Clinical Trial Protocol

A multicenter, open-label, metabolic balance study to evaluate the effects of apraglutide on intestinal absorption in adult subjects with short bowel syndrome, intestinal failure (SBS-IF), and colon-in-continuity (CIC)

Short Protocol Title: Effects of apraglutide on intestinal absorption in CIC SBS-IF

Trial Type:	Phase 2
EudraCT:	2020-005129-99
Trial Identifier:	TA799-013
Sponsor:	VectivBio AG, Aeschenvorstadt 36, 4051 Basel, Switzerland
Investigational Product:	Apraglutide (TA799)
Protocol Version and Date:	Version 4, 7 Feb 2022

Sponsor Signature Page

Trial number	Protocol number TA799-013
Protocol Title	A multicenter, open-label, metabolic balance study to evaluate the effects of apraglutide on intestinal absorption in adult subjects with short bowel syndrome, intestinal failure (SBS-IF), and colon-in-continuity (CIC)

The Sponsor has approved the current protocol and confirm hereby to conduct the trial according to the protocol, current version of the World Medical Association Declaration of Helsinki, current version of ICH-GCP guidelines and the local legally applicable requirements.

Sponsor Representative:

Printed name of Sponsor Representative: [redacted]

Feb 8, 2022 [redacted]

Date **Signature**

Statistician

Printed name of Statistician: [redacted]

Feb 8, 2022 [redacted]

Date **Signature**

International Coordinating Investigator

I have read and understood this trial protocol and agree to conduct the trial as set out in this trial protocol, the current version of the World Medical Association Declaration of Helsinki, the recent ICH-GCP guidelines and the local legally applicable requirements.

Site: [redacted]

Printed name of International Coordinating Investigator: [redacted]

Feb 8, 2022 [redacted]

Date **Signature**

Investigator Signature Page*:

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Date	Signature
Investigator Name	Printed Name
Site name and address	

**Note: This page must be individually signed by each participating Principal Investigator.*

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ABBREVIATIONS

ADA	Anti-Drug Antibodies
AE	Adverse Event
AESI	Adverse Event of Special Interest
Apo B-48	Apolipoprotein B-48
ALT	Alanine Aminotransferase
AST	Aspartate Aminotransferase
AUC	Area Under the Curve
CA	Competent Authority
CIC	Colon-in-Continuity
CKD-EPI	Chronic Kidney Disease Epidemiology
C_{max}	Maximum Observed Plasma Concentration
COVID-19	Coronavirus Disease 2019
CQ	Customer Queries
CRO	Contract Research Organization
CTCAE	Common Terminology Criteria for Adverse Events
C_{trough}	Trough plasma concentration
CTR	Clinical Trial Report
CTS	Clinical Trial Supplies
[redacted]	[redacted]
DEXA	Dual-Energy X-ray Absorptiometry
ECG	Electrocardiogram
eCRF	Electronic Case Report Form
EDC	Electronic Data Capture
eGFR	Estimated Glomerular Filtration Rate
ELISA	Enzyme-Linked Immunosorbent Assay
EOT	End of Trial
EU	European Union
FAS	Full Analysis Set
FDA	Food and Drug Administration
FSH	Follicle Stimulating Hormone
GCP	Good Clinical Practice
GGT	Gamma-Glutamyl Transferase
GH	Growth Hormone
GI	Gastrointestinal
GIP	Glucose-dependent Insulinotropic Polypeptide
GLP-1	Glucagon-like Peptide 1
GLP-2	Glucagon-like Peptide 2
HIV	Human Immunodeficiency Virus

IB	Investigator's Brochure
IBD	Inflammatory Bowel Disease
ICF	Informed Consent Form
ICH	International Council on Harmonisation
IEC	Independent Ethics Committee
I-FABP	Intestinal Fatty-Acid Binding Protein
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IMP	Investigational Medicinal Product
INR	International Normalized Ratio
ISR	Injection Site Reaction
kDa	Kilodaltons
LBP	Lipopolysaccharide-Binding Protein
LTE	Long-Term Safety and Clinical Outcomes Extension Trial
MAD	Multiple Ascending Dose
MB	Metabolic Balance
MedDRA	Medical Dictionary for Regulatory Activities
[redacted]	[redacted]
nAb	Neutralizing Antibodies
NCI-CTCAE	National Cancer Institute – Common Terminology Criteria for Adverse Events
NOAEL	No-Observed-Adverse-Effects Level
NRS	Numeric rating scale
PD	Pharmacodynamic
PGIC	Patient Global Impression of Change
PGI-PSI	Patient Global Impression of Parenteral Support Impact
PGI-SPS	Patient Global Impression of Satisfaction with Parenteral Support
PGI-TS	Patient Global Impression of Treatment Satisfaction
PGIS	Patient Global Impression of Severity
PK	Pharmacokinetic
PN	Parenteral Nutrition
POC	Proof-of-Concept
PopPK	Population PK
PRO	Patient Reported Outcomes
PS	Parenteral Support
[redacted]	[redacted]
PT	Preferred Term
PYY	Peptide YY
QoL	Quality of Life

QTcF	QT Interval corrected according to Fridericia's formula
RNA	Ribonucleic Acid
SAD	Single Ascending Dose
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SAS	Safety Analysis Set
SBS	Short Bowel Syndrome
SBS-IF	Short Bowel Syndrome with Intestinal Failure
SBS-IF-CIC-QOL	Short Bowel Syndrome with Intestinal Failure and Colon-in-Continuity Quality of Life questionnaire
SBS-IF-TI-QOL	Short Bowel Syndrome with Intestinal Failure - Treatment Impacts - Quality of Life questionnaire
SC	Subcutaneous
[redacted]	[redacted]
SIBO	Small Intestinal Bacterial Overgrowth
SMQ	Standardized MedDRA Queries
SoA	Schedule of Assessments
SUSAR	Suspected Unexpected Serious Adverse Reaction
TEAE	Treatment-Emergent Adverse Event
TEER	Transepithelial Electrical Resistance
TESAE	Treatment-Emergent Serious Adverse Event
TBL	Total Bilirubin
ULN	Upper Limit of the Normal range
USA	United States of America
VRS	Verbal Response Scale
WFI	Water For Injection

TRIAL SYNOPSIS

Sponsor	VectivBio AG
Protocol title:	A multicenter, open-label, metabolic balance study to evaluate the effects of apraglutide on intestinal absorption in adult subjects with short bowel syndrome, intestinal failure (SBS-IF), and colon-in-continuity (CIC)
Trial ID:	TA799-013
Protocol version and date:	Version 4, 07 February 2022
EudraCT number:	2020-005129-99
IND number:	Not Applicable
Clinical phase:	Phase 2
Background and rationale:	Short bowel syndrome (SBS) is a malabsorptive condition characterized by extreme reduction in the absorptive mucosal surface of the intestine, which most commonly occurs as a result of surgical resection due to mesenteric ischemia or inflammatory bowel disease (IBD), although other etiologies are also present. In patients with SBS with intestinal failure (SBS-IF), parenteral support (PS) delivered through a central venous catheter is needed to maintain an adequate balance of fluid, energy, electrolytes, trace elements, vitamins, and nutrients. After surgical resection, the remaining intestine goes through a process called intestinal adaptation by which it increases its absorptive capacity to compensate for its reduced length. It has been demonstrated that this process of intestinal adaptation can be enhanced by administering glucagon-like peptide (GLP)-2 or more stable analogs with extended half-lives such as teduglutide and apraglutide. Teduglutide, the only marketed GLP-2 analog at this time, has demonstrated significantly greater volume reduction of PS compared with placebo at 6 months in the pivotal STEPS trial. However, from a post-hoc analysis of this study, it is clear that patients with CIC behave differently from patients with an end-jejunostomy: even if the PS volume reductions at 6 months in teduglutide treated CIC patients were similar to the placebo group, they were more likely to receive days off PS or completely wean off PS after long-term treatment with teduglutide.

	Metabolic balance trials are the gold standard methodology to evaluate intestinal absorption of nutrients, fluids, and electrolytes in patients with SBS. However, these are complex, time- and labor-intensive trials, which are only available in a few centers worldwide. Most of the currently available data on GLP-2 analogs are based on subjects with a stoma in whom fluid losses are more pronounced and who have higher needs of PS in terms of fluid and energy than SBS patients with CIC. Apraglutide is a potent GLP-2 analog designed to have a long half-life due to slower absorption, decreased clearance, increased resistance to proteolysis, and increased plasma protein binding compared with teduglutide; this has been confirmed by nonclinical comparative data. These properties make apraglutide suitable for once a week dosing. In a Phase 2 study, GLY-321-2017, all subjects enrolled had either ileostomy or end-jejunostomy. In this study, apraglutide, at a weekly dose of 5 mg increased intestinal absorption after 4 weeks of treatment as evidenced by increased wet weight absorption, energy absorption, and urinary output. This trial will enroll CIC subjects exclusively and these subjects may respond differently to treatment. Therefore, the hypothesis for the current trial is that weekly dosing with the GLP-2 analog apraglutide increases energy absorption and reduces the subject's need for PS support (e.g., energy, fluids, electrolytes) in subjects with SBS-IF and CIC.
Objectives:	<u>Primary objective</u> • To evaluate the safety and tolerability of apraglutide <u>Secondary objectives</u> • To evaluate fluid, electrolytes, calories, and macronutrient absorption indicative of clinical efficacy • To evaluate the efficacy of apraglutide in reducing the administered volume per week of PS from baseline • To assess apraglutide plasma concentrations to inform the population pharmacokinetic (PopPK) model • To evaluate citrulline plasma concentrations to inform the PopPK / pharmacodynamic (PD) analysis <u>Exploratory objectives</u> • To evaluate other selected parameters indicative of clinical efficacy <u>Experimental Objectives</u>

	• To investigate predictors and underlying mechanisms on the effect of apraglutide by studying intestinal permeability, gastrointestinal (GI) motility, mucosal gene expression, and mucosa-associated and luminal microbiota

Endpoints:	PRIMARY ENDPOINTS
	• Adverse events (AEs; system organ class, frequency and severity)
	• Adverse events of special interest (AESIs):
	  ○ Injection site reaction
	  ○ Gastrointestinal obstruction
	  ○ Gallbladder, biliary and pancreatic disease
	  ○ Fluid overload
	  ○ Colorectal polyps
	  ○ Malignancies
	• Clinical chemistry, hematology, hemostasis, anti-drug antibodies (ADA), and urine analysis
	SECONDARY ENDPOINTS
	Efficacy endpoints related to PS volume
	• Relative change from baseline in actual weekly PS volume at Weeks 4, 24 and 52
	• Absolute change from baseline in actual weekly PS volume at Weeks 24 and 52
	• Subjects who achieve a reduction of at least 1 day per week of PS from baseline at Weeks 24 and 52
	• Clinical responders (20% reduction of PS volume from baseline) at Weeks 24 and 52
	• Subjects reaching enteral autonomy at Weeks 24 and 52
	• Energy reduction in the parenteral nutrition (PN) from baseline at Weeks 24 and 52
	Efficacy endpoint related to nutritional, fluid (wet weight), and electrolyte absorption
	• Change in absolute energy absorption over metabolic balance periods, from baseline to Week 48
	• Relative change in absorption of energy over metabolic balance periods from baseline at Week 48
	• Change in absorption of macronutrients over metabolic balance periods from baseline at Week 48
	• Change in absolute absorption of energy over metabolic balance periods from baseline at Week 4

	• Change in absorption of macronutrients over metabolic balance periods from baseline at Week 4 • Changes in urine output and urinary electrolytes (sodium, potassium, calcium, and magnesium) over metabolic balance periods from baseline at Week 4 and at Week 48 *Patient reported outcomes* • [redacted] • Patient Global Impression of Change (PGIC) at Weeks 24 and 52 • Changes from baseline in Patient Global Impression of Treatment Satisfaction (PGI-TS) at Weeks 24 and 52 • Changes from baseline in Patient Global Impression of Satisfaction with Parenteral Support (PGI-SPS) at Weeks 24 and 52 • Changes from baseline in Patient Global Impression of Parenteral Support Impact (PGI-PSI) at Weeks 24 and 52 *Pharmacokinetic /Pharmacodynamic related endpoints* • Trough plasma concentration (C_{trough}) • Plasma citrulline levels EXPLORATORY ENDPOINTS *Endpoints related to nutritional status* • Changes from baseline in lean body mass, bone mineral content, and fat mass by dual-energy X-ray absorptiometry (DEXA) scan from baseline to Weeks 4, 24, and 48 • Change in body weight from baseline at Weeks 4, 24 and 48 • Change in dietary intake of wet weight, energy, macronutrients, fluid, and electrolytes (sodium, potassium, calcium, and magnesium) from baseline at Weeks 4 and 48 • Change in fecal excretion of wet weight, energy, macronutrients, fluid, and electrolytes (sodium, potassium, calcium, and magnesium) from baseline at Weeks 4 and 48 *Endpoints related to efficacy* • Change from baseline in the total time per day that subjects infuse PS at Weeks 24 and 52 • Change from baseline in the total time per week that subjects infuse PS at Weeks 24 and 52

- Change in electrolytes, minerals, macronutrients, and other contents of the PS from baseline at Weeks 24 and 52

Patient reported outcomes

- Short Bowel Syndrome with Intestinal Failure and Colon-in-Continuity - Changes from baseline in Quality of Life Questionnaire (SBS-IF-CIC-QOL) at Weeks 24 and 52
- Short Bowel Syndrome with Intestinal Failure - Changes from baseline in Treatment Impacts - Quality of Life Questionnaire (SBS-IF-TI-QOL) at Weeks 24 and 52

[redacted]

Enteral autonomy is defined as a subject not receiving PS for hydration or PN for calories. Subjects may still receive minimal fluid to maintain patency of the central line or for specific elemental/micro-nutrient needs (e.g., <100mL fluid for administration of magnesium). If at the visit the subject is weaned from PS, except perhaps for minimal fluids, the information will be recorded in the eCRF. If at the next

	scheduled visit, the subject is still considered weaned, the date of the previous visit will be considered the date of enteral autonomy. If the subject is first weaned at Visit 17, this will be used for the date of weaning without a further visit needed. This is because no further visits under trial treatment will occur.
Trial design:	This is a repeated dose, open-label trial investigating efficacy, safety, PD, and PK of apraglutide in subjects with SBS-IF and CIC. The subjects will receive a subcutaneous (SC) dose of 2.5 mg or 5 mg apraglutide once weekly for 52 weeks. Safety follow-up assessments will be performed 4 weeks (±1 week) after the last dose. Subjects will undergo three 72-hour metabolic balance evaluations at baseline, Week 4, and Week 48. For the first 4 weeks, up to the completion of the second metabolic balance study, the PS should not be changed. Parenteral support volume can be reduced for safety reasons, but all possible attempts should be made to return to the baseline PS prescription for the second metabolic balance period. After each visit, starting after Week 4, the PS volume will be reviewed and adjusted by the Investigator taking into account the protocol defined algorithm for PS volume reduction, based on changes in urine output of 10% or greater. For subjects with change in urine output not higher than 10%, cases will be adjudicated by the participating investigators who will review clinical status of participants to assess if PS volume reduction is warranted. The three investigators will make recommendations for the safety of subjects and with the objective to standardize and harmonize decisions on PS volume reduction and weaning across the three sites. Interim analysis will be performed after last subject completes Weeks 4 and 24. Additional interim analysis may be performed by the sponsor at any time.
Inclusion/exclusion criteria:	<u>Inclusion Criteria:</u> Initial inclusion criteria (before baseline metabolic balance study) 1. Signed informed consent for this trial prior to any trial specific assessment 2. Male and female subjects with SBS-IF and CIC (at least 28% [Cummings, 1973] and no colostomy), receiving PS, secondary to surgical resection of the small intestine with <200 cm from duodeno-jejunal

	flexure, based on available medical/surgical records and with the latest intestinal resection leading to SBS-IF being at least 12 months prior to Screening 3. Subject considered stable with regard to PS and weight. Stability is defined as a subject meeting all the following criteria in the 3 months preceding screening: a) Weight change (increase or decrease) ≤5% b) PS volume change not exceeding 25% c) PS energy content change not exceeding 25% d) Actual PS usage in terms of volume and content matches prescribed PS (±10%) 4. Parenteral support requirement of at least 2 days per week as assessed at Screening 5. Willingness to undergo either a colonoscopy or colonography and have any identified polyps removed via colonoscopy 6. Willingness to remain in clinic for metabolic study on three occasions for 5 days 7. Adhere to an individual pre-defined drinking menu and urine measurements during the 48-hour fluid balance periods 8. No planned restorative surgery or major intestinal surgery (more than 10% intestinal resection or surgery that changes anatomy group, i.e., stoma or CIC), in the trial period 9. Age ≥18 years at Screening 10. Women of childbearing potential must agree to practice effective contraception and to use a highly effective method of contraception during the trial and for 4 weeks after the End of Trial (EOT) visit. Such methods include: combined (estrogen and progestogen containing) hormonal contraception associated with inhibition of ovulation (oral, intravaginal, transdermal); progestogen-only hormonal contraception associated with inhibition of ovulation (oral, injectable, implantable); intrauterine device; intrauterine hormone-releasing system; bilateral tubal occlusion; or a vasectomized partner. To be considered sterilized or infertile, females must have undergone surgical sterilization (bilateral tubectomy, hysterectomy or bilateral ovariectomy) or be post-menopausal (defined as at least 12 months amenorrhea, without an alternative medical cause, may be confirmed with follicle-stimulating hormone test if there is doubt). Women who do not engage in

	heterosexual intercourse, during the trial and 4 weeks after the EOT Visit, will be allowed to join the trial without contraception following a thorough discussion with the Investigator to determine if this is feasible for the subject. The following methods are not considered acceptable methods of contraception: calendar, ovulation, symptothermal, post-ovulation methods, withdrawal (coitus interruptus), spermicides only, and lactational amenorrhea method 11. Male subjects with a female partner of childbearing potential must commit to practice contraception (e.g., condoms or a vasectomy) and abstain from sperm donation during the trial and for 2 weeks after the EOT Visit. Their partners, if they are women of childbearing potential, must agree to practice effective contraception and to use a highly effective method of contraception during the trial and for 4 weeks after the EOT visit. Such methods include: combined (estrogen and progestogen containing) hormonal contraception associated with inhibition of ovulation (oral, intravaginal, transdermal); progestogen-only hormonal contraception associated with inhibition of ovulation (oral, injectable, implantable); intrauterine device; or an intrauterine hormone-releasing system Dosing inclusion criteria (after baseline metabolic balance study completion and before the first dose) 12. Average fecal wet weight excretion of ≥500 g/day during the baseline metabolic balance study 13. Average urine volume is ≥0.8 L and ≤2.5 L per day during baseline metabolic balance study <u>Exclusion criteria</u> 1. Pregnancy and/or lactation 2. Body mass index equal or higher than 30 kg/m^2 at Screening 3. Major abdominal surgery (more than 10% intestinal resection) in the last 6 months prior to screening visit. Surgery for feeding tube placement and cholecystectomy allowed 4. A history of clinically significant intestinal adhesions which have not been treated surgically, increasing the risk of GI obstruction 5. Constipation which is not adequately managed by dietary recommendations or laxatives 6. Active or untreated enterocutaneous fistula

	7. History of cancer (including colon carcinoma) or clinically significant lymphoproliferative disease within ≤5 years, except for adequately treated basal cell skin cancer 8. Acute cholecystitis or biliary obstruction left untreated within 1 month of Screening visit or during Screening period 9. Subjects with active IBD or an underlying condition who require new drug treatment or regimen changes including increased or decreased dose of a previously administered treatment for at least the previous 6 months prior to Screening and during the Screening period 10. Central venous catheter sepsis experienced within the previous 2 months, prior to Screening visit and during Screening period, or use of systemic antibiotics within the last 30 days, prior to Screening, due to catheter infection 11. Decompensated heart failure (New York Heart Association class III–IV) [NYHA] and/or known coronary heart disease defined as unstable angina pectoris and/or myocardial infarction within the previous 6 months prior to Screening 12. Radiation enteritis, scleroderma or residual evidence of intestinal dysmotility unrelated to SBS, including pseudo-obstruction and Hirschspruing's disease, and celiac disease, refractory or tropical sprue 13. History of alcohol and/or drug abuse within the previous 12 months prior to Screening 14. Child-Pugh score Class C 15. Elevated liver enzymes: a) Alanine aminotransferase (ALT) or aspartate aminotransferase (AST) >5 x upper limit of normal (ULN) b) Alanine aminotransferase or AST >3 x ULN and total bilirubin >2 x ULN or international normalized ratio (INR) >1.5 for a person not using anticoagulant drug and INR >3 for a person on anticoagulant therapy such as warfarin c) Alanine aminotransferase or AST >3 × ULN and clinical signs of fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever, rash, and/or eosinophilia

	d) Subjects with serum conjugated bilirubin >34 µmol/L or 2 mg/dL during two consecutive measurements 16. Evidence of chronic renal disease as demonstrated by inadequate renal function as defined by estimated glomerular filtration rate (eGFR) using the Chronic Kidney Disease Epidemiology (CKD-EPI) formula at Screening visit <20 mL/min/1.73m^2 17. Any treatment for a period of greater than 2 weeks of glutamine, growth factors such as growth hormone (GH) and native GLP-1, GLP-2, or GLP-1 or GLP-2 analogs in the previous 6 months prior to Screening 18. Known or suspected hypersensitivity to GLP-1 or GLP-2 analogs or apraglutide excipients 19. Known neutralizing antibodies (nAb) against GLP-1 or GLP-2 analogs 20. Participation in another clinical trial in the last 3 months and during this trial (studies with catheter locks, coronavirus disease 2019 [COVID-19] vaccines or observational trials, which are not a burden on the subject and do not interfere with the participation in this trial, are allowed) 21. Donation of blood or plasma >500 mL within 3 months prior to Screening 22. Positive results for human immunodeficiency virus (HIV), hepatitis A, B, and/or C tests[1] 23. Subject not capable of understanding or not willing to adhere to the trial visit schedules and/or other protocol requirements (including the use of the e-diary) 24. Judged not eligible by the Investigator for any other reason
Measurements and procedures:	<u>Efficacy Assessments</u> • Macronutrients and energy oral intake and output • Electrolytes oral intake and output • Wet weight excretion

[1] Subjects recovered from hepatitis B or C can be enrolled, i.e., they have markers of the infection, but the viral load is undetectable. Subjects with evidence of an acute or chronically active hepatitis B or C infection should be excluded. If a subject is positive for hepatitis A virus immunoglobulin M antibodies, this would indicate an acute infection and the subject is ineligible, but he/she may be eligible for re-screening after recovery. A subject positive for hepatitis B antigens, along with an undetectable viral load and a history of vaccination against hepatitis B would be eligible for enrollment.

	• Urine output
	• Parenteral support
	  ○ Volume
	  ○ Composition
	  ○ Days per week
	  ○ Duration
	• Drinking diary/Oral fluid intake
	• Stool diary (Bristol Stool Form Scale) and number of bowel movements
	• Change in eating/appetite
	• Investigator narrative
	• Short Bowel Syndrome with Intestinal Failure and Colon-in-Continuity - Quality of Life Questionnaire
	• Short Bowel Syndrome with Intestinal Failure - Treatment Impacts - Quality of Life Questionnaire
	• [redacted]
	• Patient Global Impression of Change
	• Patient Global Impression of Severity
	• Patient Global Impression of Treatment Satisfaction
	• Patient Global Impression of Satisfaction with Parenteral Support
	• Patient Global Impression of Parenteral Support Impact
	• Plasma drug concentration
	• Plasma citrulline concentration
	• Urinary sodium (from 48hr balance periods)
	• Dual-Energy X-ray Absorptiometry Scan
	• [redacted]

	Safety assessments • Colonoscopy* or colonography • Physical examination • Vital signs • Twelve-lead electrocardiogram • Body weight • Adverse events and AESIs • Concomitant medications • Clinical chemistry (including liver enzymes), hematology, and hemostasis • Anti-apraglutide antibodies and their neutralizing capacity (collectively ADA) • Urinalysis • Pregnancy test *Also performed for efficacy assessments
Trial product / intervention:	Apraglutide 2.5 mg or 5 mg SC once weekly
Control intervention (if applicable):	Not Applicable
Number of subjects with rationale:	This trial will enroll approximately 10 subjects. No formal sample size calculation has been performed for this trial. This number of subjects is considered sufficient to provide adequate information about the general safety, tolerability, efficacy, PD, and PK of the compound at this stage of development.
Trial duration:	Approximately 4 weeks of screening/baseline and 52 weeks on treatment, followed by a safety follow-up of 4 weeks (±1 week).
Trial schedule:	First subject in is planned for approximately May 2021 Last subject in is planned for approximately May 2022 Last subject out is planned for approximately May 2023
Principal Investigator:	[redacted]
Trial centers:	Three European sites located in Belgium, Denmark, and France.
Statistical considerations:	Given the exploratory nature of the trial, the statistical evaluation will include mostly descriptive statistics and

	simple analysis contrasting 4 weeks, 24 weeks, 48 weeks, or 52 weeks against baseline or screening as appropriate.
GCP statement:	This trial will be conducted in compliance with the protocol, the current Declaration of Helsinki, International Council for Harmonisation-Good Clinical Practice (R2), as well as all national legal and regulatory requirements.

TRIAL SCHEDULE

Table 1: Schedule of Assessments (SoA)

	Screening	Baseline	Treatment Period															End of trial
Visit No.[1]	**1**	**2[3]**	**3**	**4**	**5[3]**	**6**	**7**	**8**	**9**	**10[26]**	**11**	**12**	**13**	**14**	**15**	**16[3]**	**17**	**18**
Week (weeks unless indicated)	**-4 to -2 (Day -28 to -14)**	**-1 (Day -7 to -3)**	**0 (Day 0)**	**2 (+1)**	**4**	**8[4] (±1)**	**12 (±2)**	**16 (±2)**	**20 (±2)**	**24 (±2)**	**28 (±2)**	**32 (±2)**	**36 (±2)**	**40 (±2)**	**44 (±2)**	**48 (±1)**	**52 (±2 days)**	**4 weeks after last dose ±1 week**
General																		
Informed consent	X																	
Inclusion and Exclusion criteria	X	X																
Demographics	X																	
Medical history	X																	
Concomitant medication	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
GI health history	X	X	X															
Metabolic balance evaluation (see Table 2 for detailed schedule)		X			X											X		
Safety																		
Colonoscopy or colonography[5,7]	X									X[6]						X[23]		
Physical examination[8]	X	X			X					X						X	X	X
12-lead ECG[8]	X	X			X					X						X	X	X
Vital signs (blood pressure, heart rate, axillary temperature)[8]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Body weight and height[9]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Adverse events[10]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Investigator narrative		X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Telephone contact[11]	X		X															

	Screening	Baseline	Treatment Period															End of trial
Visit No.[1]	**1**	**2[3]**	**3**	**4**	**5[3]**	**6**	**7**	**8**	**9**	**10[26]**	**11**	**12**	**13**	**14**	**15**	**16[3]**	**17**	**18**
Week (weeks unless indicated)	**-4 to -2 (Day -28 to -14)**	**-1 (Day -7 to -3)**	**0 (Day 0)**	**2 (+1)**	**4**	**8[4] (±1)**	**12 (±2)**	**16 (±2)**	**20 (±2)**	**24 (±2)**	**28 (±2)**	**32 (±2)**	**36 (±2)**	**40 (±2)**	**44 (±2)**	**48 (±1)**	**52 (±2 days)**	**4 weeks after last dose ±1 week**
Laboratory																		
Clinical chemistry incl. liver enzymes, hematology, hemostasis, urinalysis (dipstick)	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Serum pregnancy test premenopausal women	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Blood samples for PK[12]				X	X		X			X		X		X		X	X	X
Blood sample for citrulline[12]		X		X	X		X			X		X		X		X	X	X
Blood sample for ADA[12]		X		X	X		X			X				X		X		X[13]
HIV and hepatitis test	X																	
Post-PS volume reduction safety evaluations[14,]					X	X	X	X	X	X	X	X	X	X	X	X	X	
Efficacy																		
Prescribed PS volume, composition, days per week	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X[15]
Define and confirm drinking menu[16]	X	X																
Patient Reported Outcome (PRO) questionnaires[2]		X			X		X			X			X				X	X
Change in Eating/Appetite		X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

	Screening	Baseline	Treatment Period															End of trial
Visit No.[1]	**1**	**2[3]**	**3**	**4**	**5[3]**	**6**	**7**	**8**	**9**	**10[26]**	**11**	**12**	**13**	**14**	**15**	**16[3]**	**17**	**18**
Week (weeks unless indicated)	**-4 to -2 (Day -28 to -14)**	**-1 (Day -7 to -3)**	**0 (Day 0)**	**2 (+1)**	**4**	**8[4] (±1)**	**12 (±2)**	**16 (±2)**	**20 (±2)**	**24 (±2)**	**28 (±2)**	**32 (±2)**	**36 (±2)**	**40 (±2)**	**44 (±2)**	**48 (±1)**	**52 (±2 days)**	**4 weeks after last dose ±1 week**
Urine sample for sodium analysis (from sample taken during 48-hour urine collection, balance period)[16]		X[17]		X	X[17]	X	X	X	X	X	X	X	X	X	X	X[17]	X	
DEXA Scan		X			X					X						X		
Gastroduodenoscopy	X									X[6]						X[23]		
Trial medication and diary																		
Hand out subject e-diary	X																	
Subjects complete diary prior to the visit		X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Review all subject diary information since last visit		X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	
SC injection of IMP at site			X	X	X	X	X	X	X	X	X	X	X	X	X	X		
Instruct subject in IMP handling at home and assess self-administration capability[18]			X															
Dispense IMP			X	X	X	X	X	X	X	X	X	X	X	X	X	X		

	Screening	Baseline	Treatment Period															End of trial
Visit No.[1]	**1**	**2[3]**	**3**	**4**	**5[3]**	**6**	**7**	**8**	**9**	**10[26]**	**11**	**12**	**13**	**14**	**15**	**16[3]**	**17**	**18**
Week (weeks unless indicated)	**-4 to -2 (Day -28 to -14)**	**-1 (Day -7 to -3)**	**0 (Day 0)**	**2 (+1)**	**4**	**8[4] (±1)**	**12 (±2)**	**16 (±2)**	**20 (±2)**	**24 (±2)**	**28 (±2)**	**32 (±2)**	**36 (±2)**	**40 (±2)**	**44 (±2)**	**48 (±1)**	**52 (±2 days)**	**4 weeks after last dose ±1 week**
Return home administered IMP vials to site				X	X	X	X	X	X	X	X	X	X	X	X	X	X	
[redacted]																		
[redacted]																		
[redacted]																		
[redacted]																		
[redacted]																		
[redacted]																		
[redacted]																		
[redacted]																		
[redacted]																		
[redacted]																		

ADA, anti-drug antibodies; AE, adverse event; AESI, adverse event of special interest; DEXA, dual-energy X-ray absorptiometry; ECG, electrocardiogram; EOT, end of trial; GI, gastrointestinal; HIV, human immunodeficiency virus; IMP, investigational medicinal product; LTE, long-term safety and clinical outcomes extension trial; [redacted]; PGIC, Patient Global Impression of Change; PGIS, Patient Global Impression of Severity; PGI-PSI, Patient Global Impression of Parenteral Support Impact; PGI-SPS, Patient Global Impression of Satisfaction with Parenteral Support; PK, pharmacokinetics; PRO, patient reported outcomes; PS, parenteral support, [redacted]; SBS-IF-CIC-QOL, Short Bowel Syndrome with Intestinal Failure and Colon-in-Continuity Quality of Life questionnaire; SBS-IF-TI-QOL, Short Bowel Syndrome with Intestinal Failure - Treatment Impacts - Quality of Life questionnaire; SC, subcutaneous; [redacted]

1. If at any visit the subject shows signs of significant fluid overload, leading to clinical decompensation, the Investigator should consider PS reduction. An AESI of fluid overload must be reported. In all cases this reduction must be discussed with the medical monitor either before or after reduction
2. Patient reported outcomes collected as follows (with the following preferred order):
   At Baseline: SBS-IF-CIC-QOL[redacted] PGIS, PGI-PSI, PGI-SPS, PGIC (1.0), PGIC (2.0)
   At Weeks 4, 12, and 36: SBS-IF-CIC-QOL, SBS-IF-TI-QOL,[redacted] PGIS, PGI-PSI, PGI-TS, PGI-SPS, PGIC (1.0), PGIC (2.0)
   At weeks 24, 52, and EOT visit: SBS-IF-CIC-QOL, SBS-IF-TI-QOL[redacted] PGIS, PGI-PSI, PGI-TS, PGI-SPS, PGIC (1.0), PGIC (2.0)
   Only subjects that completed version 1.0 of the PGIC and PGIS at baseline continue to complete these during the trial
3. See Table 2 Schedule of Assessments for metabolic balance periods
4. The visit window of ±1 week or ±2 weeks allows a shift in the visit 7 or 14 days (±1 day) from Baseline calculated visits. Visits should be done at the same day of the week with a window of ±1 day. Investigational medicinal product administrations during a week with a site visit must be done at the site
5. If biopsies are required a colonography will NOT be performed unless a full view of the colon to check for polyps was not possible during the colonoscopy
6. Optional assessments
7. When deemed anatomically feasible by the Investigator, colonoscopy or colonography should be performed within ± 3 weeks of end of treatment
8. ECG, physical exam and vital signs should be recorded prior to blood sampling and prior to IMP administration
9. Subjects to be asked to measure their body weight weekly and record results in the e-diary. Body height will be measured once at screening only
10. Adverse events and AESIs should be recorded starting after obtaining informed consent (see Section 6.1)
11. Telephone contact will be made every 7 days (±1 day) during screening. During each contact, AEs will be collected and changes in concomitant medications recorded. At all contacts the subject will be asked to recollect the frequency and severity of nausea, vomiting, and abdominal pain in the preceding 7 days. This information will also be collected at Visit 3, pre-dose. At Week 0, Day 1 (+1 day), the subject will be contacted to assess for fluid overload and frequency of stools
12. Pharmacokinetic, ADA, and citrulline samples are to be collected pre-dose except on the metabolic balance periods (see Table 2). An unscheduled ADA sample may be collected following an injection site reaction (ISR) or hypersensitivity event at the discretion of the Investigator
13. If a subject performs the EOT visit they will be requested to return for blood samples for ADA as detailed in Section 5.1.11
14. If PS was reduced, an additional visit and assessments will be performed as per Section 5.2.9
15. At early discontinuation, the Investigator will decide to continue documentation of PS prescription in source documents and electronic case record form until the scheduled end of trial visit for that subject, if subject consents
16. Drinking Menu defined at Screening must remain stable throughout the trial and applies to the 48-hour balance periods and the 72-hour metabolic balance periods only. This will be confirmed and agreed upon at baseline. The 48-hour balance period is defined as starting not more than 4 days before trial visit and should be completed no later than the start time of their trial visit procedure assessments (e.g. on the morning of their trial visit). For Visit 4, the 48h balance period is defined as starting not more than 2 days before trial visit and should be completed before the start time of their trial visit procedure assessments (e.g., on the morning of their trial visit). The 48-h balance period must be conducted over 48 consecutive hours
17. Sample from the 72-hour metabolic balance study collection
18. To be instructed on a continuous basis, as needed
19. [redacted]

23 [redacted]

Table 2: Schedule of Assessments for Metabolic Balance Periods (Visits 2 [Baseline], 5, and 16)

	Metabolic balance period				
Procedure	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**
Admission to hospital	X (at least 16 h prior to MB samples collection start)				
Hospital discharge					X
Confirmation of eligibility[1]	X Baseline only				X Baseline only
Review all subject diary information since last visit	X				
Adverse events	X	X	X	X	X
Concomitant medication	X	X	X	X	X
Investigator narrative	X				
GI health history	X				
Physical examination	X				
12-lead ECG	X				
Vital signs (blood pressure, heart rate, axillary temperature)[2]	X	X	X	X	X
Clinical chemistry	X				
Hematology	X				
Hemostasis	X				
Urinalysis	X				
Pregnancy test (premenopausal women)	X				
Citrulline blood sample	X				
Blood samples for PK (Visits 5 and 16)[1]		Pre-dose (within 30 min prior to dosing)	24 h (±2 h), 30 h (±2 h)	48h (±2 h)	72 h (±2 h)

	Metabolic balance period				
Procedure	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**
Blood sample ADA, nAb	X				
Fasting	X (start 22:00 ±30 min)	X (until start of MB collections)			
Urinate & defecate – prior to MB collection start[1]		X (-30 min)			
Urinate & defecate – end of MB period					X (-30 min)
Body weight[2]	X	X	X	X	X
Start of MB collection[2]		X Time of IMP[3] (+15 min)			
Collection of urine		X	X	X	X
Collection of feces		X	X	X	X
Collection of duplicate food and drinks		X	X	X	X
End of MB collection					72 h from start (±1 h)
Change in appetite	X				
Confirm drinking menu	X Baseline only				
Adhere to agreed upon drinking menu		X	X	X	X
Define baseline PS type, volume, content	X Baseline only				
Fixed baseline PS type, volume and content (Baseline & Visit 5)		X	X	X	X
DEXA Scan		X[4]			

	Metabolic balance period				
Procedure	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**
Patient reported outcomes (PRO)		X (13:00-18:00)			
Bristol Stool Form Scale and Frequency of bowel movement		X	X		
IMP administration (Visits 5 and 16)		X			
Dispense IMP (Visits 5 and 16)					X
[redacted]					
[redacted]					
[redacted]					
[redacted]					
[redacted]					
[redacted]					
[redacted]					
[redacted]					

ADA, anti-drug antibodies; [redacted]; DEXA, dual-energy X-ray absorptiometry; ECG, electrocardiogram; GI, gastrointestinal; IMP, investigational medicinal product; nAb, neutralizing antibody; PK, pharmacokinetics; PRO, patient reported outcomes; PS, parenteral support; MB, metabolic balance; MQ, [redacted]

1. At the first MB period only, confirmation of Screening eligibility to be confirmed on Day 1 and confirmation of inclusion criteria 12 and 13 to be confirmed on Day 5
2. The activities should be performed in the following sequence on Days 2-5: AE collection, weight, vital signs, pre-dose PK sample (Visits 5 and 16 only), defecate/urinate, IMP administration (Visits 5 and 16 only), start of 72-hour collection of urine, feces and food. Body weight and vital signs will be measured on Day 1 at any time, on Day 2 after defecation but before IMP administration, and on subsequent days at the same time as on Day 2 ±1 h
3. Visits 5 and 16 MB periods only
4. Same timing across all MB periods with regard to PS infusion and time of day based on first MB period ±2 h (see Section 5.2 for more details)
5. [redacted]

[redacted]

1. INTRODUCTION

1.1 Background and Rationale

Short Bowel Syndrome (SBS) is a malabsorptive condition caused by physical or functional loss of the intestine leading to reduced absorptive mucosal surface. Short bowel syndrome most commonly occurs as a result of surgical resections of the small intestine due to mesenteric ischemia or inflammatory bowel disease (IBD) although other etiologies are also present.

Short bowel syndrome with intestinal failure (SBS-IF) is defined as the reduction of gut function below the minimum necessary for the absorption of macronutrients and/or fluids and electrolytes, such that intravenous supplementation is required to maintain health and/or growth [Pironi, 2015]. Patients with chronic intestinal failure due to benign disease have a high probability of long-term survival on parenteral support (PS) [Pironi, 2012] although catheter-related sepsis is a very frequent PS-complication [Van Gossum, 2001] and liver failure is the PS-related complication with the greatest risk of death [Pironi, 2012].

However, even within the category of SBS-IF there is a spectrum of disease severity with varying degrees of malabsorption and requirements for PS. At one end of the SBS-IF severity spectrum are patients who require low volumes of PS; most of these patients retain all or part of the colon and the jejunum or ileum has been anastomosed to the remaining colon (referred hereafter as “colon-in-continuity” [CIC] patients). These patients require moderate to low PS volume, sometimes only the minimum volume required to deliver the calories needed and they have a higher likelihood of achieving enteral autonomy during treatment. At the other end of the SBS-IF spectrum are patients who have higher PS volume requirements, driven by large volume of fluids and electrolytes. Most patients in this category have jejunostomies or ileostomies and no CIC (referred to hereafter as “stoma” patients) and have a lower likelihood of achieving enteral autonomy [Joly, 2020; Lam, 2018; Pevny, 2018; Seidner, 2019]. Despite the presence of this anatomically defined spectrum of malabsorption the small bowel is the common target organ for treatment with Glucagon-like Peptide-2 (GLP-2) analogs in SBS-IF.

While the patient’s life is saved through PS, the Quality of Life (QoL) of all these patients is impaired by the time they have to spend on the PS infusions as well as the complications derived from the use of PS support, for example, catheter infections, hospitalizations and liver disease. Thus, the main therapeutic goal of SBS-IF treatment is the reduction of the volume of PS across the spectrum in 1) reduction in the time per day on PS, 2) increasing the number of days off PS per week, or 3) the ability to reach full oral/enteral autonomy.

Over time, the remaining intestine goes through a process called intestinal adaptation by which the remaining intestine increases its absorptive capacity to compensate for the reduced length. This post-resection intestinal adaptation is a spontaneous process attempting to ensure a more efficient absorption of nutrients per unit length of the remaining bowel by increasing the absorptive area (structural adaptation) and by slowing the gastrointestinal (GI) transit and/or reducing GI secretions (functional adaptation). This process usually takes place 1–2 years after the resection and

weaning off may occur in 20% to 50% of the patients [Messing, 1999]. Patients are also able to compensate for their malabsorption by increasing oral intake (hyperphagia); however, this ability varies greatly between individual patients [Jeppesen, 2013]. Interestingly, it has been shown that the PS-dependency probability in patients with stoma reaches a plateau very rapidly after surgery, whereas in CIC patients this is reached after almost 2 years, suggesting that intestinal adaptation takes much longer in CIC patients than in stoma patients [Messing, 1999].

It has been demonstrated that this process of intestinal adaptation can be enhanced by administering GLP-2 or more stable analogs with an extended half-life. Endogenous GLP-2 is produced in the enteroendocrine L cell in a nutrient dependent manner and facilitates nutrient absorption by activating pro-absorptive pathways: increasing mesenteric blood flow, inhibiting GI motility and gastric acid secretion, reducing intestinal epithelial permeability, and promoting mucosal growth. Glucagon-like peptide-2 also enhances gut barrier function and induces proliferative and cytoprotective pathways in the small bowel [Brubaker, 2018].

Teduglutide is a GLP-2 analog that has been approved for the treatment of patients with SBS-IF after a successful Phase 3 trial [STEPS trial]. The STEPS trial demonstrated that daily subcutaneous (SC) injections of 0.05 mg/kg teduglutide resulted in a reduction of the PS volume required to maintain fluid balance. Briefly, 63% of subjects on the teduglutide arm were clinical responders compared with only 30% in the placebo arm. Clinical response was defined as a reduction of at least 20% in PS at both Weeks 20 and 24 [STEPS trial; Jeppesen, 2012].

Apraglutide, the compound under investigation in this trial, is a potent GLP receptor agonist specifically designed to have slower absorption, decreased clearance, increased resistance to proteolysis, and increased plasma protein binding [Wiśniewski, 2016]. Pharmacokinetic (PK) data from two separate healthy volunteer trials show PK parameters supportive of once weekly dosing.

Dosing once weekly with apraglutide when compared against teduglutide daily dosing could substantially reduce the daily burden of therapy, improve patient compliance and adherence, and reduce the adverse reactions associated with SC administration, thereby improving the patient's QoL substantially.

In the wake of the post-hoc results of the STEPS trial and the recently published real world evidence from centers in the United States of America (USA) and Europe, a difference in the response to the GLP-2 analog teduglutide between stoma and CIC patients has become apparent [Jeppesen, 2018]. Although patients with stoma showed larger reductions in PS volume [Jeppesen, 2018], most patients who reached enteral autonomy had CIC [Joly, 2020; Lam, 2018; Pevny, 2018; Seidner, 2019]. Most likely this difference is due to better absorption of water in patients with CIC due to the presence of remnant colon as well to the PS reduction algorithm in the trial, which was based on urinary output alone.

In conclusion, SBS-IF is to be conceptualized as a disease spectrum based on the degree of malabsorption, with stoma patients at one end and CIC patients at the other end of the disease spectrum. Nevertheless, the main therapeutic objective for all patients with SBS-IF is the reduction of the PS volume, translating into 1)

reduction in the time per day on PS, 2) increasing the number of days off PS per week, or 3) the ability to reach full enteral autonomy.

The hypothesis for the current trial is that weekly dosing with the GLP-2 analog apraglutide increases energy absorption and reduces the subject's need for PS support (e.g., energy, fluids, and electrolytes) in subjects with SBS-IF and CIC.

1.2 Investigational Medicinal Product and Indication

Apraglutide is a peptide analog of GLP-2 under development for treatment of SBS-IF, which acts as a full agonist at the GLP-2 receptor with *in vitro* potency and selectivity comparable with native GLP-2 [Wiśniewski, 2016].

This peptide is designed to have a longer elimination half-life by being resistant to cleavage by dipeptidyl peptidase-4, the major GLP-2 peptidase. The systemic half-life in various animal species as well as in human healthy volunteers is significantly prolonged compared with the native human GLP-2. Apraglutide has, in various animal models, shown a trophic effect on the small intestine and maintained the mucosal barrier function.

Apraglutide has been administered to 66 healthy volunteers and 16 SBS patients and proved safe and well tolerated at single and repeated weekly doses ranging from 1 mg up to 56.9 mg.

1.3 Preclinical Evidence

Nonclinical studies performed with apraglutide showed no off-target toxicity and indicate a favorable profile for clinical development.

In a study with neonatal piglets with resected ileum and jejuno-colic anastomosis, apraglutide significantly increased small-intestinal weight, villus height and crypt depth, intestine length, reduced fecal fat, and energy losses [Slim, 2019].

In rats, apraglutide induces a greater intestinotrophic effect than teduglutide and glepaglutide using the same doses and dosing intervals, and it shows an extended duration of effect compared with teduglutide [Hargrove, 2020].

A detailed review of nonclinical results is provided in the Investigator's Brochure (IB).

1.4 Clinical Evidence to Date

To date, four clinical trials with apraglutide have been completed, including:

- Two Phase 1 trials in healthy volunteers
  - GYM-P3-698, a Single Ascending Dose (SAD) and Multiple Ascending Dose (MAD), placebo-controlled trial of apraglutide in healthy adult volunteers, to evaluate safety, tolerability, PK, and PD of single and multiple doses ranging from 2.8 to 56.9 mg
  - TA-799-002, a multiple-dose trial in healthy adult volunteers to evaluate the PK and PD effect of multiple doses of either apraglutide SC (1, 5, or 10 mg) or placebo SC once weekly for 6 weeks
- Two Phase 2 trials designed to assess the safety and tolerability and to demonstrate the proof-of-concept (POC) for apraglutide in SBS subjects
  - GLY 321-2017, a metabolic balance trial in SBS subjects testing a weekly dose of 5 mg apraglutide

- GLY-311-2017, a dose-exploration trial in SBS-IF subjects testing weekly 5 mg apraglutide against placebo and 10 mg apraglutide in an open-label phase

Key conclusions from these trials include the following:

- Apraglutide up to three doses of 56.9 mg was safe and well-tolerated in the Phase 1 study GYM-P3-698
- In TA-799-002, the PD effects of apraglutide, as measured by plasma citrulline levels (a biomarker of small intestine enterocyte mass), persisted longer than plasma drug concentrations. Apraglutide was well tolerated up to six weekly doses of 10 mg
- In the metabolic balance trial GLY-321-2017, apraglutide at a weekly dose of 5 mg increased intestinal absorption as evidenced by increased wet weight absorption, energy absorption, and urinary output while decreasing ostomy output
- The results seen with apraglutide in the dose-exploration trial, GLY-311-2017, are consistent with those seen in the metabolic balance trial GLY-321-2017. Apraglutide at 5 and 10 mg doses increased urinary output, a surrogate marker of intestinal absorption. The 10 mg dose has no different response than 5 mg
- Taken together, efficacy data and clinical findings from subjects treated in the two Phase 2 trials with 5 mg and 10 mg of apraglutide SC on a once weekly regimen for 4 weeks, support that apraglutide provides comparable clinical efficacy to once a day teduglutide
- Pharmacokinetics of apraglutide is dose-proportional within the range of 1 mg to 56.9 mg and is described by a one-compartment model with zero order absorption from the subcutaneous administration site and first order elimination from the central compartment
- Population PK (PopPK) analysis from the previously described four trials demonstrated an effect of body weight on exposure with higher exposure in subjects with lower body weight

1.5 Trial Objectives, Endpoints and Rationale

1.5.1 Trial Objectives

1.5.1.1 *Primary Objective*

- To evaluate the safety and tolerability of apraglutide

1.5.1.2 *Secondary Objectives*

- To evaluate fluid, electrolytes, calories and macronutrient absorption indicative of clinical efficacy
- To evaluate the efficacy of apraglutide in reducing the administered volume per week of PS from baseline
- To assess apraglutide plasma concentrations to inform the PopPK model
- To evaluate citrulline plasma concentrations to inform the PopPK / pharmacodynamic (PD) analysis

1.5.1.3 *Exploratory Objectives*

- To evaluate other selected parameters indicative of clinical efficacy

1.5.1.4 *Experimental Trials Exploratory Objective*

- To investigate predictors and underlying mechanisms of the effect of apraglutide by studying intestinal permeability, GI motility, mucosal gene expression, and mucosa-associated and luminal microbiota

1.5.2 Trial Endpoints

1.5.2.1 *Primary Endpoints*

- Adverse events (AEs; system organ class, frequency and severity)
- Adverse events of special interest (AESIs):
  - Injection site reaction
  - Gastrointestinal obstruction
  - Gallbladder, biliary and pancreatic disease
  - Fluid overload
  - Colorectal polyps
  - Malignancies
- Clinical chemistry, hematology, hemostasis, anti-drug antibodies (ADA), and urine analysis

1.5.2.2 *Secondary Endpoints*

Efficacy endpoints related to PS volume

- Relative change from baseline in actual weekly PS volume at Weeks 4, 24, and 52
- Absolute change from baseline in actual weekly PS volume at Weeks 24 and 52
- Subjects who achieve a reduction of at least 1 day per week of PS from baseline at Weeks 24 and 52
- Clinical responders (20% reduction of PS volume from baseline) at Weeks 24 and 52
- Subjects reaching enteral autonomy at Weeks 24 and 52
- Energy reduction in the PN from baseline at Weeks 24 and 52

Efficacy endpoint related to nutritional, fluid (wet weight) and electrolyte absorption

- Change in absolute energy absorption over metabolic balance periods, from baseline to Week 48
- Relative change in absorption of energy over metabolic balance periods from baseline at Week 48
- Change in absorption of macronutrients over metabolic balance periods from baseline at Week 48

- Change in absolute absorption of energy over metabolic balance periods from baseline at Week 4
- Change in absorption of macronutrients over metabolic balance periods from baseline at Week 4
- Changes in urine output and urinary electrolytes (sodium, potassium, calcium, and magnesium) over metabolic balance periods from baseline at Week 4 and at Week 48

Patient reported outcomes

- [redacted]
- Changes in Patient Global Impression of Change (PGIC) at Week 24 and 52
- Changes from baseline in Patient Global Impression of Treatment Satisfaction (PGI-TS) at Weeks 24 and 52
- Changes from baseline in Patient Global Impression of Satisfaction with Parenteral Support (PGI-SPS) at Weeks 24 and 52
- Changes from baseline in Patient Global Impression of Parenteral Support Impact (PGI-PSI) at Weeks 24 and 52

Pharmacokinetic/Pharmacodynamic related endpoints

- Trough plasma concentration (C_{trough})
- Plasma citrulline levels

1.5.2.3 *Exploratory Endpoints*

Endpoints related to nutritional status

- Changes from baseline in lean body mass, bone mineral content and fat mass by dual-energy X-ray absorptiometry (DEXA) scan from baseline at Weeks 4, 24, and 48
- Change in body weight from baseline at Weeks 4, 24 and 52
- Change in dietary intake of wet weight, energy, macronutrients, fluid and electrolytes (sodium, potassium, calcium, and magnesium) from baseline at Weeks 4 and 48
- Change in fecal excretion of wet weight, energy, macronutrients, fluid and electrolytes (sodium, potassium, calcium, and magnesium) from baseline at Weeks 4 and 48

Endpoints related to efficacy

- Change from baseline in the total time per day that subjects infuse PS at Weeks 24 and 52
- Change from baseline in the total time per week that subjects infuse PS at Weeks 24 and 52
- Change in electrolytes, minerals, macronutrients, and other contents of the PS from baseline at Weeks 24 and 52

Patient reported outcomes

- Short Bowel Syndrome with Intestinal Failure and Colon-in-Continuity - Changes from baseline in Quality of Life Questionnaire (SBS-IF-CIC-QOL) at Weeks 24 and 52
- Short Bowel Syndrome with Intestinal Failure - Changes from baseline in Treatment Impacts - Quality of Life Questionnaire (SBS-IF-TI-QOL) at Weeks 24 and 52

1.5.2.4 *Experimental Endpoints*

- [redacted]

Enteral autonomy is defined as a subject not receiving PS for hydration or PN for calories. Subjects may still receive minimal fluid to maintain patency of the central line or for specific elemental/micro-nutrient needs (e.g., <100 mL fluid for administration of magnesium). If at the visit the subject is weaned from PS, except perhaps for minimal fluids, the information will be recorded in the eCRF. If at the next scheduled visit, the subject is still considered weaned, the date of the previous visit will be considered the date of enteral autonomy. If the subject is first weaned at Visit 17, this will be used for the date of weaning without a further visit needed. This is because no further visits under trial treatment will occur.

1.6 Rationale of Trial Population Studied

Subjects in the population selected for this trial have SBS-IF and CIC and are receiving PS, secondary to surgical resection of the small intestine, with <200 cm from duodeno-jejunal flexure, based on available medical/surgical records and with the latest intestinal resection being at least 12 months prior to Screening.

Real world data [Joly, 2020] show that CIC patients respond positively to GLP-2 analogs when managed not only using fluid balance criteria but also overall clinical parameters with a special focus on energy balance. There are, however, very limited data on the effects of GLP-2 analogs on intestinal absorption, as previous trials have enrolled almost exclusively patients with stoma [Jeppesen, 2005; Naimi, 2019]. In addition, these CIC patients are most likely to wean completely from PS by achieving enteral autonomy [Lam, 2018; Joly, 2020; Pevny, 2018]. This may indicate that effects are present on intestinal energy absorption in these patients and that the conventional PS weaning algorithms may not be sufficient to elucidate the full potential of GLP-2 analogs. Therefore, this trial will investigate the effects of apraglutide on intestinal absorption in CIC patients.

1.7 Risk / Benefit Considerations

1.7.1 Known Risks with Use of GLP-2 Analogs

Teduglutide, the only GLP-2 analog currently on the market, has shown good tolerability and safety of once daily administration [Kochar, 2018]. There are treatment related risks reported that are related to the mode of action such as possible risk of acceleration of neoplastic growth and enhancement of colon polyp growth [Revestive, SmPC; Gattex® USPI]. The most commonly reported adverse reactions in the teduglutide clinical trials were abdominal pain and distension (45%), respiratory tract infections (28%; including nasopharyngitis, influenza, upper respiratory tract infection, and lower respiratory tract infection), nausea (26%), injection site reactions (26%), headache (16%), and vomiting (14%). Approximately 38% of the treated patients with a stoma experienced GI stoma complications. The majority of these reactions were mild or moderate [Revestive, SmPC; Gattex® USPI]. Also, due to increased absorption, there is a risk of fluid overload and of overdose with concomitant medications with a narrow therapeutic window.

1.7.2 Summary of Clinical Safety with Apraglutide Reported During Phase 1 and 2 Trials

Apraglutide has generally been safe and well tolerated in the clinical trials conducted to date as demonstrated by the following:

- A dose relationship has not been seen for any adverse event (AE) in any of the trials
- Doses up to 56.9 mg were safe and well-tolerated in the SAD and MAD parts of the Phase 1 trial GYM-P3-698 in healthy volunteers
- Apraglutide was well tolerated up to six weekly doses of 10 mg in study TA799-002 in healthy volunteers
- The most frequent AEs in SBS patients in GLY-321-2017 included nausea (75%), GI stoma output decreased (75%), and stoma complications (75%)
- Frequent AEs in SBS patients in GLY-311-2017 were primarily related to the expected efficacy effects of apraglutide, including decreased stoma output (75%) or stoma output abnormal (63%), polyuria (88%), and decreased thirst (63%)
- Serious AEs (SAE) reported by patients have primarily been disease complications that are common in SBS subjects, including device-related

sepsis and device malfunction. In GLY-311-2017, five subjects reported eight of such treatment-emergent serious adverse events (TESAEs). Three of these TESAEs occurred when they received placebo. All of these TESAEs were considered not related or unlikely related to the experimental treatments. In GLY-321-2017, two subjects had three TESAEs. All of the SAEs resolved and only one SAE, abdominal pain, was assessed by the Investigator as related to apraglutide

- For each hematology, coagulation, and chemistry parameter, mean and median change from baseline were not clinically significant. Isolated occurrences of clinically significant out of range laboratory parameters were reported as AEs, but there was no consistent pattern with the occurrence of these events
- No QT interval corrected according to Fridericia's formula (QTcF) prolongation > 500ºmilliseconds was seen in any of the clinical trials. In GLY-311-2017 and in GLY-321-2017, the changes from baseline in the QTcF values were ≤30 msec for all subjects at all time points, except for one subject at the End of Trial Visit and another subject at pre-dose of Period 3
- Low-titer ADA have been seen in five subjects (none in GYM-P3-698, one in TA799-002, three in GLY-311-2017 and one in GLY-321-2017). Anti-drug antibodies had no apparent effect on either the PD or PK of apraglutide

In summary, the safety and tolerability profile of once weekly administration with apraglutide derived from clinical trials conducted so far confirms that the expected mode of action related safety events should be anticipated to also occur with apraglutide.

1.7.3 Assessment of Known and Potential Risks and Benefits

Injection site reactions are expected to occur with apraglutide; however, since administration will be limited to once weekly, a potential beneficial impact can be anticipated through reduction of the overall tolerability burden of injection site reactions compared with existing treatment with once daily SC injections.

Once weekly administration with apraglutide is expected to result in a more stable PK exposure over time with lower fluctuations in exposure levels as with once daily administration, which potentially could lead to less discomfort in the mode of action related events that occur at peak levels of drug exposure.

Taken together, the Phase 2 clinical trials have confirmed that apraglutide has clinical effects similar to other GLP-2 analogs but with weekly dosing instead of daily. Short bowel syndrome is a condition with a known pathophysiology and a well-established physiological response. Clinical efficacy of natural GLP-2 and other GLP-2 analogs has been demonstrated in subjects with SBS [Jeppesen, 2012; Jeppesen, 2005]. Preliminary results from the two Phase 2 apraglutide trials have shown that weekly administration of apraglutide, increases intestinal absorption parameters and/or urinary output (surrogate marker of fluid intestinal absorption) in subjects with SBS (5 mg and 10 mg). In addition, weekly administration may represent a substantial additional benefit to subjects in terms of reduction in daily burden of therapy, local reactions and improvement of QoL over daily dosing. During the course of this clinical trial any AESI (serious or non-serious) of scientific and medical concern

specific to the apraglutide program will be closely monitored to detect any safety signal unknown to date as early as possible (see Section 6).

Therefore, considering the nature of the disease under investigation it is considered that potential benefit outweighs the risk.

1.8 Dose Rationale

The selection of the dose proposed for the Phase 3 trial is based on the converging evidence provided by the following:

- Phase 2 Dose Finding trial (GLY-311-2017)
- Phase 2 Proof of Mechanism trial (GLY-321-2017)
- Pharmacology results from Healthy Volunteer trials (GYM-P3-698 and TA799-002)
- Safety margins (from GLP toxicology studies)
- Population pharmacokinetic model

In the Phase 2 trial GLY-311-2017, 5 mg and 10 mg weekly doses of apraglutide were tested against placebo in SBS-IF patients. The key efficacy outcome was the ambulatory urinary output measured during a 48-hour fluid balance period. Under these conditions, urinary output is used as a surrogate marker of intestinal absorption. The average change in urinary output from baseline at 5 mg and 10 mg doses against the placebo period was an increase of 48.5% for the lower dose and 32.6% for the higher dose. In conclusion, the urinary output increased at both doses against placebo although it did not increase from 5 to 10 mg, whereas generally apraglutide was safe and well tolerated at both dose levels.

The second Phase 2 trial GLY-321-2017 showed increases in urinary output (surrogate marker of intestinal fluid absorption), wet weight intestinal absorption, and intestinal energy absorption at a dose of 5 mg once weekly. Therefore, the GLY-321-2017 trial further supports the adequacy of the proposed dose of weekly 5 mg subcutaneous injection.

Moreover, in the Phase 1 trial (GYM-P3-698), apraglutide was shown to be safe and well tolerated up to 56.9 mg after single SC doses and up to 56.9 mg when dosed weekly after three doses. Pharmacokinetics were proportional to dose at the tested dose range. The PD marker citrulline was induced after three weekly doses of apraglutide with similar response from 11.4 to 56.9 mg, suggesting that at 11.4 mg weekly an effect on citrulline also becomes saturated.

Results from TA799-002 showed that 5 mg weekly dosing of apraglutide induced significant increases in citrulline levels; steady state levels were reached at Week 2 and remained elevated with 5 mg weekly dosing, supporting that at a dose regimen of 5 mg once weekly apraglutide will sustain biological effects for at least 1 week. No statistically significant difference in plasma citrulline levels was observed between 5 mg and 10 mg doses.

The exposure margins at the no-observed-adverse-effects level (NOAEL) in the repeat-dose toxicity studies are well in excess of 200-fold in both rats and minipigs, and >40-fold at the NOAEL of 1 mg/kg/day in the 13-week mouse toxicity study relative to the exposure at the proposed clinical dose of 5 mg once a week subcutaneous injection of apraglutide. The exposure margins for apraglutide at the

NOAEL in the embryofetal development studies in rat and rabbit are 140- and 17-fold, respectively, thereby advocating for the use of highly effective methods of contraception in this trial.

A PopPK model was developed which included data from all four clinical trials. Of all the tested covariates only body weight at baseline was statistically significant, with body weight inversely correlating with area under the curve (AUC) and maximum observed plasma concentration (C_{max}). Due to the higher, non-linear increase of exposures (AUC and C_{max}) as body weight decreases, subjects with body weight below 50 kg will be dosed with 2.5 mg to prevent high exposures and mitigate safety risks. Alternatively, subjects of 50 kg or higher body weight will receive 5 mg. Although some heavy subjects may get lower exposures, it is estimated that the number of patients with SBS and heavy body weight is minimal (see for example body weight distribution in STEPS trial; Jeppesen, 2012; Jeppesen, 2018).

In conclusion, taking together all data available (nonclinical data, clinical PK, PD, safety, and clinical efficacy data from adult SBS-IF subjects) and the higher exposure in low body weight subjects, 5 mg once a week subcutaneous injection of apraglutide is proposed as the clinical dose for subjects weighing 50 kg or more and 2.5 mg for subjects with body weight lower than 50 kg.

2. TRIAL DESIGN

2.1 Rationale for the Trial Design

Completed trials investigating the effects of apraglutide enrolled subjects with stoma, whilst the ongoing blinded Phase 3 trial, TA799-007, is enrolling subjects with both stoma and CIC. The current trial will examine the safety profile of apraglutide in CIC subjects, over 1 year in an open label trial. Data from the current trial will be important for ongoing clinical trials to determine if there are differences in the safety profile of apraglutide between CIC and stoma subjects.

Balance studies quantify weight, volume, as well as the content of energy, macronutrients and electrolytes of oral intake (i.e., food and fluid intake, as well as PS) and of the output (i.e., feces and urine production). Balance studies are acknowledged as the “gold standard” to measure intestinal function and the methods used have been published [Jeppesen, 2013]

Wet weight excretion is an important secondary endpoint of this trial. Fecal output is important to assess since it is closely linked to the intestinal function and degree of malabsorption. Urinary output is another important secondary endpoint of this trial. Patients with SBS are at high risk of sodium depletion and dehydration and maintaining a proper hydration state is essential.

Sodium, and hence water, are avidly conserved by the kidney, so the hydration state of the patient can be monitored by measuring the urinary output and the urinary sodium concentration. In SBS patients, increases in urinary output and urinary sodium concentration are markers of functional intestinal rehabilitation [Jeppesen 2014].

A previous balance study has been performed using apraglutide (GLY-321-2017); however, all enrolled patients had either ileostomy or end-jejunostomy. The current study will enroll exclusively CIC patients, which may have different outcomes in the proposed endpoints. The previous study demonstrated a change in fecal excretion over the 4-week treatment period including a decreased sodium ostomy output and increased absorption of energy, fat, carbohydrate, etc. However, this previous study did not look at effects beyond the 4-week treatment period.

Studies examining the use of another GLP-2 analog, teduglutide, have demonstrated that CIC patients may have a different response to treatment. It was found that CIC may be weaned off PS support but had less of a volume of PS reduction versus patients with a stoma, reflecting the lower baseline PS volume [Joly, 2020].

Thus, the current clinical trial will examine the effect of apraglutide on metabolic parameters in CIC patients over a longer period time than previous studies. As CIC patients make up a large proportion of the SBS population it is important to investigate the effects of apraglutide in this population.

2.1.1 Rationale for the PS Volume Reduction Algorithm

In a previous 24-week treatment Phase 3 trial with the GLP-2 analog teduglutide, PS volume could be reduced by 10% at clinical visits, if urinary output increased by at least 10% [Jeppesen, 2011]. Many subjects in this trial suffered from fluid overload and stopped drinking. In a follow-up Phase 3 trial (STEPS trial) PS volume reductions of at least 10%, but not more than 30% were allowed, if urinary output increased by

at least 10% [Jeppesen, 2012]. [redacted] These fluid overloads were the results of strong drug effects on intestinal absorption that cannot be followed by adequate reductions in PS.

In the TA799-013 trial, PS volume adjustments will be performed after an increase of at least 10% of urinary output against baseline value by calculating a new PS volume (Section 4.5). This approach enables adjustment of PS volume with a lower likelihood of fluid overload.

The urinary output driven algorithm will be further expanded to include a clinical assessment of subjects which includes body weight, stools (consistency, frequency, etc.), a physician narrative of the overall well-being, oral food intake including appetite, fluid intake (oral drinking volume and PS) and daily urinary volume from 48-hour balance period which may lead to further reduction in PS.

2.2 Overall Design

This is an international, multicenter, open-label metabolic balance study to evaluate the efficacy and safety of apraglutide and effects on intestinal absorption in adult subjects with SBS-IF and CIC.

After provision of informed consent and initial confirmation of eligibility, subjects will be screened (Table 1). If they meet the eligibility criteria, they will return to site for the Baseline metabolic balance period as per Table 1. They will remain at site to complete the metabolic balance study. Dosing eligibility criteria will be confirmed after completion of the baseline metabolic balance study and before the first dose of investigational medicinal product (IMP). Subjects will return to the site on Day 0 for the first dose of IMP.

Subjects will receive a SC dose of 2.5 mg or 5 mg apraglutide once weekly for 52 weeks. Safety follow-up assessments will be performed 4 weeks (±1 week) after the last dose.

Between visits, IMP can be either self-administered at home or by a family member/caregiver, or administered at the clinic. The capabilities of the subject to self-administer the IMP will be assessed by the site after training the subject or family member/caregiver in SC administration of IMP using a training kit. The decision to select IMP administration at site or at home will be determined based on subject capability for self-administration at home, subject preference and clinical considerations (Section 4.2.1). The subject will record IMP self-administration information in their e-diary (Section 5.1.7).

If the daily urine volume of the balance period prior to the current visit is at least 10% higher than the baseline urine volume, then the weekly PS volume will be reduced as detailed in Section 2.1.1.

If the daily urine volume of the balance period prior to the current visit is less than 10% greater than baseline values, the subject's clinical status will be reviewed by all three Investigators. If in the treating Investigator's opinion an adjustment is warranted, the proposed adjustment will be sent to the other Investigators for review. Within 7 days the other Investigators will review and send their recommendation. Recommendations will be made for the safety of subjects and with the objective to standardize and harmonize decisions on PS volume reduction and weaning across the three sites.

If there is a discrepancy between the proposed adjustment by the treating Investigator and the other Investigators a telephone/video conference will be held within 7 days to resolve the differences. If no unanimous decision can be reached the treating Investigator's recommendation will be used. Final decision and summary of conferences will be filed in the subject's medical record. The treating Investigator will provide a rationale within the electronic case report form (eCRF) indicating why and which course of action was followed.

In this trial the following applies:

1) Parenteral support is defined as any intravenous infusion that contains fluids and electrolytes and may or may not include parenteral nutrition (PN). Parenteral nutrition is defined as PS that includes protein, carbohydrate, fat, vitamins, and/or trace elements
2) Fluids consumed during the balance period may be taken either by mouth or via GI tube, naso-jejunal tube, gastrostomy tube, or other type of permanent or temporary tube
3) Fluid is defined as any liquid which does not contain solids (e.g., water, soda, juice, and clear broth). If the fluid contains solids it should not be considered a liquid (e.g., soup with vegetables, noodles, and rice). Smoothies, blitzed meals, yoghurt, puddings, etc. are also considered food not fluids
4) Subjects must receive PS at least 2 times a week to be eligible for the trial. There is no minimum volume required per day but PS must be necessary for the medical treatment of the subject. An infusion of a small amount of fluid to maintain catheter patency is not considered PS
5) The volume of each urine void needs to be measured and recorded in the diary on an ongoing basis over the 48-hour balance period and will not be collected into a single container over the balance period. Further details are included in the Laboratory manual
6) During the trial, if the days of PS are reduced, then the 48-hour balance period should contain 1 day of PS support and one day without, if possible. If a subject has been weaned completely from PS, the balance period will not contain any days of PS

2.2.1 Screening Period

Initial Screening Visit

Potential subjects will be approached by the Investigator and the trial will be explained to them. It is recommended that the Investigator verifies if the subject has a history of vomiting on a frequent basis or a history of obstruction.

After obtaining informed consent, subject initial eligibility will be evaluated (Section 3.2).

All initial eligibility criteria must be met for the subject to be allowed to enter the baseline period.

If the remaining small bowel length is not specifically and clearly documented within the site's medical/surgical records to conclusively demonstrate that the small intestine is <200 cm, the Investigator will use their clinical judgement along with available surgical/medical records, to determine if the subject meets this criterion. The Investigator may also conduct additional investigations (e.g., imagistic or

endoscopic evaluations), if needed in their clinical opinion. This will be documented in the subject's site file and in the eligibility package sent to the medical monitor.

In collaboration with the Investigator, the subject will select an individual drinking menu which will have to be adhered to for all further fluid balance assessments during the course of the trial. The drinking volume must be ≥1.0 L and ≤3.5 L/day. A deviation during the 48-hour balance periods of ±10% to the defined menu is acceptable.

Subjects will receive the e-diary and the baseline metabolic balance evaluation visit will be scheduled.

After Screening, eligible subjects will be given a patient card supplied by the Sponsor that will provide information required in an emergency situation; for example, the protocol number, trial title, product name, Sponsor name and address, Investigator site's 24 hour telephone number, and the emergency contact details for the subject.

Baseline Period

Prior to subjects receiving their first dose of trial medication, they will be admitted and confined to the site for 5 days to perform the baseline metabolic evaluation. During this time the individual drinking menu and dosing eligibility criteria (average fecal wet weight excretion of ≥500 g/day and average urine volume of ≥0.8 L and ≤2.5 L per day) will be confirmed.

2.2.2 Treatment Period

All eligible subjects will begin treatment after screening and baseline period assessments have been performed. The IMP (apraglutide) should be administered on the same day of the week based on the first administration at Week 0 (Visit 3), with a window of ±24 hours, i.e., the administration could be performed up to 24 hours earlier or later than the scheduled day. For example, if the first dosing is done on a Tuesday, the following IMP administrations can be performed on Mondays, Tuesdays or Wednesdays. When the subject is admitted for the metabolic balance periods (Weeks 4 and 48), IMP will be administered as per Table 1 and Table 2.

Detailed instructions will be provided in the Trial Medication Manual for Subjects, and a Subject Visit Plan will be provided.

The IMP can be self-administered by the subject at home, as detailed in Section 4.2.1. When a subject attends site for a scheduled visit, the IMP administration must be done in the clinic to allow for appropriate scheduling of administration-related assessments (Table 1 and Table 2).

During the treatment period, visits take place 2 and 4 weeks after the first dose and then every 4 weeks ±1 week, with the final visit of the treatment period (Visit 17) taking place at Week 52 ±2 days. On Weeks 4 and 48 (Visits 5 and 16), subjects will be admitted and confined to the trial site for 5 days to complete the metabolic study. During the first week, after first dose of IMP, telephone contact must be made with the subject, to detect any signs of fluid overload.

Prior to each visit (except for Weeks -1, 4, and 48 [Visits 2, 5, 16]) and End of Trial (EOT), the subject will collect data in an e-diary during 48-hour balance periods and adhere to the drinking menu as described above. In addition, the subject will record the PS details for the week prior to every visit. Except for the period up to the second

metabolic balance study at Week 4 (Visit 5), the PS volume can be adjusted, if the criteria for adjustment are met.

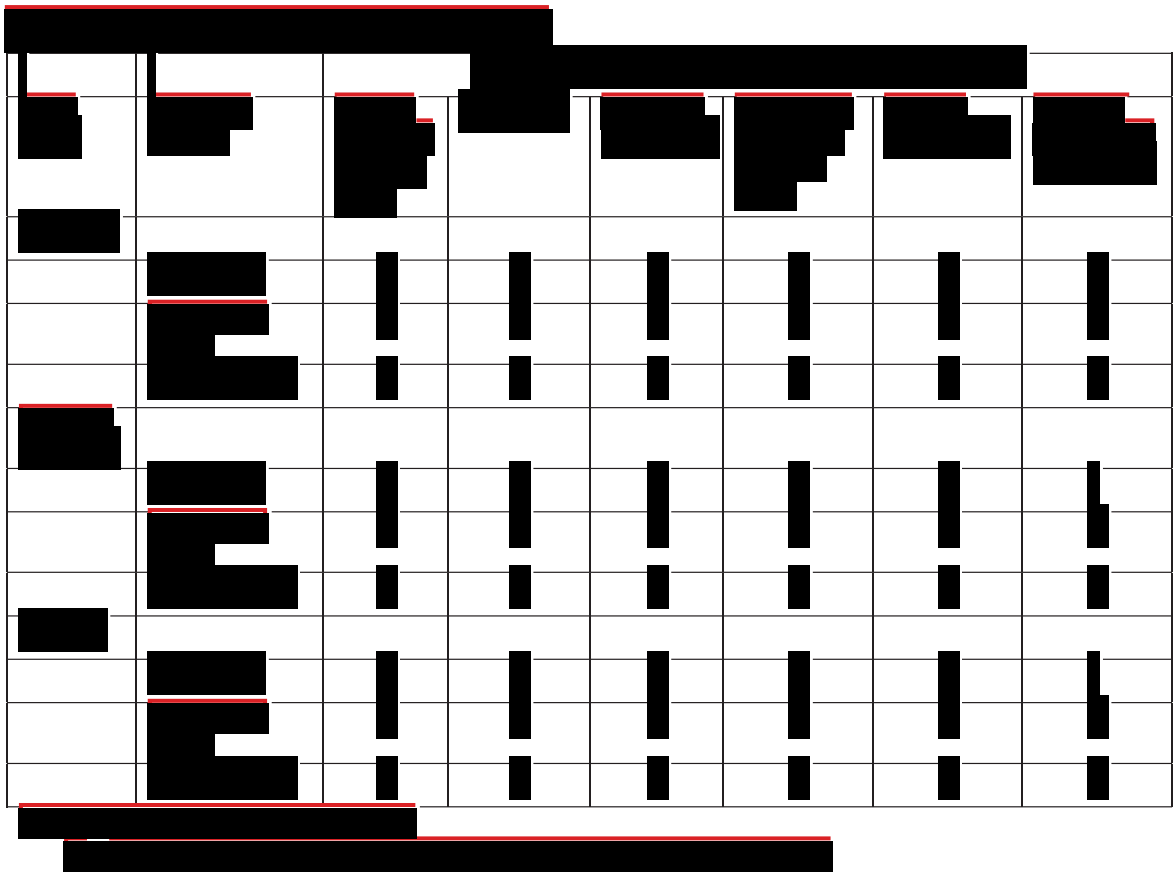
Subjects who meet individual stopping rules following IMP administration will be discontinued from the trial (Section 6.6.2).

The drinking menu must be adhered to during the 48-hour balance period and metabolic periods, but a deviation of ±10% to the defined volume is acceptable.

During the treatment period, Investigators are encouraged to increase the subject's enteral nutrition as appropriate. These changes can be entered into the Investigator Narrative.

2.2.3 [redacted]

[redacted]



2.2.4 Follow-up Period

Safety follow up assessments will be performed 4 weeks (±1 week) after the last dose (EOT), except for subjects continuing into the LTE.

2.2.5 Subject Treatment After Follow-up

Continuous treatment in a Long-Term Safety and Clinical Outcomes Extension Trial (LTE) may be offered to subjects in this trial. If a subject has not received at least 70% of the planned IMP doses they may not be eligible for the LTE. The LTE will be described in a separate protocol. All subjects will have to be eligible and consent to participate separately.

2.3 End of Trial Definition

The end of the trial is defined as the last subject last visit.

The Sponsor may terminate the trial prematurely according to certain circumstances, for example:

- Ethical concerns
- Insufficient recruitment
- When the safety of the subjects is doubtful or at risk, respectively
- Alterations in accepted clinical practice that make the continuation of a clinical trial unwise

- Evidence of harm of the experimental intervention

3. TRIAL POPULATION

3.1 Number of Subjects

This trial will enroll approximately 10 subjects. No formal sample size calculation has been performed for this trial. This number of subjects is considered sufficient to provide adequate information about the general safety, tolerability, efficacy, PD, and PK of the compound at this stage of development.

3.2 Eligibility Criteria

3.2.1 Inclusion Criteria

Initial inclusion criteria (before baseline metabolic balance study)

1. Signed informed consent for this trial prior to any trial specific assessment
2. Male and female subjects with SBS-IF and CIC (at least 28% [Cummings, 1973] and no colostomy), receiving PS, secondary to surgical resection of the small intestine with <200 cm from duodeno-jejunal flexure, based on available medical/surgical records and with the latest intestinal resection leading to SBS-IF being at least 12 months prior to Screening
3. Subject considered stable with regard to PS and weight. Stability is defined as a subject meeting all the following criteria in the 3 months preceding screening:
    a) Weight change (increase or decrease) ≤5%
    b) PS volume change not exceeding 25%
    c) PS energy content change not exceeding 25%
    d) Actual PS usage in terms of volume and content matches prescribed PS (±10%)
4. Parenteral support requirement of at least 2 days per week as assessed at Screening
5. Willingness to undergo either a colonoscopy or colonography and have any identified polyps removed via colonoscopy
6. Willingness to remain in clinic for metabolic study on three occasions for 5 days
7. Adhere to an individual pre-defined drinking menu and urine measurements during the 48-hour measuring periods
8. No planned restorative surgery or major intestinal surgery (more than 10% intestinal resection or surgery that changes anatomy group, i.e., stoma or CIC), in the trial period
9. Age ≥18 years at Screening
10. Women of childbearing potential must agree to practice effective contraception and to use a highly effective method of contraception during the trial and for 4 weeks after the EOT Visit. Such methods include one of the following: combined (estrogen and progestogen containing) hormonal contraception associated with inhibition of ovulation (oral, intravaginal, transdermal); progestogen-only hormonal contraception associated with inhibition of ovulation (oral, injectable, implantable); intrauterine device; intrauterine hormone-releasing system; bilateral tubal occlusion; or a vasectomized partner. To be considered sterilized or infertile, females must have undergone

surgical sterilization (bilateral tubectomy, hysterectomy or bilateral ovariectomy) or be post-menopausal (defined as at least 12 months amenorrhea, without an alternative medical cause, may be confirmed with follicle-stimulating hormone test if there is doubt). Women who do not engage in heterosexual intercourse during the trial and 4 weeks after the EOT Visit will be allowed to join the trial without contraception following a thorough discussion with the Investigator to determine if this is feasible for the subject. The following methods are not considered acceptable methods of contraception: calendar, ovulation, symptothermal, post-ovulation methods, withdrawal (coitus interruptus), spermicides only, and lactational amenorrhea method

11. Male subjects with a female partner of childbearing potential must commit to practice methods of contraception (e.g., condoms or a vasectomy) and abstain from sperm donation during the trial and for 2 weeks after the EOT Visit. Their partners if they are women of childbearing potential must agree to practice contraception and to use a highly effective method of contraception during the trial and for 4 weeks after the EOT visit. Such methods include: combined (estrogen and progestogen containing) hormonal contraception associated with inhibition of ovulation (oral, intravaginal, transdermal); progestogen-only hormonal contraception associated with inhibition of ovulation (oral, injectable, implantable); intrauterine device; or an intrauterine hormone-releasing system

Dosing inclusion criteria (after baseline metabolic balance study completion and before the first dose)

12. Average fecal wet weight excretion of ≥500 g/day during the baseline metabolic balance study
13. Average urine volume is ≥0.8 L and ≤2.5 L per day during baseline metabolic balance study

3.2.2 Exclusion Criteria

1. Pregnancy and/or lactation
2. Body mass index equal or higher than 30 kg/m^2 at the time of screening.
3. Major abdominal surgery (more than 10% intestinal resection) in the last 6 months prior to screening visit Surgery for feeding tube placement and cholecystectomy allowed
4. A history of clinically significant intestinal adhesions which have not been treated surgically, increasing the risk of GI obstruction
5. Constipation which is not adequately managed by dietary recommendations or laxatives
6. Active or untreated enterocutaneous fistula
7. History of cancer (including colon carcinoma) or clinically significant lymphoproliferative disease within ≤5 years, except for adequately treated basal cell skin cancer
8. Acute cholecystitis or biliary obstruction left untreated within 1 month of Screening visit or during Screening period.
9. Subjects with active IBD or an underlying condition who require new drug treatment or regimen changes including increased dose of a previously

administered treatment for at least the previous 6 months prior to Screening visit and during Screening period

10. Central venous catheter sepsis experienced within the previous 2 months, prior to Screening visit and during Screening period, or use of systemic antibiotics within the last 30 days, prior to Screening, due to catheter infection
11. Decompensated heart failure (New York Heart Association class III–IV) [NYHA] and/or known coronary heart disease defined as unstable angina pectoris and/or myocardial infarction within the previous 6 months prior to Screening
12. Radiation enteritis, scleroderma or residual evidence of intestinal dysmotility unrelated to SBS, including pseudo-obstruction and Hirschsprung's disease, and celiac disease, refractory or tropical sprue
13. History of alcohol and/or drug abuse within the previous 12 months prior to Screening
14. Child-Pugh score Class C
15. Elevated liver enzymes:
    a) Alanine aminotransferase (ALT) or aspartate aminotransferase (AST) >5 x upper limit of normal (ULN)
    b) Alanine aminotransferase or AST >3 x ULN and total bilirubin >2 × ULN or international normalized ratio (INR) >1.5 for a person not using anticoagulant drug and INR >3 for a person on anticoagulant therapy such as warfarin
    c) Alanine aminotransferase or AST >3 x ULN and clinical signs of fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever, rash, and/or eosinophilia
    d) Subjects with serum conjugated bilirubin >34 µmol/L or 2 mg/dL during two consecutive measurements
16. Evidence of chronic renal disease as demonstrated by inadequate renal function as defined by estimated glomerular filtration rate (eGFR) using the Chronic Kidney Disease Epidemiology (CKD-EPI) formula at Screening visit <20 mL/min/1.73m^2
17. Any treatment for a period of greater than 2 weeks of glutamine, growth factors such as growth hormone (GH) and native GLP-1, GLP-2, or GLP-1 or GLP-2 analogs in the previous 6 months prior to Screening
18. Known or suspected hypersensitivity to GLP-1 or GLP-2 analogs or apraglutide excipients
19. Known neutralizing antibodies (nAb) against GLP-1 or GLP-2 analogs
20. Participation in another clinical trial in the last 3 months and during this trial (studies with catheter locks, coronavirus disease 2019 [COVID-19] vaccines or non-interventional trials, which are not a burden on the subject and do not interfere with the participation in this trial, are allowed)
21. Donation of blood or plasma >500 mL within 3 months prior to Screening

22. Positive results for human immunodeficiency virus (HIV), hepatitis A, B, and/or C tests[2]
23. Subject not capable of understanding or not willing to adhere to the trial visit schedules and/or other protocol requirements (including the use of the e-diary)
24. Judged not eligible by the Investigator for any other reason

3.3 Lifestyle Considerations

During this trial, subjects will be asked to adhere to a drinking menu that will be agreed upon with the Investigator and themselves. Throughout the trial subjects will be asked to record oral consumption of beverages (including water) (refer to Section 2.2 for a definition of fluids) in a drinking diary over 48-hour periods prior to each trial visit except Screening, Visits 2, 3, 5, 16, and EOT visit.

Subjects will be confined to the site three times at Visits 2, 5, and 16, for a period of 5 days to perform the metabolic balance studies. During this time their food and drink will be monitored.

3.4 Recruitment and Screening

Recruitment will take place at three European sites located in Belgium, Denmark, and France that are specialized in the management of patients with SBS-IF.

3.5 Withdrawal/Discontinuation of Subjects

Every effort should be made to keep all subjects in the trial.

In case a subject discontinues or withdraws prematurely from the trial treatment for any reason after receiving at least one dose of IMP, the subject should be asked to perform the early termination visit and continue participation in the trial as per the Schedule of Assessments (SoA), i.e., participating in all trial assessments according to the protocol (with the exception of IMP administration) until the planned Week 52 visit.

In cases where this is not possible, the subject should be asked if they would allow the Investigator to continue collecting their weekly PS prescription and/or actual PS administration data once a month (through a phone call to the subject) until what would have been the subject's planned Week 52 visit and to continue to collect the subject's safety data (AEs).

Investigators should observe subjects who discontinue IMP treatment for potential dehydration or fluid overload, and in such case, PS should be managed according to local clinical practice.

If a subject withdraws prior to receiving IMP, the early termination visit is not required.

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[2] Subjects recovered from hepatitis B or C can be enrolled, i.e., they have markers of the infection, but the viral load is undetectable. Subjects with evidence of an acute or chronically active hepatitis B or C infection should be excluded. If a subject is positive for hepatitis A virus immunoglobulin M antibodies, this would indicate an acute infection and the subject is ineligible, but he/she may be eligible for re-screening after recovery. Patients with isolated hepatitis A virus IgG antibodies can be enrolled. A subject positive for hepatitis B antigens, along with an undetectable viral load and a history of vaccination against hepatitis B would be eligible for enrollment.

3.5.1 Withdrawals

If a subject is withdrawn from the trial, the Sponsor will be informed immediately, and the subject will be considered an early-termination subject.

If there is a medical reason for withdrawal, the subject will remain under the supervision of the Investigator until the medical situation has resolved.

Regardless of the reason for withdrawal, the Investigator will make every effort to ensure that early-termination subjects who have received any IMP complete the necessary safety follow-up assessments (EOT visit).

Reason for withdrawal and the date of discontinuation from the trial should be documented under the following categories:

1. It is the wish of the subject to withdraw
2. The Investigator judges it necessary due to non-compliance with trial procedures or administrative reasons (including if the subject loses the ability to give consent)
3. The Investigator judges it necessary to protect the subject's best interest (including safety concerns related to AEs)
4. Malignancy identified during the trial
5. Medical considerations have emerged including difficulties with obtaining or completing required protocol assessments that, in the opinion of the Investigator, potentially affect the safety of the subject. For example, this may include inability to obtain blood samples for safety assessments
6. The subject is pregnant
7. The clinical trial is terminated by the Sponsor

3.5.2 Treatment Discontinuation

If treatment is discontinued permanently, all attempts should be made to ensure a final safety visit will be conducted as per SoA (Table 1).

For all withdrawn or discontinued subjects who have been dosed with IMP, EOT assessments will be performed at a follow-up visit performed 4 weeks after the last dose. The Investigator will document the date of discontinuation of trial treatment and, if possible, the main reason for the subject's withdrawal or discontinuation in the subject's medical record.

3.5.3 Subjects "Lost to Follow-up" Prior to Last Scheduled Visit

A subject is considered lost to follow-up when the Investigator cannot reach the subject after three documented attempts to contact the subject have been made. If contact details are available and permission has been obtained, the subject's relatives should be contacted by phone to obtain a reason for the subject not to attend a visit (e.g., an AE). All contact attempts should be documented in the subject's source documents. If the subject prefers to withdraw due to an AE, the Investigator should try to motivate the subject to continue until the event has stabilized with no further change expected, or the Investigator states the AE is not clinically significant and no further follow-up is required, but still encouraged. If the subject returns to the site at a later time point, the sponsor should be informed to discuss further steps.

3.5.4 Replacement of Subjects

Subjects that do not complete the baseline and 4-week metabolic balance evaluation and/or do not receive all four doses of IMP, per protocol, may be replaced. Subjects that withdraw after the 4-week metabolic balance evaluation may not be replaced.

3.5.5 Screen Failures

All data collected during the screening assessments will be entered into the eCRF for all subjects that are screen failures.

Subjects that failed screening may be rescreened once at a later stage if the reason for screening failure has changed over time (e.g., out of range laboratory values have resolved). Re-screening should occur at least 4 weeks from time of screen failure and require re-consenting and Sponsor approval. Subjects who are re-screened will receive a new subject number.

4. TRIAL INTERVENTION/MEDICATION

4.1 Description of Investigational Medicinal Product (IMP)

All subjects will receive, based on their body weight, either 2.5 mg or 5 mg apraglutide once weekly, for 52 weeks. The IMP will be administered SC.

4.1.1 Experimental Intervention

The IMP tested in this trial is apraglutide, a GLP-2 analog composed of 33 amino acids, containing natural and unnatural amino acids from non-animal origin. Apraglutide is manufactured by solid-phase peptide synthesis using 9-fluorenylmethyloxycarbonyl as an amine-protecting group. The formulation of apraglutide is an aseptically manufactured freeze-dried powder for reconstitution with commercially available sterile water for injection. All excipients are well-known pharmaceutical excipients. When reconstituted the pH of the solution is approximately is 8.3.

Apraglutide will be supplied in vials with 10 mg freeze-dried powder for reconstitution in sterile water (to achieve an extractable dose of 2.5 mg or 5 mg) prior to SC injection.

4.1.2 Packaging, Labeling and Supply (Re-Supply)

Packaging and labeling of the IMP is performed by the Clinical Trial Supplies (CTS) vendor in accordance with Good Manufacturing Practice and applicable regulatory requirements.

The freeze-dried apraglutide powder is filled in 2 mL vials, sealed with rubber stoppers and aluminum caps. The subject boxes and individual vials will be labeled according to Annex 13, EudraLex Volume 4 [EudraLex Vol. 4, 2010] and national regulatory requirements.

The subjects will be provided with enough supply for weekly IMP administration at home between visits to the clinic. Details are included in the Pharmacy Manual and the Trial Medication Manual for Subjects.

4.1.3 Storage Conditions

The Investigator must ensure that the IMP is stored at 2–8°C in a secure location with controlled access when at the site. At the site, the temperature will be monitored at least once per hour, and recorded in a temperature log as per the policies and guidelines of the site and prior to IMP dispensation. Temperature deviations outside the allowed range must be reported and evaluated prior to use of the IMP. Details will be provided to the site in a Pharmacy Manual.

The IMP vials will be kept in an insulated cooler bag or similar during transport to the subject's home and stored in their home refrigerator. The subjects will be informed about the acceptable storage conditions for IMP that is dispensed to them and they should be reminded at each visit regarding the acceptable storage condition. Formal monitoring of the refrigerator temperature in the subject's home is not necessary and will not be recorded. Details on IMP handling by the subjects will be described in the Trial Medication Manual for Subjects.

4.2 Administration of Experimental Intervention

4.2.1 Experimental Intervention

A single 2.5 mg dose (for subjects with body weight less than 50 kg at most recent trial visit) or 5 mg dose (for subjects with body weight 50 kg or more at most recent trial visit) of apraglutide will be administered by SC injection once weekly during a treatment period of 52 weeks. Change of dosing is only allowed at each clinic visit, after obtaining the subject's current weight (Table 1 and Table 2).

The IMP should be administered on the same day of the week based on the first administration at Visit 3, with a window of ±24 hours, i.e., the administration could be done up to 24 hours earlier or later than per schedule. On days when the subject will be at site, the IMP must be administered at site. Detailed instructions will be provided in the Pharmacy Manual.

Between clinical trial visits, IMP can be administered at home. The subject or the caregiver will receive training in SC administration of IMP using a training kit containing placebo vials. The decision to select IMP administration at site or at home will be determined based on subject capability to self-administer at home, subject preference and clinical considerations. The subject will record IMP administration information in the e-diary. Detailed instructions will be provided in the Trial Medication Manual for Subjects.

4.2.1.1 *Reconstitution*

Reconstitution and preparation of the solution for SC administration will be performed using aseptic techniques following all applicable local guidelines. IMP guidelines for handling will be made available in a Pharmacy Manual that will be provided to the site.

For reconstitution, sterile water will be injected into the vial to obtain a sterile solution (Table 4). The vial will be swirled gently until its contents are completely dissolved and the contents of the vial verified to be free of foreign particles.

Table 4: Reconstitution of IMP

	2.5 mg IMP	**5.0 mg IMP**
Vial content (apraglutide)	10 mg	10 mg
Reconstitution with water for injection	0.4 mL	0.4 mL
Volume administered	0.1 mL	0.2 mL
Concentration of reconstituted solution	25 mg/mL	25 mg/mL

IMP, investigational medicinal product

After reconstitution, the IMP must be injected within a maximum of 1 hour to ensure sterility is maintained.

The solution can be drawn up into the syringe, immediately following reconstitution, and kept at room temperature until administration, or the syringe can be drawn up just before administration. The required amount of reconstituted apraglutide (0.1 mL for 2.5 mg, 0.2 mL for 5 mg) will be withdrawn from the vial into the syringe for SC

injection. After drawing up the syringe, it will be inspected for foreign particles and used if judged acceptable for administration.

4.2.1.2 *Administration*

The SC injection will be administered in the abdominal area or in the thigh. The injection site should be selected such that an injection is administered at least 5 cm away from where the last injection was administered.

The Investigator will be responsible for the preparation and administration of the IMP but can delegate this task to trained trial team members. If subjects wish to self-administer their IMP at home and are deemed capable by the trial site of adequately managing self-administration, or a family member/caregiver is able to administer the IMP, then they will be trained by site staff on the procedure. The Investigator and subject can decide that any IMP administration be performed at the site if the subject prefers to travel to the site, or if other considerations make this option preferable (Section 4.2.1).

No IMP will be administered to any person not enrolled in this trial.

4.2.1.3 *Documentation and Compliance*

The time and site of administration of IMP performed at the site will be documented in the subject's medical record. Injections performed by the subject at home will be documented in the subject e-diary and then auto uploaded into the eCRF. The Investigator or designee will check the e-diary IMP administration information recorded by the subject in order to check their compliance and understanding of procedures.

Compliance will be assessed by recording the injected volume in the eCRF and/or subject diary and will be verified by a monitor during the trial. Compliance will also be assessed during IMP accountability (see Section 4.3).

Furthermore, plasma drug concentration of apraglutide will be measured pre-dose at visits during the treatment period (see Table 1 and Table 2).

4.3 Investigational Medicinal Product Accountability

The Investigator is responsible for maintaining records of all IMP vials and sterile water for injection (WFI) ampoules received, administered, dispensed to and for IMP vials and sterile water ampoules also returned from subjects (IMP accountability). Any discrepancies between the amount of IMP (incl. sterile water) received, dispensed and returned must be reconciled.

Subjects will be provided with an e-diary to document the weekly IMP administration including the kit number used. They will be instructed to return used and partly used vials (including sterile water ampoules) to the site at each trial visit, during which the Investigator or designee will review and document the status of the IMP kit and reconcile with the subject diary, following local site policy/procedures.

4.4 Return or Destruction of Investigational Medicinal Product

All used IMP vials and WFI ampoules are to be accounted for and have to be returned to the site and kept for final reconciliation by the monitor before being returned to the CTS vendor for destruction at the end of the trial according to local site policy/procedures.

Local destruction may be considered, based on local regulations. Details are described in the Pharmacy Manual and, for IMP return only, in the Trial Medication Manual for Subjects.

4.5 Parenteral Support Volume Reduction Criteria and Process

Once the Week 4 metabolic study is completed, the Investigator will review the diary data collected from the subject and assess the urinary volume reported for the 48-hour balance period by the subject.

The average daily urinary volume (i.e., the volume reported for the 48 hours divided by 2,) will be compared with the daily average urinary volume at baseline, Visit 2 (i.e., the daily average of either 48 hours or 72-hours from metabolic balance study collection). The Investigator must decide which 48 hours of the 72 hour metabolic balance study collection will be used for the baseline measurement, prior to the metabolic balance study beginning. These must be 48 consecutive hours i.e. Days 2 and 3 or Days 3 and 4 of the metabolic balance visit.

Days 3 and 4, will be used to calculate the baseline urine average from the first metabolic balance period for all subjects enrolled into the trial prior to this amendment. For the subjects, the subsequent metabolic balance periods Days 3 and 4, will be used to calculate the average.

Parenteral support defined at Baseline should remain the same until the completion of the Week 4 metabolic balance evaluation.

[redacted]

However, if the subject shows signs of significant fluid overload, leading to clinical decompensation, then the Investigator should consider PS reduction at any point during trial period. An AESI of fluid overload must be reported. In all cases this reduction must be discussed with the Medical Monitor either before or after reduction. This must be entered into the eCRF.

If any PS volume reduction is implemented after Visit 3, the Investigator must schedule a post-parenteral support volume reduction safety evaluation as per Section 5.2.9. This visit must also be performed if a reduction is performed based on clinical criteria.

The PS volume may be increased if the subject's medical condition requires (e.g., severe dehydration or acute renal failure) or the other Investigators recommend an increase after review. The Investigator must include the reason for this increase in the eCRF. If the volume is increased no post-parenteral support volume reduction safety evaluation is required but the Investigator may schedule it at their discretion to confirm the subject is tolerating the PS increase.

4.6 Trial Specific Preventive Measures

All AESIs (see Section 6.2.2 and Section 6.3.4) will be closely monitored by the Investigator and Sponsor, and all events have to be communicated to the Sponsor within 2 weeks following the event. If an AESI is reported, the Investigator will treat the subject according to the standard of care at the site. Individual stopping rules must be followed (Section 6.6.2).

A telephone call must be performed in the first week of treatment to assess the subject for AEs, fluid load and changes in concomitant medications.

Following PS volume reduction, the Investigator must schedule a post parenteral support volume reduction safety evaluation as per Section 5.2.9.

Listings and summaries will be sent to a Data Monitoring Committee that is performing ongoing safety surveillance for Study TA799-007, using the same IMP. These data will be utilized to perform an overall safety evaluation of apraglutide.

Changes in body weight may occur during the IMP treatment phase due to fluid overload or dehydration. The subject is asked to weigh themselves weekly at home and capture the weight in their e-diary. If the weight changes by 5% in either direction from the previous trial visit assessment, the Investigator must contact the subject, assess if any immediate measures have to be taken and arrange for a body weight measure with a calibrated scale.

4.7 Prior and Concomitant Interventions (Treatments / Therapies)

4.7.1 Prior Medication / Therapy

Prior treatment includes previous medications, treatments and interventions received in the past but no longer ongoing.

Since previous use of glutamine, growth factors such as GH and native GLP-1, GLP-2 or GLP-1, GLP-2 analogs might have an impact on the production of ADA or may be associated with more serious events such as neoplastic growth and intestinal

obstruction, subjects should be specifically asked if they have been treated (including in clinical trials) with one or more of these medications (Section 3.2.2; exclusion criteria numbers 19, 20, and 21). Details of such treatments (start- and stop-date, generic name, average dose and treatment intervals) are to be recorded in the source documents for all such previous treatments and entered into the eCRF.

Use of systemic antibiotics is prohibited within 30 days prior to Screening.

Any use of antibiotics in the previous 3 months must be entered in the eCRF along with the indication/reason for prescription e.g., catheter infection, small intestinal bacterial overgrowth (SIBO). If antibiotics are given for prophylaxis, the exact reason for this must be entered in the eCRF e.g., to avoid SIBO. Information as detailed in Section 5.1.2 must be entered for each treatment period.

4.7.2 Concomitant Medication / Therapy

Concomitant treatment is any medication or therapeutic intervention being continued by the subject at trial entry and any new medication received during the trial.

At every visit the Investigator or a qualified designee will ask the subject about concomitant medication.

No clinical drug-drug interaction trials have been performed with apraglutide. An *in vitro* study indicates that apraglutide does not inhibit cytochrome P450 CYP2D6 and CYP3A4 drug metabolizing enzymes.

Based upon the PD effect of apraglutide, there is a potential for increased absorption of concomitant medicinal products. Subjects receiving oral concomitant medicinal products requiring titration or with a narrow therapeutic index (e.g., opioids, aminoglycosides, ciclosporin, carbamazepine, digoxin, digitoxin, flecainide, lithium, phenytoin, phenobarbital, rifampicin, theophylline, warfarin, or others) should be monitored closely due to potential increased absorption.

In case of pain, it is recommended that non-opioid therapies have been tried and optimized before prescribing immediate-release opioids, instead of extended-release/long-acting opioids. It is also recommended to prescribe the lowest effective dosage, below 50 morphine milligram equivalent per day, and to evaluate benefits and harms with the subject within 1 to 4 weeks of starting opioid therapy or of dose escalation.

Antibiotics may be used during the trial, if necessary, at the Investigator's discretion. All information, as detailed in Section 5.1.2, including if the antibiotic is administered as prophylaxis should be recorded in the eCRF.

Doses of concomitant medications should be kept stable during the trial unless a change in the patient's medical condition requires an adjustment. Any changes must be documented in the eCRF. No new medication should be started during the trial, unless medically necessary. The subject should be advised to consult the Investigator or designee prior to taking any prescribed or over-the-counter medications.

Routine vaccination (e.g., flu, tetanus, and varicella-zoster vaccines) is allowed during the trial but must be entered into the eCRF. Vaccination for COVID-19 is allowed, but each vaccine administration and the type of vaccine must be entered into the eCRF. All AEs attributed to vaccination, e.g., soreness at injection site, must be recorded in the eCRF.

4.7.3 Prohibited Concomitant Medication / Therapy

Any use of growth hormone (GH), enteral glutamine or growth factors such as GH and native GLP-1, GLP-2 or GLP-1, GLP-2 analogs other than the IMP under investigation in this trial is prohibited for 6 months prior to Screening and during the trial.

Citrulline containing supplements are not allowed during the trial

4.7.4 Allowed Medication/Therapy

The following concomitant medications and therapies, which are commonly used in the treatment of SBS subjects, are allowed:

- Anti-diarrheal agents
- Anti-motility drugs (e.g., loperamide, diphenoxylate, codeine, or other opiates)
- Tincture of opium
- Proton pump inhibitors
- H2 antagonists
- Bile acid sequestering agents (cholestyramine, colesevelam, cholylsarcosine)
- Diuretics
- Oral rehydration fluids
- Anti-emetics
- Routine vaccinations

Doses of concomitant medications should be kept stable during the trial unless a change in the subject's medical condition requires an adjustment. Any changes must be documented in the eCRF.

4.8 Investigational Medicinal Product of Withdrawn Subjects

Withdrawn or discontinued subjects should return used and unused IMP to the site at their EOT visit, if not done before. Further details will be described in the Pharmacy Manual. For subjects not visiting the site for any reasons (e.g., discontinuation of trial), a pick-up of used vials can be arranged. Details are described in the Site Operations Manual.

4.9 Methods of Minimizing Bias

4.9.1 Treatment Assignment

Treatment assignment is not applicable in addressing bias as all subjects will be receiving apraglutide.

4.9.2 Intent to Treat Follow-Up

All subjects will be asked to consent to be followed up even if they stop taking treatment early (Section 3.5).

4.9.3 Blinding Procedures

No blinding procedures are necessary for this trial.

4.9.4 Unblinding Procedures (Code Break)

Unblinding is not relevant to this trial as all subjects will receive apraglutide.

5. TRIAL PARAMETERS, ASSESSMENTS AND SCHEDULE

The trial visits shall be conducted according to the trial schedule (see Table 1 and Table 2) by means of a physical /personal visit of the subject to the sites.

5.1 Trial Assessments

5.1.1 Screening / Baseline Assessments

Subjects will undergo assessment of eligibility and provide informed consent at screening.

5.1.1.1 *Demography*

Demographic and baseline characteristics including:

- Age and birth year
- Gender
- Race and ethnicity

will be recorded in the subject's medical chart and entered into the eCRF. Collection of this information will be highlighted in the Informed Consent Form (ICF).

5.1.1.2 *Medical and Surgical History*

Previous and concomitant diseases and previous surgery will be documented. Only relevant medical and surgical history will be recorded in the eCRF, as judged by the Investigator. The information collected for medical and surgical history must include:

- Diagnosis
- Date of onset
- Date of resolution (if not ongoing)

Additionally, at screening any drug or alcohol abuse within the last 12 months prior to screening will be documented as medical history.

The medical history will be obtained by asking the subject and/or by inspecting his/her medical records. If a contrast study of the GI has been done within 6 months of screening it will be reviewed if subacute intestinal obstruction(s) or mild stricture(s) were present.

Any instances of SIBO within 3 months prior to Screening, including any treatment, will be entered into the eCRF.

For the documentation of the subject's SBS type and disease history, the subject will be asked, and/or his/her medical records will be inspected for information on:

- The underlying cause of SBS
- Date of PS initiation
- The length of remnant small bowel in cm
- The length of remnant colon in percent (%) according to Cummings classification Appendix 5 [Cummings, 1973] for all subjects
- Date of last intestinal resection
- All abdominal surgeries that affected the bowel anatomy and the date of such surgery

For documentation of eligibility the source documents should include details on the medical history, prior treatment, imagistic or endoscopic assessment results as applicable, to sufficiently document for each criterion the eligibility of the subject.

The subject will be asked during the visit to recollect the frequency and severity of the following in the preceding 7 days: nausea, vomiting, and abdominal pain. This will be collected weekly during the Screening period and documented in the medical notes.

5.1.2 Reporting of Prior and Concomitant Treatment

Concomitant and prior treatment, as defined in Section 4.7, will be entered into the eCRF.

At every visit the Investigator or a qualified designee will ask the subject about concomitant medication.

The following information will be recorded in the eCRF:

- Generic name (preferred) or trade name
- Indication
- Dose and frequency
- Route of administration
- Start date (if started ≥3 months prior to Visit 1, then this can be stated instead of recording the specific start date)
- Stop date (or ongoing)

For treatments/therapies

- Name of the therapy, surgical procedure or intervention
- Indication
- Start date (if started ≥3 months prior to Visit 1, then this can be stated instead of recording the specific start date)
- Stop date (or ongoing)

No new medication should be started during the trial, unless medically necessary. The subject should be advised to consult the Investigator or designee before taking any prescribed or over-the-counter medications.

5.1.3 Metabolic Balance Studies

Balance studies quantify weight, volume, as well as the content of energy, macronutrients and electrolytes of oral diet intake (i.e., food and fluid intake) from duplicate meals. During the balance studies, the subjects will collect duplicate portions of all intakes in buckets covering 24-hour periods. Likewise, all output (feces and urine production) will be collected and quantified. The content of the buckets will be weighed and homogenized. A wet and a freeze-dried sample will be processed by laboratory techniques and subsequently used for analyses. Details of the techniques used are published [Jeppesen, 2000; Prahm, 2017] and the overall procedure for the balance studies is included in Appendix 5: Section 16.5 and will be further defined in the Laboratory Manual.

5.1.3.1 *Oral Fluid Intake*

The oral liquid intake should be kept constant during the balance studies ensuring that potential changes in any output measurements are not caused by changes in liquid intake. Therefore, the subject's individual oral fluid intake ("drinking menu") will be defined at Screening and confirmed on Day 1 of the baseline metabolic balance study based on the subject's usual oral fluid intake (volume).

Solid oral food intake can be varied according to the subject's own choice.

5.1.3.2 *Parenteral Support*

A baseline PS program will be established at Screening. This baseline PS program should be followed during the first two balance periods. The baseline PS program will be based on the subject's usual weekly PS prescription. There might be daily variations in the type, content, and volume of the PS for each subject. However, the PS program will be identical during the first and second admission period. For the Week 48 metabolic balance period the prescribed PS based on the subject's medical condition will be administered.

5.1.3.3 *Wet Weight Excretion*

During each balance study, total excretion of feces over three 24-hour periods (i.e., a total of 72 hours) will be collected and weighed and entered in the eCRF.

5.1.3.4 *Urinary Output*

During each balance study, the total urinary output over three 24-hour periods (i.e., a total of 72 hours) will be collected and measured and entered in the eCRF.

5.1.3.5 *Macronutrients and Energy*

Macronutrients will be analyzed in oral intake and output over the 72-hour balance studies: carbohydrate by Englyst's method [Englyst method], nitrogen by Kjeldahl's method [Kjeldahl method], and fat by titration. Energy will be assessed by bomb calorimetry. This will be detailed in the Laboratory Manual.

5.1.3.6 *Electrolytes*

Electrolytes will be analyzed in oral intake and output (feces and urine) over the 72-hour balance studies: sodium, potassium, calcium, and magnesium will be analyzed by atomic absorptiometry. Urinary electrolytes will be analyzed at the local laboratory at the Denmark trial site. This will be detailed in the Laboratory Manual.

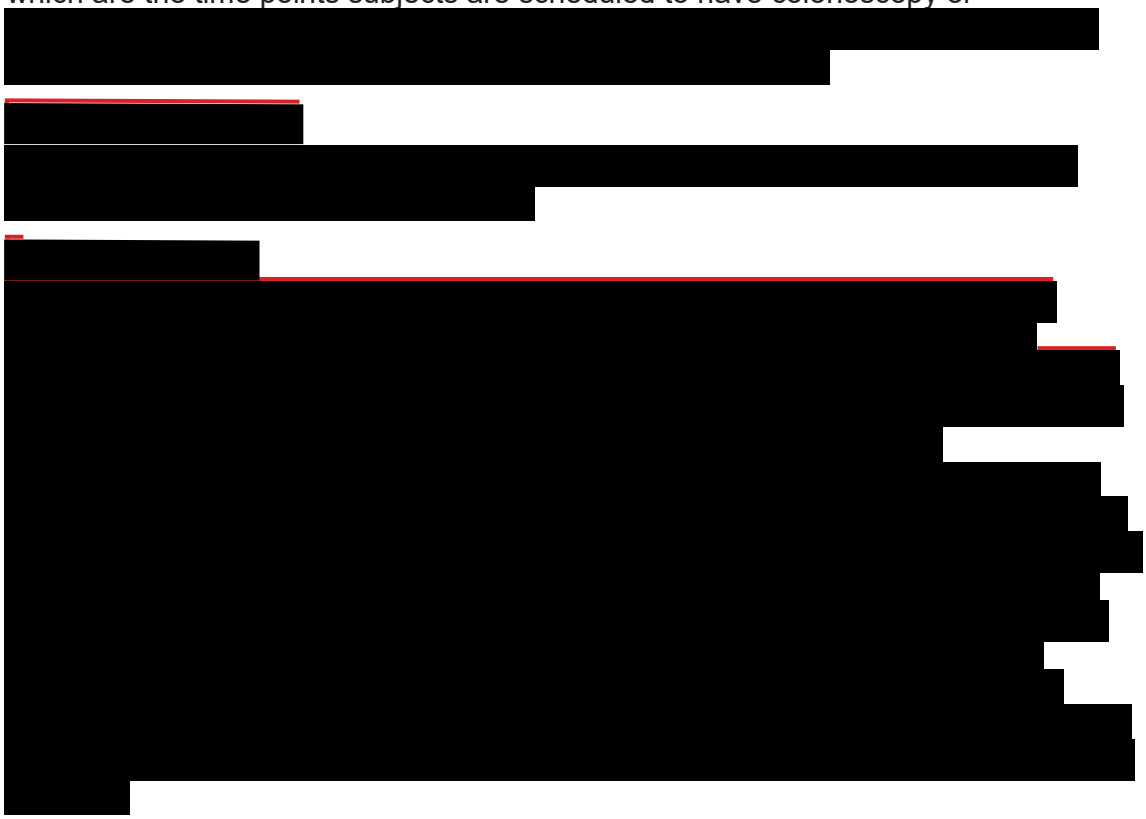
5.1.4 DEXA Scan

The total lean body mass, total fat mass, fat percent, total bone mineral content, bone mineral density as well as the T-score and Z-score will be measured using DEXA scans at visits according to the SoA (Table 1 and Table 2). The duration of each full DEXA scan will be approximately 20 minutes.

5.1.5 [redacted]

[redacted]

which are the time points subjects are scheduled to have colonoscopy or



5.1.6 Parenteral Support Prescription

The Investigator will record in the source documents and eCRF all relevant data related to the prescribed parenteral support including parenteral nutrition throughout the subject's trial participation from initial screening visit until EOT visit. For subjects who discontinue from the trial prematurely, the Investigator will collect data and complete the eCRF up to the subject's EOT Visit, if the subject consents to the collection of this data.

The PS prescription includes infusion and supplements. Data to be recorded include

- PS name
- PS volume (mL)
- PS composition such as:
    - Energy content (kcal/mL): total energy, energy from lipids, glucose, and amino acids
    - Electrolyte content: sodium, potassium, calcium, and magnesium
    - Supplement content (e.g., added vitamins and trace elements)
- PS days per week, i.e., for which days of the week the particular infusion was prescribed.

At each site visit, the Investigator will review the e-diary data with the subject, and adjust the PS as described in Section 4.5.

5.1.7 Subject Diaries

Subjects will be asked to complete an e-diary during the screening and treatment period and will receive an electronic device with an app (Medidata Patient Cloud). The e-diary is integrated into the Electronic Data Capture (EDC) system (Section 10.3) and the Investigator will be able to review data that the subject enters.

Subject diary data will be reviewed at the subjects' regular trial visits (Table 1 and Table 2) for all new entries since the last visit. The Investigator must consider re-training of subjects in case of non-compliance with an e-diary entry and/or suspicion of potential mistakes in entry (e.g., PS volume administration very different from prescribed volume).

The following information will be collected:

- For administration of the IMP the following will be documented:
    - Date and time of IMP removal from refrigerator
    - Date and time of IMP preparation (i.e., reconstitution)
    - Volume prepared
    - Vial number used
    - Date and time of injection
    - Who administered the IMP
    - Volume administered (mL)
    - Site of administration
- Body weight (weekly assessment at the subject's home, including an assessment within 2 days before the start of the drinking menu)
- Actual PS volume administered, start date and time of infusion and approximate duration of infusion, will be documented for 7 days prior to each visit (except Visit 3)
- For the 48-hour balance period prior to visits the following will be recorded:
    - Drinking diary: frequency and volume
    - Bristol Stool Form Scale (Section 5.1.7.1)
    - Approximate number of bowel movements
    - Urinary output: frequency and volume
- Following a reduction of PS, the subject will document their body weight and 24-hour urinary volume on 1 day during the time period of 2 to 7 days after the PS volume reduction (Section 5.2.9).

5.1.7.1 *Bristol Stool Form Scale*

The Bristol Stool Form Scale is a single-item questionnaire that categorizes stool consistency on a seven-point scale [Lewis, 1997]. It contains both pictures and text descriptions and was developed as a clinical assessment tool to evaluate diarrhea and constipation. The scores correlate with colonic stool transit time. Subjects will be provided with the stool chart in the e-diary and record a general assessment each day of the type of stool that they discharge, according to the scale, during the 48-hour balance periods.

5.1.7.2 *Change in Eating/Appetite*

The Investigator will ask the subject at each visit about their diet over the past week, guided by some pre-defined questions (Appendix 4, Section 16.4) in the eCRF. The Investigator will determine if the subject is eating the same, less or more and this may support the assessment of PS volume reduction.

5.1.8 Investigator Narrative

The Investigator should provide a subject narrative based on the overall evaluation of the participant during the trial visit. The Investigator should capture whether the subject has less sense of energy, lack of concentration, feeling cold, feeling of being less capable of performing daily tasks, etc. Since the symptoms may be heterogeneous among subjects, Investigators should explore whether the subject refers to symptoms following PS reduction that are different from his/her "normal situation."

5.1.9 Patient Reported Outcomes

5.1.9.1 *Short Bowel Syndrome with Intestinal Failure and Colon-in-Continuity - Quality of Life Questionnaire*

The SBS-IF-CIC-QOL was developed based on best measurement practices summarized in current regulatory guidance documents, specifically to be completed by subjects with SBS-IF without stoma, with CIC (DHHS-PRO, 2009; Patrick, 2011; DHHS-Patient Focus, 2019). This questionnaire assesses the impact of SBS-IF, on subjects' lives over the past 7 days, assessed on a 0–10 Numeric Rating Scale (NRS) with 0 representing no impact and 10 representing the worst imaginable impact. Concepts assessed include bothersomeness of diarrhea and urgency and its impact on daily activities, bothersomeness of fatigue and its impact of activities, sleep impacts, relationship impacts, and emotional impacts. The SBS-IF-CIC-QOL will be completed at the time points specified in Table 1 and Table 2. Further details are presented in Appendix 1 (Section 16.1.7).

5.1.9.2 *Short Bowel Syndrome with Intestinal Failure - Treatment Impacts - Quality of Life Questionnaire*

The SBS-IF-TI-QOL was developed to be completed by subjects with SBS-IF with or without a stoma to assess impacts associated with PS and GLP-2 analog treatments. The first 10 items related to impact of PS over the past 7 days, rated on a 0-10 NRS. The last two items are about the bother and difficulty associated with GLP-2 analog injections over the past 4 weeks, also rated on a 0-10 NRS. The SBS-IF-TI-QOL will be completed at the time points specified in Table 1 and Table 2. Further details are presented in Appendix 1 (Section 16.1.8).

5.1.9.3 [redacted]

5.1.9.4 *Patient Global Impression of Change*

Patient Global Impression of Change (version 1.0) is a single-item questionnaire using a 7-point VRS, to assess overall change in the subject's status since taking the IMP. Response options range from 3=Very much better to -3=Very much worse. Patient Global Impression of Change (version 2.0) is a single-item questionnaire using a 5-point VRS, to assess overall change in the subject's status after taking the IMP. Response options range from 2=very much better to -2=very much worse. If subjects completed the first version of the PGIC (Appendix 1, Section 16.1.1), they should complete both this version and the updated second version once it is approved by the independent ethics committees (IEC) (Appendix 1, Section 16.1.2) throughout the trial, and the
results of both should be entered into the eCRF. If subjects entered the trial after version 2.0 was available and did not complete the original version 1.0, only version 2.0 (Appendix 1, Section 16.1.2) must be completed throughout the trial.

5.1.9.5 *Patient Global Impression of Severity*

Patient Global Impression of Severity (PGIS) is a single-item questionnaire using a seven-point VRS to assess of current status of the subject's condition. Response options range from 0=Not severe to 6=Extremely severe (Appendix 1, Section 16.1.3).

This will only be completed by subjects that entered the trial and completed this questionnaire at baseline prior to this protocol amendment approval by the IEC(s). Subjects who enter the trial after this time will not complete this questionnaire.

5.1.9.6 *Patient Global Impression of Treatment Satisfaction (PGI-TS)*

This form is a single-item questionnaire assessing the subject's satisfaction with the trial medication over the preceding 7 days. Response options range from -2 to 2, very dissatisfied to very satisfied (Appendix 1, Section 16.1.6). Once approved by the IEC(s) all subjects will complete this questionnaire even if it was not included when they enrolled in the trial as defined in the SoA (Table 1).

5.1.9.7 *Patient Global Impression of Satisfaction with Parenteral Support (PGI-SPS)*

This is a single-item questionnaire assessing the subject's satisfaction with PS over the preceding 7 days. Response options range from -2 to 2, very dissatisfied to very satisfied (Appendix 1, Section 16.1.5). Once approved by the IEC(s) all subjects will complete this questionnaire even if it was not included when they enrolled in the trial.

5.1.9.8 *Patient Global Impression of Parenteral Support Impact (PGI-PSI)*

This is a three-item questionnaire assessing the impact of PS on the subject's sleep, daily activities, and QoL. All questions have response options ranging from not at all to extremely (Appendix 1, Section 16.1.4). Once approved by the IEC(s), all subjects will complete this questionnaire even if it was not included when they enrolled in the trial.

5.1.10 Safety Parameters

5.1.10.1 *Colonoscopy/Colonography*

When deemed anatomically feasible by the Investigator, a colonoscopy (including removal of any polyps) or colonography should be performed, at time points specified in the assessment schedule. Histopathology assessment of any removed polyps has to be documented in the source documents and in the eCRF prior to Baseline Visit 2. If it was not possible to view the entire length of colon for polyps during the Screening colonoscopy, a colonography may be performed as a safety check.

Subjects whose histology reports show malignancy cannot be enrolled.

5.1.10.2 *Physical Examination*

A general physical examination is conducted at time points specified in the assessment schedule in Table 1 and Table 2. Additional physical examination should be performed at any time during the treatment, if clinically indicated.

This examination serves to detect obvious and severe abnormalities and will be documented in the source documents and eCRF.

5.1.10.3 *Body Measurements*

Height will be measured and recorded in centimeters to one decimal place at the screening visit.

Body weight will be measured at each visit using the standard scales at the site, using the same scale at each assessment for a subject as far as possible and recorded in local standard metrics. During weighing, the subject should wear light clothing and no shoes.

Subjects will be asked to measure their body weight at home weekly.

5.1.10.4 *Vital Signs*

Vital signs including systolic and diastolic blood pressure, heart rate and axillary temperature will be measured at the time points specified in the assessment schedule in Table 1 and Table 2. Vital signs will be measured in a sitting or supine position after 5-minutes rest and blood pressure will be recorded in mmHg. Axillary temperature will be recorded in either Celsius or Fahrenheit.

5.1.10.5 *Electrocardiogram*

One twelve-lead resting electrocardiogram (ECG) will be recorded at the time points specified in Table 1 and Table 2 using the standard equipment and procedure at the site. Additional ECG monitoring should be performed at any time during the treatment, if clinically indicated. All ECG parameters, including intervals, QRS, and QT will be recorded. The QTcF will be calculated in the eCRF.

Additionally, an overall clinical assessment of the ECG will be made and recorded as "normal," "abnormal not clinically significant." or "abnormal clinically significant." Abnormal clinically significant findings at screening will be recorded as medical history and clinically significant new findings or worsening in ECG results during the trial must be recorded as an AE (see Section 6.5).

5.1.10.6 *Adverse Events*

All AEs will be recorded using the AE pages of the eCRF. AEs will be reported using a recognized medical term or diagnosis that accurately reflects the event. AEs will be assessed by the Investigator for severity, relationship to the investigational product or a protocol requirement, possible etiologies, and whether the event meets criteria as a SAE and thus requires immediate notification of the Sponsor. Definition of SAEs is presented in Section 6.2 and guidelines for assessment of severity and relationship are presented in Section 6.2.6 and Section 6.2.3, respectively. If the event has not recovered to baseline or Grade 1 at the end of the trial reporting period, it will be documented as ongoing for all events that are at least possibly related to trial treatment. If an AE evolves into a condition which becomes "serious" it will be reported as an SAE.

Any laboratory abnormality will be considered an AE if it is judged to be medically significant by the Investigator.

5.1.10.7 *Adverse Events of Special Interest*

The handling of AESIs is described in detail in Section 6.2.2; special reporting requirements apply (Section 6.3.4). When documenting an AE as per Section 5.1.10.6, the Investigator will assess if the AE is an AESI.

In this trial, AESIs are

- Injection site reactions
- GI obstructions
- Gallbladder, biliary and pancreatic disease
- Fluid overload
- Colorectal polyps
- Malignancies

5.1.11 Laboratory Analysis

5.1.11.1 *Laboratory Safety Parameters*

Blood samples for clinical chemistry (including liver enzymes, hematology and hemostasis) and urine samples for urinalysis will be collected at the time points specified in Table 1 and Table 2.

The total blood volume to be collected from each subject in this trial will be approximately 350mL. For Post PS Volume Reduction Safety Evaluations, an additional volume, up to a maximum of 5 mL will be withdrawn for assessments, i.e., up to 50 mL (if PS volumes were reduced at every visit). Repeat or unscheduled samples may be taken for safety reasons or if there were technical issues with a sample.

Safety blood and urine samples will be processed and analyzed at the site. Details of handling and processing are described in the Laboratory Manual. All laboratory contact details can be found in the Laboratory Manual.

All safety laboratory reports will be reviewed by the Investigator and parameters out of normal range assessed as “clinically significant” or “not clinically significant.” Clinically significant abnormal results during the trial will be appropriately followed up by the Investigator and recorded as an AE (Section 6.5). Reference ranges for the safety laboratory parameters will be provided prior to screening the first subject at each site.

The following parameters will be assessed during the trial:

Clinical Chemistry

- Total HCO_3^-(bicarbonate)
- Sodium
- Potassium
- Calcium
- Chloride
- Magnesium
- Phosphate
- Ferritin
- Glucose
- Urea
- Creatinine (estimated glomerular filtration rate (eGFR) calculated using the CKD-EPI formula)
- Uric acid
- Bilirubin (total, conjugated and unconjugated)
- Alkaline phosphatase
- Alanine aminotransferase
- Lactate dehydrogenase
- Aspartate aminotransferase
- Gamma-glutamyl transferase (GGT)
- Albumin
- C-reactive protein
- Amylase (total)
- Triacylglycerol lipase
- Additional parameters for experimental trials (see Section 5.1.11.3)

Hematology

- Erythrocytes
- Hematocrit
- Hemoglobin

- Leucocytes, automated differential counts
- Thrombocytes (platelets)

Hemostasis

- International normalized ratio

Urinalysis

- pH
- Leukocytes
- Nitrite
- Protein
- Glucose
- Ketones
- Hemoglobin
- Gravity

Urine sample from 48-hour balance period

- Urinary sodium

  Subjects will reserve one urine sample during each 48-hour balance period for analysis of urinary Na+ and will either bring the sample to the site at the upcoming trial visit.

Virology

- Human immunodeficiency virus
- Hepatitis A
- Hepatitis B
- Hepatitis C

If a subject has a positive hepatitis A immunoglobulin G (IgG) result, a hepatitis immunoglobulin M (IgM) test may be ordered to rule out acute infection.

Pregnancy Test

A serum pregnancy test will be performed for all pre-menopausal female subjects at the time points detailed in Table 1 and Table 2. To be considered sterilized or infertile, women must have undergone surgical sterilization (bilateral tubectomy, hysterectomy, or bilateral ovariectomy) or be post-menopausal (defined as at least 12 months amenorrhea without an alternative medical cause; may be confirmed with a follicle-stimulating hormone [FSH] test if there is doubt). Any pregnancies should be reported as described in Section 6.3.5.

Anti-Drug Antibodies

A blood sample for analysis of anti-apraglutide antibodies and their neutralizing capacity (collectively ADA) will be collected at the time points detailed in Table 1 and Table 2. Subjects who discontinue the trial, but do not continue into the extension trial, will be asked to have ADA samples drawn at 4 weeks and 8 weeks after the EOT visit. The Sponsor will inform the Principal Investigator of these results after trial completion.

Details on handling, storage, and shipment of the samples to the central laboratory will be provided in a trial-specific Laboratory Manual. All laboratory contact details can be found in the Laboratory Manual.

5.1.11.2 *Efficacy Laboratory Parameters*

Pharmacokinetics

Blood samples for analysis of the plasma concentration of apraglutide will be collected at the visits and time points specified in Table 1 and Table 2.

It is important to document the exact time points of previous IMP administration and PK sampling.

Biomarkers

Blood samples for analysis of citrulline will be collected at the time points specified in Table 1 and Table 2.

Samples for PK and citrulline will be collected and processed at the site and sent to the central laboratory for analysis. The total blood volume to be collected from each subject for PK and citrulline will be approximately 160mL.

Details on PK and citrulline handling, storage and shipment of the samples to the central laboratory will be provided in a trial-specific Laboratory Manual. All laboratory contact details can be found in the Laboratory Manual.

5.1.11.3 [redacted]

5.2 Procedures at Each Visit

The schedule of procedures is provided in Table 1 and Table 2. Prior to any trial-specific assessment, written informed consent must be obtained as described in Section 5.2.1 and Section 9.2.5

5.2.1 Visit 1 (Week -4 to -2, Screening Visit)

Visit 1 will take place up to 28 days and at least 14 days before Visit 2. The following will take place during the screening visit:

- The subject's informed consent to participate in the trial will be obtained prior to any trial-related procedure
- Allocation of screening number
- Review of initial inclusion and exclusion criteria
- Data will be recorded for:
  - Subject demographics
  - Medical and surgical history including alcohol or drug abuse
  - Disease history
  - Previous and concomitant medications including current PS
  - Gastrointestinal health history: vomiting, nausea, and/or abdominal pain in the preceding 7 days
  - PS prescription (volume and composition)
- Oral fluid (drinking menu) will be defined for the metabolic studies
- Measurement of height and weight
- Colonoscopy or colonography to include small bowel and colonic biopsies
- Gastroduodenoscopy to include biopsies
- A full physical examination will be performed
- A 12-lead ECG will be recorded
- Measurement of vital signs (systolic and diastolic blood pressure, heart rate, and axillary temperature)
- Blood samples will be collected for:
  - Clinical chemistry
  - Hematology
  - Hemostasis
  - Serology/virology
  - Pregnancy test (only for premenopausal women. If there is doubt about menopausal status, an FSH test may be performed)
- A urine sample will be collected for dip-stick urinalysis
- The subject will be contacted by phone every 7 days after the Screening visit and before Baseline visit. Information on AEs, concomitant medications and GI health history, such as vomiting, nausea, abdominal pain, will be entered into the eCRF
- Instruction on and handout of eDiary

5.2.2 Visit 2 (Week -1, baseline metabolic balance period)

Subjects who have met the initial screening criteria will be admitted to the clinic at Day -7 for the baseline balance study. During the baseline balance study, the following will take place:

Day of admission (Day 1)

- Initial Screening inclusion and exclusion criteria will be confirmed
- A full physical examination will be performed
- A 12-lead ECG will be recorded
- The subject's individual oral fluid intake ("drinking menu") and baseline PS prescription will be confirmed
- Blood samples will be collected for
  - Clinical chemistry
  - Hematology
  - Hemostasis
  - Pregnancy test (only for pre-menopausal women)
  - Citrulline
  - Anti-drug antibodies
- A urine sample will be collected for dip-stick urinalysis
- GI health history: Vomiting, nausea, abdominal pain in the preceding 7 days (only at baseline, Visit 2 metabolic balance period)
- Change in eating/appetite
- Investigator narrative
- Review of subject's diary
- The Investigator must decide which 48 hours will be used to calculate the baseline urine volume for the PS reduction formula

Every day during the balance study (Days 1, 2, 3, 4, and 5)

- Body weight will be measured on Day 1 at any time, on Day 2 before defecation and IMP administration, and on subsequent days at the same time as on Day 2 ±1 hour
- Vital signs (systolic and diastolic blood pressure, heart rate, and axillary temperature) will be measured on Day 1 at any time, on Day 2 before defecation and IMP administration, and on subsequent days at the same time as on Day 2 ±1 hour
- Any AEs will be recorded
- Any changes in concomitant medications will be documented

Day 2 during the balance study

- Patient reported outcomes will be performed

- Administer IMP (second and third metabolic balance periods only)
- A DEXA scan will be performed preferably on Day 2. However, if performed on another day of the baseline metabolic balance study, the time of the DEXA scan must be consistent with regard to time of PS administration and time of day for following metabolic studies. This must be consistent across all metabolic study visits
- Fasting blood samples
- Postprandial blood samples

The fasting and postprandial blood samples will be taken, on any of Days 2, 3, or 4. The fasting samples will be collected immediately before the patient consumes a standardized breakfast (-15 min). The subject has 15 minutes to consume the breakfast. The postprandial sample collection will start at 0 (immediately after the meal), 5, 15, 30, 45, 60, 120, and 180 minutes post breakfast consumption. For standardized breakfast details see the Laboratory Manual.

- [redacted]
- Hip and waist circumference
- [redacted]
- Urinate and defecate prior to metabolic balance collections start
- Start of collection

72-hour balance period (Days 2, 3, 4 and 5)

- Oral fluid intake will be kept constant according to the subject's individual drinking menu
- Type, content and volume of the PS will be kept constant according to the subject's baseline PS program (Baseline and Week 4)
- The 72-hour balance study will be performed, i.e., collection of urine, feces and duplicate food intake
- Bristol Stool Form Scale (see Section 5.1.4.1) (Day 2 and 3 only)
- Frequency of bowel movement (Day 2 and 3 only)
- Urine sample will be taken at the visit for urinalysis and one sample collected during 72-hour urine balance period for sodium analysis
- Pharmacokinetic blood samples at pre-dose (within 30 min prior to dosing), 24, 30, 48, and 72 hours post-dose (second and third metabolic balance periods only)

Last day of balance study (Day 5)

- Urinate and defecate to end the metabolic balance collections

- Magnetic resonance imaging
- Confirmation of eligibility
- Dispense IMP
- Discharge from clinic

5.2.3 Visit 3 (Day 0)

- Body weight
- Vital signs (systolic and diastolic blood pressure, heart rate, and axillary temperature)
- Data will be recorded for:
  - Changes in concomitant medications
  - Prescribed PS volume, and composition and days of week
- Any new AE will be recorded, and existing AEs will be followed up
- Gastrointestinal health history: vomiting, nausea, and/or abdominal pain in the preceding 7 days will be recorded
- Administer IMP
- Instruct subject on IMP handling and administration at home
- Dispense IMP

This visit may be delayed for up to 2 weeks, if needed, for scheduling purposes. However, the day of the week of this visit must allow the correct administration of trial medication during the following metabolic study on Day 2.

Subjects to be contacted by telephone on Day 1 (+1 day) to assess fluid overload and stool frequency, any AEs will be collected and any change in concomitant medications will be recorded.

5.2.4 Visit 4

- Data will be recorded for:
  - Changes in concomitant medications
  - Prescribed PS volume, and composition and days of week
- Body weight
- Vital signs (systolic and diastolic blood pressure, heart rate, and axillary temperature)
- Any new AE will be recorded, and existing AEs will be followed up
- Investigator narrative
- Change in eating/appetite
- Blood samples will be collected for:
  - Clinical chemistry including liver enzymes and assessment of creatinine clearance
  - Hematology
  - Hemostasis
  - Serum pregnancy test for premenopausal women
  - Citrulline
  - Pharmacokinetics
  - Anti-drug antibodies

- Urine sample will be taken at the visit for urinalysis and one sample collected during 48-hour urine balance period for sodium analysis.
- The subject e-diary will be reviewed
  - Administered PS volume, days of week
  - Duration of PS infusion
  - Drinking diary: frequency and volume
  - Bristol Stool Form Scale (see Section 5.1.4.1)
  - Frequency of bowel movement
  - Urinary output: frequency and volume
  - Body weight
- IMP administered and dispensed (home used IMP vials to be returned)

5.2.5 Visit 5 (Second metabolic balance study [Days 1–5])

Procedures as for first metabolic balance study and additionally:

- Post PS volume reduction safety evaluations (if required)
- PK blood samples pre-dose (within 30 min prior to dosing) and 24, 30, 48, and 72 hours post-dose
- Administer IMP on Day 1 (day 2 of hospital admission) of the metabolic balance period.
- Dispense IMP on Day 5 of the metabolic balance period (home used IMP vials to be returned)

[redacted]

5.2.6 Visits 6 - 15 and Visit 17

- Data will be recorded for:
  - Changes in concomitant medications
  - Prescribed PS volume, and composition and days of week
  - Post PS volume reduction safety evaluations (if required)
- Body weight
- Vital signs (systolic and diastolic blood pressure, heart rate, and axillary temperature)
- Any new AE will be recorded, and existing AEs will be followed up
- Physical Examination (Visits 10 and 17 only)
- Investigator narrative
- Change in eating / appetite
- Patient reported outcomes (Visits 7, 10, 13, and 17 only)
- Fasting blood samples to be taken immediately before breakfast (Visit 10 only)
- Standardized breakfast consumed within 15 minutes (Visit 10 only)
- Postprandial blood samples (Visit 10 only) at 0 (immediately after meal consumed) and at 5, 15, 30, 45, 60, 120, and 180 minutes
- Blood samples will be collected for:
  - Clinical chemistry including liver enzymes and assessment of creatinine clearance

- Hematology
- Hemostasis
- Serum pregnancy test for premenopausal women
- Citrulline (Visits 7, 10, 12, 14, and 17 only)
- Pharmacokinetics (Visits 7, 10, 12, 14, and 17 only)
- Anti-drug antibodies (Visits 7, 10, 12, and 14 only)

- Urine sample will be taken at the visit for urinalysis and one sample collected during 48-hour urine balance period for sodium analysis.
- [redacted]
- Hip and waist circumference (Visit 10 only)
- DEXA Scan (Visit 10 only)
- [redacted]
- [redacted]
- 12-lead ECG will be recorded (Visits 10 and 17 only)
- The subject e-diary will be reviewed
  - Investigational medicinal product administration information
  - Administered PS volume, days of week
  - Duration of PS infusion
  - Drinking diary: frequency and volume
  - Bristol Stool Form Scale (see Section 5.1.7.1)
  - Frequency of bowel movement
  - Urinary output: frequency and volume
  - Body weight
- Administer and dispense IMP (Not Visit 17) - home used IMP vials to be returned

5.2.7 Visit 16 (Third metabolic balance study [Days 1–5])

Procedures as for first metabolic balance study and additionally:

- Post PS volume reduction safety evaluations (if required)
- Pharmacokinetic blood samples pre-dose (within 30 min prior to dosing) and 24, 30, 48, and 72 hours post dose
- Anti-drug antibodies
- Administer IMP on Day 1 of the metabolic balance period.
- Dispense IMP on Day 5 of the metabolic balance period (home used IMP vials to be returned)

[redacted]

5.2.8 Visit 18 (End-of-Trial Visit)

This visit is scheduled at 4 weeks after last dose of IMP for all subjects who opt not to participate in the LTE. In case a subject terminates the trial prematurely for whatever reason, this visit will be scheduled as an EOT visit at 4 weeks after the final IMP dose.

- Data will be recorded for:
  - Changes in concomitant medications
  - Prescribed PS volume and composition
  - Change in eating/appetite
  - Patient reported outcomes
  - Investigator narrative
- A colonoscopy including removal of any polyps or colonography will be done within ±3 weeks of the EOT visit, only for subjects that terminate early. The assessment will be done only if they have received at least 8 weeks of treatment (i.e., eight doses).
- Full physical examination
- Twelve-lead ECG
- Vital signs (systolic and diastolic blood pressure, heart rate, and axillary temperature)
- Body weight
- Blood samples will be collected for:
  - Clinical chemistry including liver enzymes
  - Hematology
  - Hemostasis
  - Pharmacokinetics
  - Citrulline
  - Anti-drug antibodies
  - Serum pregnancy test for premenopausal women
- Urine sampling for urinalysis
- Any new AEs will be recorded and assessed, and existing AEs will be followed up (see Section 6)

5.2.9 Post Parenteral Support Volume Reduction Safety Evaluation

The following safety evaluations will be conducted:

- Body weight
- Twenty-four-hour urinary volume
- Blood laboratory tests
  - Total HCO_3^-(bicarbonate)
  - Sodium
  - Potassium
  - Calcium
  - Chloride
  - Magnesium

- Creatinine (and eGFR] calculated using the CKD-EPI formula)
- Albumin

- Urinalysis
  - Sodium

The Investigator will use his/her clinical judgment to determine if the subject is tolerating the PS changes well and should continue on their PS prescription or if the PS prescription should be returned to previous levels. The decision and the rationale will be included in the eCRF and when applicable an AE should be reported on the AE form.

6. SAFETY

After signing the ICF, all AEs including SAEs and AESIs regardless of relationship to IMP will be collected, fully investigated and documented in source documents and eCRFs and will be reported either until the last protocol-specific procedure or safety follow-up. The region specific, emergency contact details for the Sponsor can be found in the Investigator Site File and Site Operations Manual.

After this period, Investigators should report any SAEs, or other AEs of concern that are believed to be related to IMP.

Adverse events that occur prior to first dosing will be captured as pre-treatment AEs.

6.1 Safety Data Reporting and Collection Period

The Investigator must monitor the condition of the subject throughout the trial from the time of obtaining informed consent until the last protocol-specific procedure, whether it is the EOT visit, or a safety follow-up period.

If an Investigator becomes aware of an SAE after the subject's last visit (this includes withdrawn subjects), and he/she assesses the SAE to have a reasonable possible causality to the IMP, the case will have to be reported to the Sponsor via the contract research organization (CRO), using the contact information, regardless of the length of time that has elapsed since the end of the trial.

6.2 Definition and Assessment of (Serious) Adverse Events and Other Safety Related Events

An AE is any untoward medical occurrence in a subject or a clinical investigation subject administered a pharmaceutical product, and which does not necessarily have a causal relationship with the trial procedure. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product. [ICH E6(R2), 2016]

The sources of AEs include:

- The subject's response to questions about his/her health (a standard non-leading question such as "How have you been feeling since your last visit?" is asked at each visit)
- Symptoms spontaneously reported by the subject
- Investigations and examinations with findings that are assessed by the Investigator to be clinically significant
- Other information relating to the subject's health becoming known to the Investigator

For surgical or diagnostic procedures, the condition/illness leading to such a procedure is considered as the AE rather than the procedure itself.

An SAE is classified as any untoward medical occurrence that:

- Results in death
- Is life-threatening
- Requires in-patient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability/incapacity

- Is a congenital anomaly/birth defect in a neonate/infant born to a mother exposed to IMP (also applies if father was exposed to IMP)
- Is a medically significant event in the Investigator's judgment (e.g., may jeopardize the subject or may require medical/surgical intervention to prevent one of the outcomes listed above) [ICH E2A, 1995]

Medical conditions present at the initial trial visit that do not worsen in severity or frequency during the trial are defined as baseline medical conditions (medical history) and are not to be considered AEs.

6.2.1 Events that Do Not Meet the Definition of an SAE

Elective hospitalizations to administer, or to simplify trial treatment or trial procedures are not considered SAEs. However, all events leading to unplanned hospitalizations or unplanned prolongation of an elective hospitalization (e.g., undesirable effects of any administered treatment) must be documented and reported as SAEs.

Events that do not meet the definition of an SAE:

1. Hospitalization or prolongation of hospitalization as part of a routine procedure
2. Hospitalization for a survey visit, annual physicals or social reasons
3. Hospitalization that does not include an overnight stay
4. Elective hospitalizations for pre-existing conditions documented in the medical history that have not worsened

In case of doubt, an event should be reported within timelines.

6.2.2 Adverse Events of Special Interest

An AESI, serious or non-serious, is an AE of scientific and medical concern specific to the Sponsor's product or program, for which ongoing monitoring, additional information, and rapid communication by the Investigator to the Sponsor can be appropriate. Depending on the nature of the event, rapid communication by the trial Sponsor to other parties (e.g., regulators) might also be warranted in line with Council for International Organizations of Medical Sciences standards and local regulations.

The following are considered AESIs for this trial and should be communicated by the Investigator to the Sponsor within 2 weeks following the event:

Injection site reactions

Subjects will be monitored for injection site reactions (ISR) for at least 1 hour after each IMP administration given at site, during a trial visit at the site, or longer (i.e., until the reaction stops or the subject leaves the site), as necessary. Where IMP administration is administered by the subject, or by a family member/caregiver, the subject will be instructed to monitor and report ISRs according to instructions in the Trial Medication Manual for Subjects. Injection site reactions will be reported as AEs. The individual symptoms of the ISR (e.g., injection site pain, injection site pruritus [itching], injection site erythema [redness], injection site edema [swelling], and injection site bruising) should be reported as AEs. For administrations at the subject's home, the subject will be trained when to contact the Investigator (or a physician) immediately, depending on reaction severity.

Data on local tolerability will be collected as an AESI. The following characteristics of ISRs will be documented:

- Pain
- Erythema
- Induration
- Pruritus
- Bruising

Severity and duration of these features of the ISR will be collected by the site by direct observation when IMP is administered on site. When IMP is administered at home, ISRs should be reported to the site for documentation in the subject's source documents and entry into the eCRF.

Table 5: Grading of Severity is based on NCI-CTCAE version 5.0 in line with other AEs in this trial

Pain	
Grade 1	Mild pain
Grade 2	Moderate pain; limiting instrumental activities of daily living
Grade 3	Severe pain; limiting self-care activities of daily living
Pruritus	
Grade 1	Mild, localized reaction with only topical intervention
Grade 2	Moderate with noticeable skin change from scratching (e.g., excoriation). Oral therapy indicated
Grade 3	Widespread and resulting in treatment interruption
Induration	
Grade 1	Mild induration with skin able to slide and pinch up
Grade 2	Unable to pinch up skin but still slides
Grade 3	Severe, unable to slide or pinch, potentially limiting activity of daily living and consideration of treatment interruption
Erythema	
Grade 1	Mild, <2.5 cm
Grade 2	Moderate, 2.5–5 cm
Grade 3	Severe, >5 cm
Bruising	
Grade 1	Mild, <2.5 cm
Grade 2	Moderate, 2.5–5 cm
Grade 3	Severe, >5 cm

NCI-CTCAE, National Cancer Institute – Common Terminology Criteria for Adverse Events

Gastrointestinal Obstruction

Any GI obstructions will be treated by the Investigator according to the Investigator's judgment and carefully followed up.

Gallbladder, Biliary and Pancreatic Disease

These should be monitored by symptoms, liver enzymes (ALT, AST, GGT, alkaline phosphatase), bilirubin, lipase and amylase and subjects should be treated according to the Investigator's judgment.

Fluid Overload

Subjects will be monitored closely for signs and symptoms related to fluid overload, e.g., edema, due to increased absorption. The Investigator will document cases of substantial fluid overload and manage as per clinical practice for the subject accordingly.

Colorectal Polyps

Where deemed anatomically feasible by the Investigator, two gastroduodenoscopies and colonoscopies or colonographies will be done during the course of the trial, one before enrolment and one at the end of the trial. Any polyps found during colonoscopies must be removed, and the histology documented in the source documents and eCRF.

Malignancies

For any subject with a malignancy identified during the course of the trial, a thorough medical history will be taken and documented (e.g., smoking history for lung cancer). Information on type of malignancy, histological type and grading will be collected. The IMP treatment will be discontinued, and the subject will be closely followed up by the Investigator.

Once the clinical database containing all AEs is available, potential AESIs will be identified using the following algorithmic standardized Medical Dictionary for Regulatory Activities (MedDRA) queries (including Standardized MedDRA Queries (SMQs)):

- Standardized MedDRA Queries Functional, inflammatory and gallstone related biliary disorders
- Standardized MedDRA Queries GI perforation, ulceration, hemorrhage or obstruction
- Standardized MedDRA Queries Malignancies

In the absence of related SMQs, customer queries (CQs) will be set up from the Sponsor-defined list of preferred terms (PTs) as listed in the Statistical Analysis Plan (SAP).

6.2.3 Assessment of Causality

Both Investigator and Sponsor will assess the causality of the event in relation to the IMP, based on the criteria listed in the ICH E2A guidelines:

Investigators must also systematically assess the causal relationship of AEs to IMP(s)/trial treatment (any other non-IMPs, radiation therapy, etc.) using the following definitions. Decisive factors for the assessment of causal relationship of an AE to the IMP include, but may not be limited to, temporal relationship between the AE and the IMP, known side effects of IMP, medical history, concomitant medication, course of the underlying disease, trial procedures.

Table 6: Assessment of Causality of Adverse Events

	Assessment of Causality				
	Definitely	**Probably**	**Possibly**	**Unlikely**	**Unrelated**
Clearly due to extraneous causes	N	N	N	N	Y
Reasonable temporal association with drug administration	Y	Y	Y/N	N	N
May be produced by subject clinical state, etc.	N	N	Y	Y	Y
Known response pattern to IMP	Y/N	Y/N	N	N	N
Disappears or decreases on cessation or reduction in dose	Y	Y/N	N	N	N
Reappears on re-challenge (if possible)	Y	N	N	N	N

IMP, investigational medicinal product; Y, yes; N, no

Not related: Not reasonably related to the IMP. Adverse event could not medically (pharmacologically/clinically) be attributed to the IMP under trial in this clinical trial protocol. A reasonable alternative explanation must be available. Adverse events reported as “unlikely” related and “unrelated” will be considered unrelated for reporting purposes.

Related: Reasonably related to the IMP. An adverse event could medically (pharmacologically/clinically) be attributed to the IMP under trial in this clinical trial protocol. Table 6 can be used to differentiate between the AEs related to the IMP. Adverse events reported as definitely, probably and possibly related will be considered as “related” for reporting purposes.

6.2.4 Unexpected Adverse Drug Reaction

An “unexpected” adverse drug reaction is an adverse reaction, the nature or severity of which is not consistent with the applicable product information (e.g., Investigator’s Brochure for drugs that are not yet approved and Product Information for approved drugs, respectively) [ICH E2A, 1995].

6.2.5 Suspected Unexpected Serious Adverse Reactions

The Sponsor evaluates any SAE that has been reported regarding seriousness, causality and expectedness. If the event is related to the IMP and is both serious and unexpected, it is classified as a suspected unexpected serious adverse reaction (SUSAR).

6.2.6 Assessment of Severity

The Investigator is required to grade the severity or toxicity of each AE.

Investigators will reference the National Cancer Institute – Common Terminology Criteria for Adverse Events (NCI-CTCAE, 2017), version 5.0 (publication date: 27 November 2017), a descriptive terminology that can be used for AE reporting.

A general grading (severity/intensity; hereafter referred to as severity) scale is provided at the beginning of the above referenced document, and specific event grades are also provided.

If a particular AE's severity is not specifically graded by the guidance document, the Investigator is to use the general NCI-CTCAE definitions of Grade 1 through Grade 5 following his or her best medical judgment.

The five general grades are:

- Grade 1 or Mild
- Grade 2 or Moderate
- Grade 3 or Severe
- Grade 4 or Life-threatening
- Grade 5 or Death

6.3 Reporting of Serious Adverse Events and Other Safety Related Events

6.3.1 Reporting of AEs

All AEs must be reported to the CRO and/or Sponsor through the eCRF and within the data entry timeline defined for this trial and stipulated in the site agreement.

6.3.2 Reporting of SAEs

All SAEs must be reported immediately and within a maximum of 24 hours to the CRO and/or Sponsor of the trial through the eCRF.

The CRO/Sponsor will re-evaluate the SAE and queries will be raised in the eCRF and returned to the site, requesting clarification or follow-up information if needed. After the initial SAE report, the Investigator is required, proactively, to provide further information regarding the subject's condition. All follow-up information for other SAEs must be entered into the CRF as soon as possible, within 24 hours after it becomes available. In case e-CRF access is not possible, the Investigator must send an email with the respective filled out paper forms to: Safety.Desk@psi-cro.com. Local fax-numbers will be made available in the Investigator Site File, should email not work either.

Serious AEs resulting in death will be reported to the Ethics Committee within 7 days.

The minimum data required for a report is:

- Subject ID
- Trial code/protocol number
- Date of enrolment
- Date and time of start of SAE
- Description of SAE
- Severity of SAE
- Assessment of causality relationship to IMP
- Name and contact details of person reporting the event

6.3.3 Reporting of SUSARs

A SUSAR for an event that is life-threatening or fatal, needs to be reported to the IEC and competent authorities (CAs) within 7 calendar days,

All other SUSARs need to be reported to the IEC and CAs within 15 calendar days.

As necessary, the Sponsor must inform all Investigators, IEC and CAs participating in the clinical trial of the occurrence of a SUSAR.

6.3.4 Reporting of Safety Signals

All suspected new risks and relevant new aspects of known adverse reactions that require safety-related measures, i.e., so-called safety signals, must be reported to the Sponsor within 24 hours. The Sponsor must report the safety signals within 7 days to the IEC and CAs.

The Sponsor must immediately inform all participating Investigators about all safety signals. The other IECs and CAs involved in the trial will be informed about safety signals by the Sponsor as necessary.

6.3.5 Reporting and Handling of Pregnancies

Pregnant subjects must immediately be withdrawn from the clinical trial. Any pregnancy during the treatment phase of the trial and within 90 days after discontinuation of trial medication will be reported to the Sponsor within 24 hours. The course and endpoint of the pregnancy should be followed up carefully, and any abnormal endpoint regarding the mother or the child should be documented and reported.

A female subject must be instructed to stop taking the IMP and immediately inform the Investigator if she becomes pregnant. Pregnancies occurring in female partners of male subjects must also be reported immediately to the Investigator. Pregnancies occurring in female subjects or female partners of male subjects up to 90 days after the completion of dosing must also be reported to the Investigator.

If the Investigator becomes aware of a pregnancy occurring in the subject and/or partner of a subject participating in the trial, it should be reported to the Sponsor. The subject and/or partner should be counseled and followed as described below.

The Investigator should report all pregnancies to the Sponsor within 24 hours of becoming aware of them. The pregnancy report for a female partner of the male subject must be forwarded within 24 hours after written consent is obtained from the pregnant partner. The Investigator will make arrangements for the subject and/or partner to be seen by a specialist who can discuss any risks of continuing with the pregnancy. Monitoring of the subject and the child should continue until 4 weeks after the outcome of the pregnancy is known.

6.3.6 Periodic Reporting of Safety

An annual safety report will be submitted once a year to the local IEC via the local Investigator and to CAs via the Sponsor or Contract Research Organization.

6.4 Follow up of Serious Adverse Events

Serious AEs should be followed until resolution or stabilization. Subjects with ongoing SAEs at trial termination (including safety visit) will be followed up until recovery, return to baseline status or stabilization. If after follow-up, return to baseline status or

stabilization cannot be established, an explanation should be recorded on the eCRF SAE form.

6.5 Abnormal Laboratory Findings and Other Abnormal Investigational Findings

Abnormal laboratory findings and other abnormal investigational findings (e.g., on an ECG trace) should not be reported as AEs unless they are associated with clinical signs and symptoms, lead to treatment discontinuation or are considered otherwise medically significant by the Investigator. If a laboratory abnormality fulfills these criteria, the identified medical condition must be reported as the AE rather than the abnormal value itself.

Elevations (or new elevations from baseline) in transaminases or tests of liver function will be closely monitored. Laboratory tests (AST, ALT, alkaline phosphatase, GGT, total bilirubin, viral serologies) will be used to depart other causes of liver injury from drug-induced liver injury. Subjects with persistent or new deterioration of hepatic function will be followed-up.

6.6 Trial Specific Safety Considerations

6.6.1 Considerations on IMP dose reduction and temporary discontinuation of IMP

If the Investigator reduces the IMP dose due to safety reasons, documentation as an AE is required.

If the Investigator reduces or temporarily discontinues IMP, resumption of IMP or return to the prescribed dose must be based on the medical condition of the subject and the Investigator's clinical judgment.

Reduction of IMP can be from 5.0 mg to 2.5 mg or from 2.5 mg to 0 mg. No other dose adjustments are allowed. The Investigator should attempt to return to the per protocol dose as soon as the medical condition of the subject allows. If the subject does not continue to tolerate the per protocol dose the Investigator may choose to resume IMP administration at the lower dose of 2.5 mg weekly.

There is no limit to the number of dose reductions for an individual subject during the trial. However, the Investigator should discuss with the subject and use their clinical judgment to determine if it is safe and appropriate for the subject to continue, if numerous IMP discontinuations and/or reductions have been required.

Changes and discontinuations must be discussed with the Medical Monitor. If the subject's medical condition allows, this discussion may occur prior to changes but may occur afterwards if this is not possible (e.g., an AE necessitating an immediate halt). The final decision to reduce or discontinue IMP will be that of the Investigator.

Dose changes and the rationale will be documented in the medical records, in the subject diary (for IMP administration at the subject's home) and in the eCRF. Missed doses will not be made up for, i.e., the treatment period will not be prolonged due to temporary discontinuation. Reporting as a protocol deviation is required.

6.6.2 Individual Subject Stopping Rules

During the trial, liver toxicity will be monitored according to guidelines from the US Food and Drug Administration (FDA) (FDA Guidance for Industry: Drug-Induced Liver

Injury: Premarketing Clinical Evaluation, 2009) and treatment will be discontinued if any of the following stopping criteria are met for subjects with ALT and AST <1.5 ULN at baseline:

- Alanine aminotransferase or AST >8 × ULN once
- Alanine aminotransferase or AST >5 × ULN for more than 2 weeks
- Alanine aminotransferase or AST >3 × ULN and total bilirubin >2 × ULN or INR >1.5 for a person not using anticoagulant drug and INR >3 for a person on anticoagulant therapy such as warfarin.
- Alanine aminotransferase or AST >3 × ULN and clinical signs of fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever, rash, and/or eosinophilia

Patients with SBS-IF, who are dependent on PN, commonly develop abnormal liver function tests. This condition is known as intestinal failure-associated liver disease or PN-associated liver disease [Abu-Wasel, 2014]. In this case, ALT and or AST levels may be elevated at baseline; therefore, the stopping rules for these subjects have been adapted based on published guidelines [Treem, 2021]. For subjects with baseline AST and/or ALT values ≥ 1.5 ULN, treatment will be discontinued if any of the following stopping criteria are met:

- Alanine aminotransferase or AST ≥2 x baseline or 5 x ULN (whichever comes first) **AND** total bilirubin (TBL) 2≥ ULN (if normal at baseline) or TBL ≥1.5 x baseline (if elevated at baseline)
- Alanine aminotransferase or AST ≥2 x baseline or 5 x ULN (whichever comes first) **AND** INR ≥1.5
- Alanine aminotransferase or AST ≥3 x baseline or 8 x ULN (whichever comes first)
- Alkaline phosphatase ≥2 x baseline and direct bilirubin 1 mg/dL (17.1 mol/L) over baseline

The IMP should not be restarted until a diagnostic evaluation is completed. A re-challenge may be considered in case of IMP discontinuation due to meeting one of the above stopping criteria after a consultation and approval by the Medical Monitor. Additional visits to the site for un-scheduled laboratory samplings should be arranged in such a case, or the subject should see their local doctor for laboratory assessments as agreed with the Investigator. In case of abnormal laboratory results of CTCAE Grade 3 or higher as assessed by the subject's local doctor, a re-assessment should be scheduled at the site. All results will be filed in the medical chart, assessed by the Investigator, and entered in the eCRF accordingly.

6.6.3 Trial stopping criteria

The following trial stopping rules are based on the AE criteria of the NCI-CTCAE, 2017 version 5.0, related to ALT, AST, GGT, alkaline phosphatase, and TBL elevations.

Enrollment of new subjects should be interrupted should two or more subjects experience CTCAE Grade 3 events, or one or more subjects experience CTCAE Grade 4 events not clearly related to the underlying disease. Trial enrollment may be resumed following the review of all available safety data, if the totality of the data suggests that the event is unlikely to be related to the trial treatment. The trial may be

terminated if, following the review of the safety data, it has not been possible to demonstrate that the AE has a clear relationship to the underlying disease or associated co-morbidity (and not to IMP administration).

7. DATA ANALYSIS AND STATISTICAL CONSIDERATIONS

7.1 General Considerations

The statistical evaluation of the trial will include descriptive statistics of the primary and secondary endpoints reflecting the exploratory nature of the trial. In addition, simple analyses will be provided for the efficacy endpoints and patient reported outcomes.

7.1.1 Primary Endpoints

- Adverse events (system organ class, frequency and severity)
- Adverse events of special interest:
  - Injection site reaction
  - Gastrointestinal obstruction
  - Gallbladder, biliary and pancreatic disease
  - Fluid overload
  - Colorectal polyps
  - Malignancies
- Clinical chemistry, hematology, hemostasis, ADAs, and urine analysis

7.1.2 Secondary Endpoints

Efficacy endpoints related to PS volume

- Relative change from baseline in actual weekly PS volume at Weeks 24 and 52
- Absolute change from baseline in actual weekly PS volume at Weeks 24 and 52
- Subjects who achieve a reduction of at least 1 day per week of PS from baseline at Weeks 24 and 52
- Clinical responders (20% reduction of PS volume from baseline) at Weeks 24 and 48
- Subjects reaching enteral autonomy at Weeks 24 and 52
- Energy reduction in the PN from baseline at Weeks 24 and 52

Efficacy endpoint related to nutritional, fluid (wet weight), and electrolyte absorption

- Change in absolute absorption of energy, over metabolic balance periods from baseline to Week 48
- Relative change in absorption of energy over metabolic balance periods from baseline at Week 48
- Change in absorption of macronutrients over metabolic balance periods from baseline at Week 48
- Change in absolute absorption of energy over metabolic balance periods from baseline at Week 4
- Change in absorption of macronutrients over metabolic balance periods from baseline at Week 4

- Changes in urine output and urinary electrolytes (sodium, potassium, calcium, and magnesium) over metabolic balance periods from baseline at Week 4 and at Week 48

Patient reported outcomes

- [redacted]
- Patient Global Impression of Change at Week 24 and 52
- Changes from baseline in PGI-TS at Weeks 24 and 52
- Changes from baseline in PGI-SPS at Weeks 24 and 52
- Changes from baseline in PGI-PSI at Weeks 24 and 52

Pharmacokinetic/Pharmacodynamic related endpoints

- Trough plasma concentration
- Plasma citrulline levels

7.1.3 Exploratory Endpoints

Endpoints related to nutritional status

- Changes from baseline in lean body mass, bone mineral content, and fat mass by DEXA scan from baseline at Weeks 4, 24, and 48
- Change in body weight from baseline at Weeks 4, 24 and 52
- Change in dietary intake of wet weight, energy, macronutrients, fluid, and electrolytes (sodium, potassium, calcium, and magnesium) from baseline at Week 4 and 48
- Change in fecal excretion of wet weight, energy, macronutrients, fluid, and electrolytes (sodium, potassium, calcium, and magnesium) from baseline at Week 4 and 48

Endpoints related to efficacy

- Change from baseline in the total time per day that subjects infuse PS at Weeks 24 and 52
- Change from baseline in the total time per week that subjects infuse PS at Weeks 24 and 52
- Change in electrolytes, minerals, macronutrients, and other contents of the PS from baseline at Weeks 24 and 52

Patient reported outcomes

- Short Bowel Syndrome with Intestinal Failure and Colon-in-Continuity - Changes from baseline in Quality of Life Questionnaire at Weeks 24 and 52
- Short Bowel Syndrome with Intestinal Failure - Changes from baseline in Treatment Impacts - Quality of Life Questionnaire at Weeks 24 and 52

[redacted]

- [redacted]

7.2 Determination of Sample Size

No formal sample size calculation has been performed for this trial. From a sample of 10 enrolled subjects, eight subjects are expected to complete. This is considered sufficient to provide adequate information about the general safety, tolerability, efficacy, PD, and PK of the compound at this stage of development.

7.3 Definition of Analysis Sets

7.3.1 Full Analysis Set

The Full Analysis Set (FAS) will comprise all subjects who have received at least one dose of trial treatment and contributed at least one valid post-baseline efficacy point.

7.3.2 Safety Analysis Set

The Safety Analysis Set (SAS) will comprise all subjects exposed to trial medication.

7.4 Efficacy analysis

For all continuous efficacy endpoints mean change from baseline to end of treatment will be presented with simple 95% confidence interval and a t-test of no change from baseline. This will be done both for absolute and relative change. Due to the relatively small sample size, a non-parametric estimate of the change from baseline to end of treatment for each endpoint will be derived using the one-sample Hodges-Lehmann median estimator with corresponding 95% confidence interval.

As some of the continuous endpoints can be close to or below zero at baseline (e.g., absorption of energy), the relative change of such endpoints should be interpreted with great caution as the analysis can lead to meaningless results.

Responder endpoints will be tabulated with number of subjects and percentages of subject in FAS.

Other categorical endpoints will be tabulated with number of subjects and percentages of observed subjects. For ordinal endpoints change from baseline will also be calculated and tabulated.

For endpoints related to nutritional status and nutritional absorption sensitivity analyses will be made excluding subjects missing more than 20% of the planned

injections prior to the time of analysis. Hence the number of injections leading to exclusion will be one at Week 4, six at Week 24, and 11 at Week 52.

7.5 PK/PD Analysis

Any exploratory PK/PD modeling will be reported separately as an addendum to the Clinical Trial Report (CTR).

7.6 Safety Analysis

7.6.1 Adverse Events

All AEs occurring from ICF signature and prior to first dose of IMP will be recorded as pre-treatment AEs. All AEs occurring after first dose of IMP and up to the 4 weeks follow-up visit are to be reported as treatment-emergent adverse events (TEAEs). Adverse event summaries will be produced for TEAEs by treatment group including number of subjects with an event, percentage of subjects with an event, and total number of events. In addition, similar separate tables will be produced for each category of severity.

Similar summaries will be made for SAEs, SMQs, and CQs. The relevant SMQs and CQs will be defined in the SAP.

The verbatim terms used in the eCRF by Investigators to identify AEs will be coded using MedDRA (version 23.1 or above).

7.6.2 Concomitant Medication

The prior and concomitant medications recorded in the eCRF will be coded using the latest version of World Health Organization Drug Dictionary.

7.6.3 Safety Lab Values and Other Continuous Safety Values

All safety continuous endpoints will be presented in summary tables. All data will be used. No missing data will be replaced

7.6.4 Immunogenicity Analysis

All immunogenicity analyses will be descriptive and carried out with a special focus on neutralizing antibodies and treatment-induced or treatment-boosted ADA anti-apraglutide. The impact on PK, PD, and safety will be described.

7.7 Interim Analyses

Interim analysis will be performed after last subject completes Week 4 and another at Week 24. Additional preliminary analyses to explore the effects of apraglutide on safety and efficacy may be performed by the sponsor at the discretion of the sponsor at any time.

7.8 Handling of Missing Values

Missing values will not be replaced.

7.9 Deviation(s) From the Original Statistical Plan

Any deviations from the planned analyses will be described in the final CTR with a rationale for the deviation.

8. DATA MONITORING COMMITTEE

Listings and summaries of safety data will be sent to the data monitoring committee that will be performing ongoing safety surveillance for Trial TA799-007. These data will be utilized to perform an overall safety evaluation of apraglutide.

9. TRIAL CONDUCT

Written approval will be obtained from the IEC and CA concerning the conduct of the trial by the Sponsor before the trial commences. The clinical trial can only begin once approval from all required authorities has been received.

Steps to ensure the accuracy and reliability of data include the selection of qualified Investigators and appropriate trial sites, review of protocol procedures with the Investigator and associated personnel before the trial, periodic monitoring visits, and meticulous data management.

9.1 Ethical conduct of the trial

This clinical trial will be conducted in compliance with this protocol and in accordance with the following:

- The ethical principles stated in the locally valid version of the Declaration of Helsinki
- All applicable laws and regulations of the country where the trial is conducted
- Good Clinical Practice (GCP) Guidelines (April 1996 International Council for Harmonisation (ICH) Guidance for Industry E6 GCP (including archiving of essential trial documents) and the Integrated Addendum to ICH E6(R2), 2016)
- Clinical trial regulatory framework in the European Union (EU)

9.2 Sponsor and Investigator Responsibilities

The Sponsor is obligated to conduct the trial in accordance with strict ethical principles (Section 9.1). The Sponsor reserves the right to withdraw a subject from the trial (Section 3.5), to terminate participation of a trial site at any time, and/or to discontinue the trial prematurely (Section 2.3).

The Sponsor agrees to provide the Investigator with sufficient material and support to permit the Investigator to conduct the trial according to the trial protocol.

By signing the Investigator Signature Page (found at the start of this protocol), the Investigator indicates that he or she has read the protocol carefully, fully understands the requirements and agrees to conduct the trial in accordance with the procedures and requirements described in this protocol.

The Investigator also agrees to conduct this trial in accordance with all laws, regulations, and guidelines of the pertinent regulatory authorities, including the most current versions of the ICH E6(R2), 2016 Guidance for Industry GCP, the Integrated Addendum to ICH E6 (R1) and in agreement with the current version of the Declaration of Helsinki. While delegation of certain aspects of the trial to sub-Investigators and trial coordinators is appropriate, the Investigator will remain personally accountable for overseeing the trial closely and for ensuring compliance with the protocol and all applicable regulations and guidelines. The Investigator is responsible for maintaining a list of all persons that are/have been delegated trial-related responsibilities (e.g., sub-Investigators and trial coordinators) and their specific trial-related duties.

Investigators should ensure that all persons who are delegated trial-related responsibilities are adequately qualified and informed about the protocol and their specific duties within the context of the trial. Investigators are responsible for

providing the Sponsor with documentation of the qualifications, GCP training, and research experience for themselves and their staff as required by the Sponsor, IEC(s), and the relevant governing authorities.

9.2.1 Regulatory and Ethical Considerations

Prior to trial initiation, this protocol, the proposed subject information and ICF as well as other documents required by current regulations will be submitted to an IEC.

A signed and dated statement that this protocol and the ICF have been approved by the IEC must be filed. In accordance with GCP and applicable regulatory requirements, the trial will not start at a site before receiving the respective IEC approval which must be signed and dated.

Before commencement of the trial, the trial will be submitted to the competent authority and approval obtained.

Investigators are responsible for promptly informing the IEC and the authorities of all protocol amendments, serious adverse reactions, and SUSARs occurring during the trial that are likely to affect the safety of the subjects or the conduct of the trial. Information on pregnancies occurring during the trial and pregnancy outcomes qualifying as serious are also to be reported to the IEC. In case a vulnerable subject enters the trial, the Principal Investigator is encouraged to consult their local IEC for guidance.

The protocol may not be modified without written approval from the Sponsor. Protocol modifications or changes may not be initiated without prior written IEC/CA approval except when necessary to eliminate immediate hazards to the subjects or when the change(s) involves only logistical or administrative aspects of the trial. Such modifications will be submitted to the IEC and written verification that the modification was submitted should be obtained, as per local regulations.

Substantial Amendments are those considered “substantial” to the conduct of the clinical trial and are likely to have a significant impact on, e.g., the safety or physical or mental integrity of the subjects, the scientific value of the trial, the conduct or management of the trial or the quality or safety of the IMP used in the trial.

The constitution of the IEC must meet the requirements of the participating countries and ICH-GCP (R2). A list of the IEC/ members with names and qualifications plus a statement that it is organized according to ICH-GCP(R2) and the applicable laws must be provided to the Investigator and to the CRO for filing and archiving as per applicable local law.

9.2.2 Investigator Delegation Responsibilities

While delegation of certain aspects of the trial is appropriate, the Investigator will remain personally accountable for overseeing the trial closely and for ensuring compliance with the protocol and all applicable regulations and guidelines. The Investigator is responsible for maintaining a list of all persons that are/have been delegated trial-related responsibilities (e.g., sub-Investigators and trial coordinators) and their specific trial-related duties.

Investigators should ensure that all persons who are delegated trial-related responsibilities are adequately qualified and informed about the protocol and their specific duties within the context of the trial. Investigators are responsible for

providing the Sponsor with documentation of the qualifications, GCP training and research experience for themselves and their staff as required by the Sponsor, IEC(s), and the relevant governing authorities/IEC(s). The Investigators are responsible for keeping an up-to-date Investigator Site File, which includes all required and relevant trial documents.

9.2.3 Competent Authorities and Regulatory Considerations

The clinical trial protocol and related documents will need CA approval or notification, as required by local regulations and requirements prior to the initiation of the trial, i.e., screening or enrolment of a subject. No changes will be made to the protocol or related documents without prior approval by the CA, except for non-substantial amendments which may be implemented with notification only. Annual reporting will be done, and premature or planned trial end as well as the CTR) will be submitted to the CA as required within the appropriate timeframe (e.g., early termination of the trial within 15 days and CTR within 1 year after last subject last visit, respectively).

9.2.4 Financial Disclosure

Investigators and sub-Investigators will provide the Sponsor with sufficient, accurate financial information as requested to allow the Sponsor to submit complete and accurate financial certification or disclosure statements to the appropriate CA/IECs. Investigators are responsible for providing information on financial interests during the course of the trial and for 1 year after completion of the trial or for a longer period of time if required by local legislation.

9.2.5 Subject Information and Informed Consent

The Investigators will explain to each subject the nature of the trial, its purpose, the procedures involved, the expected duration, the potential risks and benefits and any discomfort it may entail. Each subject will be informed that the participation in the trial is voluntary and that he/she may withdraw from the trial at any time and that withdrawal of consent will not affect his/her subsequent medical assistance and treatment.

The subject must be informed that his/her medical records may be examined by authorized individuals other than their treating physician.

All subjects for the trial will be provided a subject information sheet and a consent form (this will be in the form of a paper ICF or an electronic consent form) describing the trial and providing sufficient information for subject to make an informed decision about their participation in the trial. Enough time needs to be given to the subject to ask questions and to decide whether to participate or not.

The formal consent of a subject, using the approved consent form, must be obtained before the subject is submitted to any trial specific procedure.

The subject should read and consider the statement before signing and dating the ICF and should be given a copy of the signed document. The consent form must also be signed and dated by the Investigator (or his designee) at the same time as the subject signs, and it will be retained by the Investigator as part of the trial records.

The Investigator must document the informed consent process in the subject's source documents (medical chart). Details are described in the Site Operations Manual.

In the case that a vulnerable subject enters the trial, the Principal Investigator is encouraged to consult their local IEC for guidance.

In the event of substantial changes to the trial or to the risk-benefit ratio, the Investigator will obtain the signed informed consent of subjects for continued participation in the trial using an IEC approved amendment to the ICF.

Subjects who are re-screened will undergo the consenting processes again and sign a new ICF prior to the re-screening procedures beginning.

9.2.6 Subject Privacy and Confidentiality

All sites and laboratories or entities providing support for this trial, must, where applicable, comply with the EU Regulation 2016/679 (General Data Protection Regulation) [EU 2016/679, 2016].

The confidentiality of records that may be able to identify subjects will be protected in accordance with applicable laws, regulations, and guidelines. The subject's confidentiality and privacy are to be strictly held in trust by the participating sites, Investigators, their staff, the Sponsor(s) and its designees involved in the trial. This confidentiality is extended to testing of biological samples, including biomarker testing (citrulline), and any future testing in addition to the clinical information relating to the subject. The subject's contact information will be securely stored at each clinical site for internal use during the trial.

After subjects have consented to take part in the trial, the Sponsor and/or its representative reviews their medical records and data collected during the trial. These records and data may, in addition, be reviewed by others including the following: monitors and independent auditors who validate the data on behalf of the Sponsor; third parties with whom the Sponsor may develop, register, or market apraglutide; national or local regulatory authorities; and the IEC(s), which gave approval for the trial to proceed. The Sponsor and/or its representatives accessing the records and data will take all reasonable precautions in accordance with applicable laws, regulations, and guidelines to maintain the confidentiality of subjects' identities.

Subject research data, which is for purposes of statistical analysis and scientific reporting, will be entered in the eCRF by the trial staff at each site and then transmitted to and stored at the Sponsor or its designee. This will not include the subject's contact or identifying information. Rather, individual subjects and their research data will be identified by a unique trial identification number (unique identifier). The key between the personal data and the unique identified (subject trial number) will be kept at each clinical site and this information will never leave the respective clinical site. The trial data entry and trial management systems used by clinical sites will be secured and password protected. At the end of the trial, all trial databases will be de-identified and archived by the Sponsor or its designee for a minimal period of 25 years.

Even though subjects are assigned a unique identifying number, age and birth year will also be collected and used to assist the Sponsor to verify the accuracy of the data, for example, to confirm that laboratory results have been assigned to the correct subject.

The results of trials—containing subjects' unique identifying number, relevant medical records, and age and birth year—will be recorded. Subject to adequate safeguards, they may be transferred to, and used in, other countries that may not afford the same level of protection that applies within the countries where this trial is conducted. The purpose of any such transfer would include, but not be limited to, to support regulatory submissions, to conduct new data analyses to publish or present the trial results, or to answer questions asked by regulatory or health authorities, or for activities that are otherwise connected to the trial.

9.3 Trial Documents

All documentation and materials provided by Sponsor for this trial are to be retained in a secure location and treated as confidential material.

9.4 Early Termination of the Trial

The Sponsor may terminate the trial prematurely according to certain circumstances, for example:

- Ethical concerns
- Insufficient subject recruitment
- When the safety of the subjects is doubtful or at risk, respectively
- Alterations in accepted clinical practice that make the continuation of a clinical trial unwise
- Early evidence of benefit or harm of the experimental intervention

Individual subject stopping rules and trial stopping criteria are described in Section 6.6.2 and Section 6.6.3, respectively.

9.5 Trial Site Closure

At the EOT, all trial sites will be closed. The Sponsor may terminate participation of a trial site at any time. Examples of conditions that may require premature termination of a trial site include, but are not limited to, the following:

- Non-compliance with the protocol and/or applicable regulations and guidelines
- Inadequate subject enrollment
- Discontinuation of the trial, as decided by the Sponsor
- Institution and/or Investigator breaches the clinical trial agreement, or the agreement is terminated
- The trial is not initiated or suspended at the institution for any reason

9.6 Protocol Amendments

The protocol may not be modified without written approval from the Sponsor. Protocol modifications or changes may not be initiated without prior written IEC/CA approval except when necessary to eliminate immediate hazards to subjects or when the changes involve only logistical or administrative aspects of the trial. Such modifications will be submitted to the IEC/CA and written verification that the modification was submitted should be obtained.

In the event that an amendment to this protocol is required, it will be classified into one of the following categories:

- Substantial Amendments are those considered “substantial” to the conduct of the clinical trial and are likely to have a significant impact on, e.g., the safety or physical or mental integrity of the subjects, the scientific value of the trial, the conduct or management of the trial or the quality or safety of the IMP used in the trial
- Non-substantial Amendments are amendments which are not considered to meet the definition of substantial

Investigators are responsible for promptly informing the IEC of any amendments (substantial only in the EU) to the protocol.

Documentation of IEC/CA approval must be sent to the Sponsor immediately upon receipt.

10. DATA HANDLING AND RECORD KEEPING/ARCHIVING

10.1 Confidentiality and Access to Source Data

All documentation and materials provided by Sponsor to the investigational site for this trial are to be retained in an Investigator Site File, which should be stored in a secured location and treated as confidential material.

All local legal requirements regarding protection of personal data must be adhered to.

The trial protocol, documentation, data, and all other information generated during the trial will be held in strict confidence. No information concerning the trial or the data will be released to any unauthorized third party without prior written approval of the Sponsor.

10.2 Record Keeping/Archiving

Each clinical site will retain in a secured location, trial records and documents pertaining to the conduct of this trial and the distribution of IMP, including eCRFs, ICFs, laboratory test results, and medication inventory records, for at least 25 years after completion or discontinuation of the trial, or for the length of time required by relevant national or local health authorities, whichever is longer. Additionally, record retention may be dependent on the reviewing IEC, institutional policies, respective country legislation, specifications in trial contract, or Sponsor requirements.

The Sponsor is to be informed about any re-location of documents. After the 25-year archiving period, the documents may be destroyed, subject to local regulations.

No records may be disposed of without the written approval of VectivBio. Written notification should be provided to VectivBio prior to transferring any records to another party, moving them to another location or destroying them.

10.2.1 Storage of Biological Material and Related Health Data

Biomarker samples (citrulline) and PK samples will be kept for a maximum period of 15 years after completion of the bioanalytical report, and ADA samples will be kept for a maximum of 15 years after the completion of the bioanalytical report to use later for refinement and optimization of the bioanalytical methods or for additional research involving apraglutide, GLP-1/2 biology, and gastroenterology, and thus make informed decisions as applicable to optimize product use and further development. Therefore, biological samples will be stored and analyzed for up to 15 years after the end of the trial. Analyses will not include any genetic testing, but may include additional evaluation of biomarkers related to the study population or antibody responses induced by apraglutide.

Leuven and Copenhagen site laboratories will be used for the long-term storage of human biologic specimens, including body fluids, solid tissues, and derivatives thereof (e.g., deoxyribonucleic acid, RNA, proteins, and peptides). The collection and analysis of these specimens will facilitate the rational design of new pharmaceutical agents, the development of diagnostic tests, and research into disease mechanisms.

Blood, biopsies, and stool samples will be collected from patients during the study and will be used to achieve the following objectives:

- To investigate predictors and underlying mechanisms of the effect of apraglutide by studying intestinal permeability, GI motility, mucosal gene expression, and mucosa-associated and luminal microbiota

After the above objectives are completed the remainder of the samples will be stored for further research.

For all samples, dates of specimen collection must be recorded in the eCRF. For sampling procedures, sample coding, storage conditions, and shipment instructions, see the Laboratory Manual.

Specimens will be destroyed no later than 15 years after the date of final closure of the associated clinical database (with the exception of the Leuven Lab; see below). The specimen storage period will be in accordance with the IEC-approved ICF and applicable laws (e.g., health authority requirements).

Samples, metadata, and results of microbiota analyses are de-identified, and stored and processed in coded form. Coded metadata and results of sample analyses are stored in an independent secured database, accessible by Leuven lab researchers only. This database will not contain data that would allow participant identification without decoding. Samples, genetic/biological derivatives thereof and metadata can be stored for 30 years at the Leuven lab and used for additional analyses upon approval by the IEC.

10.3 Case Report Forms

Each of the trial sites will be assigned a unique site number, and every subject enrolled into this trial will receive a unique subject number that identifies the subject throughout the trial. The subjects enrolled into the trial will be identified in a clinical trial database by subject number. The Investigator or delegate will enter subject data from the source documents into an EDC system.

All relevant data are entered directly into the clinical database via an eCRF. Access to the eCRF is restricted to staff participating in the trial and the extent of access will depend on the subjects' user role in the trial. Following training, the trial staff will be given access to the eCRF and be able to enter data. The eCRF is designed to capture all required information in compliance with GCP standards.

The clinical database will be designed based on the final protocol, system configuration and consistency check specifications.

Subjects will receive an electronic device with the e-diary ([redacted]) installed and will be recording data into this e-diary at home as described in Section 5.1.4.

[redacted] will be used as an eCRF and the subject's e-diary is integrated into Rave.

All eCRF data is to be entered in English. Data recorded in the eCRFs will be accessible to the trial staff throughout the trial.

All sections of the eCRF are to be electronically approved by the Investigator or designee after the data has been entered and all queries have been resolved, signifying that the data entered in the eCRF is complete and accurate. Any subsequent changes to any eCRF page require a new approval signature.

10.3.1 Data Collection

The Investigators' authorized site personnel must enter the information required by the protocol on the eCRF. A trial monitor will visit each site in accordance with the monitoring plan and review the eCRF data against the source data for completeness and accuracy. Data entry, corrections and addressing of discrepancies will be made by qualified site personnel.

10.3.2 Clinical Data Management

Data are to be entered into a clinical database in a timely manner of the subject's visit in accordance with the trial specific data management plan and in accordance with the instructions received from Sponsor/CRO to that end. Quality Control and data validation procedures are applied to ensure the validity and accuracy of the clinical databases.

Data are to be reviewed and checked for omissions, errors, and values requiring further clarification using computerized and manual procedures.

10.4 Specification of Source Documents

Source documents provide evidence for the existence of the participating subjects and substantiate the integrity of the data collected. Source documents (apart from e-diary) are filed at the Investigator's trial site.

Data entered in the eCRF that are transcribed from source documents must be consistent with the source documents and the discrepancies must be explained. The Investigator may need to request medical records from other healthcare professionals, if appropriate. Also, current medical records must be available. Electronic source data should be handled in compliance with FDA Guidance [FDA, 2013].

All entries into the eCRF performed by site personnel must be verifiable by source documents. Additionally, the following data entered into the eCRF, should be verifiable by source documents in the subject's medical record, or other records, at the trial site, as applicable:

- Details of trial participation (Trial ID and unique identifier)
- Date(s) of subject's informed consent
- Date of each trial visit including signature and/or initials of person(s) conducting the trial visit
- Results of blood tests and other examinations

Information recorded in the eCRF system should be supported by corresponding source documentation, which is attributable, legible, contemporaneous, original, accurate, and complete. Changes to source data should be traceable, should not obscure the original entry, and should be explained if necessary (e.g., via an audit trail). Examples of acceptable source documentation include, but are not limited to, hospital records, e-diary, clinic and office charts, laboratory notes, and recorded data from automated instruments, and memoranda.

Clinical laboratory data received from a local laboratory for entry into the eCRF will be considered as source documentation.

Entries into the eCRF will be verified with source documentation by the monitor who will perform 100% source data verification. The location of source data for all

pertinent data will be defined in the relevant source data location form prior to the start of the trial.

11. QUALITY ASSURANCE AND QUALITY CONTROL

This trial will be performed in compliance with the clinical trial protocol, the current version of the Declaration of Helsinki, ICH-GCP (R2) and applicable regulatory requirements.

The accuracy, consistency, completeness and reliability of the trial data produced under this protocol will be assured through quality control and quality assurance activities performed in accordance with the standard operating procedures of the Sponsor or of the Sponsor representative (CRO). The Investigator agrees, when signing this protocol, to fully cooperate with compliance checks by allowing direct access to all clinical trial related documentation by authorized individuals.

11.1 Audits and Inspections

The trial may be subject to audit by Sponsor or its designee, IEC and/or regulatory authority inspections. Audits may be performed to check compliance with ICH GCP E6 in its most current version, guidelines and other applicable regulations, and may include:

- Site audits
- (Electronic) Trial Master File/Investigator Site File audits
- Database audits
- Document audits

The Sponsor or its designee may conduct additional audits on a selection of trial sites, requiring access to subject's notes/medical records, trial documentation, and facilities or laboratories used for the trial.

The trial site, facilities, all data (including source data), and documentation will be made available for audit by quality assurance auditors and for IEC or regulatory authority inspections according to ICH E6(R2) guidelines. The Investigator agrees to cooperate with the auditor during the visit and will be available to supply the auditor with eCRF or other files necessary to conduct that audit. Any findings will be strictly confidential.

If a regulatory authority informs the Investigator that it intends to conduct an inspection, the Investigator shall notify the Sponsor immediately.

11.2 Monitoring

The Sponsor and/or its representatives will conduct on-site monitoring visits to monitor the trial and ensure compliance with the protocol, GCP, and applicable regulations and guidelines. Remote monitoring may be done, if needed. There will be a Clinical Monitoring Plan that will provide guidelines for monitors concerning how to monitor the trial.

To ensure data accuracy, completeness, and compliance, monitoring visits will be performed to review source data versus eCRF. The assigned monitor will visit the Investigator and trial site at periodic intervals and maintain periodic communication. The Investigator agrees to allow the Monitor(s) and other authorized Sponsor personnel access to trial facilities and all source documents, as and when needed. The Monitor(s) will maintain current personal knowledge of the trial through observation, review of trial records and source documentation, and discussion of the

conduct of the trial with the Investigator and staff. While on site, the Monitor(s) will review the following:

- Source documents, directly comparing entries in the EDC system with the source documents
- Consenting procedures
- Investigator Site File [ICH E6(R2), 2016]
- Other documentation verifying the activities conducted for the study

The monitor will ask for clarification and/or correction of any noted inconsistencies. (Procedures for correcting eCRF are described in the eCRF completion guideline.) As representatives of the Sponsor, monitors are responsible for notifying project management of any noted protocol deviations.

In compliance with ICH guidelines, the Investigator, or person designated by the Investigator, should document and explain any deviation from the approved protocol. All important deviations relating to trial inclusion/exclusion criteria, conduct of the trial, patient management, or patient assessment should be described and an appropriate summary provided per center [ICH E3, 1995].

All laboratory data from citrulline biomarker samples and from future analyses of the residuals of the citrulline biomarker samples will be available only to the Sponsor.

By signing the Investigator's Agreement (found at the start of this protocol), the Investigator agrees to meet with the monitor during trial site visits; to ensure that trial staff is available to the monitor as needed; and to provide the monitor access to all trial documentation, if requested. Further, the Investigator agrees to allow Sponsor to assist the inspectors in their duties, if requested.

12. PUBLICATION AND DISSEMINATION POLICY

Data will be reported in a CTR in compliance with the requirements of the current version of ICH E3, 1995: 'Structure and Content of Clinical Trial Report' [ICH E3, 1995].

The CTR will be completed within 12 months after the end of the trial and distributed as required by local regulations.

A description of this clinical trial is available on clinical trial registries such as ClinicalTrials.gov and the EU Clinical Trials Register (clinicaltrialsregister.eu). A summary of results, whether positive, negative, or inconclusive, will be published on ClinicalTrials.gov.

The trial results may be published and presented to the public and used for educational purposes. Information that could identify subjects will not be used in any publication or presentation.

All information concerning the Sponsor's operations, patent applications, basic scientific data, and information supplied by the Sponsor or its designee to the Investigator and not previously published, is considered confidential and remains the sole property of the Sponsor. Case report forms also remain the property of the Sponsor.

The information developed in this trial will be used by the Sponsor in connection with the future development of an investigational product and thus may be disclosed as required to other clinical Investigators or government regulatory agencies.

The results generated by this trial are the property of the Sponsor who will disclose the trial results in accordance with applicable regulatory requirements and laws. The results of this trial may be published or presented at scientific meetings. If this is foreseen, the Investigator agrees to submit all manuscripts or abstracts to the Sponsor at least 30 days before submission for the Sponsor to provide comments and approval prior to the relevant publication submission deadline. If requested during the initial 30-day review period the sponsor requests, the Investigator shall delay the publication or presentation for up to 60 additional days which allows the Sponsor to protect proprietary information. If more than one trial site will be participating, data from individual trial sites must not be published separately.

Authorship will be determined in line with International Committee of Medical Journal Editors authorship requirements.

The publication policy with respect to the Investigator and clinical trial site will be further detailed in a separate document (e.g., clinical trial agreement). In the event of any inconsistency between the Agreement and the Protocol, the Protocol shall prevail for scientific matters and patient care issues, while the Agreement shall prevail for all other matters.

13. INSURANCE

The Sponsor will cover this trial by means of an adequate insurance of the participating subjects that will be in place prior to the start of the trial.

As per local regulations, details about the insurance are described in the subject information sheet and informed consent form. A copy of the insurance certificate is filed in the Investigator Site File and the subject can request a copy.

14. FUNDING AND SUPPORT

A contract/agreement will be made (as appropriate) with the Investigator and/or Institution.

The Sponsor is paying the trial site as per the signed contracts between sponsor and site. The signed contract details the amount to be paid (including overhead fee, if applicable) to cover site staff work in relation to the trial. Furthermore, the signed contract details that Sponsor will reimburse the following expenses for trial subjects: travel expenses, meals, subject hotel stay (where relevant). All amounts will be paid to a research bank account held by the trial site. Bank account details are specified in the signed contract.

Details on payment to site and reimbursement payments for subjects are also given in the Subject Information Letter and ICF.

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16. APPENDICES

16.1 Appendix 1 Scales and Assessments

16.1.1 Patient Global Impression of Change v1.0

PATIENT GLOBAL IMPRESSION OF CHANGE (PGIC)

Please mark an "X" in the box (☒) that best describes the ***change in your experience*** with short bowel syndrome with intestinal failure (SBS-IF), including treatment with parenteral support (PS), since you ***began taking the study medication***.

- ☐$_{3}$ **Very much better**
- ☐$_{2}$ **Much better**
- ☐$_{1}$ **A little better**
- ☐$_{0}$ **The same (no change)**
- ☐$_{-1}$ **A little worse**
- ☐$_{-2}$ **Much worse**
- ☐$_{-3}$ **Very much worse**

16.1.2 Patient Global Impression of Change v 2.0

PATIENT GLOBAL IMPRESSION OF CHANGE (PGIC)

How has your experience taking parenteral support (PS) changed since you began taking the Trial Medication?

- ☐$_{2}$ **Much better**
- ☐$_{1}$ **A little better**
- ☐$_{0}$ **No change**
- ☐$_{-1}$ **A little worse**
- ☐$_{-2}$ **Much Worse**

VectivBio PGIC v2_0 08 Apr 2021

16.1.3 Patient Global Impression of Severity

PATIENT GLOBAL IMPRESSION OF SEVERITY (PGIS)

Please mark an "X" in the box (☒) that best describes your ***current experience*** with short bowel syndrome with intestinal failure (SBS-IF), including treatment with parenteral support (PS).

Right now, the severity of my SBS-IF is:

☐0 **Not severe** (well-controlled)

☐1 **Minimal**

☐2 **Mild**

☐3 **Moderate**

☐4 **Moderately severe**

☐5 **Severe**

☐6 **Extremely severe**

16.1.4 Patient Global Impression of Parenteral Support Impact (PGI-PSI)

PATIENT GLOBAL IMPRESSION OF PARENTERAL SUPPORT IMPACT (PGI-PSI)

1. Overall, how much has your **parenteral support (PS)** interfered with your **sleep** over the past 7 days?

- ☐$_0$ **Not at all**
- ☐$_1$ **A little bit**
- ☐$_2$ **Moderately**
- ☐$_3$ **Quite a bit**
- ☐$_4$ **Extremely**

2. Overall, how much has your **parenteral support (PS)** interfered with your **daily activities** over the past 7 days?

- ☐$_0$ **Not at all**
- ☐$_1$ **A little bit**
- ☐$_2$ **Moderately**
- ☐$_3$ **Quite a bit**
- ☐$_4$ **Extremely**

3. Overall, how much has your **parenteral support (PS)** interfered with your **quality of life** over the past 7 days?

- ☐$_0$ **Not at all**
- ☐$_1$ **A little bit**
- ☐$_2$ **Moderately**
- ☐$_3$ **Quite a bit**
- ☐$_4$ **Extremely**

VectivBio PGI-PSI v1_0 08 Apr 2021

16.1.5 Patient Global Impression of Satisfaction with Parenteral Support Impact (PGI-SPS)

PATIENT GLOBAL IMPRESSION OF SATISFACTION with PARENTERAL SUPPORT (PGI-SPS)

Overall, how satisfied were you with your **parenteral support (PS)** over the past 7 days?

- ☐ $_{2}$ **Very satisfied**
- ☐ $_{1}$ **Satisfied**
- ☐ $_{0}$ **Neither satisfied nor dissatisfied**
- ☐ $_{-1}$ **Dissatisfied**
- ☐ $_{-2}$ **Very dissatisfied**

VectivBio PGI-SPS v1_0 08 Apr 2021

16.1.6 Patient Global Impression of Treatment Satisfaction (PGI-TS)

PATIENT GLOBAL IMPRESSION OF TREATMENT SATISFACTION (PGI-TS)

Overall, how satisfied were you with the **Trial Medication** over the past 7 days?

- ☐ $_{2}$ **Very satisfied**
- ☐ $_{1}$ **Satisfied**
- ☐ $_{0}$ **Neither satisfied nor dissatisfied**
- ☐ $_{-1}$ **Dissatisfied**
- ☐ $_{-2}$ **Very dissatisfied**

VectivBio PGI-TS v1_0 08 Apr 2021

16.1.7 Short Bowel Syndrome with Intestinal Failure and Colon-in-Continuity - Quality of Life Questionnaire (SBS-IF-CIC-QOL)

Short Bowel Syndrome with Intestinal Failure and Colon-in-Continuity – Quality of Life Questionnaire (SBS-IF-CIC-QOL)

Instructions: The following questions are about your intestinal failure due to short bowel syndrome (SBS-IF) ***without a stoma***. Please clearly mark the number that best describes your experience ***during the past 7 days***. Please select only one answer for each question. Please answer all of the questions and do not skip any. There are no right or wrong answers to any of these questions.

	0	1	2	3	4	5	6	7	8	9	10
1. During the past 7 days, how much did **diarrhea** due to SBS-IF interfere with your daily activities?	☐ Did not interfere	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐ Completely interfered
2. During the past 7 days, how much did the **urgency of your bowel movements** due to SBS-IF interfere with your daily activities?	☐ Did not interfere	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐ Completely interfered
3. During the past 7 days, how much did **having to be close to a bathroom** limit your daily activities?	☐ Not limited at all	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐ Extremely limited
4. During the past 7 days, how **worried** were you about **having an accident** due to your SBS-IF?	☐ Not worried at all	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐ Extremely worried
5. During the past 7 days, how much did **fatigue** due to SBS-IF interfere with your daily activities?	☐ Did not interfere	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐ Completely interfered
6. During the past 7 days, how much did SBS-IF **interfere with your sleep**?	☐ Did not interfere	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐ Completely interfered
7. During the past 7 days, how much did SBS-IF **interfere with your relationships with others (e.g., family and friends)**?	☐ Did not interfere	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐ Completely interfered

SBS-IF-CIC-QOL v1.0 11 May 2020

8. During the past 7 days, how much did SBS-IF **interfere with your family life (e.g., activities with your family such as meals or outings)**?	0 ☐ Did not interfere	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐	7 ☐	8 ☐	9 ☐	10 ☐ Completely interfered
9. During the past 7 days, how much did SBS-IF **limit your ability to act spontaneously (without planning)**?	0 ☐ Not limited at all	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐	7 ☐	8 ☐	9 ☐	10 ☐ Extremely limited
10. During the past 7 days, how much did SBS-IF **limit your leisure activities**?	0 ☐ Not limited at all	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐	7 ☐	8 ☐	9 ☐	10 ☐ Extremely limited
11. During the past 7 days, how much did your SBS-IF **limit your social activities?**	0 ☐ Not limited at all	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐	7 ☐	8 ☐	9 ☐	10 ☐ Extremely limited
12. During the past 7 days, how **worried** were you due to your SBS-IF?	0 ☐ Not worried at all	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐	7 ☐	8 ☐	9 ☐	10 ☐ Extremely worried
13. During the past 7 days, how **self-conscious** were you about your **appearance** due to your SBS-IF?	0 ☐ Not self-conscious at all	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐	7 ☐	8 ☐	9 ☐	10 ☐ Extremely self-conscious
14. During the past 7 days, how **sad** were you due to your SBS-IF?	0 ☐ Not sad at all	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐	7 ☐	8 ☐	9 ☐	10 ☐ Extremely sad

SBS-IF-CIC-QOL v1.0 11 May 2020

16.1.8 Short Bowel Syndrome with Intestinal Failure - Treatment Impacts - Quality of Life Questionnaire (SBS-IF-TI-QOL)

Short Bowel Syndrome with Intestinal Failure – Treatment Impacts – Quality of Life Questionnaire (SBS-IF-TI-QOL)

Part 1: Parenteral support (PS)

Instructions: The following questions are about your treatment with parenteral support (PS). Please clearly mark the number that best describes your experience with your PS ***during the past 7 days***. Please select only one answer for each question. Please answer all the questions and do not skip any. There are no right or wrong answers to any of these questions.

1. During the past 7 days, how bothered were you by the **diarrhea** you experienced because of PS?	0 ☐ Not bothered at all 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐ 8 ☐ 9 ☐ 10 ☐ Extremely bothered ☐ N/A: I have a stoma
2. During the past 7 days, how bothered were you by the **frequent urination** you experienced because of PS?	0 ☐ Not bothered at all 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐ 8 ☐ 9 ☐ 10 ☐ Extremely bothered
3. During the past 7 days, how bothered were you by **how long** your PS treatment lasted?	0 ☐ Not bothered at all 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐ 8 ☐ 9 ☐ 10 ☐ Extremely bothered
4. During the past 7 days, how much did your PS treatment **disturb your sleep**?	0 ☐ Not disturbed at all 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐ 8 ☐ 9 ☐ 10 ☐ Extremely disturbed
5. During the past 7 days, how bothered were you by **physical tiredness** because of PS?	0 ☐ Not bothered at all 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐ 8 ☐ 9 ☐ 10 ☐ Extremely bothered

SBS-IF-TI-QOL v1.0 11 May 2020

6. During the past 7 days, how much did your PS treatment **limit the time you had available for your daily activities**?	0 ☐ Not limited at all	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐	7 ☐	8 ☐	9 ☐	10 ☐ Extremely limited	
7. During the past 7 days, how much did your PS treatment **limit your leisure activities**?	0 ☐ Not limited at all	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐	7 ☐	8 ☐	9 ☐	10 ☐ Extremely limited	
8. During the past 7 days, how much did your PS treatment **limit your social activities**?	0 ☐ Not limited at all	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐	7 ☐	8 ☐	9 ☐	10 ☐ Extremely limited	
9. During the past 7 days, how **difficult was it to carry your PS infusion backpack**?	0 ☐ Not difficult at all	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐	7 ☐	8 ☐	9 ☐	10 ☐ Extremely difficult	☐ N/A: I do not have a PN infusion backpack
10. During the past 7 days, how **difficult was it to manage your PS equipment**?	0 ☐ Not difficult at all	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐	7 ☐	8 ☐	9 ☐	10 ☐ Extremely difficult	

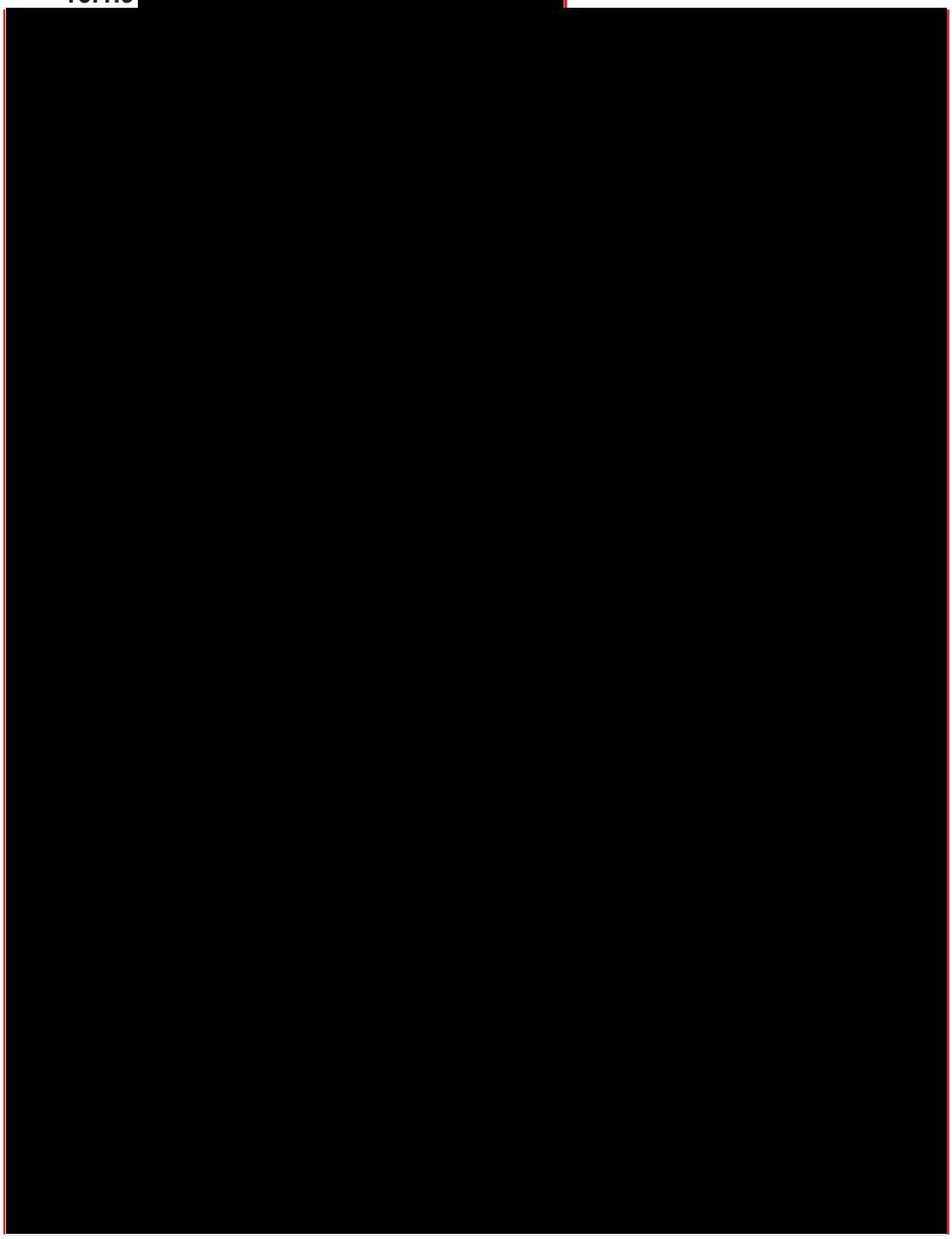
Short Bowel Syndrome with Intestinal Failure – Quality of Life Questionnaire for Treatment Impacts (SBS-IF-QoL-TI)

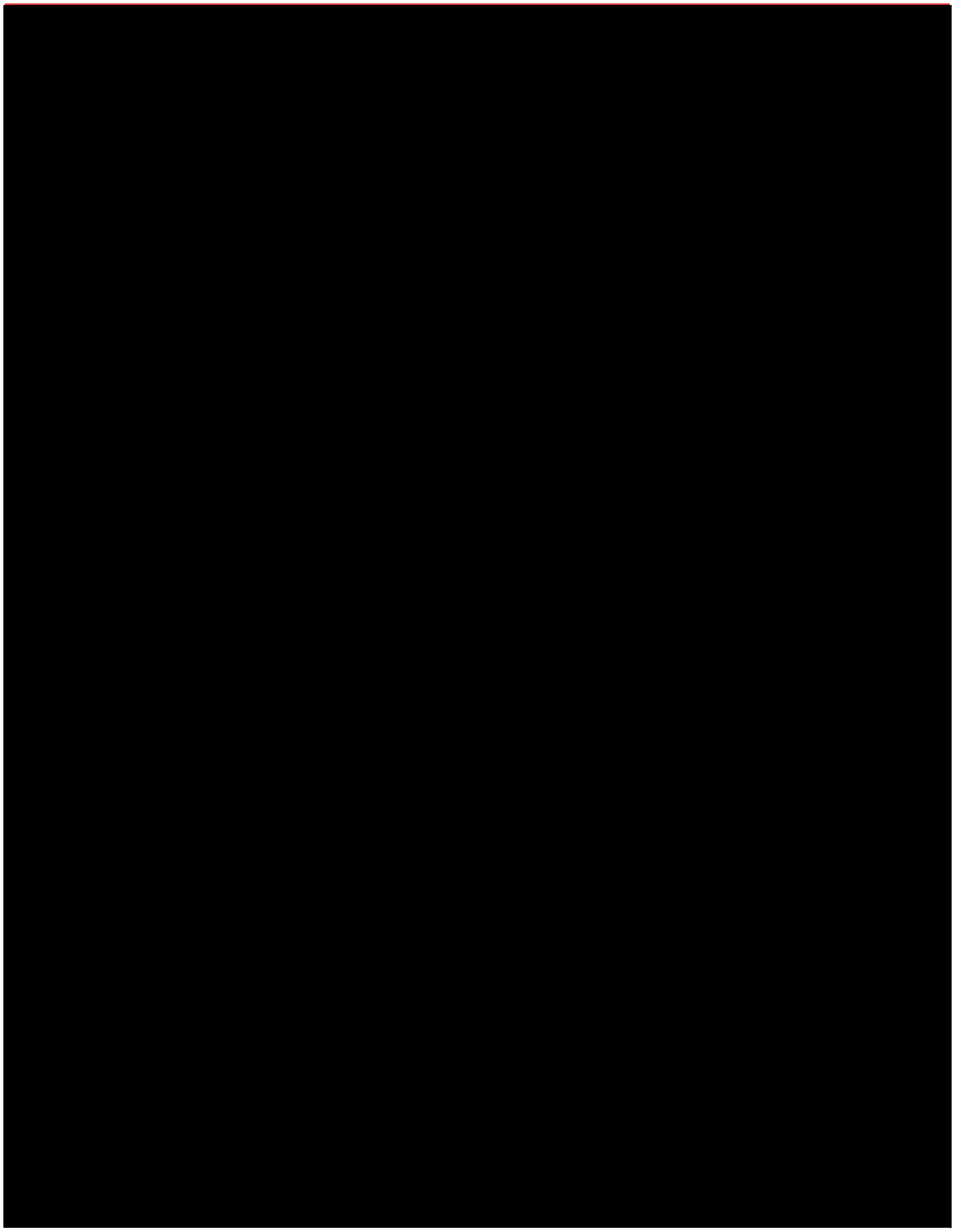
Part 2: GLP-2 analogue administration

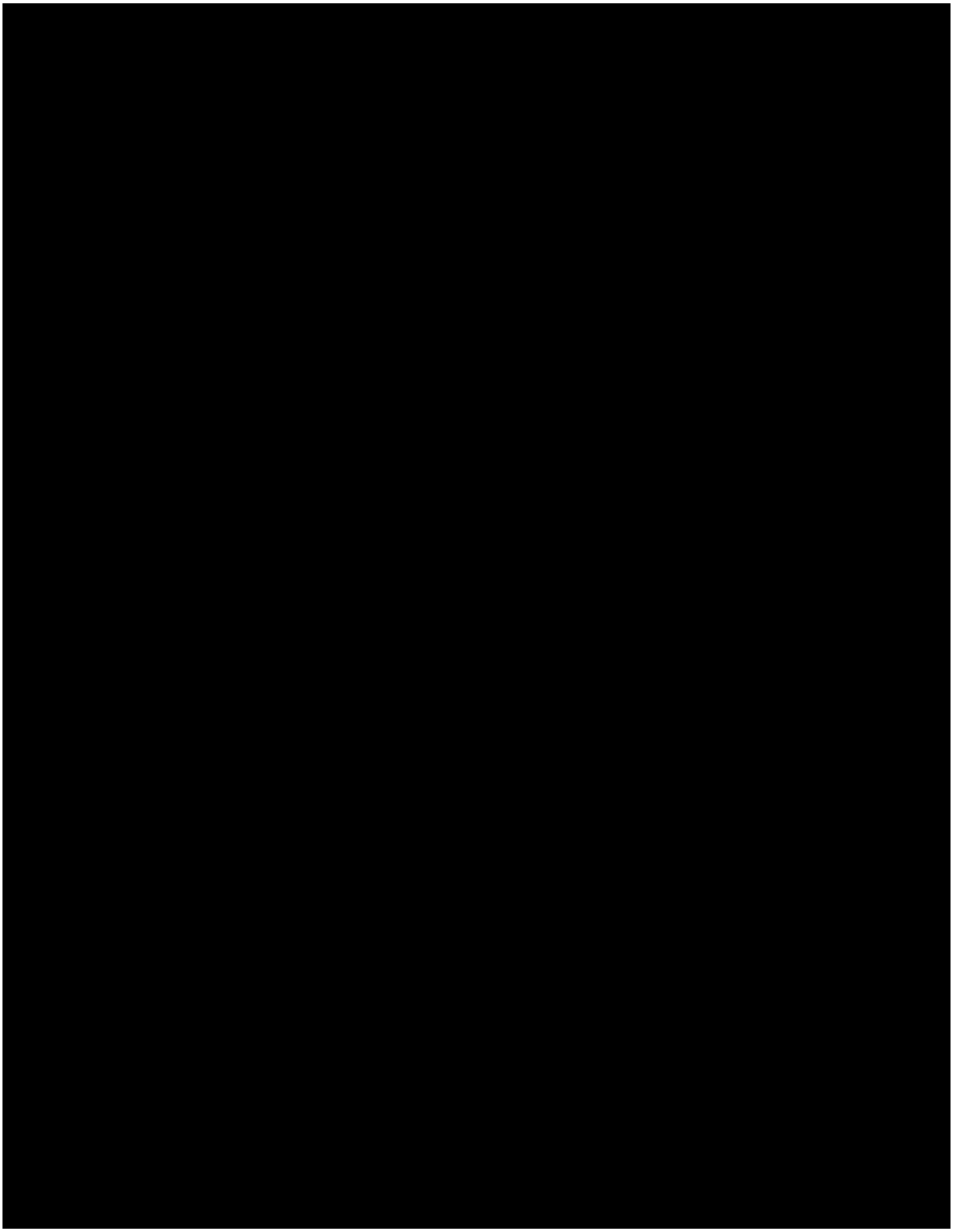
Instructions: The following questions are about your GLP-2 analogue (e.g., teduglutide, Gattex) injections for your intestinal failure due to short bowel syndrome (SBS-IF). Please clearly mark the number that best describes your experience with your GLP-2 analogue injection ***over the past 4 weeks***. Please select only one answer for each question. Please answer all of the questions and do not skip any. There are no right or wrong answers to any of these questions.

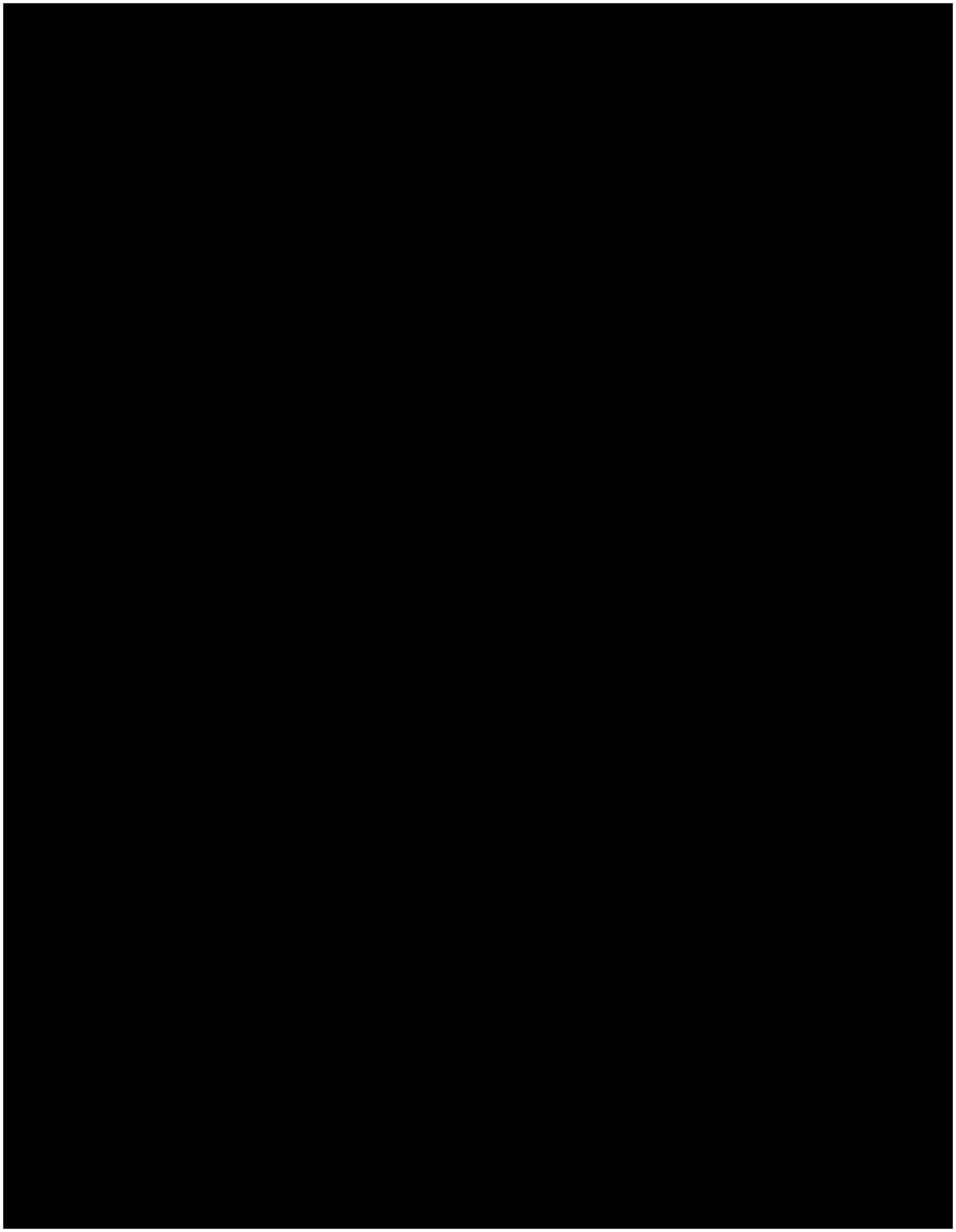
1. During the past 4 weeks, how bothered were you because of your GLP-2 **injection**?	0 ☐ Not bothered at all	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐	7 ☐	8 ☐	9 ☐	10 ☐ Extremely bothered
2. During the past 4 weeks, how difficult was it to **prepare and administer your** GLP-2 injection?	0 ☐ Not difficult at all	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐	7 ☐	8 ☐	9 ☐	10 ☐ Extremely difficult

16.1.9 [redacted]









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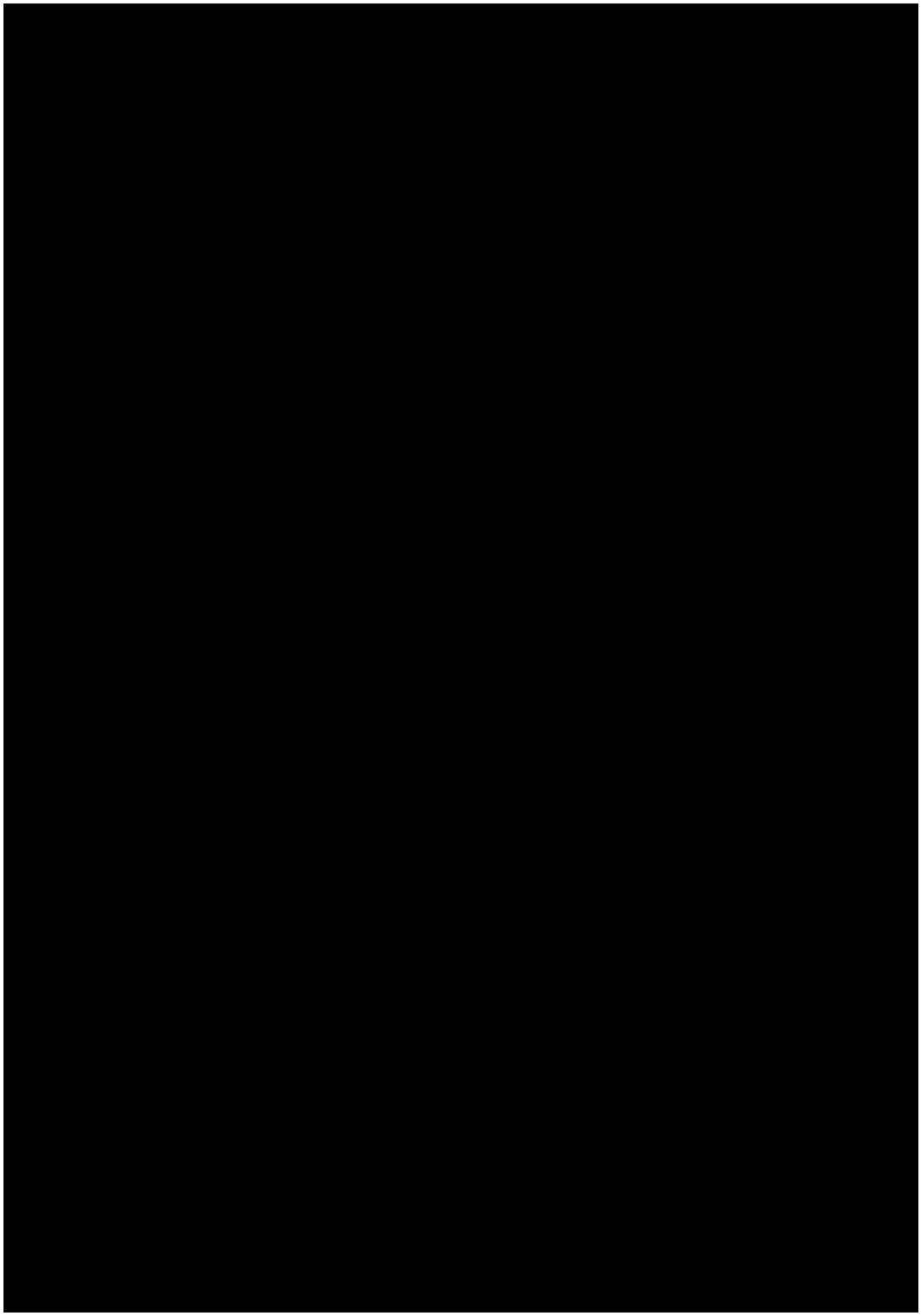

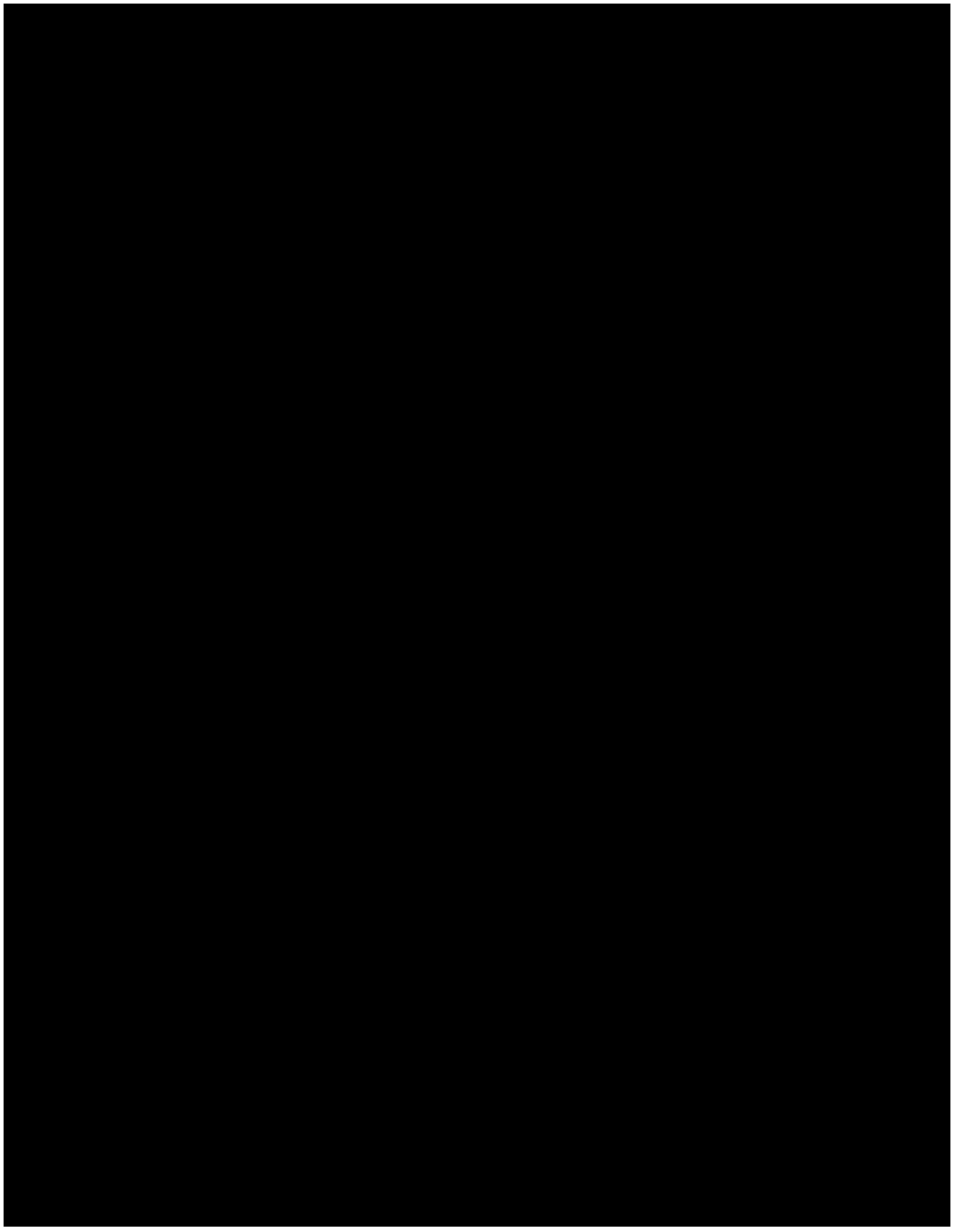

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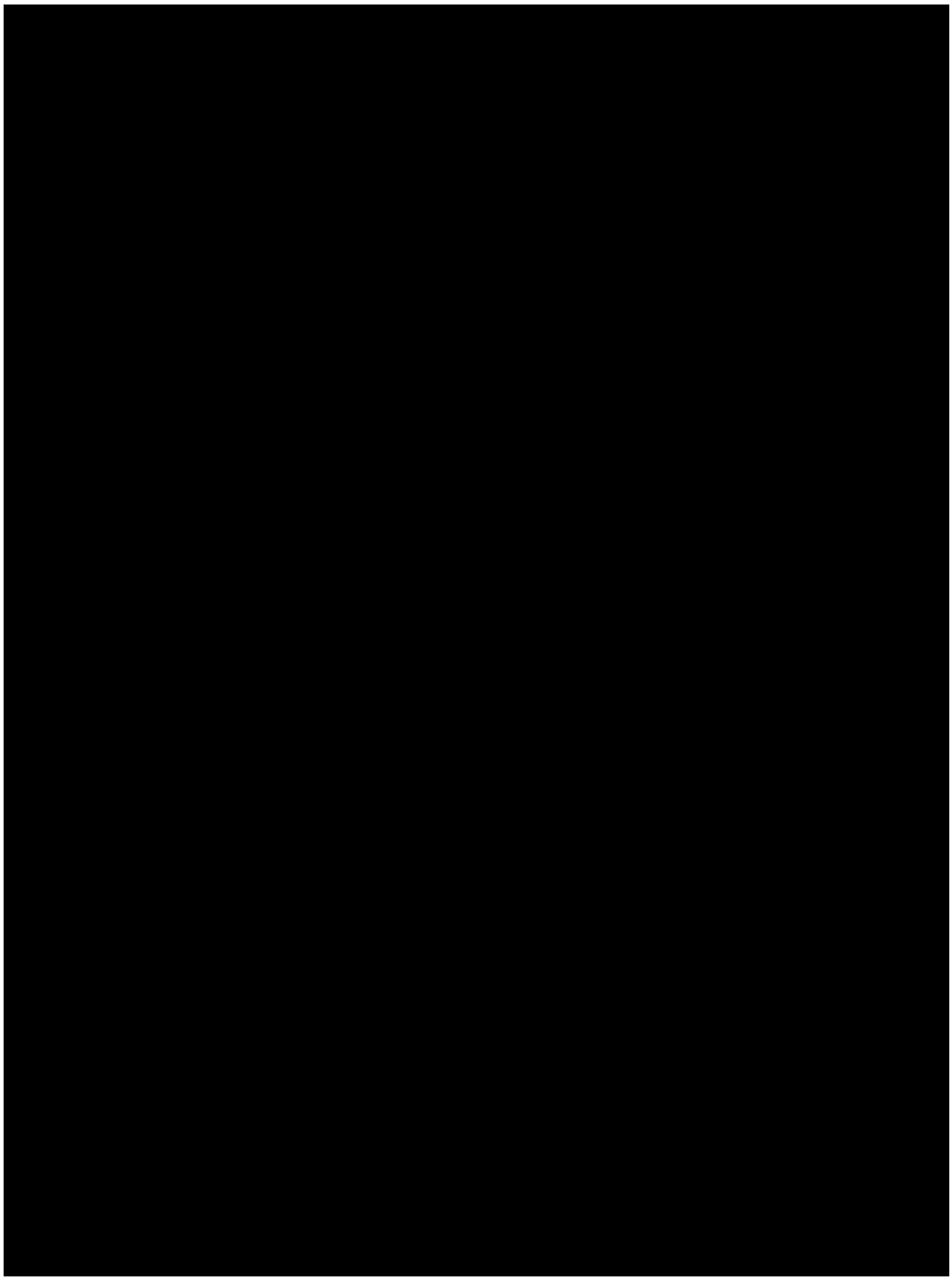

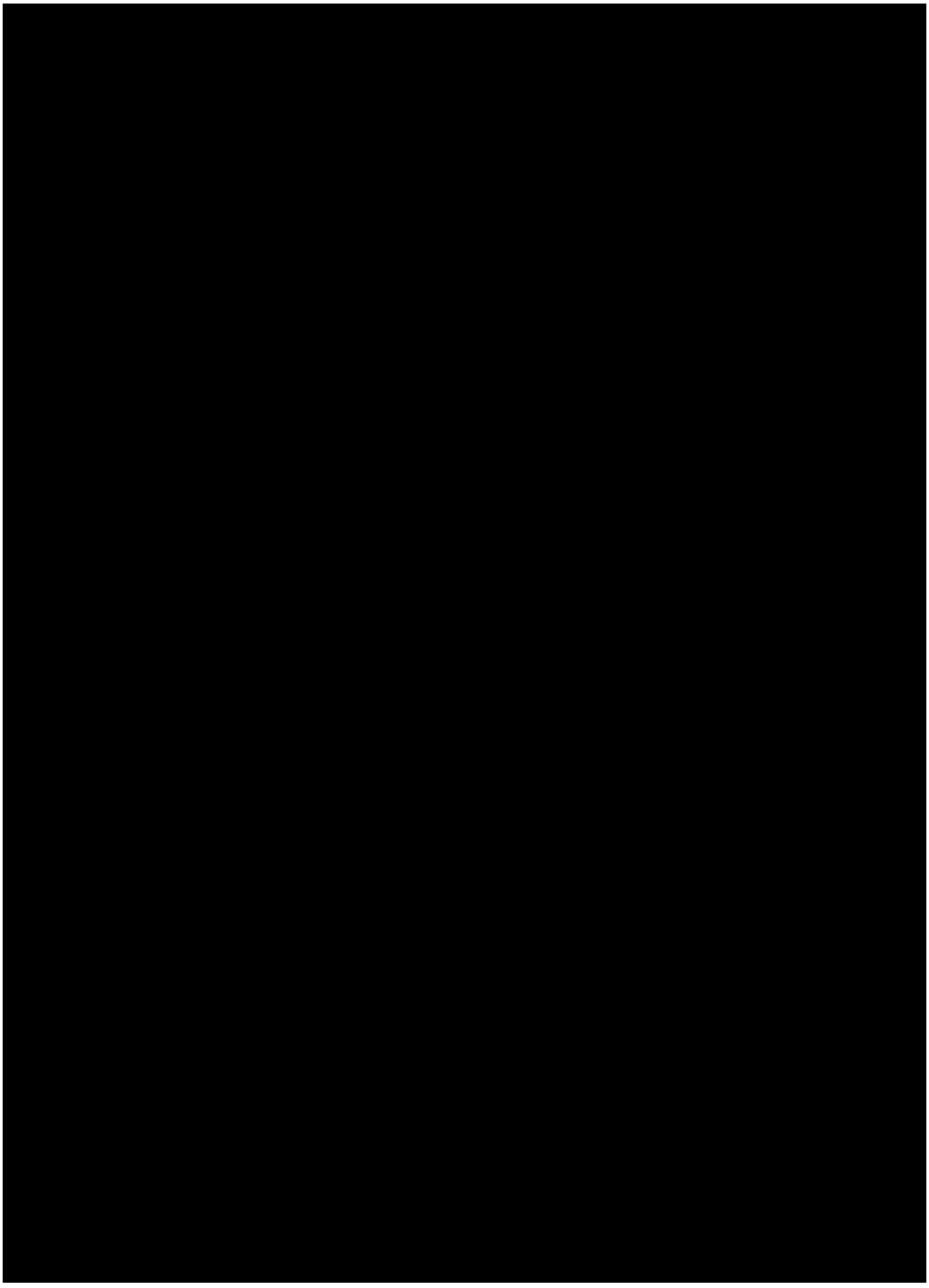

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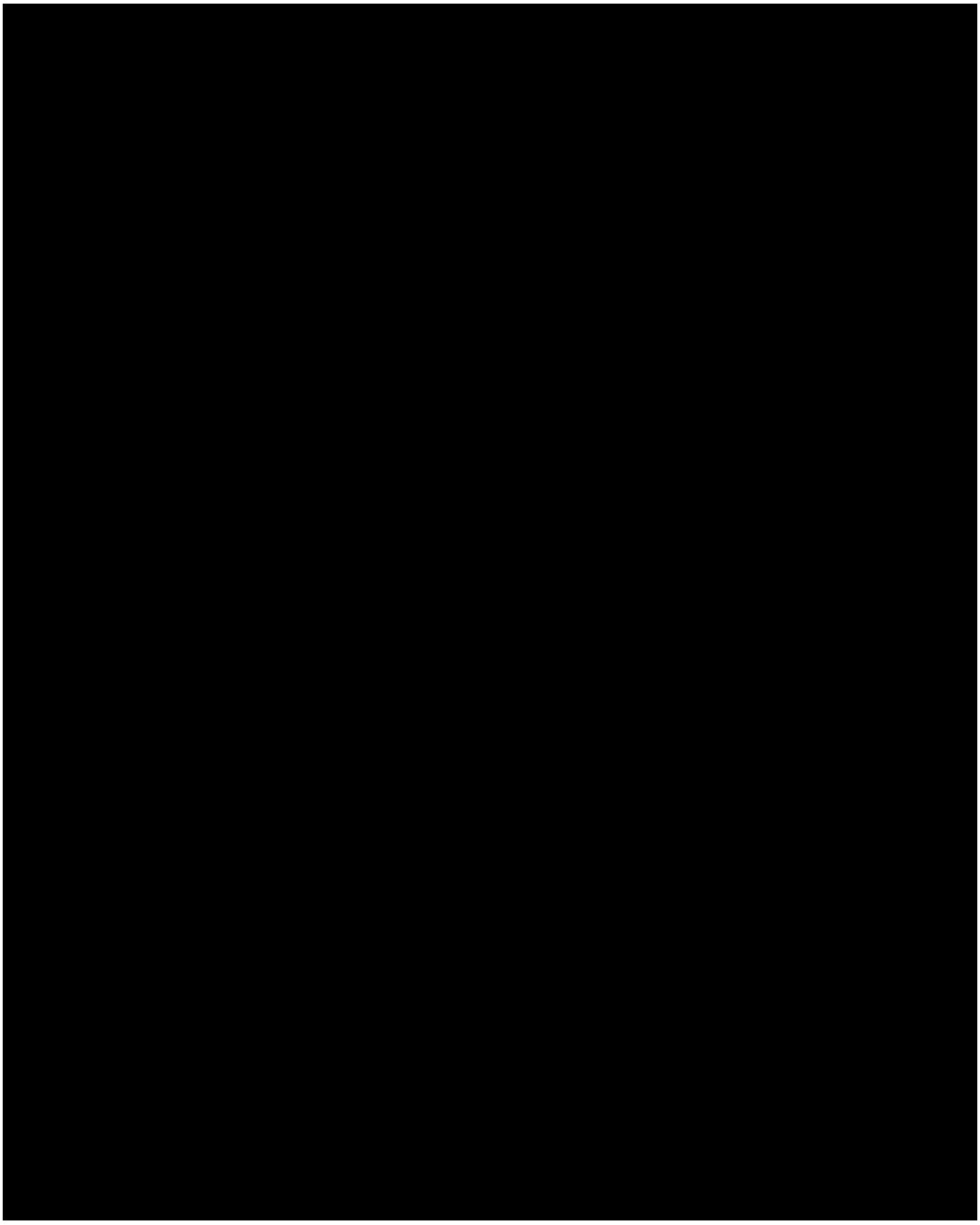
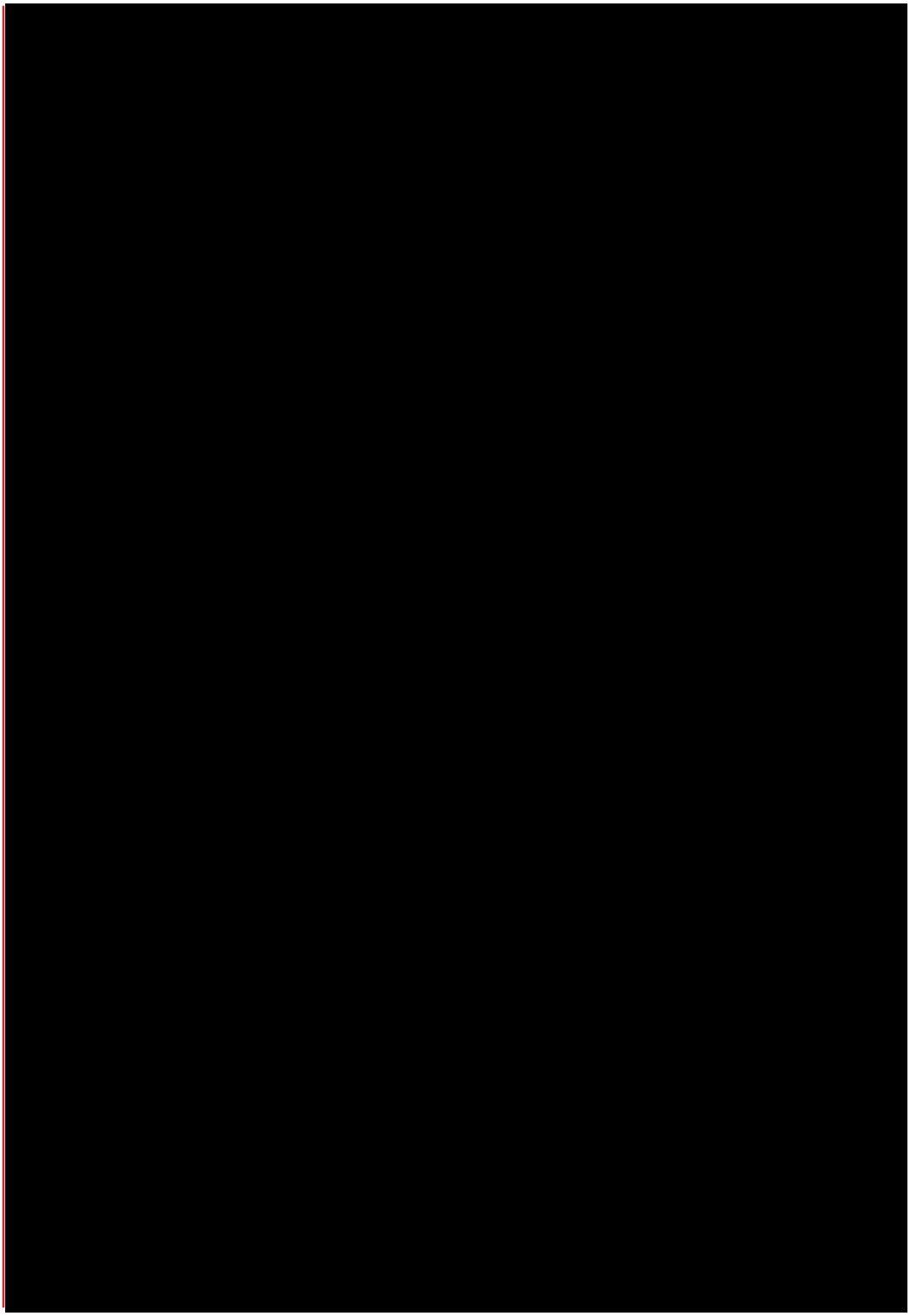
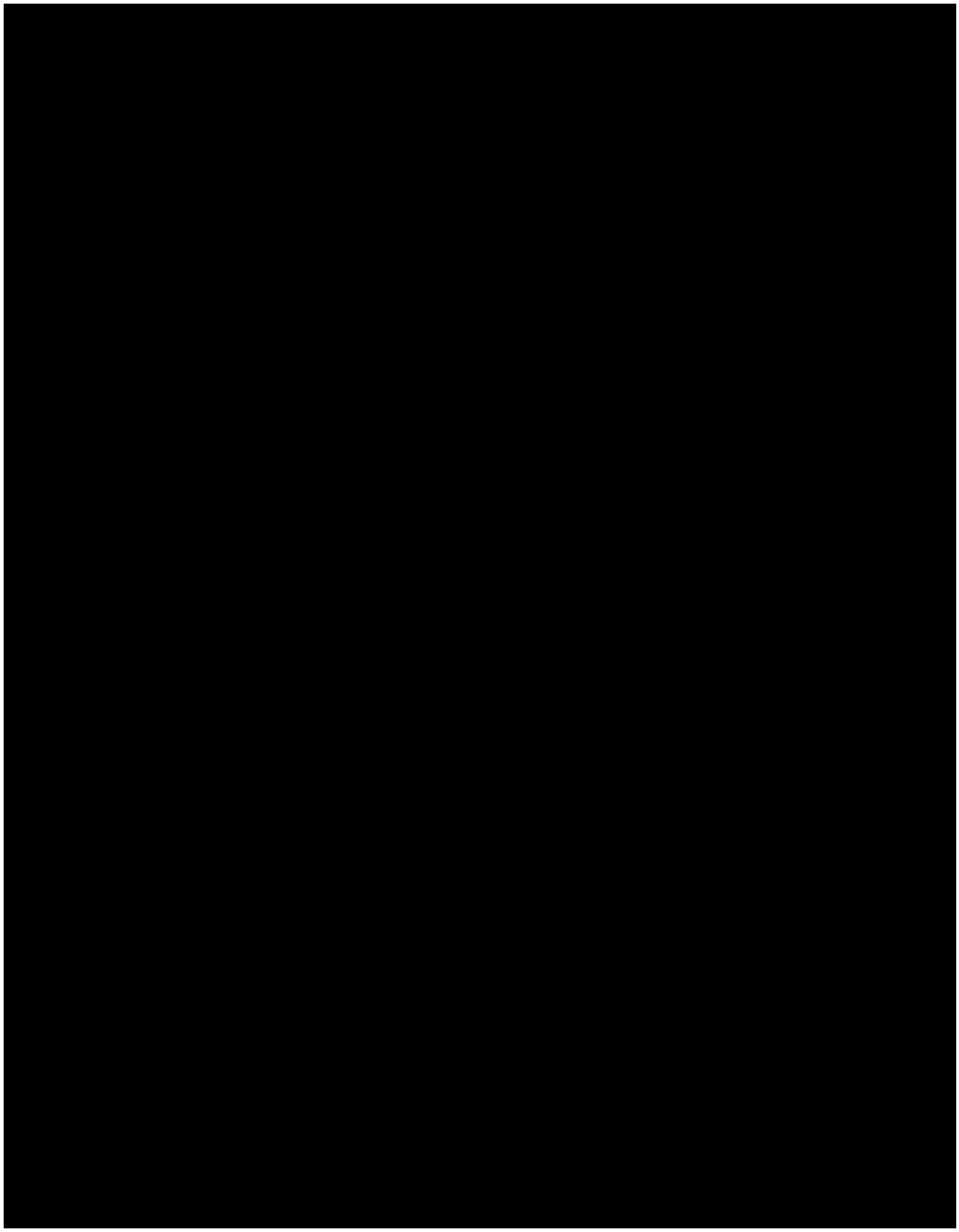
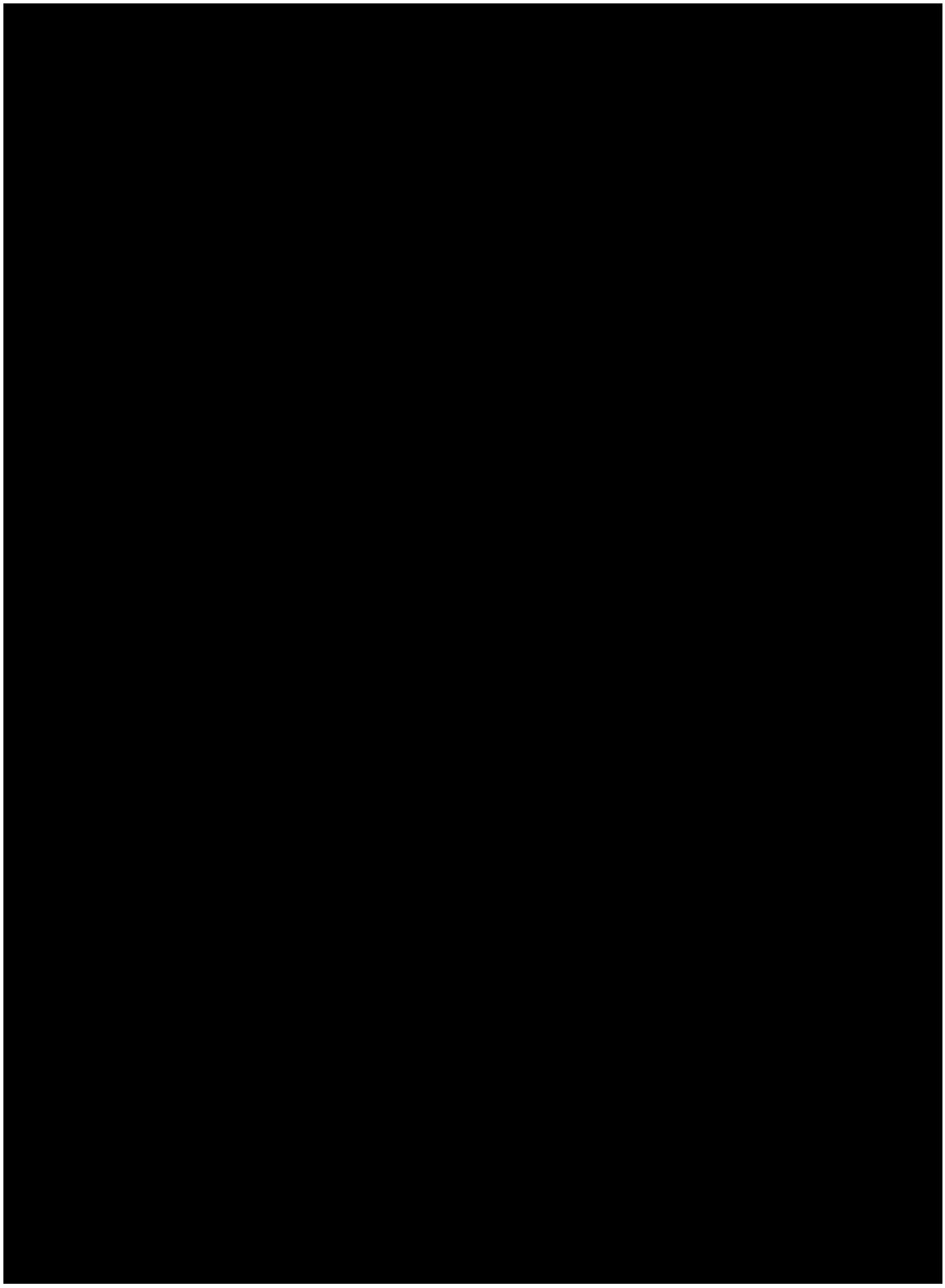
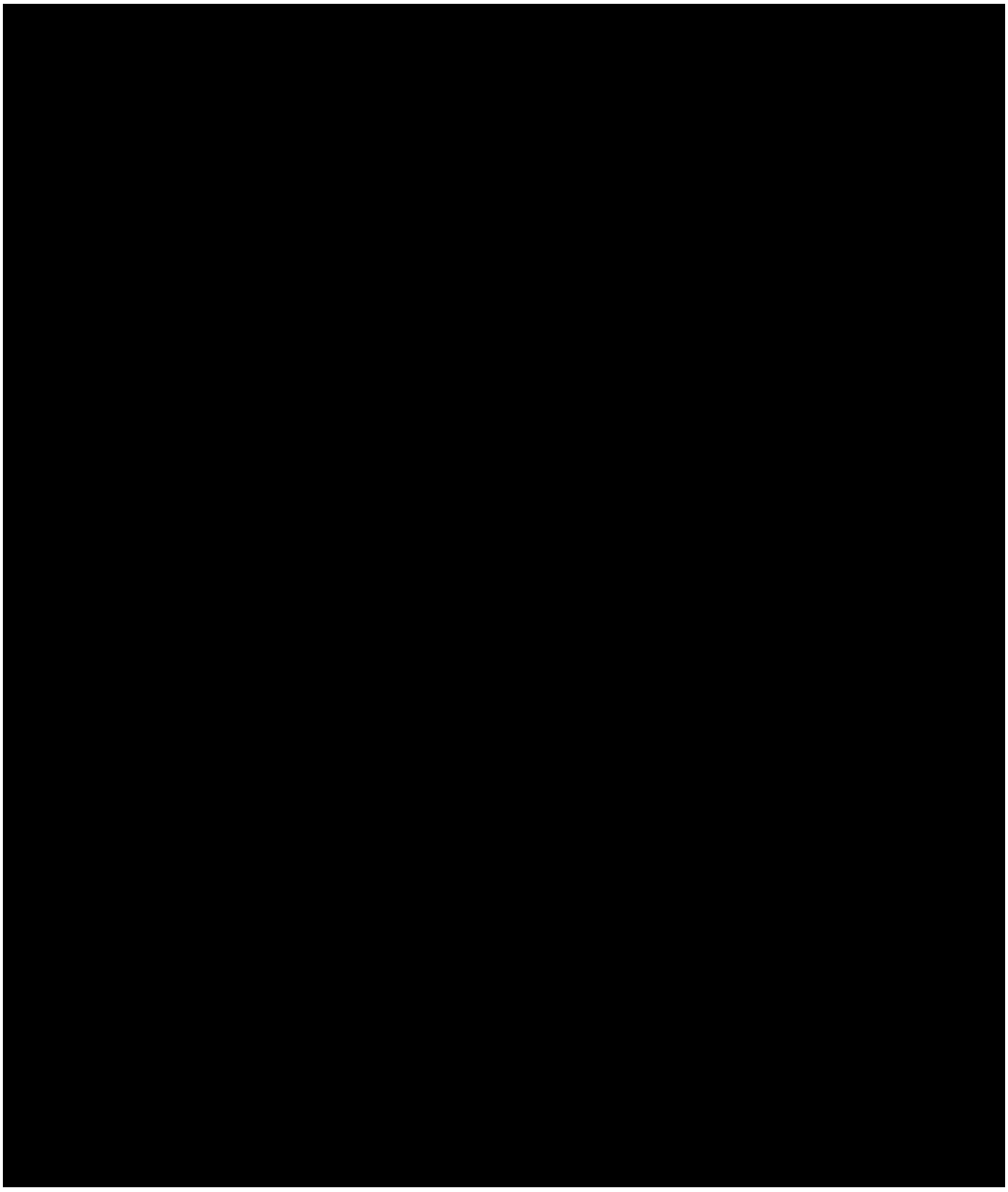
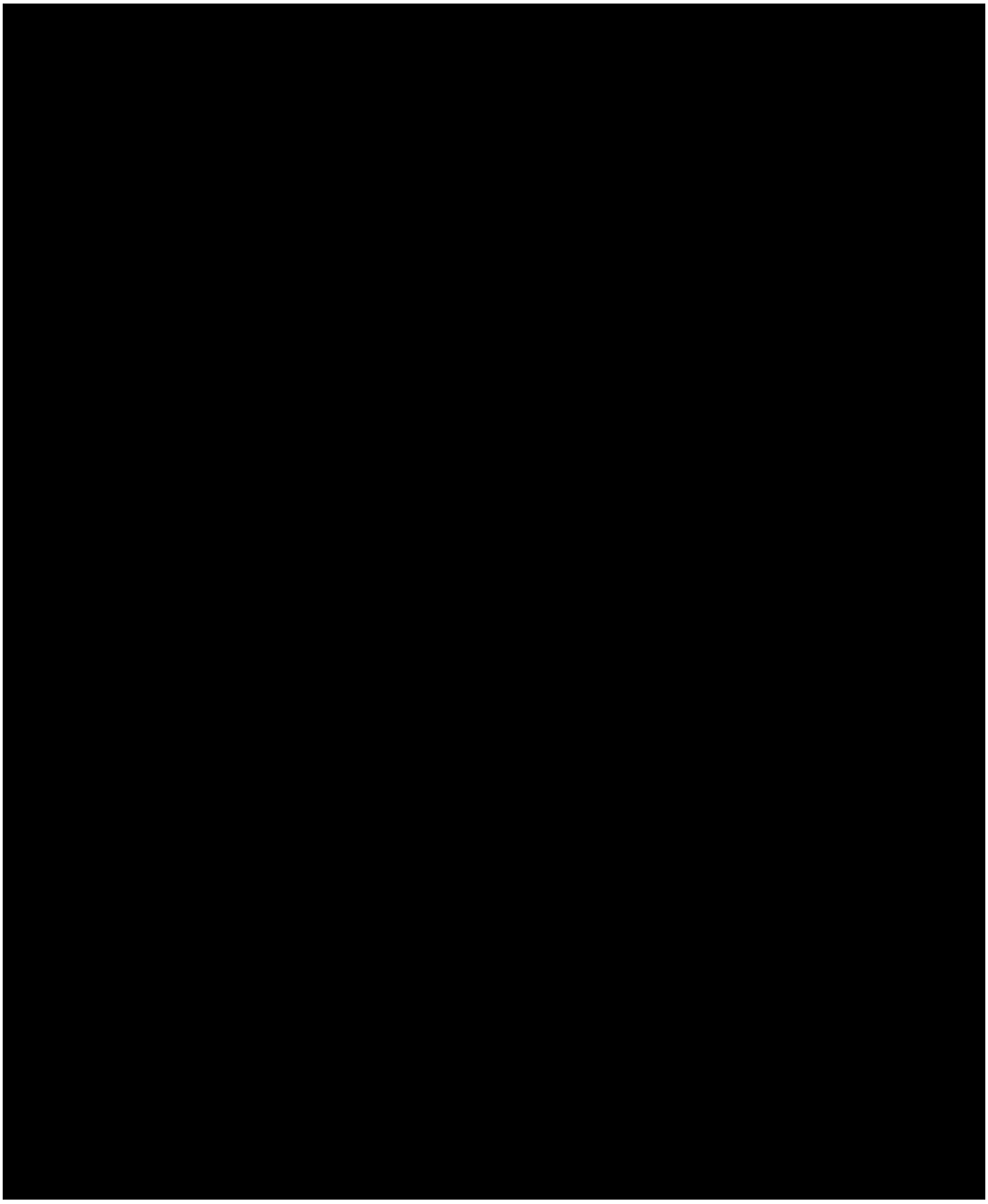


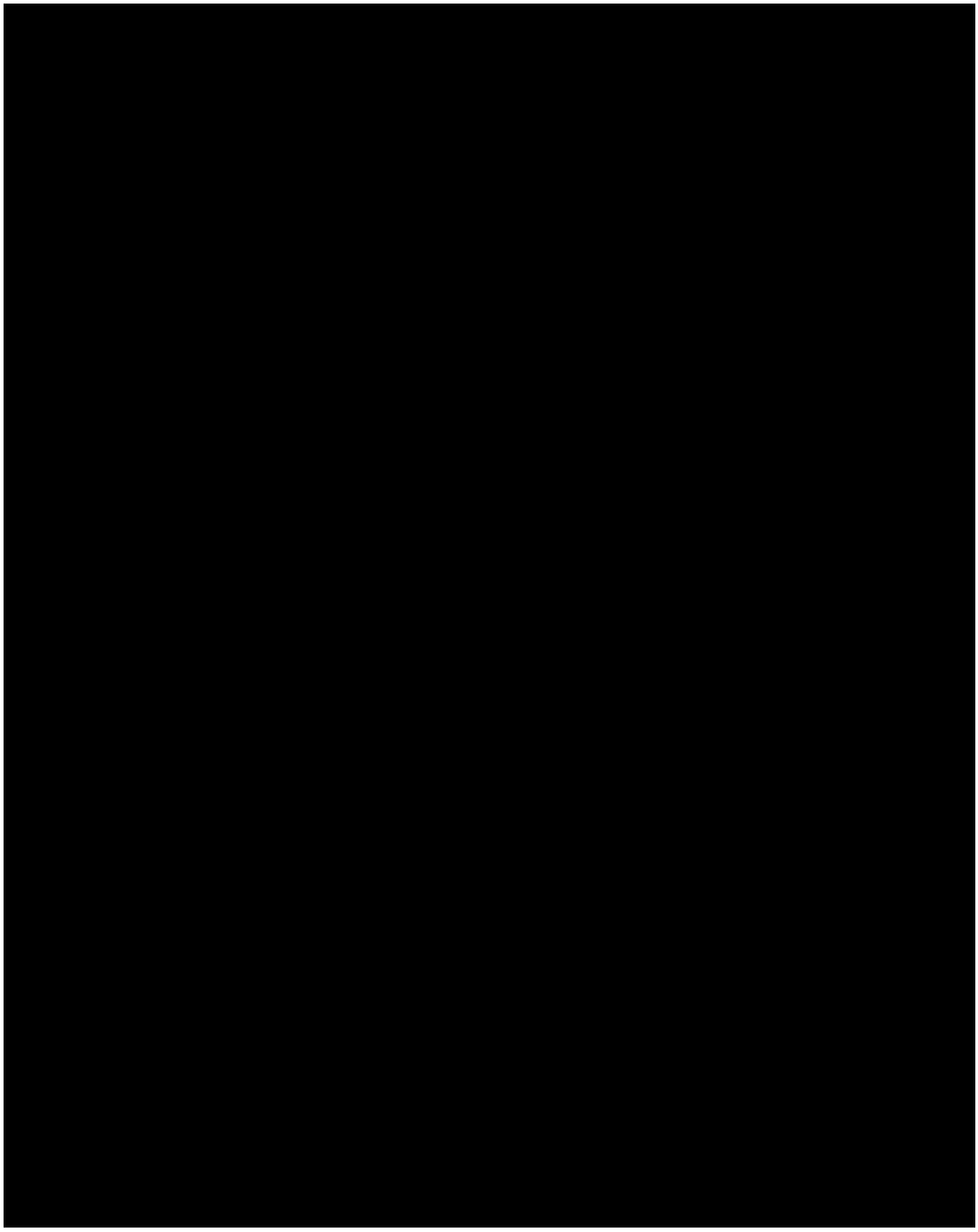


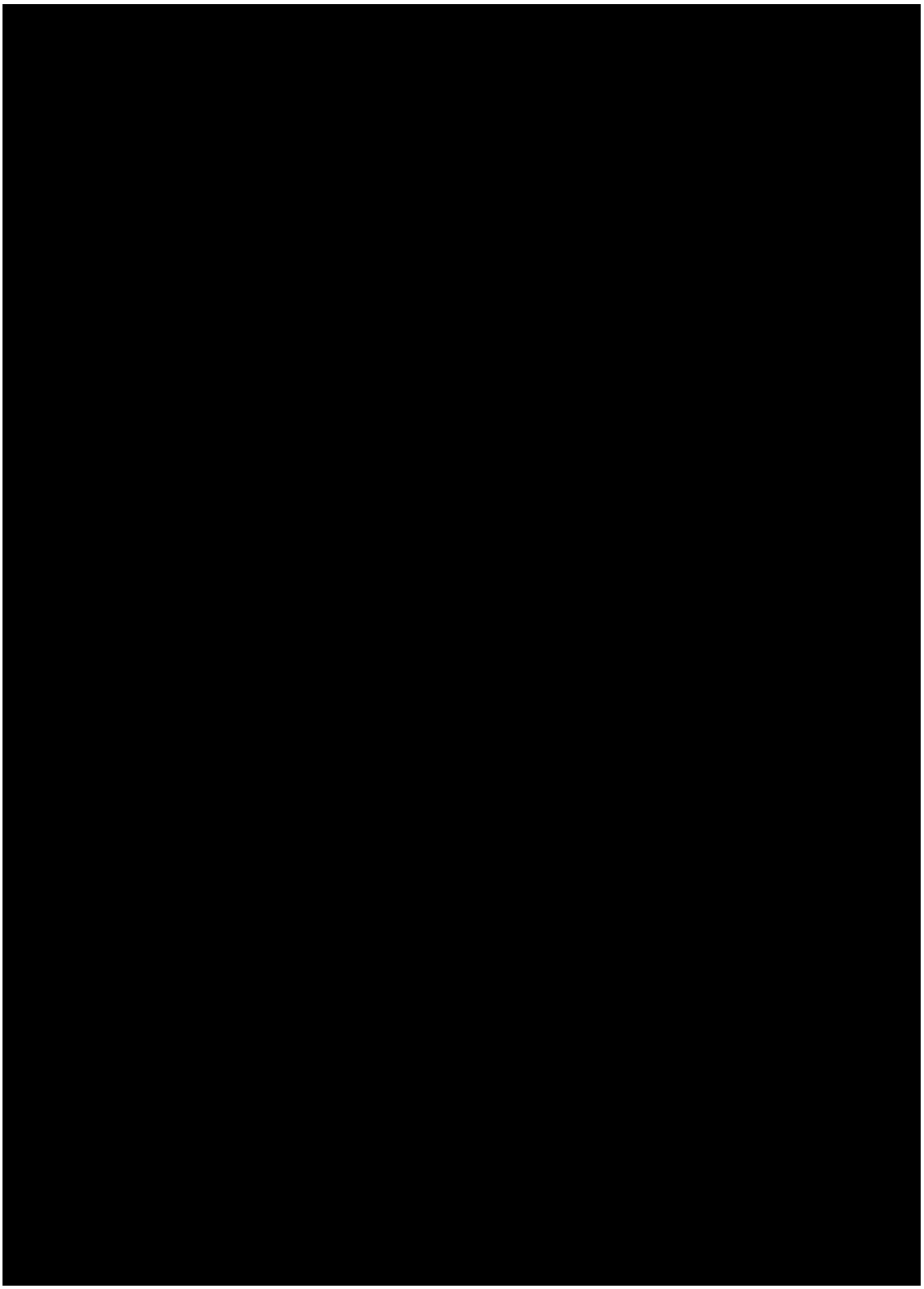


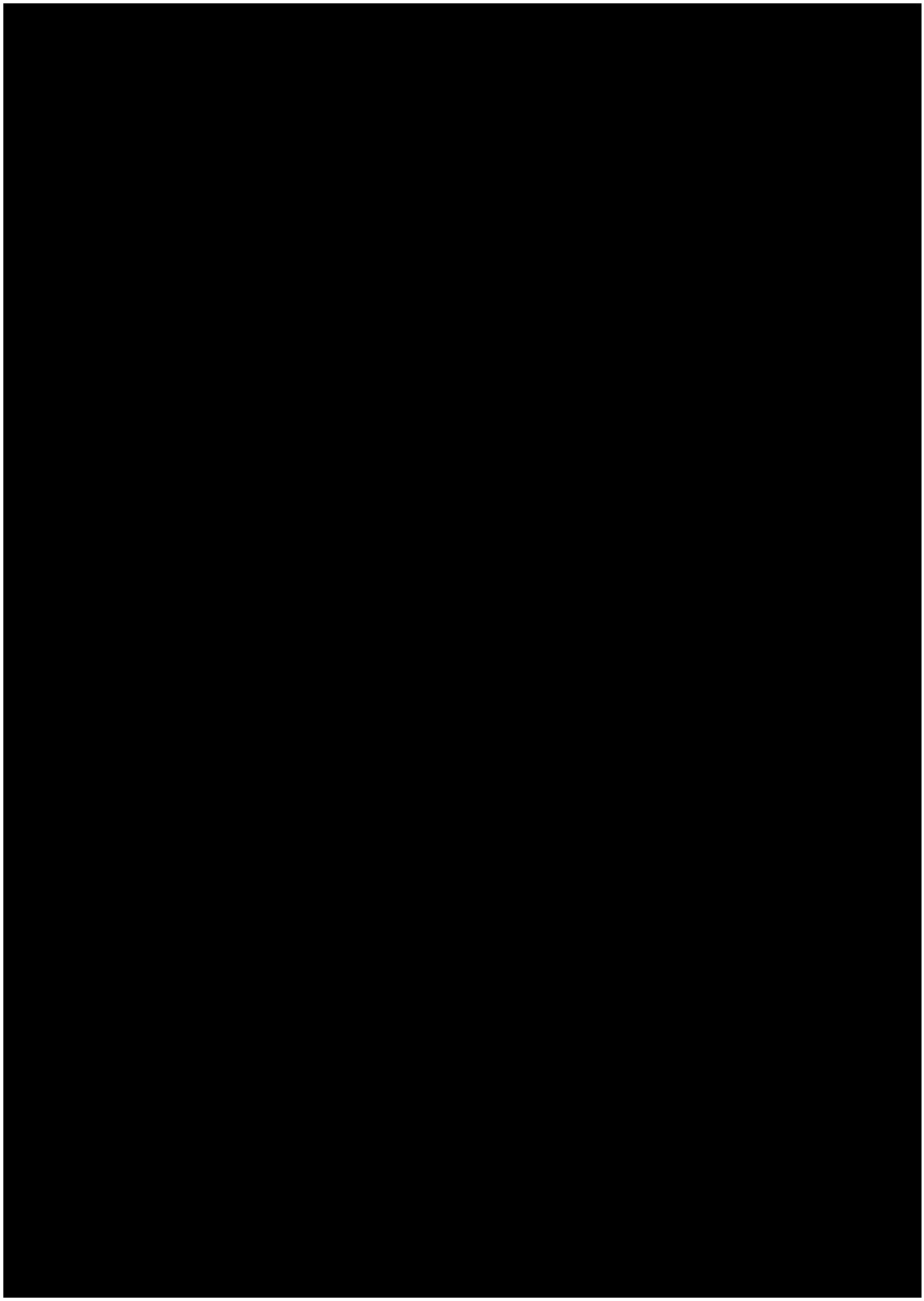


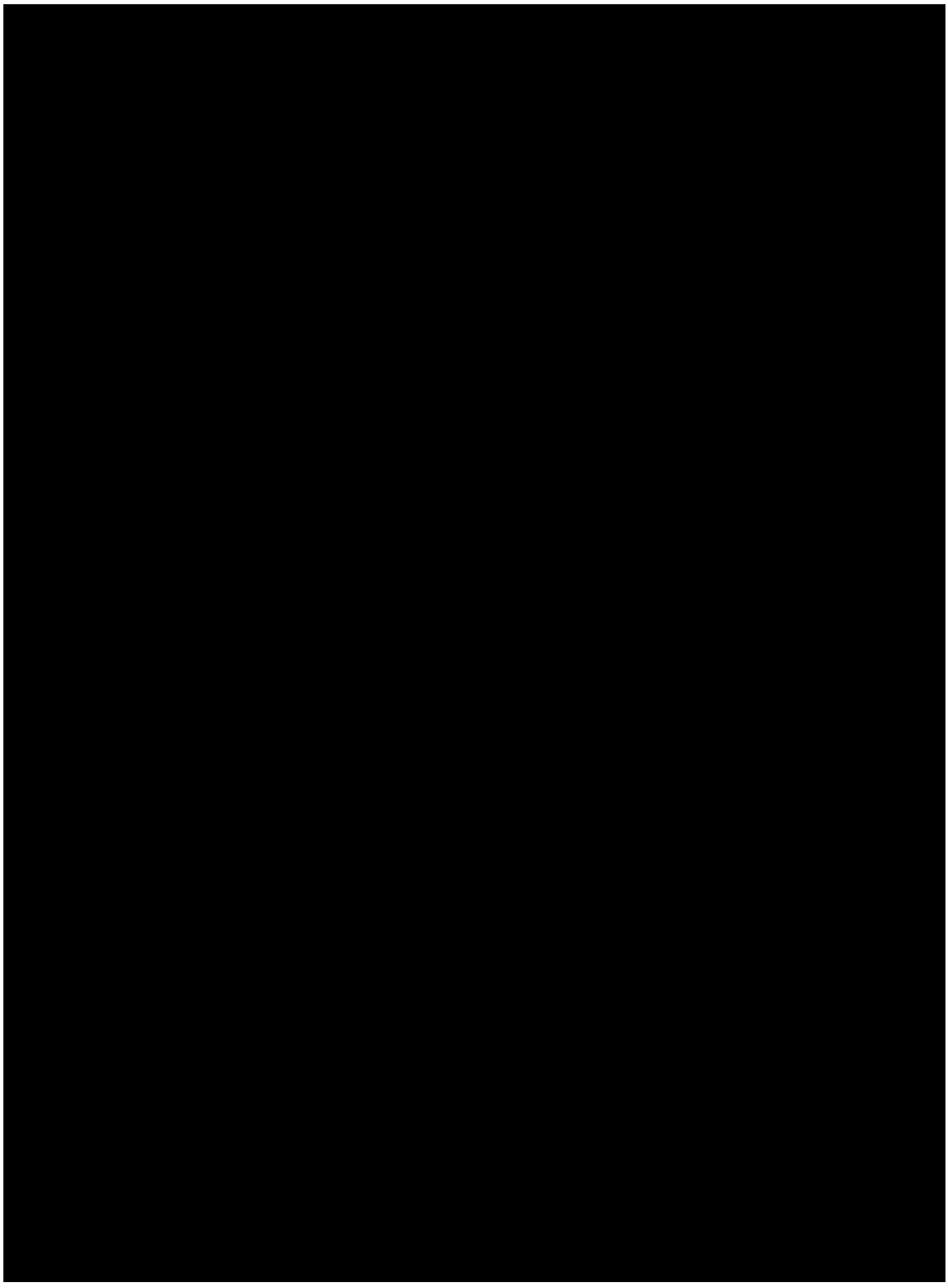


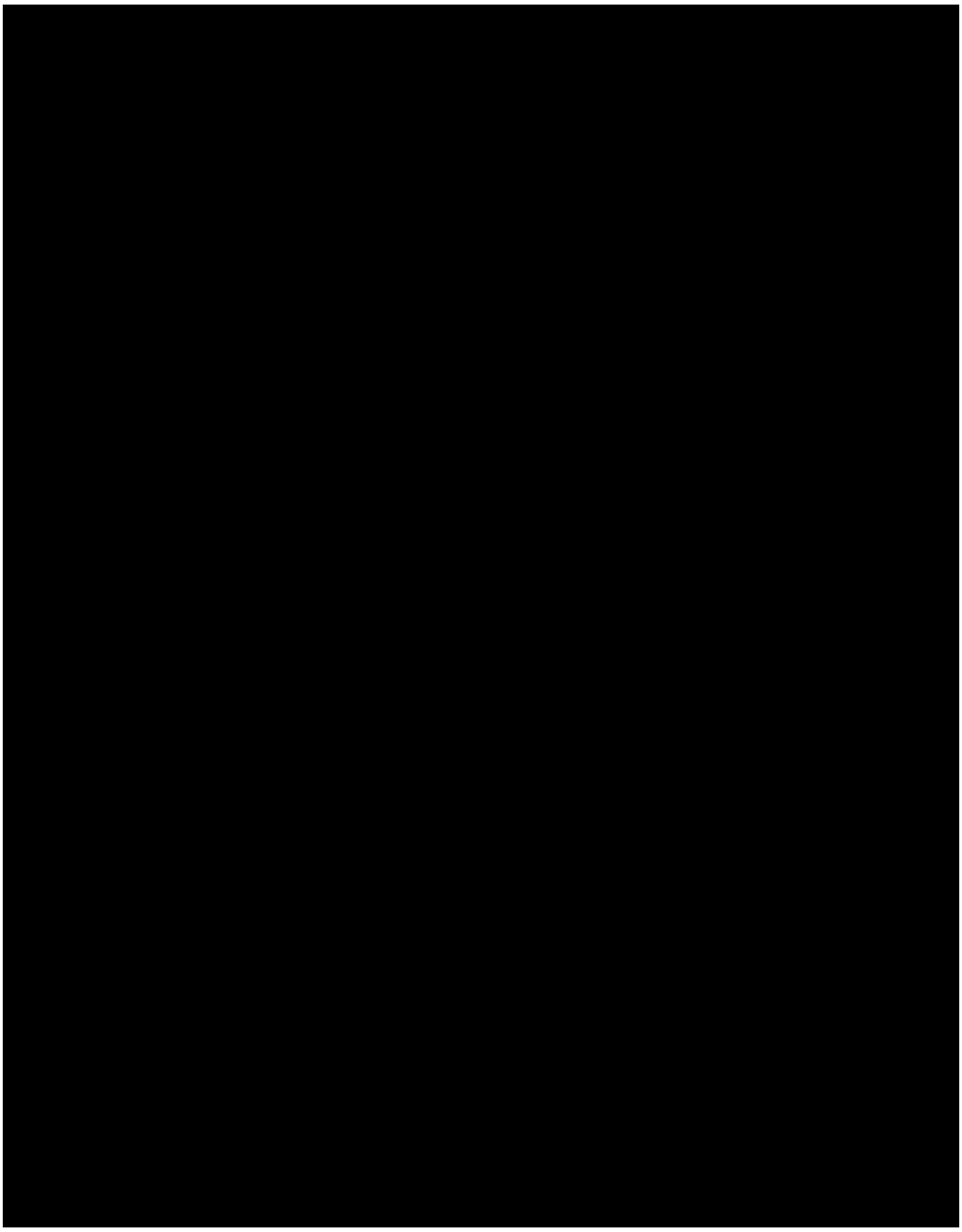


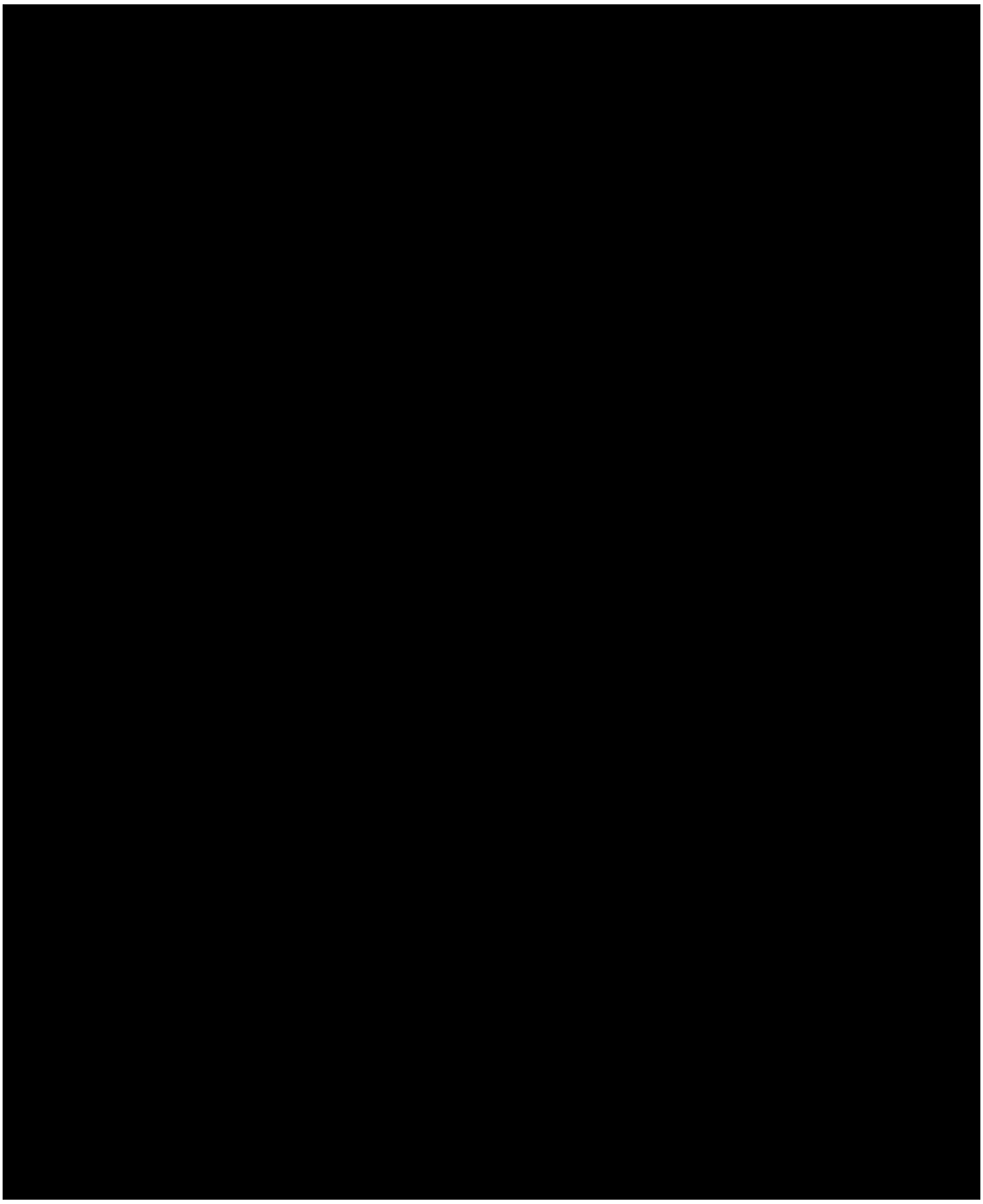


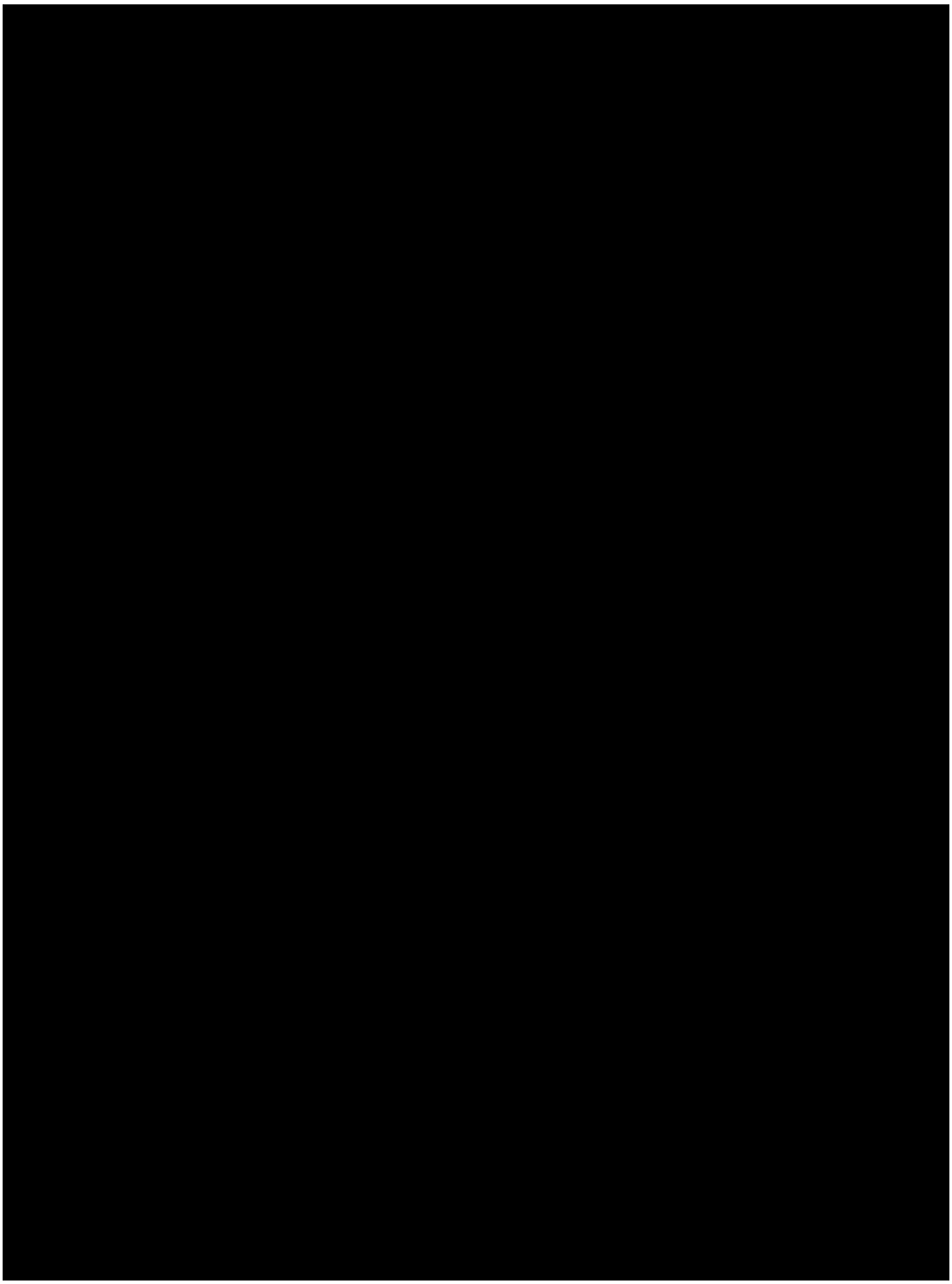


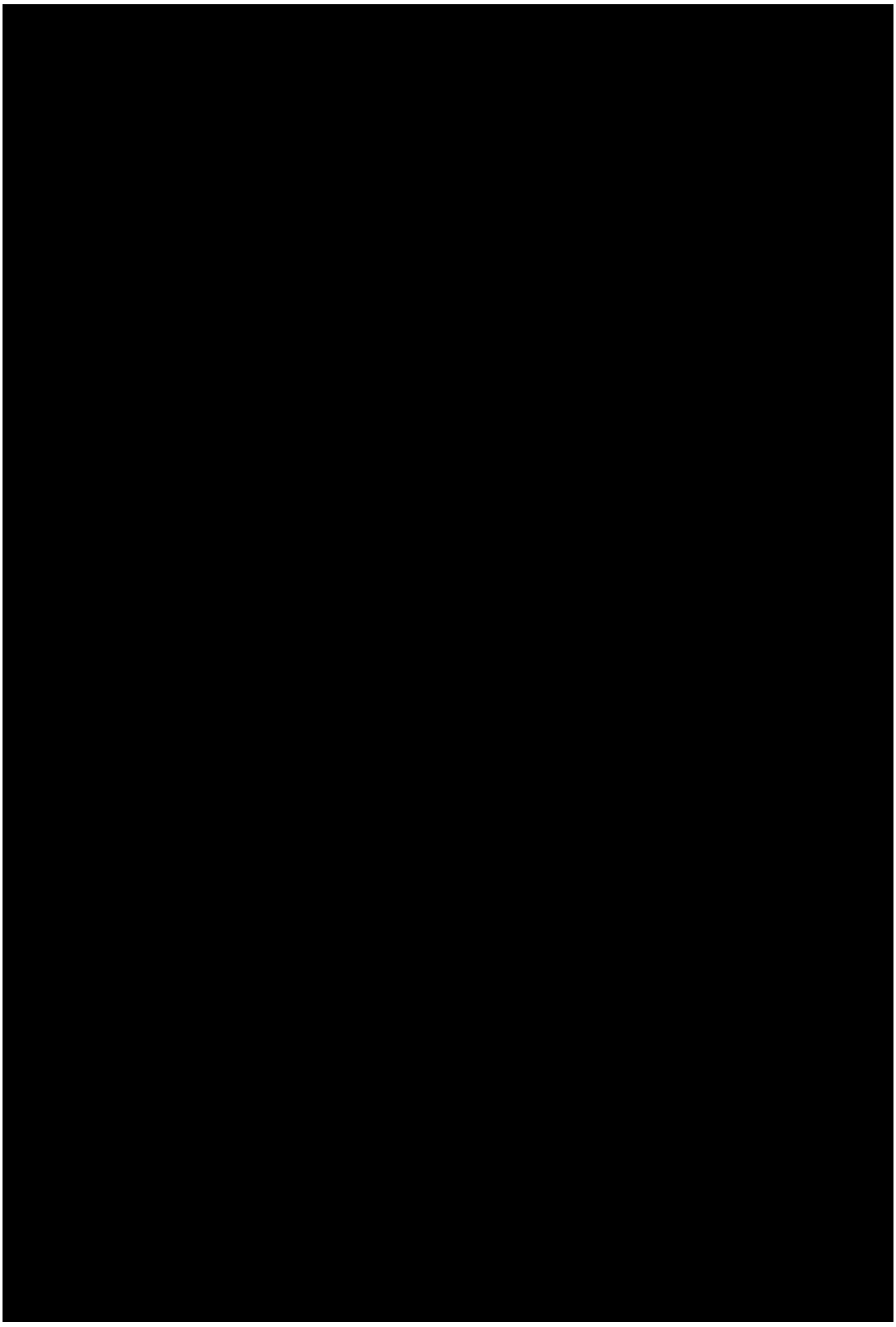


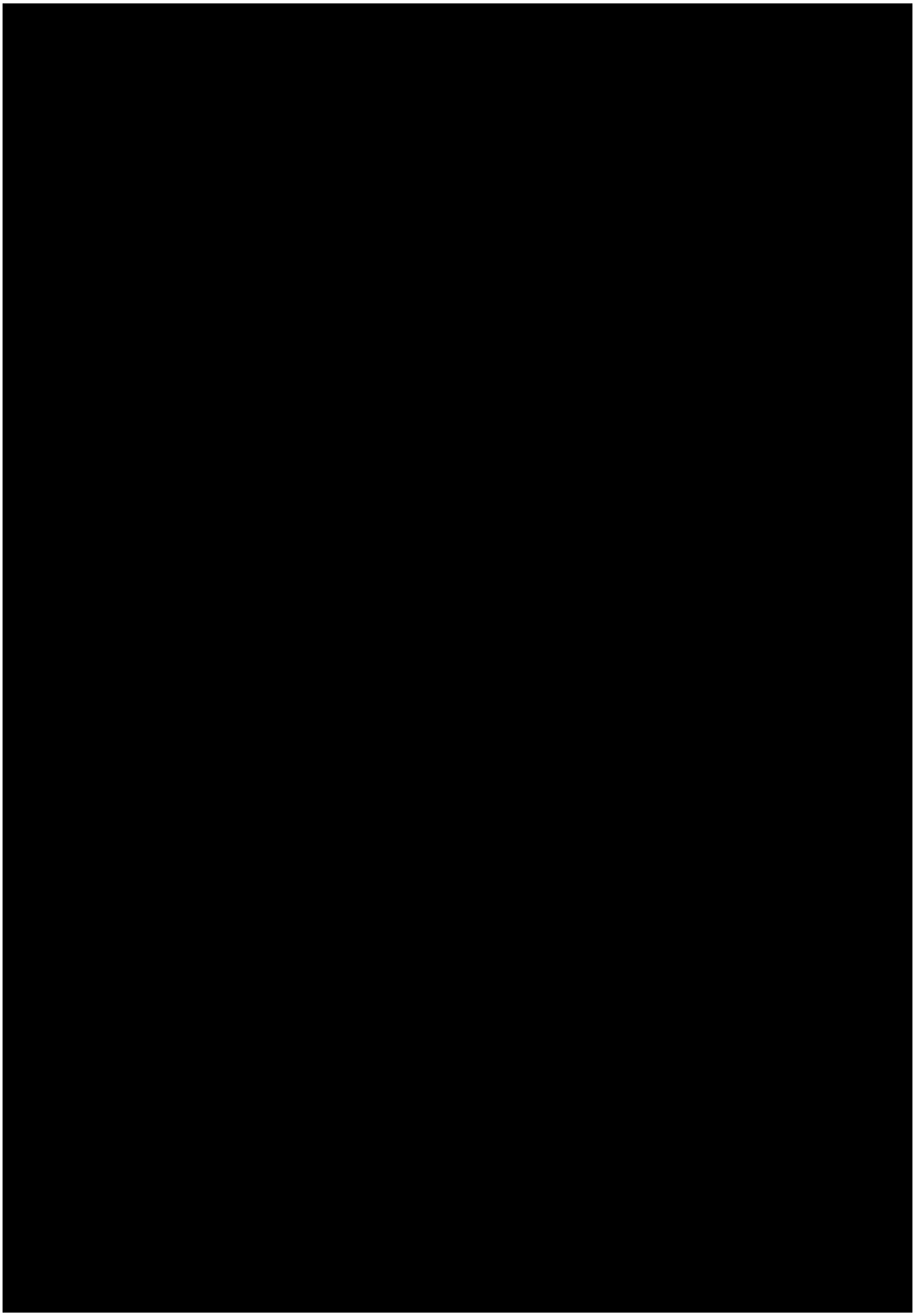


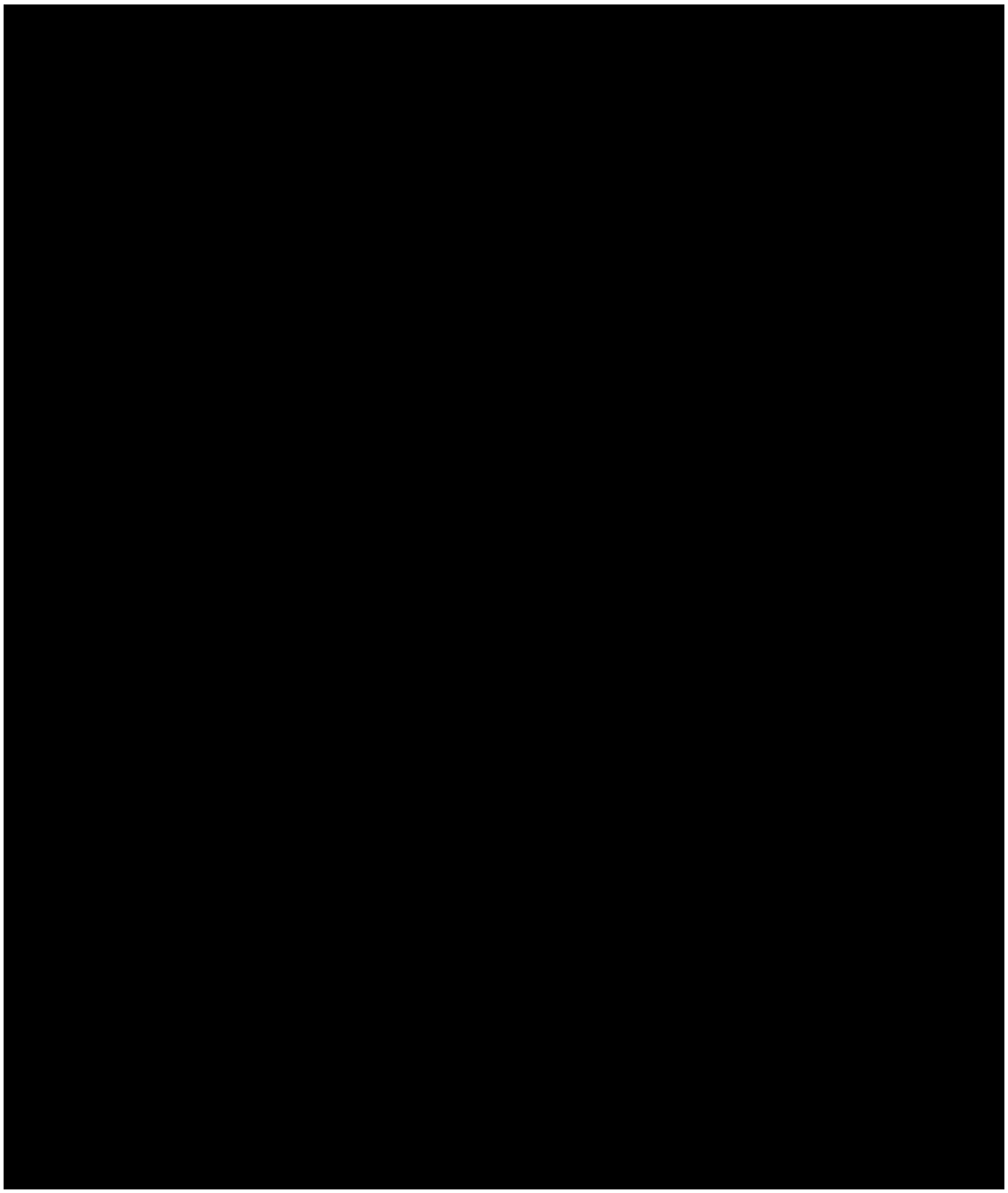


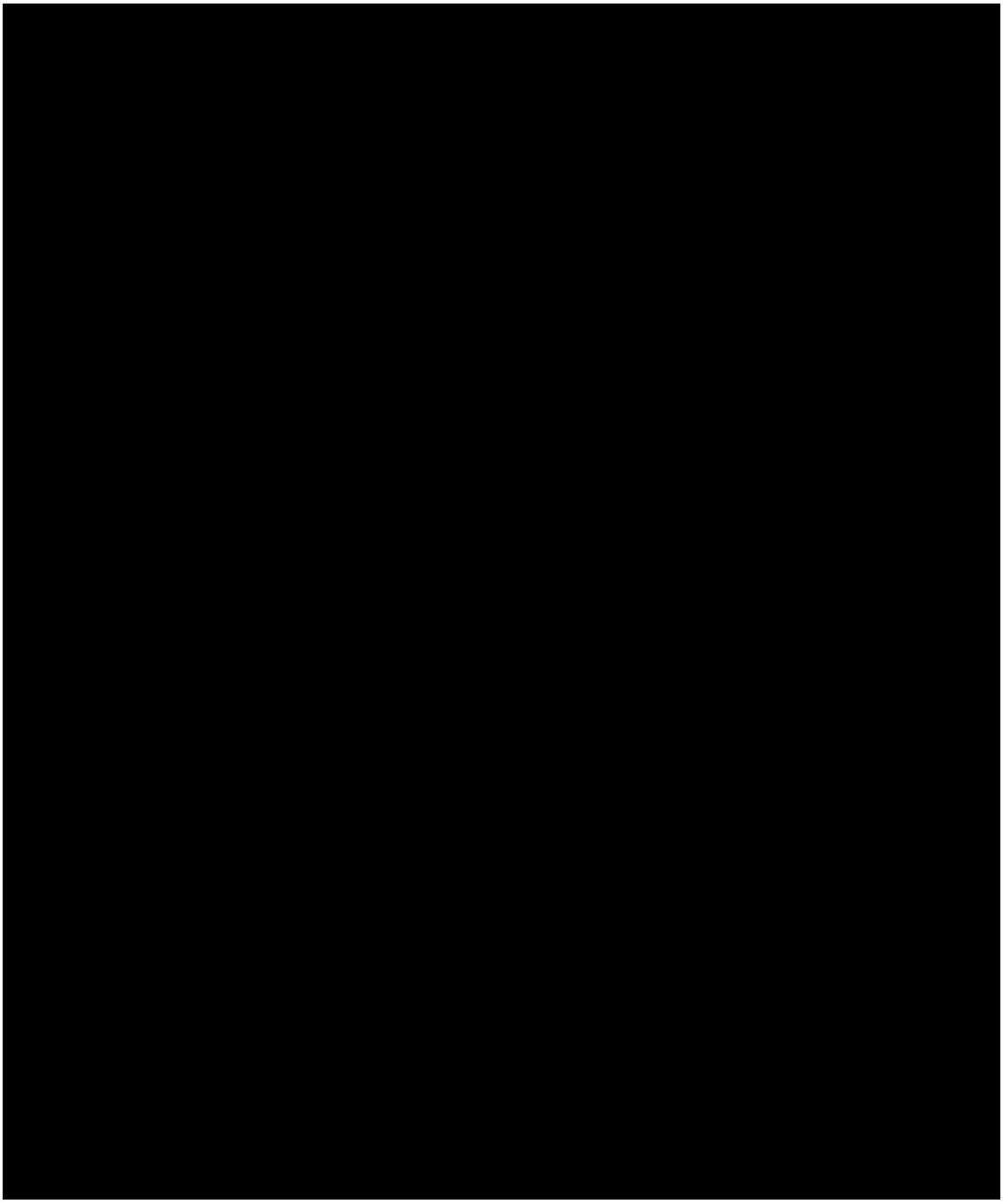


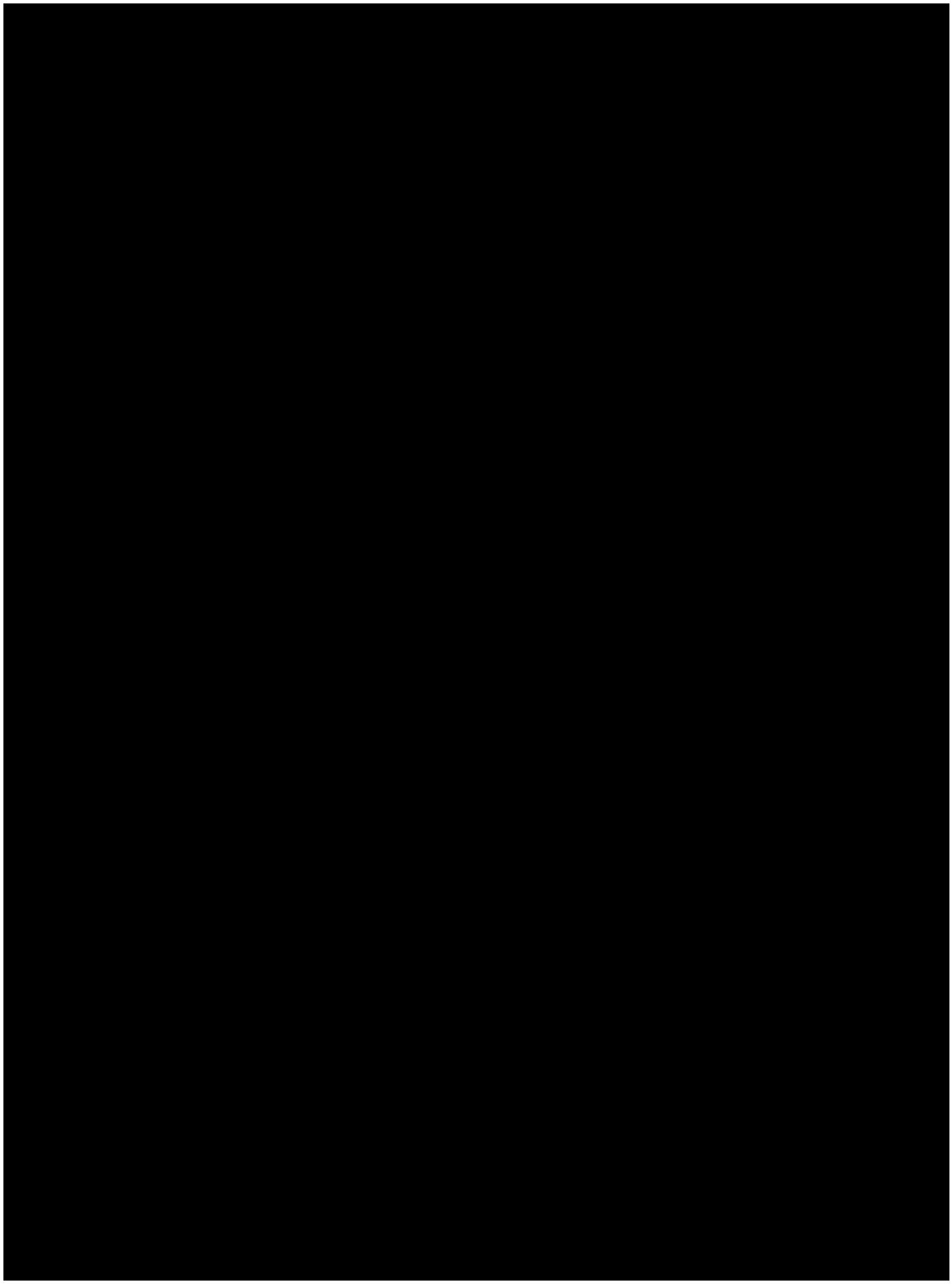


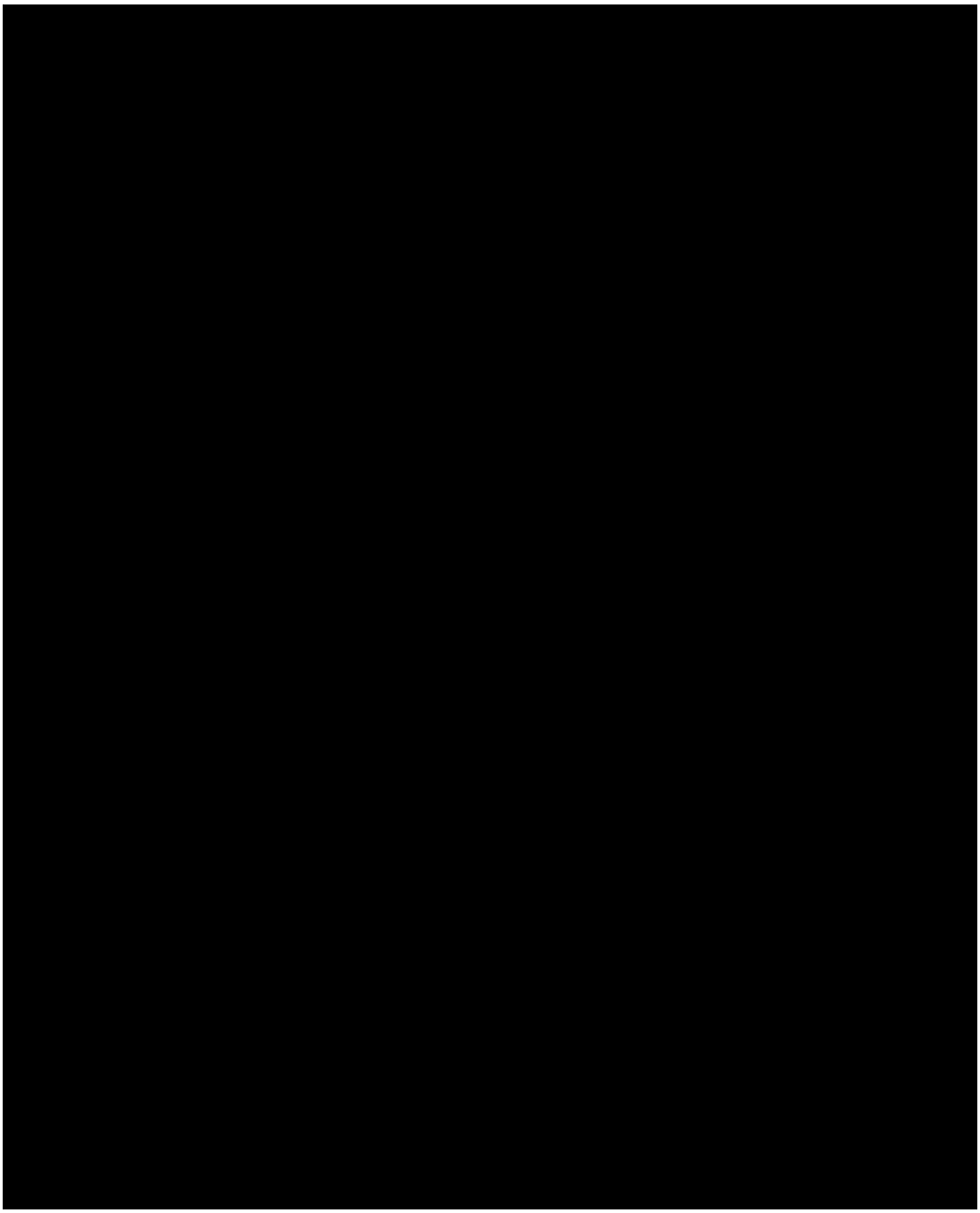


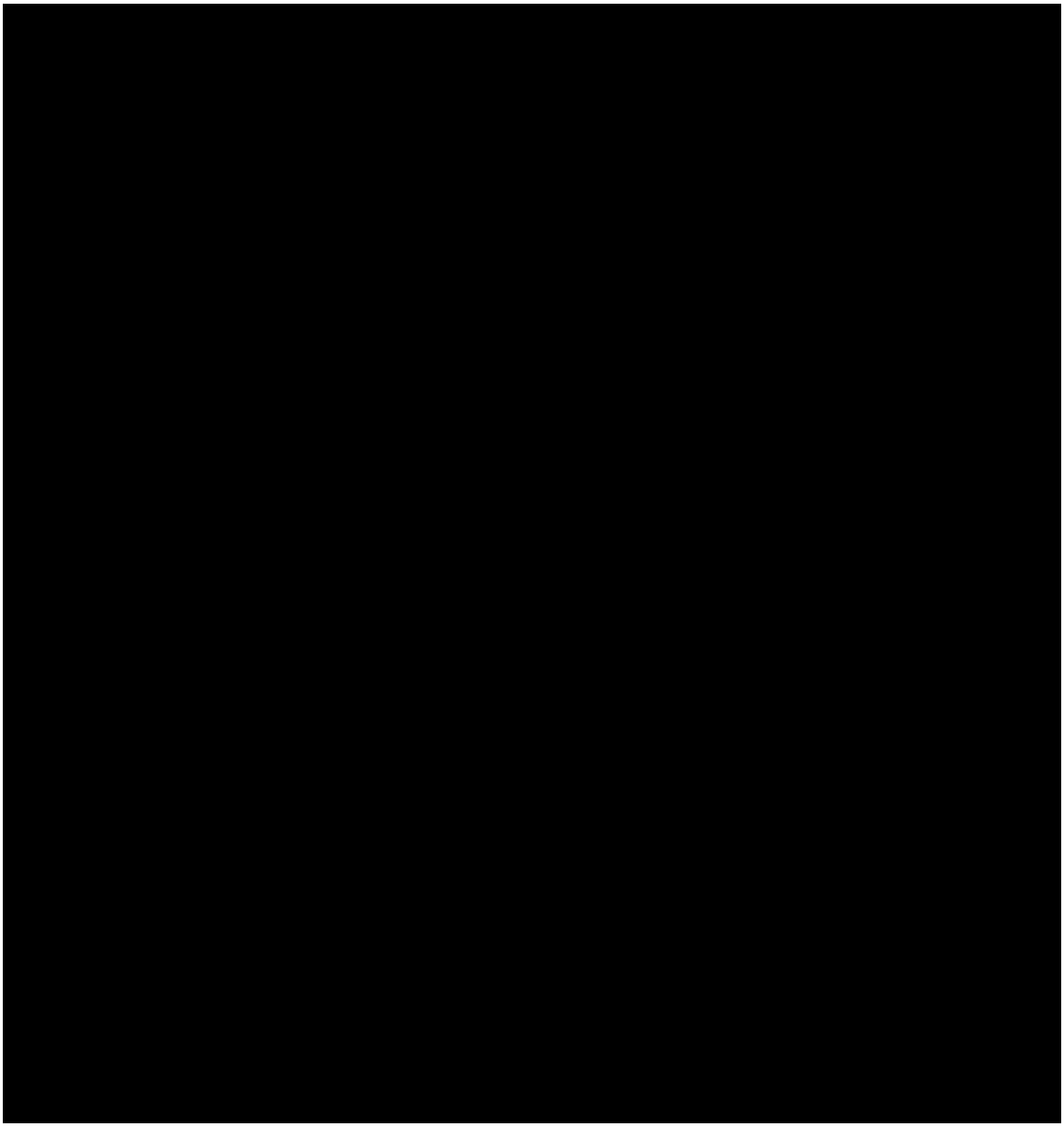












16.2 Appendix 2 Estimated creatinine clearance rate (eCrCl) using the Chronic Kidney Disease Epidemiology (CKD-EPI) formula

The eCrCl is to be used to calculate the eGFR in mL/min. This will be achieved by using the CKD-EPI formula [CKD-EPI, 2009]. It employs min and max serum creatinine measurements and a subject's age to predict the creatinine clearance.

The formula is:

$eGFR = 141 \times \min(Cr/\kappa,1)^{\alpha} \times (\max(Cr/\kappa,1)^{-1.209}) \times (0.993^{Age}) \times 1.018$ [if female] $\times$ 1.159 [if black]

Where:

Cr- is serum creatinine in mg/dL;

k equals 0.7 for females, 0.9 for males;

α is –0.329 for females and –0.411 for males

This formula expects creatinine to be measured in mg/dL, as is standard in the USA. The resulting value is multiplied by a constant of 1.018 if the subject is female and 1.159 if the subject is black.

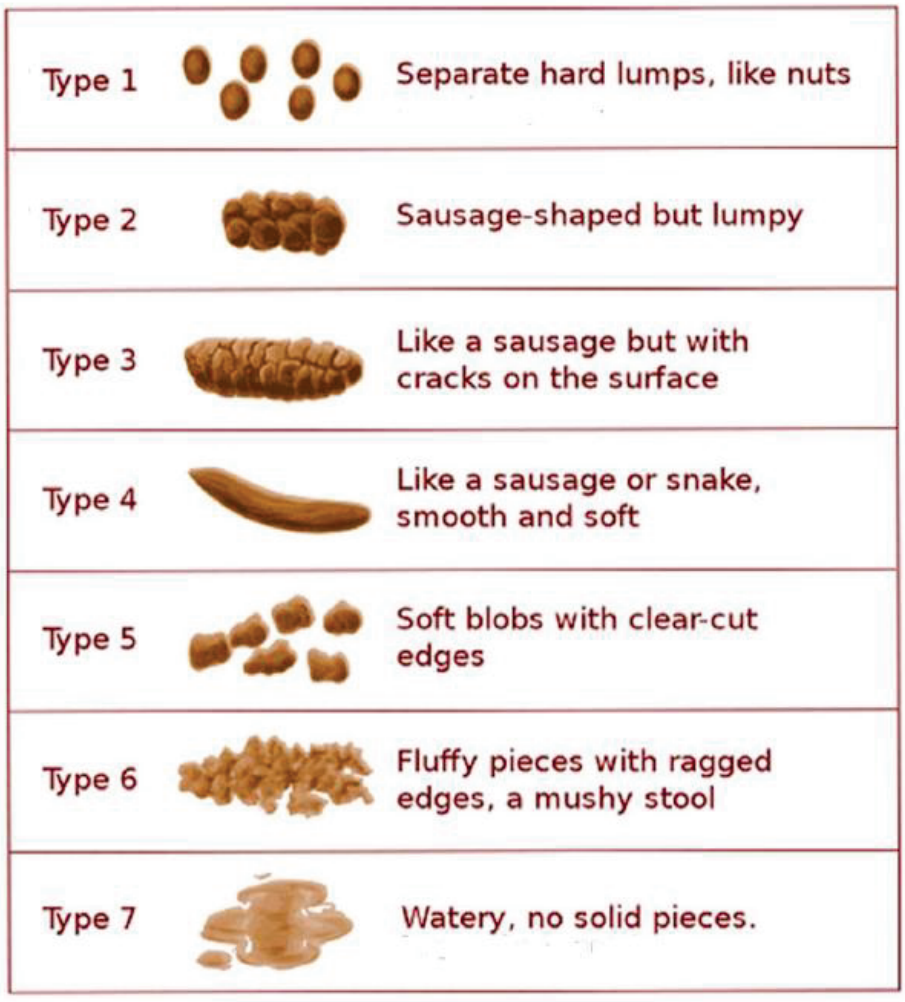
16.3 Appendix 3: Bristol Stool Form Scale

Bristol Stool Form Scale Diary

Date of Bowel movement

Select Date:

Overall, how would you describe the shape and consistency of your bowel movements today?



16.4 Appendix 4: Change in Eating / Appetite

The Investigator will ask the subject at each visit for changes that the subject observed in his eating habits and appetite during the course of the week prior to the visit (7-day recall period). The following questions should guide the interview:

1. During the past week, did you experience any change or variation in your appetite?
    a. Yes
    b. No
2. During the past week, did you change any habits in your eating menu?
    a. Yes
    b. No
3. During the past week, did you eat less, the same, or more compared to the week before?
    a. Less
    b. Same
    c. More

16.5 Appendix 5: Procedure for Balance Studies

A balance study measures the total intake and the output of energy, lipids, carbohydrates, nitrogen (as a marker for proteins) and micronutrients.

During a balance study, the following will be performed:

- Subjects will be informed about collection of diet intake, urine and feces and hand out bottles and buckets that will be used for the collection.
- The collection will be performed over the course of 72 hours (in three 24-hour periods).
- The subjects will collect oral diet intake (i.e., food and fluid) corresponding to the type and amount ingested. Wrapping paper, plum stones, chicken bones and similar will not be collected.
- Each day, bottles and buckets will be picked up by the laboratory technician.

The following will be collected:

Sample	To be collected in	Handling and storage
Urine	Bottle	No special handling or precautions
Feces	Bucket	Feces will be stored in a freezer[1]
Oral liquid intake	Bottle	No special handling or precautions
Oral food intake	Bucket	No special handling or precautions

[1] The subjects will have a freezer at their hospital ward where the feces is stored during the entire 72-hour period and only removed from the freezer to add new stools and/or diarrhea

The content of the bottles/buckets will be weighed, processed into dry matter by laboratory techniques and subsequently analyzed at the laboratory. Details of the techniques used are published [Jeppesen, 2000]. The following analyses will be performed on the collected material:

Analysis	Samples	Method
Weight	Urine, feces and food	Weight
Energy content	Feces and food	Bomb calorimetry
Lipids (aliphatic compounds)	Feces and food	Titration
Carbohydrates	Feces and food	Englyst's method [Englyst method]
Nitrogen (as a marker of protein)	Feces and food	Kjeldahl's method [Kjeldahl method]
Sodium, potassium, calcium and magnesium	Urine, feces and food	Atomic absorptiometry

16.6 Appendix 6: Summary of Changes to previous Protocol Amendments

Version No.	Version date	Changes to previous Protocol (amendments and indication if substantial or not)
1.0	10Nov2020	Not applicable, first version
2.0	24Feb2020	- Corrected inconsistencies and typos - Corrected the wording of the primary endpoint, as Week 48 is not end of treatment - Inclusion criteria: separated into initial criteria and dosing criteria. Clarified that the ≤5% weight change is either an increase or a decrease. Additional criterion on willingness to undergo colonoscopy/colonography - Exclusion criteria: specified that surgery for cholecystectomy is not exclusionary; and fistula is exclusionary only if active or untreated. Additional criterion on elevated liver enzymes. Updated wording on cholecystitis, biliary obstruction, and IBD. Removed positive viral serology exception for subjects recovered from hepatitis A (i.e., exception is now for subjects recovered from hepatitis B or C only) - Updated planned trial dates (first subject first visit, etc.) - Added glutamine to the list of prior medications to be specifically recorded - [redacted] - Specified that body temperature is to be taken in the axilla for all vital signs assessments; added GGT to the list of safety clinical chemistry parameters - Additional clinical chemistry parameters to be tested (experimental trials). Updated the total blood volume to be collected from each subject for the experimental trials - [redacted] - Updated the number of biopsies required for the experimental trials and clarified the distinction between standard and single-cell RNA sequencing - [redacted]

		- Added details of the considerations on IMP dose reduction and temporary discontinuation of IMP - Specified that in the case that a vulnerable subject enters the trial, the Principal Investigator is encouraged to consult their local IEC/IRB for guidance
3.0	03Sep2021	- Corrected inconsistencies and typos - Minor wording changes for clarification and to remove duplication - Sponsor representative changed - Abbreviations list updated - Synopsis – clarified definition of short bowel syndrome and deled PS definition - Secondary endpoints and Section 5.1.9: PROs: added PGI-PSI. PGI-TS & PGI-SPS, PGIC updated, PGIS removed for subjects enrolled after this amendment - Inclusion criteria 3 updated b) and c) to read '…not exceeding 25%' - Inclusion criteria No. 8: Definition of planned surgery amended during the trial period - Exclusion criteria No. 5, 8, 9, 10 & 11: minor wording changes to clarify the criteria - Exclusion criteria 15: deleted criteria defining Gilbert's disease subjects and subjects with PPN associated liver disease - Removed exclusion criteria No 17: unplanned hospitalizations - Removed exclusion criteria No 18:Changes in systemic corticosteroids - Exclusion criteria footnote 1: Further definition details added - Table 1 – Days added to Weeks -1 to -2 (Day -28 to -14) - Table 1 – Inclusion & Exclusion criteria check removed from Visit 3 - Table 1 – Telephone contact time point clarified at Week 0, Day 1(+1 day) to assess fluid overload - Table 1 – row added to include subjects completion of diaries - Table 1 – Order of PROs defined in footnote 2 - Table 1 – Footnote added detail regarding ADA sample collection following ISR at discretion of Investigator

		- Table 2 – Confirmation of eligibility added on Day 5 Baseline MB period - Section 2.2 – wording added to include definitions of PS, fluids and other parameters during the trial - Section 2.2.1 – inclusion of wording regarding history of vomiting - Section 4.5 – Added 'The maximum PS volume reduction allowed at any individual visit is 50%' and further details on the PS reduction procedure - Section 4.7 and exclusion criteria – use of somatostatin analogs removed - Section 4.7.1 & 4.7.2 – Definition of use of antibiotics updated. Added routine vaccinations allowed - Section 5.1.11 – Anti-drug antibodies follow up procedures post end of treatment clarified - Section 5.2.9 – Total HCO_3^- added to blood lab tests at safety evaluation post PS reduction - Section 6.6.2 - Updated the stopping rules for subjects that have baseline AST or ALT >1.5 ULN and if a subject reaches a LFT stopping rule restarting of IMP must be discussed with the medical monitor - Section 8 – Added section regarding Data Monitoring Committee - Section 10.2.1 – Updated that biomarker samples will be stored for 15 years - Section 16.5 – Electrolytes all now analyzed by Atomic absorptiometry
4.0	07Feb2022	- General changes to improve clarity and minor formatting issues - International Coordinating Investigator changed - Primary objective changed to evaluate the safety and tolerability of apraglutide - Evaluation of calories added to secondary objectives - Primary endpoints updated to reflect the change in primary objective by including AEs, AEs of special interest, clinical laboratory assessment and anti-drug antibodies - Addition of the Week 4 assessment in relative change from baseline in actual weekly PS volume - Changes to secondary endpoints, including change in absolute energy absorption over metabolic balance

		periods, from baseline to Week 48; changes in urine output and urinary electrolytes and magnesium) over metabolic balance periods from baseline at Week 4 and at Week 48 - Section 1.5.2.4 – Definition of enteral autonomy added - Inclusion criterion 2 clarified to include subjects with intestinal resection leading to SBS-IF at least 12 months prior to Screening - Table 1 – Addition of definition of balance period - Table 2 – Addition of Bristol Stool Form Scale on Days 2 and 3 - Section 2.1 – Additional background added - Section 2.2 – Confirmation that infusion of a small amount of fluid to maintain catheter patency is not considered PS - Section 2.2.1 – Added that investigator can use clinical judgment to demonstrate that the small intestine is <200 cm - Section 2.2.2 – Additional instructions relating to drinking menu and enteral nutrition - Section 4.5 – Clarifications on PS volume reduction criteria and process and clarification on method of calculation baseline urine average

TA799-013_20220207_Clinical_Trial_Protocol_v4.0

Final Audit Report 2022-02-08

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