylureas, metformin, or SGLT-2 inhibitor; triple therapy with sulphonylureas/metformin/SGLT-2 inhibitor. In addition, patients in Part B are allowed to take hypolipidaemic and/or antihypertensive treatments, provided that the doses have not been altered within the 4 weeks prior to entering the study. Other medications may be allowed if the Investigator and Sponsor both agree that they will not affect the outcome of the study or the safety of the subject.
16. Subjects who have used over the counter medication excluding routine vitamins and paracetamol but including megadose (intake of 20 to 600 times the recommended daily dose) vitamin therapy within 7 days of first dosing, unless agreed as not clinically relevant by the Principal Investigator and Sponsor;
17. Subjects who have donated blood within 3 months prior to screening;
    Subjects who have donated plasma within the 7 days prior to screening;
    Subjects who have donated platelets within the 6 weeks prior to screening
18. Subjects who have used any investigational drug in any clinical trial within 3 months of their first admission date;
19. Subjects who have received the last dose of investigational drug greater than 3 months ago but who are on extended follow-up;
20. Subjects who have previously received G3215;
21. Subjects who are vegans or have any dietary restrictions;
22. Subjects who cannot communicate reliably with the Investigator;
23. Subjects who are unlikely to co-operate with the requirements of the study;
24. History or evidence of abnormal eating behaviour, as observed through the Dutch Eating Behaviour (DEBQ) and SCOFF questionnaires [24,25] at screening.

2.2.3 Restrictions

In Part A (SAD), subjects will be admitted to the clinical unit on Day -1 and will remain in the clinical unit under supervision until discharge on Day 4 (at least 72 h postdose) or when C_{max} is achieved, whichever is later. Subjects will be required to attend outpatient visits.

In Part B (MAD), subjects will be admitted to the clinical unit for 6 inpatient stays: Day -2 (admission) to Day 4 (discharge), Day 8 (admission) to Day 9 (discharge), Day 15 (admission) to Day 16 (discharge), Day 22 (admission) to Day 23 (discharge), Day 29 (admission) to Day 31 (discharge), and Day 36 (admission) to 37 (discharge). Subjects will return to the clinical unit for outpatient visits.

In Part C, subjects will be admitted to the clinical unit for 1 inpatient stay, comprising 5 nights, 6 days: Day -1 (admission) to Day 5 (discharge). They will also attend a final outpatient review on Day 7 (± 1 day). Subjects will return to the clinical unit for outpatient visits as described in Table 16.4.

For both Part A, Part B and Part C, the inpatient stays and outpatient visits may be amended upon review of safety, tolerability and PK data from previous cohorts or study part.

Subjects will receive a standard diet whilst resident in the clinical unit and will only consume foodstuffs provided by the clinical unit. Subject's energy intake at each meal will be recorded during the inpatient stays.

For Part A, Part B and Part C subjects should refrain from the following:

- Strenuous exercise for 48 h before screening, 48 h prior to each admission, throughout each inpatient stay and for 48 h prior to each outpatient visit.
- Alcohol and/or xanthine containing products (e.g. caffeine) for 48 h prior to screening and each admission, during the inpatient stay, and for 48 h prior to each outpatient visit.
- Smoking for the duration of the study (until after the final follow-up visit).
- Eating foodstuffs that contain poppy seeds, as these may result in a positive urine test for opiate consumption.
- Taking medications during the study as described in Section 3.5.

2.2.3.1 Avoidance of Pregnancy

Instructions for Male Subjects

There is no information about effects that G3215 could have on the development of the foetus in humans. Therefore, it is important that the partners of male subjects do not become pregnant during the study and for a total period of 3 months after the male subject has taken the last dose of G3215.

As a precaution, all male subjects should avoid fathering a child by either true abstinence or the use of 2 highly effective means of contraception (see Section 2.2.3.2).

As there is no information about G3215 being secreted in the ejaculate, male subjects (including men who have had vasectomies) whose partners are currently pregnant should use barrier methods for the duration of the study and for a suitable time afterwards (e.g. 3 months). This is to ensure that the foetus is not exposed to the investigational medicinal product (IMP) in the ejaculate.

2.2.3.2 Acceptable Forms of Contraception

Highly effective methods of birth control are defined as those which result in a low failure rate (i.e. less than 1% per year) when used consistently and correctly.

These include female sterilisation (i.e. documented bilateral tubal ligation), hormonal methods of contraception (oral, implanted or transdermal) or an intrauterine device (IUD) in combination with a barrier method (condom, diaphragm).

Where there is a possibility of pregnancy, 2 effective methods should be used. Effective forms of contraception are:

1. Established use of oral, transdermal, injected or implanted hormonal methods of contraception.
2. Placement of an IUD or intrauterine system (IUS).
3. Barrier methods of contraception: condom or occlusive cap (diaphragm or cervical/vault caps) with spermicidal foam/gel/film/cream/suppository. The use of barrier contraceptives should always be supplemented with the use of a spermicide. The following should be noted:
    - Failure rates indicate that, when used alone, the diaphragm and condom are not highly effective forms of contraception. Therefore the use of additional spermicides does confer additional theoretical contraceptive protection.
    - However, spermicides alone are inefficient at preventing pregnancy when the whole ejaculate is spilled. Therefore, spermicides are not a barrier method of contraception and should not be used alone.
4. Male sterilisation (the subject should have received medical assessment to confirm surgical success).
5. True abstinence: when this is in line with the preferred and usual lifestyle of the subject. Periodic abstinence (e.g., calendar, ovulation, symptothermal, post-ovulation methods) and withdrawal are not acceptable methods of contraception.

2.2.3.3 Time Period for the Collection of Pregnancy Information

All pregnancies in female partners of male subjects receiving at least one dose of IMP will be recorded from first dose to 3 months after the final dose.

2.2.3.4 Follow-up in the Event of a Pregnancy

If the female partner of a male subject who has received G3215 becomes pregnant within the time window in Section 2.2.3.3, the pregnancy will be recorded. The ethics committee and the Sponsor will be informed. The subject will be asked to provide information on the outcome of the pregnancy, including premature termination should the case arise.

Spontaneous miscarriage and congenital abnormalities will be reported as SAEs.

The follow-up period will be deemed to have ended when the health status of the child has been determined on its birth.

2.2.4 Subject Withdrawals

A subject may withdraw for any reason. The Investigator will advise the Sponsor of the withdrawal of any subject.

A subject may be withdrawn in any of the following circumstances:

- Adverse events;
- Protocol violation;
- Withdrawal of consent;
- Termination of the study by the Investigator or Sponsor.

Subjects who voluntarily withdraw are termed dropouts. Dropouts may be replaced following discussion with the Investigator and Sponsor. Subjects withdrawn due to an adverse event which is thought to be related to the study drug will not be replaced.

If a subject is withdrawn or withdraws during the inpatient stay, the discharge procedures should be performed and a follow-up visit should be scheduled. If a subject is withdrawn or withdraws after the inpatient stay is completed a follow-up visit should be scheduled.

If a subject is withdrawn due to an adverse event, appropriate medical care should be provided and the adverse event should be followed to resolution. Follow-up procedures should be conducted as scheduled.

3 STUDY TREATMENT

3.1 Investigational Product(s)

G3215 is a 35 natural amino acid linear peptide. G3215 does not contain any products of human or animal origin.

The bulk drug product (20 mg/vial) is manufactured as a sterile lyophilised powder supplied in a clear glass 3 mL vial sealed with a fluoro-coated lyophilisation stopper and an aluminium crimp seal. G3215 will be reconstituted in sterile diluent supplied in a glass vial with a fluoro-coated rubber stopper and an aluminium crimp seal. The details of the diluent and reconstituent steps will be provided in the Study Reference Manual.

The placebo is sterile 0.9% (w/v) saline.

3.1.1 Supply, Packaging and Labelling

G3215 bulk Drug Product and Diluent will be supplied to Clinical CRO by Symbiosis Pharmaceutical Services.

A release document signed by a legally authorised Qualified Person (QP) at CRO will be placed in the appropriate section of the Trial Master File to document labelling and dispensing of the study drug to the subject. A technical agreement between CRO and Sponsor will be in place to cover all pharmacy related activities, detailing roles and responsibilities prior to receipt of the IMPs at CRO.

These supplies will be packaged in accordance with Annex 13 of "The Rules Governing Medicinal Products in European Community, Volume IV Good Manufacturing Practice for Medicinal Products".

All documents required to perform GMP activities at CRO will be supplied as per the Technical Agreement. The Technical Agreement will outline the roles and responsibilities between the contract giver and the contract acceptor.

The placebo (sterile 0.9% [w/v] saline) will be commercially sourced by CRO.

3.1.2 Storage and Handling Procedures

The G3215 lyophilised powder will be stored at -15 to -25°C. The diluent will be stored at +2 to +8°C. All study medication will be stored in the CRO Pharmacy.

Details of how the IMP will be prepared for administration will be included in a separate laboratory manual.

The Sponsor will be permitted upon request to audit the supplies, storage, dispensing procedures and records provided that the blind of the study is not compromised.

3.1.3 Accountability

In accordance with GCP, the clinical unit will account for all supplies of G3215 and placebo. Details of receipt, storage, assembly and return will be recorded.

All unused supplies of G3215 and placebo will either be destroyed by CRO or returned to the study Sponsor at the end of the study in accordance with instructions by the Sponsor.

3.2 Dosage and Administration

G3215 or placebo will be administered by SC injection or continuous infusion into the abdomen. The maximum individual injection volume will not exceed 1.0 mL, though multiple injections (up to 5) may be given for a single dose. The maximal SC infusion volume will not exceed 20 ml per 24 hr. The injection site(s) should be circled with indelible marker and inspected at each AE check during Day 1. Reactions should be described according to the grading table (Table 4.5). Where multiple doses or infusions of G3215 are given to the volunteer, the injection sites must rotate, i.e. given at different sites in the abdomen.

In the event of injection site reactions thought to be related to the volume of the injection, the dose of G3215 or placebo may be fractionated and up to 5 injections may be given.

If skin irritation/reaction occurs at the infusion site, the infusion set and infusion site will be changed. Subjects will receive G3215 or placebo after a standardised breakfast, while in the supine/semi-supine position. Subjects will remain confined to their beds for a period of 4 h after injection, but will be permitted to visit the bathroom to use the toilet if needed.

In Part A (SAD) the proposed doses are: 0.1 mg, 0.5 mg, 1.5 mg, 4.0 mg, 10 mg, 20 mg and 40 mg. Depending on emerging safety, tolerability and PK data, the doses given may be modified. Dose levels administered in Part B (MAD) will be selected following review of the data from Part A. In Part B, dose titration for an individual subject may be necessary within a dosing regimen depending on the activity profile and tolerability, but will not exceed the maximum dose of 48 mg tested in the SAD trial. In Part C, subjects will receive a continuous dose of G3215 by SC infusion, with a dose increment every 24 hours. The starting dose will be 0.8 mg/24 hr and if there are no safety and tolerability issues, the dose will be increased after 24 hr to 1.6 mg/24 hr and then 3.2 mg/24 hr. Dose titration can go up or down for an individual subject within a dosing regimen depending on the activity profile and tolerability. The highest total dose given in Part C (over the entire dosing interval) will not exceed the maximum dose of 48 mg tested in the SAD trial.

For Parts A and B, subjects will not eat for 4 h postdose after which time lunch will be served. Dinner will be served 10 h postdose. Water will be restricted for 1 h pre and 1 h postdose. For Part C, subjects will eat a standardised breakfast. For each subject, lunch will be served 3 hours after the start of the infusion on each day, and dinner served 8 hours after the start of the infusion on each day. There will be no restriction to the amount of water the

subjects drink. With regards to eating and drinking when subjects have been discharged from the clinical unit during Parts B and C, study participants will be provided with a dietary advice sheet providing information with regards to a healthy eating diet which they are recommended to follow when they have been discharged from the clinical unit.

3.3 Treatment Strategy

The clinical staff at Covance Clinical Research Unit, Leeds, are responsible for the ongoing safety and well-being of the volunteers while they are in the clinical unit. There is a paging system to alert the clinical staff to any area in the unit where a subject may need medical attention. In the case of an emergency, cardiac resuscitation trolleys are found in the main ward areas of the clinical unit. These trolleys contain drugs, equipment for airway insertion, circulation lines, defibrillation etc, together with oxygen cylinders and portable suction machines. There is a physician on-call 24 hours a day (this can be off site) and all physicians are Advanced Life Support (ALS) trained. In addition, the clinical staff can contact further on-call physicians or public emergency services, including the ambulance service, in the event of a serious medical event. Equipment and emergency drugs are available to treat common medical emergencies that might occur in a Phase I study.

3.4 Warnings and Precautions

As this is the first administration of G3215 to man, all effects cannot be reliably predicted. The preclinical data suggest an acceptable safety margin. Facilities and staff for resuscitation and the treatment of other medical emergencies will be provided.

3.5 Prior and Concomitant Medication

The participants are not allowed to take any prescription medication within 4 weeks of first dosing apart from those for diabetes in Part B. Subjects are not allowed to take any non-prescription medication (excluding paracetamol) or megadose (intake of 20 to 600 times the recommended daily dose) vitamins within 7 days of first dosing prior to entrance into the clinical research facility and for the duration of the study.

In the interests of subject safety and acceptable standards of medical care the Investigator will be permitted to prescribe treatment(s) at his/her discretion. All treatments must be recorded in the subjects' case report form (CRF) (medication, dose, treatment duration and indication).

3.6 Method of Assigning Subjects to Treatment Groups

At screening, potential study subjects will be assigned a screening number. Following confirmation of eligibility, at study drug administration, subjects will be assigned a subject number in the order in which they are enrolled in the study, as shown in Table 3.1. The subject number will determine the allocation of treatment.

Table 3.1 Assignment of Subjects to Treatment

Study Part	Dose Level(s)	Subject Numbers
A	1, 2, 3	[redacted]
	4	[redacted]
	5	[redacted]
	6	[redacted]
	7	[redacted]
	Additional[1]	[redacted]
B	1	[redacted]
	2	[redacted]
	3	[redacted]
	Additional[1]	[redacted]
C	1	[redacted]
	Additional[1]	[redacted]

1 – Eight additional cohorts of up to 6 subjects in Part A (up to an additional 48 patients), three additional cohorts of up to 8 subjects in Part B (up to an additional 24 patients), three additional cohorts of up to 4 subjects each in Part C (up to an additional 12 patients) may be enrolled in each part if agreed by the Safety Committee.

Any replacement subjects will receive the same treatment allocation as those whom they replace.

Subjects who are replaced (dropouts) will be allocated the same treatment number with the number 1 in the fourth digit position, e.g., if subject number 10001 is replaced then the replacement subject's number will be 11001.

3.7 Randomisation Procedures

Allocation to treatment will be according to a predetermined random order. Randomisation of G3215 or placebo will take place for each group separately. In Part A (SAD), each pair of sentinel subjects in Cohort 1 will be randomised such that 1 subject receives G3215 and 1 receives placebo.

The randomisation list will be generated by Covance using the statistical analysis system (SAS®) computer package.

3.8 Maintenance of Randomisation Codes

Randomisation codes will be provided to the pharmacist and bioanalyst in a list. Individual treatment disclosure envelopes (code break) will also be provided to the clinical unit. The pharmacist will use the randomisation code list for preparing subject doses throughout the study. The individual disclosure envelopes will be used if it is necessary to break the blind for an individual subject.

3.9 Blinding

Part A (SAD) will be conducted double-blind (with the exception of Cohort 1 where the volunteers taking the 3rd and 4th injections within a treatment period are guaranteed to be given G3215); subjects and clinical staff will remain blinded until the Safety Committee meeting.

Part B (MAD) will be conducted double-blind. Treatments will be administered double blinded; subjects will be blinded with regard to treatment and clinical staff will remain blinded with regard to treatment until the Safety Committee meeting.

For the purposes of blinding in Parts A and B "clinical staff" refers to staff at the investigator site; all sponsor staff will be unblinded to study treatments.

Part C (Ascending dose study of G3215 via continuous infusion) will be conducted single blinded to enable the clinical staff to titrate the dose according to tolerability.

In the event of a medical emergency when management of a subject's condition requires knowledge of the trial medication, the sealed "code-break" envelope provided may be opened by personnel authorised by the Principal Investigator to determine the nature of the trial medication dispensed. If possible, such emergencies should be discussed with the study monitor and the Sponsor's safety manager prior to disclosure of the treatment allocation. Reasons for breaking a code must be clearly explained and justified in the CRF. The date on which the code was broken together with the identity of the person responsible must also be documented.

The bioanalyst and the PK scientist who will be unblinded, will be required to provide coded data to the Principal Investigator so that the blind is maintained for clinical staff connected with the study.

With the exception of the CRO pharmacy department, the statistician preparing the randomisation, the bioanalytical assay group and the quality assurance auditors where necessary, all clinical and non-clinical staff, with the exception of the PK scientist and PK/PD statistician, will remain blinded to the treatment allocation (where applicable) until after the database is locked unless there is a medical event that requires code break.

4 STUDY PROCEDURES

The blood volumes to be collected from each subject are detailed in Table 4.1 for Part A (SAD), Table 4.2 for Part B (MAD), and Table 4.3 for Part C. The total volume to be collected per subject will be no greater than 550 mL, staggered over the duration of the study. No more than 15% of the estimated blood volume of a subject will be taken on any one occasion as per JPAC guidelines (http://www.transfusionguidelines.org/). Laboratory sample handling details will be presented in a separate laboratory manual.

Table 4.1 Blood Volumes: Part A (SAD)
Cohort 1

Assessment	Sample Volume (mL)	No. of Samples	Total Volume (mL)
Safety			
Serum Biochemistry, Thyroid Function, LH, FSH, testosterone	3.5	11	38.5
Coagulation	1.8	11	19.8
Serology	3.5	1	3.5
Haematology	4.0	11	44
Tryptase[1]	3.5	2	7
Bedside blood glucose	0.1	Up to 23	2.3ml
Pharmacokinetic			
G3215	5.0	Up to 38	190
Pharmacodynamics			
Immunogenicity[3]	6.0	2	12.0
Total[2]			**317.1**

[1] In the event of an allergic reaction additional samples will be collected.
[2] Sample volumes are based on direct venepuncture; where a cannula is used, an extra 1 mL will be drawn and discarded.
[3]Immunogenicity samples from cohort 1 only.

Cohort 2 onwards

Assessment	Sample Volume (mL)	No. of Samples	Total Volume (mL)
Safety			
Serum Biochemistry, Thyroid Function, LH, FSH, testosterone	3.5	5	17.5
Coagulation	1.8	5	9.0
Serology	3.5	1	3.5
Haematology	4.0	5	20.0
Tryptase[1]	3.5	2	7
Bedside blood glucose	0.1	Up to 11	1.1ml
Pharmacokinetic			
G3215	5.0	Up to 16	80.0
Total[2]			**138.1**

[1] In the event of an allergic reaction additional samples will be collected.
[2] Sample volumes are based on direct venepuncture; where a cannula is used, an extra 1 mL will be drawn and discarded.

Table 4.2 Blood Volumes: Part B (MAD)

Assessment	Sample Volume (mL)	No. of Samples	Total Volume (mL)
Safety			
Serum Biochemistry including Thyroid function (TSH, FT4), LH, FSH, testosterone	3.5	16	56.0
Serology	3.5	1	3.5
Coagulation	1.8	16	28.8
Haematology	4.0	16	64.0
Tryptase[1]	3.5	2	7.0
Bedside glucose monitoring	0.1	54	5.4
Pharmacokinetic			
G3215	5.0	37	185.0
Pharmacodynamics			
Immunogenicity	6.0	2	12.0
Oral glucose tolerance test glucose and insulin levels	3.5	21	73.5
Enteropancreatic hormones (GLP-1, PYY, ghrelin, leptin)	5.0	21	105
Total[2]			540.2

[1] In the event of an allergic reaction additional samples will be collected.
[2] Sample volumes are based on direct venepuncture; where a cannula is used, an extra 1 mL will be drawn and discarded.

Table 4.3 Blood Volumes: Part C

Assessment	Sample Volume (mL)	No. of Samples	Total Volume (mL)
Safety			
Serum Biochemistry including Thyroid function (TSH, FT4), LH, FSH, testosterone	3.5	5	17.5
Serology	3.5	1	3.5
Coagulation	1.8	5	9.0
Haematology	4.0	5	20.0
Tryptase[1]	3.5	2	7.0
Bedside glucose monitoring	0.1	23	2.3
Pharmacokinetic			
G3215[3]	9.0	26	234
Pharmacodynamics			
Immunogenicity	6.0	2	12.0
Total[2]			305.3

[1] In the event of an allergic reaction additional samples will be collected.
[2] Sample volumes are based on direct venepuncture; where a cannula is used, an extra 1 mL will be drawn and discarded.
[3] Two 9ml baseline sample will be collected, and one 9ml PK sample at all other time points

4.1 Pharmacokinetic Assessments

4.1.1 Plasma Samples

Blood sample collection times are included in the schedule of events.

Details of how samples will be collected, processed and stored will be included in a separate laboratory manual.

Bioanalysis

The bioanalysis will be performed by [redacted]. They will be responsible for the GLP validated bioanalysis, which will utilise a validated high performance liquid chromatography-tandem mass spectrometry (LC-MS/MS) assay in human plasma.

Any remaining plasma may be sent to the sponsor for analysis of G3215 using an exploratory non-GLP compliant, non-validated radioimmunoassay. If this analysis is performed the results from this analysis will not be reported in the clinical study report for this study.

4.2 Pharmacodynamic/Efficacy Assessments

Time points for collection of PD variables are included in the schedule of events (see Section).

4.2.1 Visual Analogue Scales

Assessments of nausea and satiety will be performed using VAS. Subjects will be requested to rate their responses to a series of questions on a VAS from 0 to 100 mm.

4.2.2 Energy Intake

Energy intake will be recorded for Part A and Part B.

A formal 24 h food intake study will be carried out on Part A from Cohort 3 onwards, on Day -1 (measuring lunch and dinner on Day -1 and breakfast on Day 1) and Day 1 (measuring lunch and dinner on Day 1 and breakfast on Day 2).

For Part B, the food intake studies will be carried out on Days -1, 2, and 30. The food intake study on Day -1 will measure the ad libitum intake of food during breakfast, lunch and dinner on Day -1. The food intake study on Day 2 will measure the ad libitum intake of food during breakfast, lunch and dinner on Day 2. The food intake study on Day 30 will measure the ad libitum intake of food during breakfast, lunch and dinner on Day 30. The timing of the food intake study will be altered according to emerging PK data if the t_{max} is delayed beyond 24 hours. If the timing of the food intake studies are changed, the times of the OGTT samples may also be changed to ensure they do not happen on the same day.

For Part C, semiquantitative food intake measurements will take place throughout the admission.

While resident in the clinical unit during 24 h food intake assessment days, subjects will be individually provided meals in a designated area with individual cubicles per subject. Meals will be presented with an excess of food and subjects will be asked to eat until they feel "comfortably full" within a period of 20 minutes. The energy value of each component of

the meal will be determined. Food will be weighed pre- and post-meal to determine consumption.

For other days in Part A and Part B and throughout Part C energy intake will be carried out semiquantitively i.e. clinical research unit staff will record the timing, types and amounts of food eaten by subjects. For the semiquantitative measurement, at the end of each meal the percentage of food left will be assessed to be either <10%, 10-24%, 25-49% or ≥50%. Snack boxes will also be provided at certain timepoints whilst resident in the clinical unit (to be specified in the Meal Manual).

In Part B, subjects will be admitted on Day -2 (2 days prior to dosing) in order to acclimatise to the clinical research unit and obtain baseline food consumption measurements.

4.2.3 Body Weight

In Part A (SAD), Part B and Part C, body weight will be recorded AM and PM each day of the inpatient stay and once on every outpatient visit (Part B and Part C).

Body weight for each subject should be measured on the same calibrated scale in light clothing, after voiding urine, before breakfast (AM) and before dinner (PM). At each timepoint the weight will be measured in triplicate and the mean calculated. The weight should be measured in kg to one decimal place.

4.2.4 Immunogenicity

For assessment of immunogenicity, plain tube serum samples (6 mL blood volume) will be collected predose and at the final follow-up visit for SAD Group 1 and the MAD study, as subjects will be receiving multiple doses of the same peptide in these studies. Samples will be analysed using a GLP compliant and validated assay method utilising an ELISA (bridging assay format) using rabbit positive control antibodies raised against G3215.

4.2.5 Oral glucose tolerance testing and enteropancreatic hormones (Part B)

To assess the effect of G3215 on enteropancreatic hormone secretion, a 75 g oral glucose tolerance test will be carried out on the designated days as per the schedule of events. Subjects will fast for 10 hours before they are given 75 g of glucose to drink. Blood samples will be taken for glucose and enteropancreatic hormones (including but not exclusively PYY, GLP-1, ghrelin, insulin, glucagon) at the following timepoints: before glucose, 30 mins, 60 mins, 90 mins, 120 mins, 150 mins, 180 mins.

4.3 Safety Assessments

4.3.1 Adverse Events

Definitions:

Adverse Event (AE)

Any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have to have a causal relationship with this treatment.

An AE can therefore be any clinically significant sign (including an abnormal laboratory finding, for example), symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.

Adverse Drug Reaction (ADR)

Any untoward and unintended response in a subject to an investigational medicinal product which is related to any dose administered to that subject.

Serious Adverse Event (SAE)

An adverse reaction is 'serious' if it:

1. Results in death;
2. Is life-threatening;
3. Requires hospitalisation or prolongation of existing hospitalisation;
4. Results in persistent or significant disability or incapacity;
5. Consists of a congenital anomaly or birth defect;
6. Is a medically important event.

   Pre-planned surgeries and hospitalisations for elective treatment of a pre-existing condition do not need reporting as SAEs.

Unexpected Adverse Reactions

An adverse reaction is 'unexpected' if its nature and severity are not consistent with the information about the medicinal product in question set out:

1. In the case of a product with a marketing authorisation, in the summary of product characteristics for that product;
2. In the case of any other investigational medicinal product, in the Investigator's Brochure relating to the trial in question.

Suspected Unexpected Serious Adverse Reaction (SUSAR)

Any suspected adverse reaction related to an IMP that is both unexpected and serious.

4.3.2 Reporting of Adverse Events

All adverse events must be fully recorded in the adverse event book throughout the entire study period and will be transcribed into the subjects' CRF, whether or not they are considered to be drug-related. Signs and symptoms of each AE should be described in detail: onset time and date, offset time and date, description of event, severity, relationship to investigational product, action taken and outcome.

Adverse events should be followed until recovery to the normal state has been achieved. In the event of a subject not returning to the clinical unit, the outcome of this event will be recorded as lost at follow up.

Reporting of SAEs and SUSARs

An SAE form should be completed and faxed to the Principal Investigator and the Sponsor within 24 hours.

In the case of a SUSAR, the staff at the site should:

Complete the SAE case report form & send it immediately (within 24 hours, preferably by fax or email), signed and dated to the Principal Investigator and Sponsor together with relevant treatment forms and anonymised copies of all relevant investigations.

The delegated site member centre will notify the MHRA, REC and the Sponsor of all SUSARs occurring during the study according to the following timelines; fatal and life-threatening within 7 days of notification and non-life threatening within 15 days. All investigators will be informed of all SUSARs occurring throughout the study.

4.3.3 Categorisation of Adverse Events

The intensity of an AE will be categorised as follows:

Mild:	Mild events are those which are easily tolerated with no disruption of normal daily activity.
Moderate:	Moderate events are those which cause sufficient discomfort to interfere with daily activity.
Severe:	Severe events are those which incapacitate and prevent usual activity.

4.3.4 Causal Relationship Assessment

Causal relationship assessment to drug treatments is required for purposes of reporting AEs. To promote consistency, the following guidelines should be taken into consideration along with good clinical and scientific judgment when determining the relationship of drug treatments to an AE:

Definitely Related:	A clinical event, including laboratory test abnormality, occurring in a plausible time relationship to the medication administration,

	and which cannot be explained by concurrent disease or other drugs or chemicals. The response to withdrawal of the drug should be clinically plausible.
Possibly Related:	A clinical event, including laboratory test abnormality, with a reasonable time sequence to the medication administration, but which could also be explained by concurrent disease or other drugs or chemicals. Information on the drug withdrawal may be lacking or unclear.
Unlikely Related:	A clinical event, including laboratory test abnormality, with little or no temporal relationship to medication administration, and for which other drugs, chemicals or underlying disease provide plausible explanations.
Not Related:	A clinical event, including laboratory test abnormality that has no temporal relationship to the medication or has more likely alternative aetiology.

4.3.5 Action Taken

Action taken will be defined as:

- None;
- Medication given;
- Dosing interrupted (this applies to Parts B and C only);
- Dosing stopped (this applies to Parts B and C).

4.3.6 Outcome

Outcome will be defined as:

- Resolved;
- Ongoing;
- Lost to follow up.

4.3.7 Coding of Adverse Events

All adverse events will be coded using the current version of Medical Dictionary for Regulatory Activities (MedDRA).

4.4 Clinical Laboratory Safety Tests

Sample collection times are included in the schedule of events

Additional and repeat testing may be performed at the discretion of the Principal Investigator. Safety laboratory variables to be assessed are presented in Table 4.4.

Table 4.4 Safety Laboratory Variables

Haematology	White blood cells	Haematocrit
	Red blood cells	Neutrophils (absolute and %)
	Mean corpuscular volume	Lymphocytes (absolute and %)
	Mean corpuscular hemoglobin	Monocytes (absolute and %)
	Mean corpuscular hemoglobin concentration	Eosinophils (absolute and %)
	Haemoglobin	Basophils (absolute and %)
		Platelets
Biochemistry	Sodium	Total bilirubin
	Potassium	Calcium
	Urea	Chloride
	Creatinine	Total protein
	Alkaline phosphatase	Globulin
	Alanine aminotransferase	Cholesterol
	Creatine kinase	Triglycerides
	Gamma-glutamyltransferase	Uric acid
	Lactate dehydrogenase	Phosphate
	Aspartate aminotransferase	Albumin
	Glucose	Bicarbonate
	Lipase	C-reactive protein[1]
	Amylase	HbA1c[1]
	FSH[2]	LH[2]
	Testosterone[2]	
Coagulation	Prothrombin time	Activated partial thromboplastin time
	International normalized ratio	
Thyroid Function	TSH, FT4	
Biomarkers	Tryptase	
Urinalysis	Protein	Leukocytes
	Bilirubin	Red blood cells
	Urobilinogen	pH
	Ketones	Nitrite
	Glucose	Specific gravity
Urine Microscopy	Microscopic investigation of sediment in case of pathological findings [performed if clinically indicated]	
Viral Serology	HIV I and II	Hepatitis C virus
	HBsAg	
Drugs of Abuse and Alcohol Screen	Amphetamine / Ecstasy	Benzodiazepines
	Ethanol	Methadone
	Cannabinoids	Barbiturates
	Cocaine	Cotinine
	Opiates	

[1]At pre-screening and screening only, in Parts B and C (cohorts 2 onwards for Part C) HbA1c may be remeasured at any time up to and including check in on Day -2 to confirm stability of glycaemia.
[2] At screening and follow up in Part A. At screening, Day 15, Day 29 and follow up in Part B. At screening and follow up in Part C.

Unless otherwise specified in the schedule of assessments all clinical laboratory tests will be performed by CRO Pathology Lab, which is GLP Accredited Laboratory. Details of all methodology and reference ranges are provided in the Trial Master File.

4.5 Clinical Safety Assessments

Assessment times are included in the schedule of events (see Section 16). Time points of assessments may be amended based on emerging safety, tolerability and PK data.

4.5.1 Vital Signs

Blood pressure and pulse rate will be measured using an automated instrument with the subject in the supine position after resting comfortably for 10 minutes. Body temperature will be measured orally in degrees Celsius using an automated thermometer. Additional vital signs measurements may be added for safety of the subjects.

Measurements will be reported in the subject's CRF.

4.5.2 Bedside glucose testing

Blood glucose level will be monitored at the times detailed in the schedule of events (Section 16). The Investigator may at their discretion vary the number and timing of such blood glucose measurements.

4.5.3 12-Lead ECG

Computerised 12-lead ECG recordings will be obtained after 5 minutes supine rest. Each lead shall be recorded for at least 3 beats at a speed of 25 mm/sec.

The following parameters will be recorded: ventricular rate, PR interval, QRS duration, QT and QTc.

4.5.4 Medical History

A complete medical history will include evaluation (past or present) of the following: general, head and neck, eyes, ears, nose, throat, chest/respiratory, heart/cardiovascular, gastrointestinal/liver, gynaecological/urogenital, musculoskeletal/extremities, skin, neurological/psychiatric, endocrine/metabolic, haemotologic/lymphatic, allergies/drug sensitivities, past surgeries, substance abuse or any other diseases or disorders.

4.5.5 Physical Examination

Physical examinations will be performed by a physician and will include the examination of the following: general appearance, eyes, ears, nose, throat, chest/respiratory, heart/cardiovascular, gastrointestinal/liver, musculoskeletal/extremities, dermatological/ skin, thyroid/neck, lymph nodes, neurological/psychiatric.

4.5.6 Local Injection Site Reactions

Injection sites will be monitored while the subject is resident in the clinical unit for redness, swelling, pain, tenderness and bruising. Reaction grades are provided in Table 4.5.

In the event that there are injection site reactions thought to be related to the volume of the injection, subsequent doses may be split into a maximum of 5 smaller injections (see Section 3.2).

Injection site reactions with a Grade ≥1 will be recorded as an AE.

Table 4.5 Injection Site Reaction Grading Scheme

Reaction	Grade 0	Grade 1	Grade 2	Grade 3	Grade 4
Redness[1]	0–24 mm	25–50 mm	51–100 mm	More than 100 mm	Requires medical intervention greater than analgesia
Swelling[2]	0–24 mm	25–50 mm and does not interfere with activity	51–100 mm or interferes with activity	More than 100 mm and prevents daily activity	Requires medical intervention greater than analgesia
Pain	None	Does not interfere with activity	Interferes with activity or repeated use of non-narcotic pain reliever	Prevents daily activity or repeated use of narcotic pain reliever	Requires medical intervention greater than analgesia
Tenderness	None	Mild pain to touch	Moderate pain to touch	Severe pain to touch	Requires medical intervention greater than analgesia
Bruising	None	Present			
Ulceration ± tissue necrosis	None	None	None	None	Present

1 Assessed by estimating the size of the red patch at the injection site across its widest point
2 Assessed by estimating the size of the raised area around the injection site across its widest point; also take into account how much it affects the subject in their routine daily activities.

5 DATA COLLECTION

An electronic data capture system will be used in this trial. Data will be captured onto source data documents (Workbooks) and will be entered into the Remote Data Capture system by staff at the clinical site. Covance will perfom 100% QC of the safety data for the dose escalation and safety meeting. Any discrepancies will be resolved in the database. The sponsor's independent monitor will perform 100% SDV for 3 subjects at each dose level plus 100% SDV of all safety data.

Following all data validation steps, the Principal Investigator, or designee, will electronically sign each eCRF prior to database lock.

6 EVALUATION OF STUDY DATA

6.1 Evaluation of Pharmacokinetic Parameters

Pharmacokinetic (PK) parameters of G3215 will include: maximum observed concentrations (C_{max}), time of occurrence of C_{max} (t_{max}), area under the plasma concentration-time curves ($AUC_{0-\infty}$, AUC_{0-t}, $AUC_{0-\tau}$), the apparent terminal rate constant (λ_z), the apparent terminal half-life ($t_{½}$) and the extent of accumulation in plasma (R_O). Pharmacokinetic parameters will be calculated, where appropriate, by non-compartmental analysis using WinNonlin Pro Version 5.2.1 (or higher version). Full details of the pharmacokinetic analysis, associated statistics and reporting will be documented in a separate Pharmacokinetic Analysis Plan which will be distributed to the Sponsor and Principal Investigator for approval.

PK parameters will be calculated using the data from the GLP qualified LC-MS/MS assay.

6.2 Evaluation of Pharmacodynamic Measures

6.2.1 Visual Analogue Scales

Change from baseline values will be calculated using Day -1 values as baseline.

6.2.2 Energy Intake

The energy value per unit of weight will be determined for each individual component of a meal. Each component of the meal will be weighed pre and post-meal and the weight consumed will be calculated (i.e. weight pre meal − weight post meal = weight consumed). Total energy intake will be calculated from the weight of each component consumed.

6.2.3 Body Weight

Time-matched change from baseline values will be calculated using Day -1 (AM and PM) values as baseline.

6.2.4 Immunogenicity

Samples will be analysed using a GLP compliant and validated assay method utilising an ELISA (bridging assay format) using rabbit positive control antibodies raised against G3215.

6.3 Evaluation of Safety

The safety evaluation will include blood pressure, pulse rate, ECG parameters, clinical laboratory tests (haematology, serum biochemistry, coagulation, urinalysis and urine microscopy). Tryptase determinations will only be performed after relevant adverse events and analysed retrospectively to inform the determination of the possible mechanism underlying AEs.

7 STATISTICAL METHODS

7.1 Primary and Secondary Target Variables

Primary Target Variables

- Safety and tolerability as assessed by adverse events, vital signs, physical examination, clinical laboratory safety assessments, and ECG parameters.

Secondary Target Variables

- Pharmacokinetics of G3215 as measured by plasma concentrations and derived pharmacokinetic parameters.

Exploratory Target Variables

- The effects of multiple doses of G3215 on food consumption.
- The effects of multiple doses of G3215 on VAS (nausea and satiety).
- The effects of multiple doses of G3215 on body weight.
- The effects of multiple doses of G3215 on enteropancreatic hormone levels.
- The effects of a continuous subcutaneous infusion of G3215 on food consumption.
- The effects of a continuous subcutaneous infusion of G3215 on VAS (nausea and satiety).
- The effects of a continuous subcutaneous infusion of G3215 on body weight.
- The effects of a continuous subcutaneous infusion of G3215 on enteropancreatic hormone levels.
- The comparative analytical performance of an Imperial College radioimmunoassay versus the LC-MS/MS assay for G3215.

7.2 Sample Size Determination

This is a Phase I study to investigate the safety and tolerability of a novel compound, an appropriate sample size cannot be statistically determined and hence the sample size chosen was based on previous experience in Phase I studies.

7.3 Subject Population for Analyses

Safety Population

All subjects who receive study medication (G3215 or placebo) will be included in the safety population.

Pharmacokinetic Population

All subjects who receive G3215 and have data from at least 1 PK blood draw will be included in the PK population.

Pharmacodynamic Population

All subjects who receive study medication and have data from at least 1 PD blood draw or measurement, as appropriate will be included in the PD population.

7.4 Pharmacokinetic Analysis

Pharmacokinetic consultancy will be responsible for the pharmacokinetic analysis. The pharmacokinetic analysis will be conducted at a Good Laboratory Practice compliant facility which operates a Quality Management System (QMS). The QMS has been designed to be compatible with the Organisation for Economic Co-operation and Development (OECD) Principles of GLP and GCP requirements. The pharmacokinetic analysis will be reported at [redacted] and the report will be included as an appendix to the clinical report. All data generated at [redacted] will be dispatched to the Sponsor and archived according to their archiving procedures.

A review of dosing information will be performed to consider excluding data in any period or on any day where a subject was judged to have received <80% or >120% of the scheduled dose of the investigational product; resulting exclusions will be documented in advance of issuing the draft report and agreed with the Sponsor. Plasma concentration data will be excluded for the affected period or day if concentrations are extremely low relative to other subjects' data; in these cases, plasma concentrations will be excluded from all or part of the profile, as appropriate.

PK parameters will be excluded from the analysis and summary statistics, where there are insufficient plasma concentration data available.

Plasma concentration data will be summarised by sampling time, dose level and Day, as appropriate; pharmacokinetic parameters (e.g. $AUC_{0-\infty}$, AUC_{0-24}, AUC_{0-t}, $AUC_{0-\tau}$, C_{max}, t_{max}, $t_{½}$ and R_O) will be summarised by dose level and Day, as appropriate.

All individual plasma and pharmacokinetic parameter estimates will be listed and summarised. Mean and individual plasma concentration versus time profiles will be illustrated using both linear-linear and logarithmic-linear scales.

Summary statistics will include number of subjects (n), arithmetic mean and standard deviation (SD). Summaries for the pharmacokinetic parameters will also display the median, minimum and maximum. In addition, the geometric mean and geometric coefficient of variation

($CV = \sqrt{\exp(SD_{ln}^{2}) - 1} * 100$, where SD_{ln} is the standard deviation of the natural logarithmically transformed data) will be reported for all pharmacokinetic parameters except t_{max}.

Between-subject variability will be based on geometric mean coefficients of variation (CVs).

A non-linear power model will be used to assess dose-proportionality. The proportional relationship between each parameter and dose is written as a power function:

$y = a \times dose^{b}$ (Equation 1)

where 'a' is a constant, 'b' is the proportionality constant and 'y' is the parameter of interest ($AUC_{0-\infty}$, AUC_{0-t}, $AUC_{0-\tau}$ or C_{max}). $AUC_{0-\tau}$ (after repeated dosing) $AUC_{0-\infty}$ (or AUC_{0-t} if $AUC_{0-\infty}$ could not be reliably estimated in all subjects) and C_{max} will be each plotted against dose. The exponent, b, will be estimated by performing a linear regression of the logged parameter on log dose. The exponent, b, is the estimated slope of the resulting regression line since taking logs of Equation (1) gives the linear relationship, $\log y = \log a + b \times \log dose$. The relationship is dose-proportional when $b = 1$. The exponents and 95 % confidence intervals (CIs), b_{lower} (b_l) and b_{upper} (b_u), are presented. There would be evidence of non dose-proportionality if this CI excludes one. The estimate of the fold increase in exposure for a doubling in dose (with 95 % CI) will also be presented. The increase in exposure expected for a doubling in dose will be calculated as 2^{b} (95 % CI: 2^{bl}, 2^{bu}).

Full details of the PK analysis and reporting will be included in a PK Analysis Plan.

7.5 Pharmacodynamic Analysis

Observed values for all pharmacodynamic parameters will be listed and where appropriate, summarised with descriptive statistics.

Change-from-baseline may be calculated if appropriate.

7.6 Safety Data Analysis

Safety data analysis will be performed by Covance. Individual and summary blood pressures, pulse rate, ECG parameters and clinical laboratory data (haematology, serum biochemistry, urinalysis and coagulation) will be presented in tabular form with mean, median, standard deviation and range (min and max) as appropriate. Continuous variables will be presented in tabular form with mean, median, standard deviation and range (minimum and maximum) as appropriate. Categorical variables will be summarised in frequency tables (frequency and proportion).

Tryptase values will be listed if the analysis has been performed and data are available.

For the laboratory safety data, out of range values will be flagged in the data listings and a list of clinically significantly abnormal values will be presented.

Adverse events will be tabulated and summarised according to the current version of Medical Dictionary for Regulatory Activities (MedDRA).

8 REGULATORY AND ETHICAL ISSUES

8.1 Regulatory and Ethics Review and Approval

The study will be submitted to the MHRA (UK) for review and approval and to an National Research Ethics Service for ethical review and approval. The documents submitted will include but are not limited to;

MHRA:

1. The final protocol;
2. The Investigators Brochure (IB);
3. The Investigational Medicinal Product Dossier (IMPD); and
4. Annex 1: Clinical Trial Application Form (Request for authorisation of a clinical trial on a medicinal product for human use to the competent authorities and for opinion of the ethics committees in the community).

Ethics:

1. The final protocol;
2. The Investigators Brochure (IB);
3. The ethics application form; and
4. The Informed Consent Form (ICF)

The study will not commence unless the following conditions are satisfied:

1. An ethics committee has given a favourable opinion in relation to the clinical trial; and
2. The clinical trial has been authorised by the licensing authority (MHRA).

8.2 Informed Consent

Subjects will sign the pre-screening/generic ICF in the presence of a suitably trained CRU clinical operations staff member prior to the pre-screening procedures being performed (if applicable).

For the main study, informed consent will be given freely after the subject has been informed of the nature, significance, implications and risks of the trial; and consent is evidenced in writing, dated and signed, or otherwise marked, by that person so as to indicate his/her consent, prior to the start of the study. The nature of the informed consent will comply with the current version of the Declaration of Helsinki, the current requirements of GCP (CPMP/ICH/135/95) and local regulation (The requirements are set out in Schedule

1 of Statutory Instrument 1031 and amendments) whichever provides the greater subject protection.

8.3 Indemnity and Compensation

In accordance with Statutory Instrument 1031 and amendments section 15 (5i, j) and the EU Clinical Trials Directive 2000/20/EC Article 3 (2f), provision is to be made for:

1. The indemnity or compensation in the event of injury or death attributable to the clinical trial; and
2. Insurance or indemnity to cover the liability of the Investigator or Sponsor.

Therefore the Sponsor, Imperial College, will indemnify the Investigator, Covance, from all and any claims arising out of this study except for their negligence or malpractice and providing that the study is conducted according to the standards established by the protocol.

In the event that it can be demonstrated that a subject suffers any significant deterioration in health or well-being or any harmful susceptibility or toxicity as a direct result of their participation in this study then Imperial College will agree to abide by the current Association of the British Pharmaceutical Industry Guidelines with regard to compensation payable to the subject. The amount of compensation will be calculated by reference to the level of damages commonly awarded in the UK for similar injuries at the time when such injury occurred.

The Investigator, Covance, declare to having insurance cover for the malpractice and/or negligence of their employees and agents.

9 STUDY MANAGEMENT

9.1 Quality Assurance and Quality Control

In accordance with the guideline for ICH GCP, the Sponsor has responsibility for implementing and maintaining quality assurance and quality control systems, and the ultimate responsibility for the quality integrity of the trial data resides with the Sponsor.

Authorised representatives of the Sponsor may perform audits or inspections during the study. The purpose of an audit is to independently assure that the study is conducted and that data is collected and reported according to the protocol, Good Clinical Practice (GCP), guidelines of the International Conference on Harmonisation (ICH), and any applicable regulatory requirements.

9.2 Protocol Adherence

The protocol must be read thoroughly and the instructions followed exactly. Any deviations should be agreed by both the Sponsor and the Investigator, with the appropriate written and approved protocol amendments made to reflect the changes agreed upon. Where the deviation occurs for the well-being of the subject, the Sponsor must be informed of the action agreed upon.

9.3 Documents Necessary for Initiation of Study

The following documents will be available prior to the first administration of the drug to the first subject:

1. Regulatory authorisation;
2. Copy of current Investigator's Brochure;
3. Risk assessment report;
4. Completed and signed investigator agreement/contract;
5. Signed original of the final protocol;
6. Ethics Committee approval;
7. Copy of the constitution of the Research Ethics Committee;
8. A list of members of the Ethics Committee;
9. A copy of the consent form and subject information to be used;
10. The curriculum vitae of all Investigators;
11. The Qualified Person's certification for the release of each batch of bulk IMP;

12. A technical agreement between the Sponsor and CRO defining the responsibilities of the Sponsor, CRO and any third parties significantly involved in the supply chain of the IMP, where applicable;

9.4 Study Monitoring

In accordance with ICH GCP, the Sponsor will be responsible for monitoring the conduct of the study. An independent study monitor will be appointed before the study begins.

The study monitor, will conduct pre-study and start-up meeting site visits and in addition will visit the site during the conduct of the study, review the study data and conduct a post-study visit. Study data recorded on source documents will be made available to the study monitor for the purpose of source document verification. The monitor will verify that the:

- data are authentic, accurate, and complete.
- safety and rights of subjects are being protected.
- study is conducted in accordance with the currently approved protocol and any other study agreements, GCP, and all applicable regulatory requirements.

The Investigator will allow trial-related monitoring audits, IEC review, and regulatory inspection allowing direct access to the source data/documents.

9.5 Study Closure

The Sponsor will be responsible for the close out visit which will be conducted on clinical completion of the study.

9.6 Study Record Retention

In accordance with SI 1928, the Sponsor and the Principal Investigator shall ensure that the documents contained, or which have been contained, in the trial master file are retained for at least 15 years after the conclusion of the trial and that during that period are:

- Readily available to the licensing authority on request; and
- Complete and legible.

All data derived from the study will remain the property of the Sponsor. The study will be the subject of a final clinical study report compiled by, or by order of the Sponsor.

All correspondence (e.g. with the Sponsor, ethics committee) relating to this study should be kept in the appropriate file folders.

Records of subjects' source documents, CRFs, IMP inventory, pertaining to the study must be kept on file. Records must be retained according to the current ICH Guidelines on GCP.

The Sponsor and Principal Investigator shall ensure that the medical files of trial subjects are retained for at least 15 years after the conclusion of the trial. The Sponsor shall appoint

named individuals within his organisation to be responsible for archiving the documents which are, or have been, contained in the trial master file. Access to those documents shall be restricted to those appointed individuals. If there is transfer of ownership of data or documents connected with the clinical trial:

- The Sponsor shall record the transfer; and
- The new owner shall be responsible for data retention and archiving in accordance Statutory Instrument 1031 and amendments.

If the Investigator moves, withdraws from an investigation or retires, the responsibility for maintaining the records may be transferred to another person who will accept responsibility. Notice of transfer must be made to and agreed by the Sponsor.

9.7 Publication Policy

After completion of the study, the Investigator may prepare a joint publication with the Sponsor. The Investigator must undertake not to submit any part of the individual data from this protocol for publication without prior consent of the Sponsor at a mutually agreed time.

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