ALFASIGMA

STUDY PROTOCOL

Velusetrag for the treatment of Chronic Intestinal Pseudo-Obstruction (CIPO). A multicenter, double-blind, placebo-controlled, cross-over, multiple (n=1) trial

Study code:	VE-CIP2001/2021
EudraCT number:	2021-000854-24
Phase:	II
Name of the investigational product:	Velusetrag
Coordinating Investigator	Prof. [redacted]
Sponsor:	Alfasigma S.p.A. Via Ragazzi del '99, n. 5 40133 Bologna, Italy.
CRO:	[redacted]
Status/Date:	Final version 1.0-24 May 2021

SIGNATURE PAGE (1/4)

The signatories are obliged to comply in all respects with:

- this clinical study protocol,
- the standards of Good Clinical Practice as defined in the " Guideline for Good Clinical practice E6 (R2)" (EMA/CHMP/ICH/135/1995)" and related Guidelines,
- all applicable regulatory requirements including national drug law and data protection law.

[redacted], MD
Alfasigma S.p.A.

27-05-2021 | 4:27 PM CEST

Date (dd/mm/yyyy) Signature

DocuSigned by:
[redacted]
Signer Name: [redacted]
Signing Reason: I approve this document
Signing Time: 27-05-2021 | 4:27 PM CEST
[redacted]

[redacted], MD
Alfasigma S.p.A.

27-05-2021 | 3:00 PM CEST

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[redacted], MD
Clinical Trial Operations Head
Alfasigma S.p.A.

27-05-2021 | 3:35 PM CEST

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[redacted]
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27-05-2021 | 3:06 PM CEST

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27-05-2021 | 3:00 PM CEST

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[redacted] Alfasigma S.p.A.

27-05-2021 | 3:45 PM CEST

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DocuSigned by:
[redacted]
Signer Name: [redacted]
Signing Reason: I approve this document
Signing Time: 27-05-2021 | 3:45 PM CEST
[redacted]

[redacted]
Alfasigma S.p.A.

28-05-2021 | 9:30 AM CEST

Date (dd/mm/yyyy) Signature

DocuSigned by:
[redacted]
Signer Name: [redacted]
Signing Reason: I approve this document
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[redacted]

SIGNATURE PAGE (2/4)

STUDY COORDINATOR'S SIGNATURE

Declaration of Coordinating Investigator

Title: Velusetrag for the treatment of Chronic Intestinal Pseudo-Obstruction (CIPO). A multicenter, double-blind, placebo-controlled, cross-over, multiple (n=1) trial

This study protocol was subjected to critical review. The information it contains is consistent with current knowledge of the risks and benefits of the investigational product, as well as with the moral, ethical, and scientific principles governing clinical research as set out in the Declaration of Helsinki, the guidelines on Good Clinical Practice, and other applicable national and local laws and regulations.

Prof. [redacted], MD
[redacted]
Tel. [redacted]
E-mail [redacted]

Date (dd/mm/yyyy) Signature

SIGNATURE PAGE (3/4)

STEERING COMMITTEE

[redacted]

Prof. Vincenzo Stanghellini, MD
[redacted]
Tel. [redacted]
E-mail [redacted]

Date (dd/mm/yyyy) Signature

Prof. [redacted], MD, PhD
[redacted]
Phone: [redacted]
Email: [redacted]

Date (dd/mm/yyyy) Signature

SIGNATURE PAGE (4/4)

Declaration of the Investigator

Title: Velusetrag for the treatment of Chronic Intestinal Pseudo-Obstruction (CIPO). A multicenter, double-blind, placebo-controlled, cross-over, multiple (n=1) trial

All documentation for this study supplied to me, which has not been previously published, will be kept in the strictest confidence. This documentation includes this study protocol, Investigator's Brochure, Case Report Forms, and other scientific data.
The study will not be commenced without the prior written approval of a properly constituted Institutional Review Board (IRB) or Independent Ethics Committee (IEC).
No changes will be made to the study protocol without the prior written approval of the Sponsor and the IRB or IEC, except where necessary to eliminate an immediate hazard to the subjects.
I have read and understood and agree to abide by all the conditions and instructions contained in this protocol.

Responsible Investigator of the local study center:

XXXXXXXXX, MD
Director of Department of
XXXXXXXXX
Address
Telephone

Date (dd/mm/yyyy)	Signature

EMERGENCY INSTRUCTIONS

1. All Serious adverse events (SAEs)
2. All adverse events (AEs) that could affect the safety of the study participants or the conduct of the trial must be reported within 24 hours to Alfasigma Pharmacovigilance:

Information about all SAEs will be reported using the SAE tool of the eCRF. In case of technical difficulties, SAE notification can be carried out filling paper SAE Report form and it must be mailed or faxed using the following contact details:

Alfasigma S.p.A., Corporate Pharmacovigilance - Clinical Safety Unit
E-mail: [redacted]
Fax: [redacted]

Definition of Serious Adverse Events and Serious Adverse Drug Reaction:

A serious adverse event (SAE) or serious adverse drug reaction (SADR) is any untoward medical occurrence that at any dose is:
1. fatal
2. life-threatening
3. resulting in or prolonging in-patient hospitalization
4. severely or permanently disabling or incapacitating
5. a congenital anomaly/birth defect
6. other event(s) considered medically important

During an emergency the Investigator may unblind the patient via e-CRF if the knowledge of the assigned treatment is necessary to treat the patient adequately. In case unblinding via e-CRF is not functioning properly, opening of the individual sealed envelopes containing the randomization code may be used for unblinding.

For consultation on safety issues or in case of need of more detailed safety information, the investigators can refer to [redacted], MD, PhD: [redacted]

SYNOPSIS

Study Title:	Velusetrag for the treatment of Chronic Intestinal Pseudo-Obstruction (CIPO). A multicenter, double-blind, placebo-controlled, cross-over, multiple (n=1) trial
Study Code:	VE-CIP2001/2021
Coordinating Investigator:	Prof. [redacted]
Study Center(s):	Up to 5 centers
Study Phase:	Phase II
Study Rationale:	Chronic intestinal pseudo-obstruction (CIPO) is a rare, severe condition characterized by an impairment of coordinated propulsive activity in the intestinal tract resulting in a clinical picture similar to that of mechanical intestinal obstruction, although in the absence of any lesion occluding the gut. It may be due to an underlying neuropathic, myopathic disorder, or abnormality in the interstitial cells of Cajal, either alone or in combination. CIPO can be *idiopathic*, when no primary underlying disorder is demonstrated (i.e., chromosomal abnormalities), or secondary, when related to systemic diseases (e.g., neurodegenerative, endocrine/metabolic, autoimmune etc.). The clinical picture, characterized by disabling digestive symptoms (i.e., abdominal pain, bloating, nausea and vomiting, constipation, heartburn, fullness, early satiety) associated with "sub-occlusive episodes", contributes to a significant deterioration of quality of life of the subjects. CIPO is one of the most important causes of chronic intestinal failure. Subjects with CIPO are often unable to maintain normal body weight and/or normal oral nutrition. Prokinetics (e.g., erythromycin, prucalopride, cisapride, tegaserod), symptomatic drugs (e.g., antisecretory drugs, antispasmodics, laxatives, analgesics, etc.), antibiotics (to treat small intestinal bacterial overgrowth, SIBO) as well as invasive procedures such as surgery or small bowel transplant are used to treat subjects with CIPO but no treatment is currently licensed for this indication. Velusetrag is a selective 5-hydroxytryptamine receptor 4 (5-HTR_4) agonist. 5-HTR_4 agonists are able to stimulate the release of acetylcholine from enteric motor neurons and calcitonin gene-related peptide from sensory neurons. Velusetrag has been shown to enhance the peristaltic reflex, stimulate intestinal secretion, and inhibit gastrointestinal visceral sensitivity.

Study Objectives:	The objective of this study is to investigate the safety, tolerability and efficacy of velusetrag in improving the symptoms severity associated with CIPO, in subjects with *idiopathic CIPO* and *CIPO secondary to neurodegenerative or demyelinating conditions (e.g., Parkinsonian Syndromes, multiple sclerosis, etc.)*.
Experimental design:	This is a phase II, multicenter, double-blind, placebo-controlled, two-treatment four period cross-over, multiple (n=1) trial. Eligible subjects will be treated for 4 periods of 4 weeks each with either velusetrag 15 mg (2 periods) or placebo (2 periods) with a wash-out period of 2 weeks between treatment periods. After an up to 7-day screening period (from Day -7 to Day -1, V1), at randomization visit (V2), eligible subjects will be randomly allocated to one of the following four sequences: *A.* VEL-PLA-VEL-PLA *B.* PLA-VEL-PLA-VEL *C.* VEL-PLA-PLA-VEL *D.* PLA-VEL-VEL-PLA Where: VEL= velusetrag 15 mg once a day for 4 weeks. PLA= matching placebo once a day for 4 weeks. There will be a 2-week wash-out period between each treatment period and a final 2-week follow-up period at the end of the last treatment period. [redacted] will be registered at Day–1 (referring to the last 7 days including Day-1) and weekly during the study, as appropriate according to the schedule of assessment using a subject's e-diary. [redacted] will be also assessed at screening and during the study according to the schedule of assessments. Number of [redacted] will be assessed at each study visit as well as the [redacted]. At the screening visit and at the end of the first 4-week treatment period, [redacted] will be performed in order to assess [redacted]. Subjects under treatment with a [redacted] must have a 5-day washout period before randomization (note: symptomatic treatments other than [redacted] will be permitted through the study, see below).

	In case, during a treatment period or during a washout period, a subject should experience an exacerbation of the CIPO related gastrointestinal symptoms that is not controlled by permitted medications and is not judged as an adverse event, he/she will attend an early switch visit (ESV) at the clinical site. After being reassessed, he/she will start the next scheduled treatment period (SOT-2 or SOT-3 or SOT-4) without completing the ongoing treatment or washout period. Only one early switch is possible during the study.
Number of Subjects:	16 (4 subjects per treatment sequence). [redacted]
Study Population:	Subjects with a diagnosis of *idiopathic CIPO* or *CIPO secondary to primary neurodegenerative or demyelinating conditions (e.g., Parkinsonian Syndromes, multiple sclerosis, etc.).*
Inclusion Criteria:	1. Men or women aged 18-80 years. 2. Subjects with history of chronic idiopathic intestinal pseudo-obstruction or CIPO secondary to neurodegenerative or demyelinating disease. 3. Subjects with estimated oral caloric intake of at least 30% of the daily age- and sex-recommended caloric intake (stage 0, 1 or 2 of the "[redacted], see 21.3 Appendix 3: [redacted] for CIPO patients). 4. Subjects with at least 2 out of 4 CIPO gastrointestinal symptoms (i.e., abdominal pain, bloating, nausea and vomiting), each of the 2 with a score ≥3 (on a 0 to 4 scale) collected on the gastrointestinal symptom questionnaire at Day -1 5. Subjects accepting to provide and legally capable of providing free and informed consent to all procedures included in the protocol. 6. All sexually active male participants who are partner of women of childbearing potential must use condom during intercourse until the 90th day after the end of the entire study. 7. All female participants must be: • of non-childbearing potential, i.e.: i) post-menopausal (at least 2 years without spontaneous menses), or ii) surgically sterile (bilateral tubal occlusion, or hysterectomy), or iii) ablation of both ovaries or • of childbearing potential with a negative pregnancy test result at screening and randomization AND agreeing to use a highly effective method of contraception (i.e., with failure rate of less than 1% per year) until the end of the entire study.

	Note 1. Based on the EU Clinical Trial Facilitation Group recommendations, a highly effective method of contraception is one of the following: • Intrauterine device (IUD). • Intrauterine hormone-releasing systems (IUS). • Combined hormonal contraceptives (i.e., estrogen and progestogen) in oral, intravaginal or transdermal form, with inhibition of ovulation as primary mode of action. • Progestogen-only hormonal contraceptives in oral, injectable or implantable form, with inhibition of ovulation as primary mode of action. • True and absolute abstinence: when this is in line with the preferred and usual lifestyle of the subject. Note 2. Periodic abstinence, such as calendar, ovulation, symptom thermal, post ovulation methods, and withdrawal are not acceptable methods of contraception. Note 3. In each case of delayed menstrual period (over one month between menstruations), female participants of child-bearing potential will be strongly recommended to provide a confirmation of absence of pregnancy. This recommendation applies also to women of child-bearing potential with infrequent or irregular menstrual cycles.
Exclusion Criteria:	1. Subjects with primary CIPO or CIPO secondary to other known endocrine/metabolic, autoimmune diseases and neurologic conditions other than neurodegenerative or demyelinating diseases. 2. Subjects with conditions characterized by mechanical intestinal obstruction. 3. Nasogastric tube, gastrostomy tube, or jejunostomy feeding tube in place at randomization or planned throughout the duration of the study, or [redacted] stage 3 (“total non-oral nutrition”, see 21.3 Appendix 3: Artificial Food Need" (AFN) Scale for CIPO patients). 4. Presence of untreated clinically relevant thyroid dysfunction or known thyroid dysfunction not well controlled by treatment (e.g., subjects with abnormal thyroid stimulating hormone [TSH], and, if available, triiodothyronine [T3] and thyroxin [T4] levels) deemed clinically significant by the Investigator. 5. Subjects with history of diabetes at screening. 6. Clinically significant ECG abnormalities (e.g., ST segment elevation or depression suggestive of ischemia, partial or complete left bundle branch block [LBBB]) at Screening and randomization. 7. Screening ECG with a QTcF >450 msec in males or >470 msec in females or family history of sudden cardiac death. 8. Subjects requiring a low galactose diet.

	9. Hypersensitivity or documented intolerance to lactulose, lactose or any excipient of the lactulose preparation to be used for [redacted]. 10. History of sensitivity to velusetrag, or any of the velusetrag or placebo excipients. 11. Use of scopolamine or erythromycin within 2 weeks prior to Screening and/or planned throughout the duration of the study. 12. Use of [redacted] within 5 days prior to randomization and/or planned throughout the duration of the study 13. Use of opioids within 8 weeks from screening and/or planned throughout the duration of the study. 14. Received strong cytochrome P450-isozyme 3A4 (CYP3A4) inhibitors (e.g., clarithromycin, indinavir, itraconazole, ketoconazole, nefazodone, nelfinavir, ritonavir, saquinavir, telithromycin, grapefruit juice) or strong CYP3A4 inducers (e.g., rifampin, phenytoin, carbamazepine, phenobarbital, St. John's wort) within 2 weeks prior to screening and/or planned throughout the duration of the study. 15. Received strong P-glycoprotein (P-gp) transporter inhibitors (e.g., captopril, carvedilol, diltiazem) within 2 weeks prior to Screening and/or planned throughout the duration of the study. 16. Received strong breast cancer resistance protein (BCRP) transporter inhibitors (e.g., curcumin, cyclosporine A, eltrombopag) within 2 weeks prior to Screening and/or planned throughout the duration of the study. 17. Current swab-positive or suspected (under investigation) COVID-19 infection. 18. Cancer (excluding non-melanoma skin cancer) and/or need of any anti-cancer treatment (also including radiotherapy) within the last 5 years. 19. Severe kidney impairment (i.e., estimated glomerular filtration rate <30 ml/min). 20. Aspartate aminotransferase (AST) or alanine transaminase (ALT) levels >2.5 times the upper limit of normal (ULN); bilirubin (unless deemed to be due to Gilbert's Syndrome) or alkaline phosphatase (ALP) >1.5 times ULN. 21. Severe hepatic impairment defined as Child-Pugh C. 22. History of any of the following cardiac disorders: • Torsade de pointes, ventricular tachycardia, ventricular fibrillation. • Previous myocardial infarction, unstable angina pectoris, acute coronary syndrome, coronary artery or cerebral revascularization procedure or stroke within the previous 18 months. • Angina pectoris class 2-4 during the last 12 months prior to

	screening. • Congestive heart failure NYHA class III-IV during the last 18 months prior to screening. 23. History of any alcohol or drug abuse or dependence within the last year (Investigator's judgement). 24. Any current significant health condition (e.g., cardiovascular, respiratory, renal, hepatic, neurologic, psychiatric, hematologic, oncologic, immune, muscle and joint, etc.) that in the Investigator's judgement may: • jeopardize the subject's safe participation in the trial; or • make unlikely the subject's completion of the study; or • make unlikely the subject's compliance with the study procedures (e.g., highly anticipated need of non-permitted treatments, significant disability, terminal illness, etc.). 25. Pregnant or breastfeeding woman. 26. Use of any experimental drug within 12 weeks prior to screening.
Test product, dose and mode of administration:	Velusetrag 15 mg ([redacted] mg [redacted] once a day to be taken orally approximately at the same time in the morning, on an empty stomach, with water.
Reference therapy, dose and mode of administration:	Placebo [redacted] ([redacted]) once a day to be taken orally approximately at the same time in the morning, on an empty stomach, with water.
Study duration:	A maximum of 4 weeks of velusetrag (or placebo) followed by a maximum of 2-week wash-out period, 4 weeks of placebo (or velusetrag) and 2 weeks of wash-out. This cycle will be repeated twice. A follow up visit will be performed after 2 weeks from the end of the last treatment period. Overall, each subject will be involved in the study for a maximum of a 7-day screening period and approximately a 24 -week period after randomization.
Criteria for evaluation:	<u>EFFICACY</u> Rating of symptoms (i.e., abdominal pain, bloating, nausea and vomiting) is using a recall period of 7 days and a Likert scale with the following categories: 0 - Absent 1 - Mild (not influencing usual activities) 2 - Moderate (diverting from, but not urging modification of, usual activities) 3 - Severe (influencing usual activities markedly enough to urge modifications) 4 - Extremely severe (precluding daily activities) <u>Primary Efficacy Endpoint</u> Change in weekly global gastrointestinal symptoms average index score from start to the end of each treatment period.

	The weekly global gastrointestinal symptoms average index score is obtained by averaging the scores for each of the 4 symptoms assessed weekly: abdominal pain, bloating, nausea and vomiting.
	[redacted]

	[redacted]
Concomitant treatments:	Prohibited treatment: Use of the following medications is not allowed within 2 weeks prior to Screening and throughout the duration of the study: • Strong CYP3A4 inhibitors or strong CYP3A4 inducers. • Strong P-gp transporter inhibitors. • Strong BCRP transporter inhibitors. • Scopolamine. • Erythromycin.

	• Use of [redacted] is not allowed throughout the duration of the study starting from 5 days before randomization. In addition, use of opioids is not allowed within 8 weeks from screening and throughout the duration of the study. <u>Permitted treatments:</u> Medication(s) used to relieve main symptoms of CIPO are allowed and the use of the following concomitant medications will be registered in the e-diary daily: • Treatments for nausea and vomiting and/or non-serotoninergic prokinetics (e.g., metoclopramide, domperidone, pharmaceutical ginger preparations, pyridostigmine prochlorperazine, promethazine, ondansetron and aprepitant, etc.). • Treatments for constipation (e.g., macrogol, bisacodyl linaclotide, laxative enemas, etc.). • Treatments for diarrhea (e.g., tannate, loperamide, etc.) • Treatments for abdominal pain (e.g., paracetamol. NSAIDs, trimebutine, mebeverine, gabapentin, duloxetine, amitriptyline, etc.). • Others (e.g., octreotide, somatostatin, pancreatic enzymes, probiotics, rifaximin, metronidazole, fluconazole, etc.). Permitted medications for CIPO gastrointestinal symptoms taken right before the start of the first treatment period (Day-1) as well as all changes in concomitant treatment doses (increased/reduced) or number of concomitant drugs (added/removed) during all the treatment and washout periods will be daily recorded in the e-diary by the subject.
Proof of Absorption	[redacted]
Statistical Methods	SAMPLE SIZE The sample size is based on the main analysis (t-test) of the primary endpoint that consists of the differences between velusetrag and placebo within each paired treatment cycle (2 per subject). A total of 16 subjects [redacted] will be randomized leading to 32 pairs. Accounting for 25% dropouts/missing pairs, 18 pairs should be available for the primary analysis on the subgroup of subjects with history of benefit from [redacted] or naïve to [redacted], and 24 pairs should be available for the analysis on the overall population.

With a two-sided significance level of 5%, the planned sample size will lead to power levels above 80% to detect effect size above 0.7 (see table below).

Power for different values of Effect Size (ES)* and number of pairs			
Number of pairs	ES: 0.6	ES: 0.7	ES: 0.8
18	67%	80%	89%
24	80%	91%	96%
32	91%	97%	99%

** More than medium to moderate effect size (Cohen, 1988)*

If based on a blinded review of the data, the rate of missing pairs is found to be higher the sample size might be increased by the Sponsor.

RANDOMIZATION

Block randomization will be used to randomly assign subjects in a 1:1:1:1 manner in each of the 4 treatment sequences. Randomization will be stratified into 3 strata by CIPO diagnosis (idiopathic or secondary to neurodegenerative or demyelinating disease) and by [redacted] responder status (responder/naive or non-responder) as follows:

- [redacted] non-responder
- [redacted] responder/naïve and idiopathic CIPO
- [redacted] responder/naïve and CIPO secondary to neurodegenerative or demyelinating disease.

Non responders are defined as all subjects that based on Investigator's judgement, have an history of a lack of benefit from [redacted].

A centralized randomization service, IWRS, will be used.

STUDY POPULATIONS

- Screened Population, defined as the set of all subjects who provided informed consent.
- Safety Set (SS), defined as the set of subjects treated (i.e., having received at least one [redacted] of investigational product).
- Full Analysis Set (FAS), defined as the set of all subjects randomized and treated.
- Modified Full Analysis Set 1 (mFAS1), defined as the set of subjects responder/naïve to [redacted] and treated who reported data on the primary endpoint at least once during a velusetrag treatment period and at least once during a placebo treatment period.
- [redacted]

[redacted]

- Per Protocol Set (PPS), defined as the set of all subjects in the mFAS1 with no major deviations that may affect the analysis of the primary endpoint.

Analysis of the efficacy endpoints will be performed on the mFAS1 (primary analysis), [redacted] PPS and FAS. Analysis of the safety and tolerability endpoints will be performed on the SS.

STATISTICAL ANALYSIS

Descriptive statistics per sequence, visit/period, and stratification factor will be presented as number of observations, number of missing observations, mean, standard deviation, median, minimum and maximum and 25th and 75th percentiles for continuous variables; frequency distribution (n, %) for categorical variables.

Graphical visualization for each subject overtime, including all assessments, will be used for the evaluation of efficacy and safety.

All statistical analysis will be two-sided at a nominal level of 5%. Confidence intervals will be set at the 95% level.

SAS software (V. 9.3 or subsequent) will be used for all statistical analyses.

<u>Primary efficacy endpoint</u>

The primary endpoint is the change in weekly global gastrointestinal symptoms average index score from pre -treatment to the end of each treatment period.

The primary analysis is performed on the mFAS1.

The average weekly global gastrointestinal symptoms index score is obtained by averaging the scores for each of the 4 symptoms assessed weekly: abdominal pain, bloating, nausea and vomiting.

Descriptive statistics will be provided for each treatment sequence along with summaries of the paired differences (velusetrag minus placebo within paired treatment cycle – 2 cycles per subjects). Plots will be made by subjects, by cycle and by treatment. A stratified t-test will be used to analyze the treatment effect

[redacted]

<u>Other efficacy endpoints</u>

Continuous efficacy endpoints will be summarized and analyzed in the same way as the primary endpoint.

	Binary outcome will be compared between treatments with an odds ratio and confidence interval using logistic model and Cochran-Mantel-Haenszel test. Poisson model will be used for count data.
	[redacted]
	Analysis of safety
	[redacted]

STUDY SCHEDULE OF ASSESSMENTS

	Screening Period	1st Treatment Period[a]		2nd Treatment Period[a]		3rd Treatment Period[a]		4th Treatment Period[a]		EFU[n]	ETV	ESV[o]
		Randomiz. SOT-1	EOT-1	SOT-2	EOT-2	SOT-3	EOT-3	SOT-4	EOT-4	End of Follow-up	Early Termination Visit	Early switch Visit
Visit	**V1**	**V2**	**V3**	**V4**	**V5**	**V6**	**V7**	**V8**	**V9**	**V10**	**ETV**	**ESV**
Day	*-7 to -1*	*1*	*28(±1)*	*43(±1)*	*70(±1)*	*85 (±1)*	*112(±1)*	*127(±1)*	*154(±1)*	*169(±1)*		*Any time between Day 1 and 126*
Informed Consent	X											
On Site Visit	X	X	X	X		X		X				X
On Site Visit or home visit[b]					X		X		X	X	X	
Medical, surgical history and previous medications	X											
Assessment of responder status to [redacted]	X											
Demographics	X											
[redacted]												

	Screening Period	1st Treatment Period[a]		2nd Treatment Period[a]		3rd Treatment Period[a]		4th Treatment Period[a]		EFU[n]	ETV	ESV[o]
		Randomiz. SOT-1	EOT-1	SOT-2	EOT-2	SOT-3	EOT-3	SOT-4	EOT-4	End of Follow-up	Early Termination Visit	Early switch Visit
Visit	V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	ETV	ESV
[redacted]		[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]		[redacted]	[redacted]
Inclusion/ Exclusion Criteria Evaluation	X	X										
Randomization		X										
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]		[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]		[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]		[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]											
[redacted]	[redacted]	←——————————————————————→									[redacted]	[redacted]
[redacted]		[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]		[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]		[redacted]	[redacted]
Drug dispensing		X		X		X		X				X
Drug administration time recording[k]		X	X	X	X	X	X	X	X			X
Drug accountability[l]			X		X		X		X		X	X
[redacted]		[redacted]	[redacted]									

SOT: Start of Treatment; EOT: End of Treatment; EFU: End of Follow up; ETV: Early Termination Visit; [redacted]; ESV: Early Switch Visit

a. Subjects will undergo a 2-week wash-out period between treatment periods.
b. If the subject cannot go back to the clinical site for one or more of the following visits: Visit 5, 7, 9 and 10 or ETV, they can be performed at the subject's home.
c. Assessment of responder status to [redacted] will be based on Investigator's opinion and medical history.

[redacted]

k. Dose to be taken under observation at each scheduled visit at the clinical center. The exact time of dose administration should be recorded at all visits.
l. If the subject cannot go back to the clinical center for Visit 5, 7, 9 or ETV, the empty packs and the unused investigational product of the relevant period will be collected by the study nurse during the home visit.
m. [redacted].
n. The final 2 week follow up period are considered also as final 2 week wash out period for the 4th treatment period.
o. In case, during a treatment period or during a washout period, a subject should experience an exacerbation of the CIPO related gastrointestinal symptoms that is not controlled by permitted medications and is not judged as an adverse event, he/she will attend an early switch visit (ESV) at the site. After being reassessed, he/she will start the next scheduled treatment period (SOT-2 or SOT-3 or SOT-4), without completing the ongoing treatment or washout period. ESV will be Day 1 of the relevant period (SOT-2 or SOT-3 or SOT-4) based on the timing of early switch. Only one early switch is allowed during the study.

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[redacted]

1 LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

Abbreviation	Description
5-HT_4	5-hydroxytryptamine receptor 4
AE	Adverse event
ALP	Alkaline phosphatase
ALT	Alanine transaminase
API	Active pharmaceutical ingredient
AST	Aspartate aminotransferase
BCRP	Breast cancer resistance protein
BMI	Body mass index
BP	Blood pressure
bpm	Beats per minute
CH_4	Methane
CIC	Chronic idiopathic constipation
CIPO	Chronic intestinal pseudo-obstruction
C_{max}	Maximum concentration
CRF	Case report form
CYP(3A4)	Cytochrome P450 (isozyme 3A4)
ECG	Electrocardiogram
eCRF	Electronic-case report form
EFU	End of follow up
EOT	End of treatment
ES	Effect size
ESV	Early switch visit
ETV	Early termination visit
FAS	Full analysis set
GCP	Good clinical practice
GE $t_{1/2}$	Gastric emptying half time
GI	Gastrointestinal
GMP	Good manufacturing practice
GP	Gastroparesis
H_2	Hydrogen
HDPE	High-density polyethylene
[redacted]	[redacted]
IB	Investigator's brochure
ICC	Interstitial cells of Cajal

Abbreviation	Description
ICF	Informed consent form
ICH	International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use
IEC	Independent ethics committee
IMP	Investigational medicinal product
IRB	Institutional review board
IWRS	Interactive web response system
LOCF	Last observation carried forward
MAA	Marketing approval authorization
MedDRA	Medical Dictionary for Regulatory Activities (MEDDRA®)
mFAS	Modified full analysis set
NYHA	New York Heart Association
P-gp	P-glycoprotein
PI	Principal investigator
PK	Pharmacokinetic(s)
PPS	Per protocol set
PT	Preferred term
QP	Qualified person
[redacted]	[redacted]
[redacted]	[redacted]
SAE	Serious adverse event
SAP	Statistical analysis plan
SBM	Spontaneous bowel movement
[redacted]	[redacted]
SOC	System organ class
SOT	Start of treatment
SS	Safety set
SS	Safety set
T3	Triiodothyronine
T4	Thyroxine
TEAE	Treatment emergent adverse event
T_{max}	Time of maximum concentration
TSC	Trial steering committee
TSH	Thyroid stimulating hormone
ULN	Upper Limit of Normal

2 ETHICS

2.1 INDEPENDENT ETHICS COMMITTEE (IEC) OR INSTITUTIONAL REVIEW BOARD (IRB)

Before initiating the trial, Alfasigma S.p.A. (AL) and the investigator(s)/institution(s) must obtain written and dated approval/favorable opinion from the IRB(s)/IEC(s) for the trial protocol, written informed consent form (ICF), subject recruitment procedures (e.g., adverts), and any other written information to be provided to subjects. As part of the investigator(s)'/institution(s)' written application to the IRB/IEC, Alfasigma will provide the IRB(s)/IEC(s) with a current copy of the Investigator's Brochure (IB). If the IB is updated during the trial, AL will supply a copy of the updated IB to the IRB(s)/IEC(s).

Alfasigma S.p.A and the investigator(s)/institution(s) must obtain approval/favorable opinion from the IRB(s)/IEC(s) for change(s) to any aspect of the trial, such as modification(s) of the protocol, written ICF, written information to be provided to subjects, and/or other procedures.

Alfasigma S.p.A must promptly report any new information that may affect the safety of the subjects or the conduct of the trial to the IRB(s)/IEC(s).

Alfasigma S.p.A will submit safety update reports to the IRB(s)/IEC(s) and to the Competent Authorities periodically, in accordance with the applicable laws. Upon completion of the trial, Alfasigma S.p.A will provide the IRB(s)/IEC(s) and the Competent Authorities with a brief report of its outcome (synopsis).

(See also section: "Emergency Instructions")

2.2 ETHICAL CONDUCT OF THE STUDY

The Guidelines of the World Medical Association's Declaration of Helsinki in its revised edition (64th WMA General Assembly, Fortaleza, Brazil, October 2013), the Guidelines for Good Clinical Practice E6 (R2) (EMA/CHMP/ICH/135/1995, 1 December 2016) and the Directives 2001/20/EC and 2005/28/EC as well as demands of the national drug and data protection laws will be strictly followed.

(See also sections: "18.9 Insurance for Subjects, "17.4 Data Protection" and "17.3 Documentation of Subjects' Participation").

2.3 SUBJECT INFORMATION AND CONSENT

The investigator is responsible for not admitting subjects to the trial before informed consent has been given. Consent means that the person involved has the legal capacity to give consent and is able to exercise free power of choice. Consent should be given as written informed consent after receiving detailed information. The subjects who refuse to give informed consent must not be included in this trial.

Subjects will be given a written "Subject information and consent form".

Before signing the ICF the subject will be informed in detail by a physician about the following items:

A. the aim and rationale of the trial
B. the nature of the treatment and the allocation of subjects to the different treatment groups
C. other therapeutic alternatives
D. expected therapeutic effects of the medication
E. known adverse drug reactions and other risks or inconvenience during the clinical trial
F. the trial procedures to be followed, including all invasive procedures
G. compensation and/or treatment available to the subject in the event of trial-related injury

H. anticipated prorated payment and expenses to the subjects for participating in the trial (if applicable)
I. subject's right to withdraw at any time without justification and without penalty or loss of benefits to which the subject is otherwise entitled
J. the subject's responsibilities
K. availability of more detailed information before and during the trial
L. information about data protection
M. person(s) to contact in the event of trial-related injury
N. foreseeable circumstances and/or reasons under which the subject's participation in the trial may be terminated
O. the expected duration of the subject's participation in the trial
P. the approximate number of subjects involved in the trial

The subject must be given ample time to inquire about the details of the trial. The consent form is signed by the informing physician and by the subject. Persons who withdraw their informed consent must not continue the trial.

The filled-in and signed ICFs will be kept and archived in original by the investigator in the "Investigator's Site File". (See section 17.1 Documentation of Essential Documents/Supplements at Study Centre during the Trial). The subject will be provided with a copy.

3 GENERAL INFORMATION

Sponsor

Alfasigma S.p.A., Via Ragazzi del '99, 5, I-40133 Bologna, Italy

Sponsor Personnel:

[redacted]
Tel.:
e-mail:

[redacted]
Tel.:
e-mail:

[redacted]
Tel.:
e-mail:

[redacted]
Tel.:
e-mail:

[redacted]
Tel.:
e-mail:

CRO:

[redacted]

Investigators

A list of all Investigators involved in the Study will be provided as a separate document.

Central Laboratory

[redacted]

Home nursing and travel agency

[redacted]

Central [redacted] reading laboratory

[redacted]

Tel.: [redacted]

Bioanalytical Laboratory

[redacted]

4 BACKGROUND AND RATIONALE

Chronic intestinal pseudo-obstruction (CIPO) is a rare, severe condition characterized by an impairment of coordinated propulsive activity in the intestinal tract resulting in a clinical picture similar to that of mechanical intestinal obstruction, although in the absence of any lesion occluding the gut (Antonucci 2008). It may be due to an underlying neuropathic, myopathic disorder, or abnormality in the interstitial cells of Cajal (ICC), either alone or in combination (Antonucci 2008).

CIPO is a rare condition and most estimates of the incidence and prevalence are from tertiary referral centers. In a national survey in Japan, the estimated prevalence of CIPO was 0.80 to 1.00 per 100,000, with an incidence of 0.21 to 0.24 per 100,000 (Lida, 2013). The mean age at diagnosis is 63.1 years for males and 59.2 for females (Camilleri 2021).

Neuropathic, myopathic, or ICC abnormalities may be idiopathic or secondary to another disease. Approximately half of the cases of CIPO are secondary to neurologic (e.g., Parkinson disease and Shy-Drager) syndromes, paraneoplastic, autoimmune, metabolic/endocrine, and infectious diseases. (Camilleri 2021). The clinical picture, characterized by disabling digestive symptoms (i.e., abdominal pain, bloating, nausea and vomiting, constipation, heartburn, fullness, early satiety) associated with “sub-occlusive episodes”, contributes a significant deterioration of quality of life of the patients. CIPO is one of the most important causes of chronic intestinal failure. Patients with CIPO are often unable to maintain normal body weight and/or normal oral nutrition (Antonucci 2008).

The diagnosis of CIPO is based on the presence of longstanding symptoms of mechanical obstruction in the absence of an anatomic cause on radiologic examination and endoscopy, and evidence of impaired motility. Confirmation of the diagnosis requires exclusion of mechanical obstruction and other causes of dysmotility by performing imaging studies, endoscopy, and scintigraphy to assess motility. (Camilleri 2021).

Prokinetic drugs (e.g., [redacted], erythromycin, etc.) are often used in CIPO patients to improve gastrointestinal motility.
[redacted], accelerates transit through the stomach, small bowel, and colon. In a randomized, double-blind, crossover study, [redacted] appeared to provide symptom relief in 4 out of 7 patients with CIPO. In 3 patients with visceral myopathy and 1 with visceral neuropathy, 2 to 4 mg [redacted] (relative to placebo) significantly improved pain in 3 out of 4 patients, nausea in 2, vomiting in 1, and bloating in 4, whereas bowel function was not changed substantially. In contrast to [redacted] appears to have much lower risk of cardiac arrhythmia (Emmanuel 2012).
[redacted]. In randomized trials, oral [redacted] at a dose of 20 mg three times a day was effective in improving gastric emptying but did not provide symptomatic relief in patients with CIPO (Abell 1991). [redacted] has been associated with a number of drug interactions and fatal cardiac arrhythmias, prompting the manufacturer to severely limit its availability in many countries.
Intravenous erythromycin is effective during acute exacerbations of CIPO, acting at least in part by stimulation of the motilin receptors (Catnach 1992, Emmanuel 2004). Such patients are typically hospitalized and require intravenous fluids. Intravenous erythromycin lactobionate at a dose of 3 mg/kg every eight hours should be continued for at least five to seven days. Erythromycin has not been very effective for chronic therapy and has only been tried in a small number of patients (Chami 1991).
Along with pharmacokinetics agents, symptomatic drugs (e.g., antisecretory drugs, antispasmodics,

laxatives, analgesics, etc.), antibiotics (to treat small intestinal bacterial overgrowth, SIBO) as well as invasive procedures such as surgery or small bowel transplant are used to treat patients with CIPO (Camilleri 2021) but no pharmacological treatment is currently licensed for this indication.

Velusetrag is a selective 5-hydroxytryptamine receptor 4 (5-HTR_4) agonist. 5-HTR_4 agonists are able to stimulate the release of acetylcholine from enteric motor neurons and calcitonin gene-related peptide from sensory neurons. Velusetrag has been shown to enhance the peristaltic reflex, stimulate intestinal secretion, and inhibit gastrointestinal visceral sensitivity.
Velusetrag contracts the guinea pig colonic longitudinal muscle–myenteric plexus and relaxes human colonic circular muscle in vitro. Data from in vivo nonclinical studies have shown that velusetrag increases colonic transit in guinea pigs, relaxes the esophagus in rats, and increases contractility in various portions of the GI tract of dogs.

A description of the clinical profile of velusetrag can be found in the current version of the velusetrag IB. Velusetrag has been evaluated in ten Phase 1 studies in healthy subjects, one Phase 2 study in subjects with chronic idiopathic constipation (CIC), and two Phase 2 studies in subjects with gastroparesis.

Dosages studied in clinical trials range from 0.1 to 90 mg as single doses, from 15 to 50 mg administered once daily for up to 28 days, and from 5 to 30 mg administered once daily for 12 weeks. Overall, more than 800 subjects have received one or more velusetrag doses of 5 mg or higher.

In a Phase 1 study in healthy subjects assessing GI transit using scintigraphy, single and multiple doses of velusetrag were associated with acceleration of colonic transit and multiple doses with acceleration of gastric emptying (Manini, 2010]). Velusetrag also achieved statistically and clinically significant increases in stool frequency, including weekly frequency of spontaneous bowel movements (SBM) and weekly frequency of SBM resulting in a sensation of complete evacuation (complete spontaneous bowel movement, CSBM), relative to subjects receiving placebo, in a Phase 2 study of 401 subjects with CIC of 1 month duration (Golberg, 2010).

In addition, a Phase 2 study of velusetrag in 34 subjects with diabetic or idiopathic gastroparesis (GP) exhibited clinically significant relevant improvements in gastric emptying half times (GE $t_{1/2}$) at doses of 5 mg (-35 minutes), 15 mg (-34 minutes), and 30 mg (52 minutes) compared to placebo (-13 minutes) (Ahn, 2015). Similar treatment effects were observed in both diabetic and idiopathic gastroparesis subjects treated with velusetrag.

A Phase 2b study was conducted in 233 subjects with diabetic or idiopathic GP (~50%/~50% allocation) to assess GP symptom improvement with velusetrag up to 12 weeks of dosing. A reverse dose-response pattern was noted with the most robust improvements in symptoms observed at 5 mg. Velusetrag at 5 mg was observed to reduce all symptoms of GP including nausea, vomiting, upper abdominal pain, fullness, bloating, and epigastric burning. In addition, after 4-week treatment a statistically significant reduction in gastric emptying time was observed in all 3 velusetrag dose groups. Gastric emptying normalization, defined as a 4-hour retention percentage < 10%, was observed in 44%, 65% and 71% of subjects for 5, 15 and 30 mg, respectively, compared to no subjects in the placebo group. Furthermore, besides the expected adverse events of nausea, abdominal pain, headache, and diarrhea of this drug class, no other safety signal was detected.

As a high-affinity, potent agonist with high intrinsic activity at the human 5-HT_4 receptor, velusetrag offers promise for the treatment of GI disorders, such as CIPO, in which enhanced motility may be beneficial.

The rationale for velusetrag treatment in patients with CIPO is that increased stomach small and large and intestine motility, together with putative visceral antihyperalgesic activity, will be associated with an improvement in CIPO symptoms.

Because velusetrag is highly selective for the 5-HT_4 receptor and displays no significant affinity at a

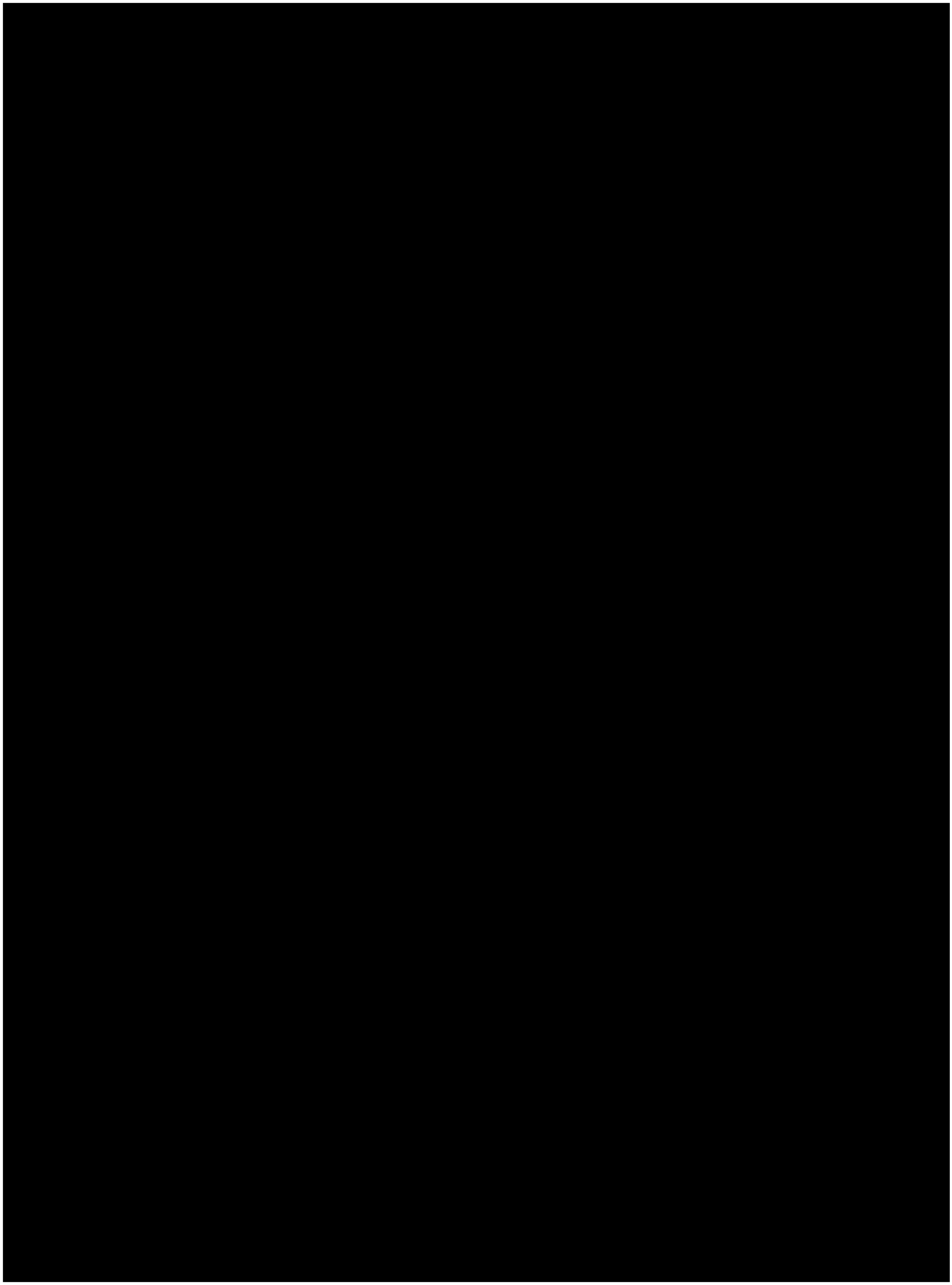
variety of G protein coupled receptors, enzymes, and ion channels (including other 5-HT receptors or dopamine receptors), the potential for unwanted activity (i.e., side effects) is minimized.

5 STUDY OBJECTIVES AND ENDPOINTS

5.1 PRIMARY STUDY OBJECTIVE AND ENDPOINT

Primary Study Objective	Primary Study Endpoint
To investigate the efficacy of velusetrag in improving the symptoms severity associated with CIPO, in subjects with idiopathic CIPO and CIPO secondary to neurodegenerative *or demyelinating* conditions (e.g., Parkinsonian Syndromes, multiple sclerosis etc.).	Change in weekly global gastrointestinal symptoms average index score* from start to the end of each treatment period. **The weekly global gastrointestinal symptoms average index score is obtained by averaging the scores for each of the 4 symptoms assessed weekly: abdominal pain, bloating, nausea and vomiting.* Rating of symptoms (i.e. abdominal pain, bloating, nausea and vomiting) is using a recall period of 7 days and a Likert scale with the following categories: 0 – Absent 1 - Mild (not influencing usual activities) 2 - Moderate (diverting from, but not urging modification of, usual activities) 3 - Severe (influencing usual activities markedly enough to urge modifications) 4 - Extremely severe (precluding daily activities)

[redacted]



6 OVERALL STUDY DESIGN AND PLAN

This is a phase II, multicenter, double-blind, placebo-controlled, two-treatment four-period cross-over, multiple (n=1) trial to evaluate the efficacy and safety of velusetrag 15 mg once a day, compared to placebo, in subjects with a diagnosis of idiopathic CIPO or CIPO secondary to primary neurodegenerative *or demyelinating* conditions (e.g., Parkinsonian Syndromes, multiple sclerosis, etc.).

Eligible subjects will be treated for 4 periods of 4 weeks each with either velusetrag 15 mg (2 periods) or placebo (2 periods), once daily, with a wash-out period of 2 weeks between treatment periods.

After an up to 7-day screening period (from Day -7 to Day -1, V1), at randomization visit (V2), eligible subjects will be randomly allocated to one of the following four sequences:

A. VEL-PLA-VEL-PLA
B. PLA-VEL-PLA-VEL
C. VEL-PLA-PLA-VEL
D. PLA-VEL-VEL-PLA

Where:

VEL= velusetrag 15 mg once daily for 4 weeks.

PLA= matching placebo once daily for 4 weeks.

There will be a 2-week wash-out period between each treatment period and a final 2-week follow-up period at the end of the last treatment period.

Subjects will be assessed during a total of 10 visits during the study, as detailed in the Study Schedule of Assessments, including the screening visit, a start of treatment visit (SOT) and an end of treatment visit (EOT) for each of the 4 treatment periods, for a total of 8 visits, and a final End of Follow (EFU) up Visit, 2 weeks after the end of the last treatment period.

If the subject cannot go back to the clinical center for one or more of the following visits: EOT- 2 (Visit 5), the EOT- 3 (Visit 7), the EOT- 4 (Visit 9), End Of Follow Up (EFU)- Visit (Visit 10) or in case of an early termination visit (ETV), the visit will be scheduled at the subject's home and it will be performed by a home nursing service. The clinical study staff at the clinical center may also attend the visit remotely.

Overall, each subject will be involved in the study for a screening period of up to 7 days before randomization and approximately a 24-week period after randomization.

Subjects under treatment with a [redacted] must have a 5-day washout period before randomization (note: symptomatic treatments other than [redacted] will be permitted throughout the study, see section 10.8 Prior and Concomitant Therapy).

In case, during a treatment period or during a washout period, a subject should experience an exacerbation of the CIPO related gastrointestinal symptoms that is not controlled by permitted medications and is not judged as an adverse event, he/she will attend an early switch visit (ESV) at the clinical site. After being reassessed, he/she will be offered to directly switch to the next scheduled treatment period (SOT-2 or SOT-3 or SOT-4) without completing the ongoing treatment or washout period. Only one early switch is possible during the study. ESV will be Day 1 of the relevant period (SOT-2 or SOT-3 or SOT-4) based on the timing of early switch.
In case of ESV/SOT-2 or ESV/SOT-3 or ESV/SOT-4, the subsequent visits will follow the relevant sequential number (see Study Schedule of Assessments).

[redacted] will be registered on Day-1 (taking into consideration the symptoms relevant to the last 7 days including Day-1) and weekly after randomization, during both treatment and wash out periods, as appropriate according to the Study Schedule of Assessments using a subject's e-diary, until End of Follow-up or in case of ETV or ESV.

[redacted]

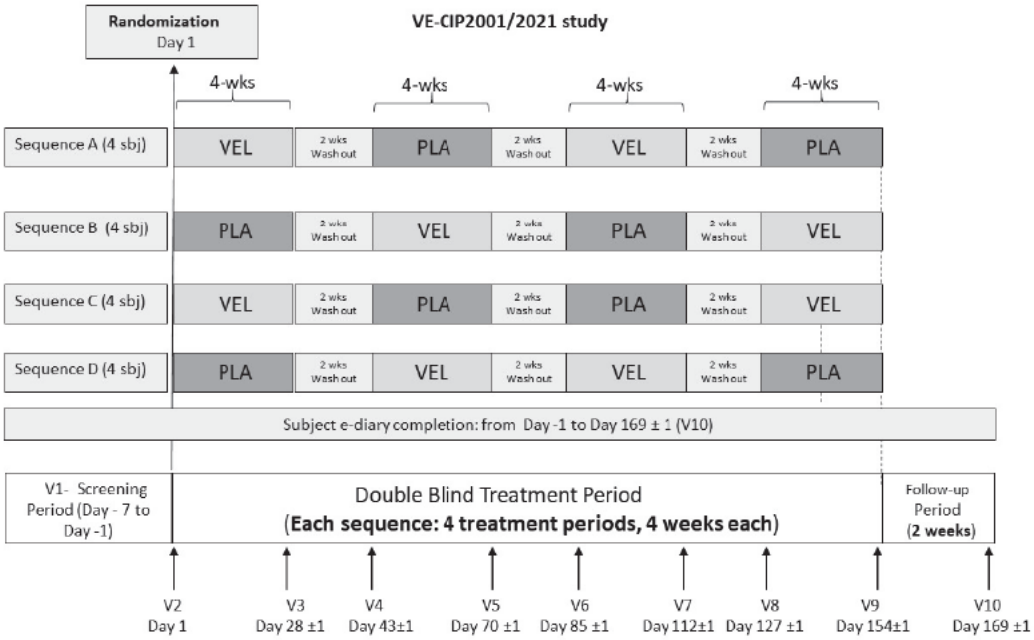
In addition, investigational product intake and time will be registered by the subjects in the e-diary during the treatment periods daily.

[redacted] will be also assessed at screening and then, after randomization during the study according to "Study Schedule of Assessments ". [redacted]

At the screening visit and at the end of the first 4-week treatment period, [redacted] will be performed in order to assess [redacted].

In order to assess [redacted] in all subjects on Visit 2 (Day 1) and Visit 3 (Day 28 ± 1), blood samples for assessment of plasma velusetrag and THRX 830449 (metabolite) concentrations will be collected [redacted].

Figure 1: Schematic study design



7 DISCUSSION OF STUDY DESIGN, INCLUDING THE CHOICE OF CONTROL GROUPS

This study will evaluate the safety, tolerability and efficacy of velusetrag 15 mg once a day versus placebo, in improving the symptoms severity associated with CIPO, in subjects with idiopathic CIPO and CIPO secondary to neurodegenerative conditions (e.g., Parkinsonian Syndromes, etc.).

CIPO is a chronic, rare disease, with fluctuating symptoms and differences in underlying pathology, which may respond differently to active treatment. Therefore, a standard parallel group placebo-controlled study may not allow to detect clear benefit due to the large variability of clinical presentation.

A n=1 cross over, placebo-controlled study design, as the one chosen for the present study, allows to assess the treatment effect in every subject, thereby allowing individual subjects to act as their own control and evidencing a positive effect even in a single subject, avoiding under-estimation of the therapeutic efficacy (Emmanuel, 2012).

Subjects will be evaluated over a total of 16 weeks, receiving either velusetrag 15 mg or placebo, once daily, for four randomized periods of 4 weeks each. The planned treatment time period is considered enough to demonstrate a difference between active treatment and placebo. The wash-out periods' duration has been selected based on velusetrag and active metabolite half-life.

Efficacy will be assessed after each 4 weeks of treatment for primary analysis. The choice of placebo as comparator will allow for estimation of the real treatment effect of velusetrag and is considered acceptable due to: i) the lack of any approved treatment for CIPO; ii) the fact that symptomatic rescue therapies are allowed, and iii) the close monitoring during a relatively short treatment period. Moreover, participants, that during the study should experience significant exacerbation of CIPO-related gastrointestinal symptoms that are not controlled by permitted medications and are not judged as adverse events, after being reassessed may decide to continue the study and start the subsequent treatment period, or discontinue the study.

8 BENEFIT-RISK EVALUATION

Velusetrag was generally well tolerated in healthy subjects, elderly subjects, subjects with CIC, and subjects with gastroparesis.

- In the Phase 2 study in CIC, clinically significant improvements in symptoms associated with CIC as well as increased bowel movement frequency were observed over 4 treatment weeks.
- In the Phase 2 studies in gastroparesis, improvement in GE $t_{1/2}$ or higher rates of normalization of gastric emptying (44%-71%) compared to placebo (0%) were observed for all doses of velusetrag (both in diabetic and idiopathic patients).
- In the Phase 2b study, velusetrag at 5 mg was observed to reduce all gastroparesis symptoms including nausea, vomiting, post-prandial fullness, early satiety, bloating, abdominal pain and epigastric burning with higher response rates compared to placebo particularly in the idiopathic gastroparesis group.
  Changes from baseline were similar in the idiopathic and diabetic subgroups for GCSI-24H domain scores, however a larger placebo effect was observed in diabetic subjects compared to idiopathic subjects on the GCSI-24H resulting in a smaller treatment effects in the diabetic subgroup compared to the idiopathic subgroup.

On the basis of these clinical studies, velusetrag was well tolerated without relevant AEs. The majority of observed AEs could be interpreted within its pharmacodynamic activities and most are common to the underlying disease.

No ischemic colitis, cerebrovascular, or peripheral vascular events were noted in the prior clinical studies with this compound.

No clinically significant changes from pre-treatment have been observed in clinical laboratory test results, [redacted] measurements, or [redacted] besides an increase in [redacted] after the first drug intake; however, increases in [redacted] were observed after dosing on subsequent visits. These changes were detected only on [redacted] and not on [redacted] evaluation.

In the thorough [redacted]

In summary, based on PK and PD of Velusetrag, the following potential warnings may be anticipated:

- Subjects receiving velusetrag should be monitored for exaggerated responses to the GI prokinetic activity of velusetrag (GI AEs), especially upon initiation of treatment.
  The onset and resolution of GI AEs in the Phase 2 CIC study subjects typically occurred within 1 to 2 days of treatment initiation.
- Investigators should be aware of the potential for increased heart rate (HR).
  A modest increase in [redacted] was observed at both 15 mg and 90 mg in the tQTc study on the first 2 days of dosing, but it was not observed after 6 days of dosing. [redacted] evaluation in the Phase 2b gastroparesis study suggested an average increase in [redacted] compared to baseline (Day 1 predose). However, upon [redacted] analysis, there was no obvious sign of increase in [redacted] The clinical significance of the transient effects observed on [redacted] is unclear, but it was [redacted] on average at all doses, even on Day 1.
- There is limited experience regarding administration of velusetrag to subjects who are taking another pharmacologic treatment.
- Co-administration of strong CYP3A inhibitors may result in an increase in exposure to velusetrag and a decrease in exposure to the active metabolite THRX 830449..
  Co-administration of strong CYP3A inducers may result in a decrease in exposure to velusetrag and an increase in exposure to the active metabolite THRX 830449.
  Co-administration of strong P-glycoprotein (P-gp) transporter inhibitors may result in an increase in exposure to velusetrag and the active metabolite THRX 83044.
  Co-administration of strong breast cancer resistance protein (BCRP) transporter inhibitors may result in an increase in exposure to velusetrag and the active metabolite THRX 83044.
- There are no data from the use of velusetrag in pregnant women.
  Animal studies do not indicate direct or indirect harmful effects with respect to reproductive toxicity.
  As a precautionary measure, it is preferable to avoid the use of velusetrag during pregnancy.
  It is unknown whether velusetrag/metabolites are excreted in human milk.
  A risk to the suckling child cannot be excluded.

These data, overall, support further evaluation of velusetrag for treatment of CIPO.

9 SELECTION OF STUDY POPULATION

9.1 NUMBER OF PARTICIPANTS

Sixteen male and female subjects with a diagnosis of idiopathic CIPO or CIPO secondary to primary neurodegenerative or demyelinating conditions (e.g., Parkinsonian Syndromes, multiple sclerosis, etc.), who meet all the inclusion and none of the exclusion criteria will be enrolled in this study.

[redacted]

Subjects will be enrolled in up to 5 centers.

9.2 INCLUSION CRITERIA

1. Men or women aged 18-80 years.
2. Subjects with history of chronic idiopathic intestinal pseudo-obstruction or CIPO secondary to neurogenerative or demyelinating disease.
3. Subjects with estimated oral caloric intake of at least 30% of the daily age- and sex-recommended caloric intake (Stage 0, 1 or 2 of the [redacted], see 21.3 Appendix 3: [redacted] for CIPO patients).
4. Subjects with at least 2 out of 4 CIPO gastrointestinal symptoms (i.e., abdominal pain, bloating, nausea and vomiting), each of the 2 with a score ≥3 (on a 0 to 4 scale) collected on the gastrointestinal symptom questionnaire at Day -1.
5. Subjects accepting to provide and legally capable of providing free and informed consent to all procedures included in the protocol.
6. All sexually active male participants who are partner of women of childbearing potential must use condom during intercourse until the 90th day after the end of the entire study.
7. All female participants must be:

   - of non-childbearing potential, i.e.: i) post-menopausal (at least 2 years without spontaneous menses), or ii) surgically sterile (bilateral tubal occlusion, or hysterectomy), or iii) ablation of both ovaries

or

   - of childbearing potential with a negative pregnancy test result at screening and randomization AND agreeing to use a highly effective method of contraception (i.e. with failure rate of less than 1% per year) until the end of the entire study.

Note 1. Based on the EU Clinical Trial Facilitation Group recommendations, a highly effective method of contraception is one of the following:

- Intrauterine device (IUD).
- Intrauterine hormone-releasing systems (IUS).
- Combined hormonal contraceptives (i.e. Estrogen and progestogen) in oral, intravaginal or transdermal form, with inhibition of ovulation as primary mode of action.
- Progestogen-only hormonal contraceptives in oral, injectable or implantable form, with inhibition of ovulation as primary mode of action.
- True and absolute abstinence: when this is in line with the preferred and usual lifestyle of the subject.

Note 2. Periodic abstinence, such as calendar, ovulation, symptom thermal, post ovulation methods, and withdrawal are not acceptable methods of contraception.

Note 3. In each case of delayed menstrual period (over one month between menstruations), female participants of child-bearing potential will be strongly recommended to provide a confirmation of absence of pregnancy. This recommendation applies also to women of child-bearing potential with infrequent or irregular menstrual cycles.

9.3 EXCLUSION CRITERIA

1. Subjects with primary CIPO or CIPO secondary to other known endocrine/metabolic, autoimmune diseases and neurologic conditions other than neurodegenerative or demyelinating diseases.
2. Subjects with conditions characterized by mechanical intestinal obstruction.
3. Nasogastric tube, gastrostomy tube, or jejunostomy feeding tube in place at randomization or planned throughout the duration of the study, or [redacted] stage 3 (“total non-oral nutrition”, see 21.3 Appendix 3: [redacted] for CIPO patients).
4. Presence of untreated clinically relevant thyroid dysfunction or known thyroid dysfunction not well controlled by treatment (e.g., subjects with abnormal thyroid stimulating hormone [TSH], and, if available, triiodothyronine [T3] and thyroxin [T4] levels) deemed clinically significant by the Investigator.
5. Subjects with history of diabetes at screening.
6. Clinically significant ECG abnormalities (e.g., ST segment elevation or depression suggestive of ischemia, partial or complete left bundle branch block [LBBB]) at screening and randomization.
7. Screening ECG with a QTcF >450 msec in males or >470 msec in females or family history of sudden cardiac death.
8. Subjects requiring a low galactose diet.
9. Hypersensitivity or documented intolerance to lactulose, lactose, or any excipient of the lactulose preparation to be used for [redacted].
10. History of sensitivity to velusetrag, or any of the velusetrag or placebo excipients.
11. Use of scopolamine or erythromycin within 2 weeks prior to Screening and/or planned throughout the duration of the study.
12. Use of [redacted] within 5 days prior to randomization and/or planned throughout the duration of the study.
13. Use of opioids within 8 weeks from screening and/or planned throughout the duration of the study.
14. Received strong cytochrome P450-isozyme 3A4 (CYP3A4) inhibitors (e.g., clarithromycin, indinavir, itraconazole, ketoconazole, nefazodone, nelfinavir, ritonavir, saquinavir, telithromycin, grapefruit juice) or strong CYP3A4 inducers (e.g., rifampin, phenytoin, carbamazepine, phenobarbital, St. John's wort) within 2 weeks prior to screening and/or planned throughout the duration of the study.
15. Received strong P-glycoprotein (P-gp) transporter inhibitors (e.g., captopril, carvedilol, diltiazem) within 2 weeks prior to Screening and/or planned throughout the duration of the study.
16. Received strong breast cancer resistance protein (BCRP) transporter inhibitors (e.g., curcumin, cyclosporine A, eltrombopag) within 2 weeks prior to Screening and/or planned throughout the duration of the study.
17. Current swab-positive or suspected (under investigation) COVID-19 infection.
18. Cancer (excluding non-melanoma skin cancer) and/or need of any anti-cancer treatment (also including radiotherapy) within the last 5 years.
19. Severe kidney impairment (i.e., estimated glomerular filtration rate <30 ml/min).

20. Aspartate aminotransferase (AST) or alanine transaminase (ALT) levels >2.5 times the upper limit of normal (ULN); bilirubin (unless deemed to be due to Gilbert's Syndrome) or alkaline phosphatase (ALP) >1.5 times ULN.
21. Severe hepatic impairment defined as Child-Pugh C.
22. History of any of the following cardiac disorders:
    - Torsade de pointes, ventricular tachycardia, ventricular fibrillation.
    - Previous myocardial infarction, unstable angina pectoris, acute coronary syndrome, coronary artery or cerebral revascularization procedure or stroke within the previous 18 months.
    - Angina pectoris class 2-4 during the last 12 months prior to screening.
    - Congestive heart failure NYHA class III-IV during the last 18 months prior to screening.
23. History of any alcohol or drug abuse or dependence within the last year (Investigator's judgement).
24. Any current significant health condition (e.g., cardiovascular, respiratory, renal, hepatic, neurologic, psychiatric, hematologic, oncologic, immune, muscle and joint, etc.) that in the Investigator's judgement may:
    a. jeopardize the subject's safe participation in the trial; or
    b. make unlikely the subject's completion of the study; or
    c. make unlikely the subject's compliance with the study procedures (e.g., highly anticipated need of non-permitted treatments, significant disability, terminal illness, etc.).
25. Pregnant or breastfeeding woman.
26. Use of any experimental drug within 12 weeks prior to screening.

9.4 PREMATURE DISCONTINUATION FROM THE STUDY PER SUBJECT

Any subject (or his/her legally authorized representative) may withdraw the consent to participate in the study at any time without prejudice. The investigator must withdraw from the study any subject who requests to be withdrawn. A subject's participation in the study may be discontinued at any time at the discretion of the investigator and in accordance with his/her clinical judgment. When possible, the tests and evaluations listed for the ETV should be carried out.

If a subject withdraws before completing the study, the Investigator should determine the primary reason for premature discontinuation of the study, and the reason and date of withdrawal will be recorded on the e-CRF. The Sponsor will be notified of all subject withdrawals.

Reasons for which the investigator or the Sponsor MAY withdraw a subject from the study, or a subject MAY choose to terminate participation before completion of the study include, but are not limited to, the following:

- Treatment failure including the use of [redacted] and scopolamine, erythromycin and opioids for analgesic use during the study.
- Positive COVID-19 test
- Adverse event: any (significant) adverse event that in the opinion of the investigator, sponsor, or subject is not compatible with study continuation.
- Death.
- Loss to follow-up: the loss or lack of continuation of a subject to follow-up.
- Significant non-compliance with investigational product: an indication that a subject has not agreed with or followed the instructions related to the study medication.

- Physician or sponsor's decision: any other reason that in the opinion of the Investigator or Sponsor may affect the safe participation of a subject in the trial or significantly jeopardize the study design.
- Subject's withdrawal of the informed consent: study discontinuation requested by a subject for whatever reason.
- Significant protocol violation.
- Other: different than the ones previously specified.

In case of premature study discontinuation, a final evaluation should be completed at the time of discontinuation as per section 11.2.7 Early Termination Visit (ETV), when applicable. Subjects who discontinue treatment due to SAE will be followed up until the event resolves or stabilizes.

Subjects withdrawn from the study will not be replaced. The data of subjects who are withdrawn will be considered for evaluation.

The Sponsor reserves the right to discontinue this study at any time for any reason.

10 TREATMENTS

10.1 TREATMENTS TO BE ADMINISTERED

Eligible subjects will be randomly allocated in a 1:1:1:1 manner (4 subjects per treatment sequence), in a blinded fashion, to one of the following four treatment sequences:

A. VEL-PLA-VEL-PLA
B. PLA-VEL-PLA-VEL
C. VEL-PLA-PLA-VEL
D. PLA-VEL-VEL-PLA

Where:

VEL= velusetrag 15 mg ([redacted] mg [redacted] once a day for 4 weeks.

PLA= matching placebo ([redacted]) once a day for 4 weeks.

There will be a 2-week wash-out period between each treatment period and a final 2-week follow-up period at the end of the last treatment period.

Randomization will be stratified into 3 strata by CIPO diagnosis (idiopathic or secondary to neurodegenerative or demyelinating disease) and by [redacted] responder status (responder/naive or non-responder) as follows:

- [redacted] non-responder
- [redacted] responder/naïve and idiopathic CIPO
- [redacted] responder/naïve and CIPO secondary to neurodegenerative or demyelinating disease.

Non responders are defined as all subjects that based on Investigator's judgement, have an history of a lack of benefit from [redacted]

The Investigator at each study site will be responsible for the handling and storage of the study material (in accordance with the Sponsor's indications).

Important note: Investigational products are only allowed to be administered to subjects selected for this clinical trial according to the protocol.

10.2 INVESTIGATIONAL PRODUCTS

10.2.1 Test preparation

Velusetrag

Active product ingredient: velusetrag 5 mg. Excipients: [redacted]

Velusetrag drug substance is manufactured [redacted] strength: 5 mg. Velusetrag [redacted] are packaged in 35-count (high-density polyethylene HDPE induction sealed bottles.

Placebo

Active product ingredient: absent. Excipients: [redacted]

10.2.2 Labelling and Packaging

Manufacturing of velusetrag API was performed by [redacted]

Test drug and matched placebo bulk manufacturing and primary packaging in 30 mL HDPE bottles, each containing 35 [redacted] were performed by [redacted]

Alfasigma S.p.A Bologna performed the EU import of the investigational medicinal products (IMPs).

The secondary packaging and labelling will be performed in Alfasigma S.p.A. Pomezia. Final Batch Certification (according to the Annex XIII to GMP Vol IV requirements) will be executed by Alfasigma S.p.A. Bologna.

All investigational clinical materials will be labelled and packaged in boxes ("Medication Packs"). A medication pack will be dispensed to the subject at each of the 4 start of treatment visits (SOT): randomization visit (Visit 2- Day 1), Start of treatment -2 (SOT-2; Visit 4), Start of treatment -3 (SOT-3; Visit 6), Start of treatment -4 (SOT-4; Visit 8) or at ESV, which will be considered as SOT-2 or SOT-3 or SOT-4, as appropriate. In case of ESV/SOT-2, ESV/SOT-3, or ESV/SOT-4, the subsequent visits will follow the relevant sequential number (see Study Schedule of Assessments)

Each medication pack will consist of 3 bottles for a treatment period of 4 consecutive weeks (28 days of therapy, plus 7 extra days).

Each bottle contains 35 [redacted] of velusetrag 5 mg or matching placebo.

Each subject randomized to each of the 4 treatment sequences will receive, during the full study duration, a total of 2 medication packs, each containing 3 bottles of velusetrag 5 mg and 2 medication packs, each containing 3 bottles of placebo. Each bottle (primary packaging) will be labelled at least with the following information:

Study Code; Sponsor name and address; CRO name; bottle content description; IMP Batch number; Packaging Code; Kit number.

The "Medication Packs" (secondary packaging) will be labelled with at least the following information (secondary label): Study Code; Sponsor name, address, phone number; CRO name, address; box content description; IMP Batch number; Packaging Code; Kit number; IMP Expiry date; directions

for the IMP use; storage conditions; empty fields to be filled in by the Investigator: randomization N., Site number, Investigator's Name, dispensing date.

Each medication pack will be labelled according to Volume 4, Good Manufacturing Practices, Annex 13, Manufacture of Investigational Medicinal Products, 3rd February 2010.

10.2.3 Storage, Dispensing, Use and Disposal of the compound during and at the end of the study

All investigational products will be kept in a locked place with restricted access and maintained under controlled temperature conditions not above 25°C (do not refrigerate or freeze).

At the end of each of the 4 treatment periods: Visit 3 (EOT-1), Visit 5 (EOT-2), Visit 7 (EOT-3), Visit 9 (EOT-4) or in case of ETV or ESV, each subject will return to the Investigator the empty packs and the unused investigational product of the relevant period. In case the subject cannot go back to the clinical center for one or more EOT Visit (i.e. Visit 5, 7 and/or 9 or in case of ETV, the empty packs and the unused investigational product of the relevant period will be collected by the study nurse during the home visit.

The Investigator undertakes not to supply the investigational product to persons other than enrolled subjects or the authorized study personnel in charge of distribute it. Under no circumstances, the Investigator will supply the investigational product to other Investigators or clinics or allow the drug to be used other than as directed in the actual version of the protocol, without authorization by the Sponsor.

The Investigator must store the study medication separately from any other medication. The investigational products should be stored safely and properly, and they must not be used after the expiry date.

The investigational products will be self-administered by the subject, following instruction provided by the Investigator or personnel authorized by the Investigator and according to the treatment scheme detailed in section 10.1 Treatments to be Administered.

The investigator or designee is responsible for maintaining accountability records for all investigational product(s) received from the Sponsor, in accordance with applicable government regulations and study procedures.

The final accountability records will reconcile shipment records with those of used and returned investigational product. Any discrepancy will be accounted for.

All unused investigational products will be returned or destroyed locally, based on the local regulation, but return and destruction will not occur until authorized by the Sponsor. Copies of the study medication accountability records will be provided to the Sponsor at completion of the study and will be made available for review by the site monitor during the course of the study.

The Investigator must report any defect in the manufacturing and packaging or any deviation with respect to the prescribed storage conditions of the drugs immediately to the clinical monitor who will immediately inform the Sponsor QP.

10.3 Method of Assigning Subjects to Treatment Groups/Randomization

A unique subject's identification number, obtained concatenating the center number and the screening number, will be assigned to all subjects consenting to be enrolled in the study.

A subject who fails to meet the protocol eligibility criteria will be identified as screening failure; his/her identification number will not be reallocated.

Randomization will occur at Visit 2 (V2- Day 1), after all screening procedures have been performed and eligibility for the study has been assessed, prior to dispensing the first study medication.

To each elected subject a unique Randomization Number will be assigned. Randomization will be

stratified by CIPO diagnosis (idiopathic or secondary to neurodegenerative or demyelinating disease) and [redacted] responder status (responder/naive or non-responder) as follows:

- [redacted] non-responder
- responder/naïve and idiopathic CIPO
- responder/naïve and CIPO secondary to neurodegenerative or demyelinating disease.

Non responders are defined as all subjects that based on investigator's judgement, have an history of a lack of benefit [redacted].

A centralized randomization service, Interactive Web Response System (IWRS), will be used.

Block randomization will be used to randomly assign subjects in a 1:1:1:1 manner in each of the 4 treatment sequences.

At V2, according to a computer-generated randomization list, subjects will be randomized 1:1:1:1 to one of the following four sequences:

A. VEL-PLA-VEL-PLA
B. PLA-VEL-PLA-VEL
C. VEL-PLA-PLA-VEL
D. PLA-VEL-VEL-PLA

Where:

VEL= velusetrag 15 mg once a day for 4 weeks.

PLA= matching placebo once a day for 4 weeks

There will be a 2-week wash-out period between each treatment period and a final 2-week follow-up period at the end of the last treatment period.

The assigned medication pack will be dispensed to each subject, for each of the 4 treatment periods of each sequence, at randomization visit (Visit 2- Day 1), Start of treatment -2 (SOT-2; Visit 4), Start of treatment -3 (SOT-3; Visit 6), Start of treatment -4 (SOT-4; Visit 8) or in case of ESV (which will be considered as SOT-2, SOT-3 or SOT-4, as appropriate) recording the subject's assigned kit number in the e-CRF.

Per each subject and period all the kit numbers are independent of each other. The enrolment will be competitive.

Unblinding in case of emergency is described in section 11.5 Unblinding of randomization in case of emergency.

10.4 Selection of Doses in the Study

Velusetrag 15 mg once daily, have been selected for this study based on the following evidence:

- Prokinetic activity demonstrated in healthy subjects and in patients with CIC and results from a Phase 2 study in subjects with CIC which showed a statistically significant increase in the weekly frequency of SBM at 15, 30 and 50 mg velusetrag respectively, compared with placebo and the difference was statistically significant ($p < 0.0001$) for all three doses of Velusetrag. The average increase from baseline over the 4-week treatment period was 3.6 SBMs/week at 15 g, 3.3 SBMs/week at 30 mg, and 3.5 SBMs/week at 50 mg Velusetrag compared with 1.4 SBMs/week for placebo. The 50 mg dose was least well tolerated, showing a clinically significantly greater number of reports of headache, diarrhea, nausea,

and vomiting. Diarrhea was reported by 11% to 15% of the Velusetrag subjects compared with 1% of placebo subjects. The incidence of headache was similar for the Velusetrag 15 mg and placebo treatment groups (6% each) and higher for the 30-mg and 50-mg treatment groups (10% and 21%, respectively).

- Results from a Phase 2a and Phase 2b study in subject with idiopathic or diabetic gastroparesis suggested that velusetrag improves gastric emptying. The Phase 2b study was a multicenter, randomized, double-blind, placebo-controlled study to evaluate three doses (5, 15, or 30 mg) of Velusetrag compared to placebo administered once daily for 12 weeks. Velusetrag 15 mg demonstrated improvement in gastric emptying delay on scintigraphy without unexpected concerning safety signals in 65% of subjects, compared to no subjects in the placebo group.

10.5 Selection and Timing of Dose for each Subject

Subjects will take velusetrag 15 mg [redacted] mg [redacted] or placebo ([redacted]) once daily during the 4 weeks of each of the 4 treatment periods. Subjects will be instructed to take orally three [redacted] of blinded study medication, once daily at approximately the same time each morning, on an empty stomach, with water.

Investigational product will be administered under the supervision of the study personnel at randomization (V2) and on all other study visits, if the visit is performed at the clinical site and not in case of home visits. Each 4-week treatment period will be followed by a 2-week wash-out period.

10.6 Blinding

Blinding is intended to limit the occurrence of conscious and unconscious bias in the conduct and interpretation of a clinical trial arising from the influence which the knowledge of treatment may have on the recruitment and allocation of subjects, their subsequent care, the attitudes of subjects to the treatments, the assessment of end-points, the handling of withdrawals, the exclusion of data from analysis, and so on.

Velusetrag 5 mg [redacted] and matching placebo [redacted] are identical in appearance and will be packaged in individual treatment bottles.

The Sponsor, CRAs, CROs, site staff and subjects will be blinded to treatment allocation. All the IMP packaging will not contain any information that could potentially unblind the treatment to the subjects and investigators.

Only in case of emergency, when knowledge of the study medication is essential for the safety of the subject, the investigator may unblind a subject's treatment assignment (see section 11.5 Unblinding of randomization in case of emergency).

10.7 Treatment Compliance

Compliance with the dosing regimen will be assessed by reconciliation of used and unused investigational product. Compliance will be calculated at the end of each treatment period, counting the unused returned [redacted] and taking into consideration the total number of treatment days occurred from the previous visit in which the treatment kit has been delivered to the subject.

Any discrepancies will be reviewed by the site staff and the subject retrained on proper dosing as needed.

Compliance to the study treatment is defined as subjects who are considered to have taken at least 80% of the total number of doses that should have been administered during the study.

10.8 Prior and Concomitant Therapy

Use of the following medications is not allowed within 2 weeks prior to Screening and/or planned throughout the duration of the study:

- Scopolamine.
- Erythromycin
- Strong CYP3A4 inhibitors or strong CYP3A4 inducers (Table 10.1).
- Strong P-gp transporter inhibitors (Table 10.1).
- Strong BCRP transporter inhibitors (Table 10.1).

In addition, use of:

- Opioids is not allowed within 8 weeks from screening and/or planned throughout the duration of the study.
- [redacted] are not allowed starting from 5 days before randomization and throughout the duration of the study
- Orally poorly absorbed opioids (i.e., loperamide) that could be used to treat potential adverse events, such as diarrhea, may be used if medically indicated.

In case a [redacted] or scopolamine, or erythromycin and an opioid for analgesic use is taken during the study, the subject will be considered a treatment failure and will be withdrawn from the study.

[redacted]

Additional prescription and over-the-counter medications are permitted, provided that such agents are not known to be strong inducers or inhibitors of CYP3A4, P-gp and BCRP activity.

Any addition or change in regimen of concomitant medications that affect CYP3A4, P-gp and BCRP activity should be in accordance with inclusion or exclusion criteria and recorded in the source documents and the electronic-case report form (eCRF).

Medications taken 30 days prior to the Screening visit through the end of the Follow-up period should

be recorded. If subjects have previously taken a [redacted], the last treatment period (even if occurred more than 30 days before the screening visit) and the efficacy of such treatment (responder/not responder) for each subject will be recorded in the eCRF.

If clinically permitted, subjects should be encouraged not to change their current regimen (dose, frequency) of concomitant medications or not to start new concomitant medications.

Permitted treatments: Medication(s) used to relieve main symptoms of CIPO are allowed and the use of the following concomitant medications will be registered in the e-diary daily:

- Treatments for nausea and vomiting and/or non-serotoninergic prokinetics (e.g., metoclopramide, domperidone, pharmaceutical ginger preparations, pyridostigmine, prochlorperazine, promethazine, ondansetron and aprepitant, etc.).
- Treatments for constipation (e.g., macrogol, bisacodyl linaclotide, laxative enemas, etc.).
- Treatments for diarrhea (e.g., tannate, loperamide, etc.).
- Treatments for abdominal pain (e.g., paracetamol. NSAIDs, trimebutine, mebeverine, gabapentin, duloxetine, amitriptyline, etc.).
- Others (e.g., octreotide, somatostatin, pancreatic enzymes, probiotics, rifaximin, metronidazole, fluconazole, etc.).
- Permitted medications for CIPO gastrointestinal symptoms taken right before the start of the first treatment period (Day -1) as well as all changes in concomitant treatment doses (increased/reduced) or number of concomitant drugs (added/removed) during the entire treatment and washout periods, until end of follow up, will be daily recorded in the e-diary by the subject.

11 STUDY PROCEDURES

11.1 VISIT SCHEDULE

Subjects will be assessed during a total of up to 10 visits during the study, as detailed in the following sections (see also Study Schedule of Assessments).

11.2 STUDY EVALUATIONS/PROCEDURES

11.2.1 Screening Period – Collectively named Visit 1 – from Day -7 to Day -1

The screening visit can be performed within Day -7 and Day -1 before randomization and screening activities will comprise the following:

1. Obtain written informed consent, signed and dated by the subject, after the nature of the study has been explained and before any study procedure is performed.
2. Demographic data: age, gender, ethnicity.
3. Record of relevant medical and surgical history including subject's history of CIPO, number of pseudo-obstruction episodes and [redacted] in the previous 6 months.
4. Assessment of responder status [redacted] (e.g., [redacted]) according to investigator's judgement and medical history (subjects who have history of benefit [redacted] or never treated (naïve) [redacted] and subjects non responders [redacted]).
5. Record of relevant medication history:

- all medications (i.e., prescription and over-the-counter medications, herbals, vitamins, and supplements) used within 30 days of the screening visit to be recorded. (Attention will be paid to prohibited treatments)
- Subject's history of [redacted] within 30 days of the screening visit will also be recorded including the name of medication, date started and stopped, route of administration, indication, dose and reason for stopping, to be recorded in the e-CRF. Last [redacted] intake (e.g., [redacted]), if previously taken by the subject, should be recorded as previous medication, even if taken more than 30 days before the screening visit.

6. [redacted]
7. [redacted]
8. COVID-19 rapid swab virus testing (to be performed on the same day of [redacted] and results to be available before starting [redacted]).
9. [redacted]
10. Biometric measures: weight in kilograms, height in centimeters, body mass index (BMI) to be calculated.
11. [redacted]
12. [redacted]
13. Blood collection (in fasting condition):
    - [redacted]
14. [redacted]
15. 4h Lactulose Breath Test (L-BT).
16. Dispense e-diary and instruct the subject on how to complete the electronic diary (e-diary), which encompasses the [redacted] These questionnaires should be completed by the subject on Day -1, before randomization and then weekly afterwards.

In addition, subjects will be instructed on how to record [redacted] of the day before randomization (Day -1) and daily throughout the study duration.

17. Verify inclusion and exclusion criteria.
18. Record any adverse events occurred since signing the ICF.
19. Schedule Randomization visit (V2, if eligible).

11.2.2 Visit 2 (Randomization Visit- V2)

The following procedures will be performed at the randomization visit:

Pre-Randomization:

1. [redacted]
2. [redacted]
3. [redacted]
4. [redacted]
5. Review and record any AEs that occurred since the previous visit.
6. Review and record any concomitant medications taken since the previous visit (attention will be paid to prohibited treatments).
7. Re-instruct the subject on how to complete the electronic diary (e-diary), which encompasses the [redacted]
8. e-diary review for eligibility according to inclusion **criterium n 4**. Questionnaires will be completed at the clinical center before randomization if not already completed by the subject.
9. Confirmation of inclusion and exclusion criteria.
10. [redacted] completion by the subject.
11. Randomization (if still eligible).
12. Blood collection (in fasting condition) sample (30 mins prior to drug administration) for proof for absorption.
13. Treatment dispensing (if subject has been randomized).

Dosing:

First investigational product dose will be administered under the supervision of the study personnel in the morning, on an empty stomach, with water. Time of dosing will be registered.

After the investigational product administration, the following procedures will be completed:

Post-dose:

14. [redacted]
15. [redacted]
16. [redacted]
17. Retrain the subject on how to use the electronic diary (e-diary), instructing to complete:
    - [redacted]
    - The subject will be instructed to record, starting from Day 1, investigational product intake information daily.
18. Schedule next visit and remind the subject to not to take the investigational product in the morning of the next visit, but to take the bottle to the clinic.

11.2.3 Visit 3 (V3; Day 28±1)

The following procedures will be performed at V3:

Pre-dose

1. COVID-19 rapid swab virus testing (to be performed on the same day of [redacted] and results to be available before starting [redacted]).
2. [redacted]
3. [redacted]
4. [redacted]
5. [redacted]
6. [redacted]
7. Blood collection (in fasting condition):
   - [redacted]
   - [redacted]
8. [redacted]
9. [redacted]
10. [redacted]
11. Review and record any concomitant medications taken since the previous visit (attention will be paid to prohibited treatments).
12. [redacted]
13. [redacted]
14. [redacted]
15. Review and record any AEs that occurred since the previous visit.
16. Review the e-diary to assess the completeness and retrain the subject as needed.
17. [redacted]

Dosing:

Investigational product dose will be administered under the supervision of the study personnel in the morning, on an empty stomach, with water. Time of dosing will be registered.

After the investigational product administration, the following procedures will be completed:

Post-dose:

18. [redacted]
19. [redacted]
20. Blood samples for analysis for proof of absorption: [redacted]
21. Collect all investigational product bottles and account investigational product.
22. Schedule next visit.

11.2.4 Start of Treatment (SOT) Visits: Visit 4 (V4, Day 43 ± 1), Visit 6 (V6, Day 85 ± 1) and Visit 8 (V8, Day 127 ± 1)

The following procedures/assessments will be completed at V4, V6 and V8:

Pre-dose

1. [redacted]

2. [redacted]
3. [redacted]
4. [redacted]
5. [redacted]
6. Blood collection (in fasting condition):
   - [redacted]
7. [redacted]
8. [redacted]
9. [redacted]
10. [redacted]
11. [redacted]
12. [redacted]
13. Review and record any AEs that occurred since the previous visit.
14. Review the e-diary to assess the completeness and retrain the subject as needed. Questionnaires not yet completed should be completed by the subject at the clinical center before investigational product administration
15. [redacted]
16. Dispense investigational product.

Dosing:

Investigational product dose will be administered under the supervision of the study personnel, in the morning, on an empty stomach, with water. Time of dosing will be registered. After the investigational product administration, the following procedures will be completed:

Post-dose:

17. [redacted]
18. Schedule next visit and remind the subject not to take the investigational product in the morning of the next visit, but to bring the bottle to the clinic, if the visit is scheduled at the clinical center; If the subject cannot go back to the clinical center for the next visit (EOT period), a home visit will be scheduled for the subject.

11.2.5 End of Treatment (EOT) Visits: Visit 5 (V5, Day 70 ± 1), Visit 7 (V7, Day 112 ± 1) and Visit 9 (V9, Day 154 ± 1)

The following procedures/assessments will be completed at V5, V7 and V9, **if the subject can attend the visit at the clinical center:**

Pre-dose

1. [redacted]
2. [redacted]
3. [redacted]
4. [redacted]

5. [redacted]
6. Blood collection (in fasting condition):
   - [redacted]
7. [redacted]
8. [redacted]
9. [redacted]
10. [redacted]
11. [redacted]
12. [redacted]
13. Review and record any AEs that occurred since the previous visit.
14. Review the e-diary to assess the completeness and retrain the subject as needed.
15. [redacted]

Dosing:

The last dose of the investigational product period will be administered under the supervision of the study personnel, in the morning, on an empty stomach, with water. Time of dosing will be registered.

After the investigational product administration, the following procedures will be completed:

Post-dose:

16. [redacted]
17. Collect all investigational product bottles and account investigational product.
18. Schedule next visit.

Home visit: In case the subject cannot attend the visit at the clinical center, this visit can be scheduled at the subject's home and a home nursing service will perform the visit. The clinical staff at the study center may attend the visit remotely. The data collected (source documents) during the home visits by the nurse will be transmitted on the same day to the clinical center in order to allow the study doctor to perform a preliminary clinical evaluation regarding safety. In case of home visits the subject will take the investigational product about 2 hours before the nurse's visit in the morning and the same procedures scheduled for the visit at the clinical center will be performed with the exception of physical examination that will not be performed. [redacted]

The nurse will collect the empty packs and the unused investigational product of the relevant period during Visit 5, Visit 7 and Visit 9

11.2.6 End of Follow-up (EFU; Day 169 ±1)

The following procedures/assessments will be completed during End of Follow up Visit, **if the subject can attend the visit at the clinical center:**

1. [redacted]
2. [redacted]

3. [redacted]
4. [redacted].
5. [redacted]
6. Blood collection (in fasting condition):
   - [redacted]
7. [redacted]
8. [redacted]
9. [redacted]
10. [redacted]
11. [redacted]
12. Review and record any AEs that occurred since the previous visit.
13. Review the e-diary to assess the completeness.

Home visit: In case the subject cannot attend the visit at the clinical center, this visit can be scheduled at home and a home nursing service will perform the visit. The clinical staff at the study center may attend the visit remotely. The data collected (source documents) during the home visits by the nurse will be transmitted on the same day to the clinical center in order to allow the study doctor to perform a preliminary clinical evaluation regarding safety. In case of home visits, the same procedures scheduled for the visit at the clinical center will be performed with the exception of physical examination that will not be performed.

11.2.7 Early Termination Visit (ETV)

The following procedures/assessments will be completed during the ETV. if the subject agrees to perform the visit and can attend the visit at the clinical center:

1. [redacted]
2. [redacted]
3. [redacted]
4. [redacted]
5. [redacted]
6. Blood collection (in fasting condition):
   - [redacted]
7. [redacted]

8. [redacted]
9. [redacted]
10. [redacted]
11. [redacted]
12. [redacted]
13. Review and record any AEs that occurred since the previous visit.
14. [redacted]
15. Review the e-diary to assess the completeness. Weekly questionnaires will be completed during the ETV.
16. Collect all investigational product bottles and account investigational product.

Home visit: In case the subject cannot attend the visit at the clinical center, this visit can be scheduled at home and a home nursing service will perform the visit. The clinical staff at the study center may attend the visit remotely. The data collected (source documents) during the home visits by the nurse will be transmitted on the same day to the clinical center in order to allow the study doctor to perform a preliminary clinical evaluation regarding safety. In case of home visit the same procedures scheduled for the visit at the clinical center will be performed with the exception of physical examination that will not be performed.

The nurse will collect the empty packs and the unused investigational product of the relevant period during the ETV.

11.2.8 Early Switch Visit (ESV; anytime between Day 1 and Day 126)

The following procedures/assessments will be completed in case of ESV occurrence (see section 6 Overall Study Design and Plan): The ESV will be considered as Day 1 of SOT-2 or SOT-3 or SOT-4, as appropriate.

Pre-dose

1. [redacted]
2. [redacted]
3. [redacted]
4. [redacted]
5. [redacted]
6. Blood collection (in fasting condition):
   - [redacted]
   - [redacted]
7. [redacted]
8. [redacted]
9. [redacted]
10. [redacted]
11. [redacted]
12. [redacted]

13. Review and record any AEs that occurred since the previous visit.
14. Review the e-diary to assess the completeness and retrain the subject as needed. Weekly questionnaire will be completed during this visit. Questionnaires not yet completed should be completed by the subject at the clinical center before investigational product administration
15. [redacted].
16. Collect all investigational product bottles and account investigational product (if applicable).
17. Dispense investigational product.

Dosing:

Investigational product dose will be administered under the supervision of the study personnel, in the morning, on an empty stomach, with water. Time of dosing will be registered. After the investigational product administration, the following procedures will be completed:

Post-dose:

18. [redacted]
19. Schedule next visit and remind the subject not to take the investigational product in the morning of the next visit, but to bring the bottle to the clinic, if the visit is scheduled at the clinical center; If the subject cannot go back to the clinical center for the next visit (EOT), a home visit will be scheduled for the subject.

After this visit, the subsequent visits will follow the relevant sequential number (see Study Schedule of Assessments).

11.2.9 Contingency activities in case of travel restrictions due to SArS-COV2 pandemic

In case the subject or the nurse cannot travel to attend/perform any of the scheduled visit due to travel restrictions due to sanitary emergency, the clinical site will implement all the procedures able to maintain the subject in the study, always taking in primary consideration the safety of the subject. Tele-medicine visits could be implemented in order to collect as much as possible information, as foreseen by the specific study schedule, and if the case will organize the investigational product delivery at the subject's home, throughout a dedicated courier able to trace the transportation and guarantee the appropriate transport condition.

11.3 LABORATORY TESTS

During the study period, it can be estimated that a total volume of up to approximately 300 ml of blood will be drawn for each subject.

[redacted]

- [redacted]

Additional safety laboratory tests may be performed, as required for extra study assessments.

[redacted]

[redacted] will be performed locally, and results assessed locally at the clinical center or samples will be sent to [redacted], Bologna, Italy for analysis and result assessment.

Handling of Biological Samples

Blood and urine samples for hematology, clinical chemistry, and urinalysis collected during the home visits will be handled according to a centralized laboratory procedure.

Blood and urine samples for hematology, chemical chemistry, and urinalysis collected during the visits performed at the clinical center will be handled according to the local clinical laboratory procedures.

Plasma samples for proof of absorption will be stored at -20°C/-80°C before shipment to the bioanalytical laboratory. Additional details on the collection, processing, storage and shipment of these samples can be found in a "Proof of absorption sample handling laboratory manual".

Samples are shipped within qualified shippers (these range from Ambient to dry ice) that meet and maintain the storage temperature for the samples whilst in transit.

11.4 SUBJECT DIARY

Diary data will be collected and reviewed throughout the course of the study electronically until the end of the follow up period (2-weeks after last dose). The subjects will collect weekly information regarding [redacted]. Investigational product intake and time will be recorded daily during the treatment periods. [redacted] daily during all the study duration (4-week treatment periods, 2-week wash out periods and 2-week follow up period).

This e-diary is integrated in the electronic data capture system utilized for the collection of all the other study data. The clinical site staff will review periodically the e-diary data collected and in case of missing data will be responsible for reminding the subject about appropriate completion. The diary data collected during the different study periods will be also revised by the clinicians in occasion of each scheduled study visit.

11.5 UNBLINDING OF RANDOMIZATION IN CASE OF EMERGENCY

In accordance with the double-blind design, both the Investigator and the subject will be unaware of the treatment actually being dispensed in each case. The overall randomization code will be broken at the study end. This will occur once Blind Data Review Meeting has been held and clinical database has been locked.

During the study, the blind should be maintained for persons responsible for the ongoing conduct of the study (such as management, Monitors, Investigators).

ONLY the PV-Clinical Safety of the Sponsor may unblind, and ONLY for safety reasons upon Clinical Safety Manager and EU-QPPV decision or for regulatory purposes (when SUSAR has to be submitted as expedited to RAs and ECs).

The PV-Clinical Safety of the Sponsor can break the blind using the online tool embedded in the e-CRF system.

The Investigator, ONLY in the case of an emergency safety issue, can unblind a subject's treatment assignment, when knowledge of the study medication is essential for the clinical management or welfare of the subject. Most often, investigational treatment discontinuation and knowledge of the possible treatment assignments are sufficient to treat a study subject who presents with an emergency condition. The investigator will inform the subject how to contact their backup in cases of emergency when he/she is unavailable. Should a situation arise where unblinding is required, the investigator at that site may perform immediate unblinding without the need for communication with the Sponsor.

As a rule, the Investigator can break the blind using the online tool embedded in the e-CRF system.

However, in case of system malfunction, the Investigator can at any time open the specific treatment envelope assigned to the subject.

After the emergency blind-breaking, the Investigator must notify the unblind, as soon as possible, to the Sponsor Clinical Safety Manager without sharing the treatment information to other people involved in the study (see contact details in section 15 Safety Aspects).

The investigator must record the date and time the blind was broken and the reason that treatment assignment information was required.

The blind should be maintained for persons responsible for the ongoing conduct of the study (such as sponsor and CRO representatives, monitors, sub-investigators); if not the subject must be withdrawn from the study and procedures accompanying withdrawal are to be performed.

In cases where there are ethical reasons for the subject to remain in the study, the Investigator must obtain specific approval from the Sponsor for the subject to continue in the study.

12 EFFICACY AND SAFETY ASSESSMENT

12.1 ASSESSMENT OF EFFICACY

During the present study the assessment of the efficacy of velusetrag versus placebo will be based on collection of the [redacted] using a subject's e-diary as described below.

In addition, [redacted] will be also assessed during the study, according to the Study Schedule of Assessments in order to assess the treatment impact on subject's nutritional status. [redacted]

The [redacted] will be evaluated at the screening visit as well as at the end of the first 4-week treatment period in order to assess the investigational product impact on [redacted].

12.1.1 Primary Efficacy Parameters

The primary efficacy parameter will be the change in weekly global gastrointestinal symptoms average index score from start to the end of each treatment period.

The weekly global gastrointestinal symptoms average index score is obtained by averaging the scores for each of the 4 symptoms assessed weekly: abdominal pain, bloating, nausea and vomiting.

Symptom severity will be graded 0 – 4 according to its influence on subjects' usual activity, as previously described (Barbara, 2004) (0 – Absent; 1 - Mild (not influencing usual activities); 2 - Moderate (diverting from, but not urging modification of, usual activities); 3 - Severe (influencing usual activities markedly enough to urge modifications; 4 - Extremely severe (precluding daily activities).

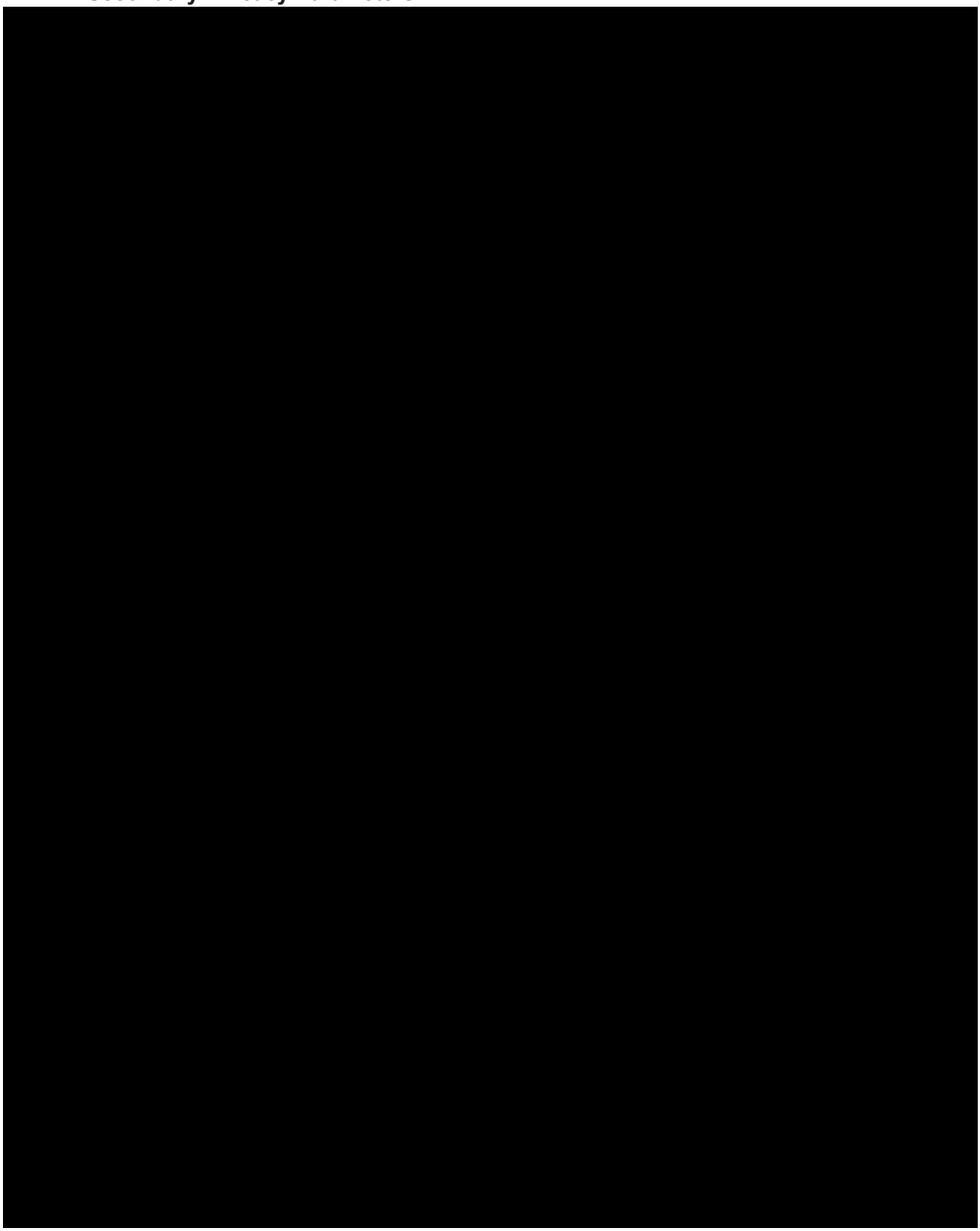
Predominant symptoms of CIPO include nausea and vomiting, associated with weight loss, when the functional derangement primarily affects the upper gastrointestinal tract, while diffuse abdominal pain, abdominal distension and constipation are suggestive of a more distal involvement of the gut (Antonucci, 2008)

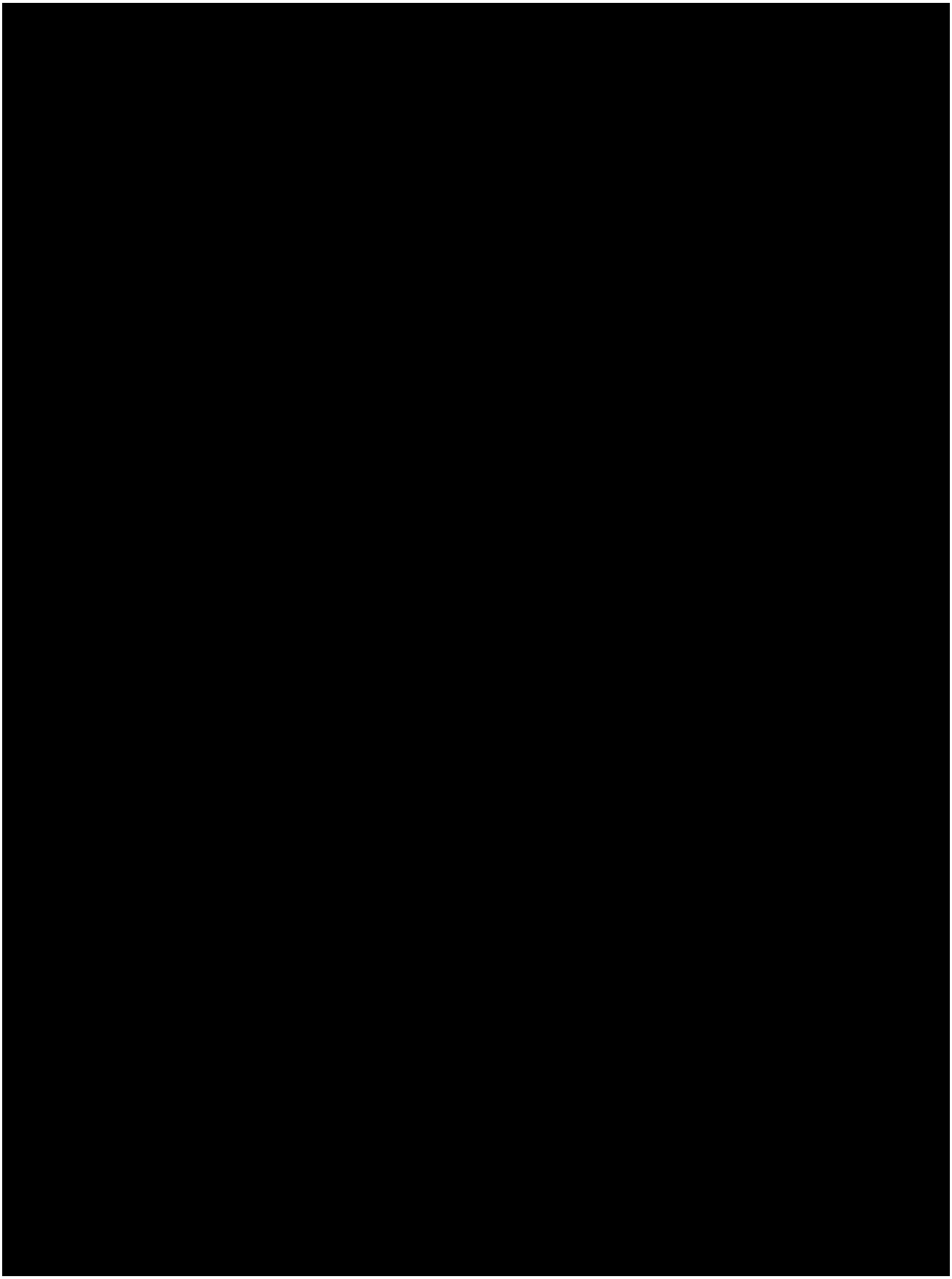
In order to assess the efficacy of velusetrag on major gastrointestinal CIPO symptoms, subjects will be asked to score severity of their symptoms: abdominal pain, bloating, nausea and vomiting over the last week (Appendix 2).

Subjects will complete the gastrointestinal symptom severity questionnaire, including 4 questions, to assess the following symptoms of CIPO: abdominal pain, bloating, nausea and vomiting during the study, starting on day -1 and then weekly during each treatment period and wash out period and

during the 2-week follow up period. Gastrointestinal symptom severity questionnaire will be also completed in case of ETV or ESV.

12.1.2 Secondary Efficacy Parameters





[redacted]

12.3 Appropriateness of Measurements

Not Applicable

13 PHARMACODYNAMIC ENDPOINTS

13.1 Description and Time Points/Intervals for Measuring the Pharmacodynamic Endpoint(s)

Not Applicable

14 PHARMACOKINETIC ENDPOINTS

Not Applicable

14.1 Blood Sampling for Proof of Absorption

[redacted]

Actual collection time will be recorded for each blood collection. Blood samples for plasma concentration analysis of velusetrag and the active metabolite, THRX-830449, will be collected, processed and stored at the study site and shipped for analysis at a bioanalytical laboratory.

Blood will be drawn using an indwelling intravenous cannula inserted into a forearm vein.

The blood sample will be collected with a 4-mL vacutainer (K2EDTA). The dead space of the cannula will be flushed using a small amount of sterile saline following each blood sample to ensure cannula patency. Prior to withdrawal of blood sample, 2 mL of fluid will be drawn and discarded.

[redacted]

Refer to the relevant Proof of absorption sample handling laboratory manual and Appendix 1: Collection and storage of samples for proof of absorption for specific information on the collection, storage and processing of plasma samples.

15 SAFETY ASPECTS

15.1 ADVERSE EVENTS

15.1.1 Definition of Adverse Events

An AE is any untoward medical occurrence in a patient or clinical trial patient administered a medicinal product which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of an investigational product, whether or not related to the investigational product.

For recording purpose, the Investigator must consider that:
An AE does include any:

- Symptoms associated with disease not previously reported by the patient.
- Exacerbation of pre-existing illness.
- Increase in frequency or intensity of a pre-existing episodic event or condition.
- Condition detected or diagnosed after investigational product administration even though it may have been present prior to the start of the study.
- Continuous persistent disease or symptoms present at baseline that worsen following the start of the study,

An AE does not include a/an:

- Medical or surgical procedure (e.g., surgery, endoscopy, tooth extraction, transfusion); the event that leads to the procedure is an AE.
- Planned medical or surgical procedures.
- Pre-existing diseases or conditions present or detected at the start of the study and that do not worsen during the study.
- Situations (e.g., hospitalization for cosmetic elective surgery, social and/or convenience admissions); where an untoward medical occurrence has not occurred.
- Signs, symptom and/or altered laboratory values identified as study endpoints, unless more severe than expected for the patient's condition.
- **Symptoms often associated with the disease under study** (other than those mentioned above) that are consistent with the patient usual clinical course unless the symptom(s) meet(s) the criteria for "serious".
- Overdose of concurrent medications without signs or symptoms.

Observations matching an adverse event after ICF is signed but occurring when the study medication is not administered (during the screening/washout/follow-up period), by definition represent AEs, and should be recorded as such. In the report these observations will be described separately.

15.1.2 Definition of Serious Adverse Events

A SAE is any untoward medical occurrence in a patient or clinical trial patient administered a medicinal product which does not necessarily have a causal relationship with this treatment. A SAE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of an investigational product, whether or not related to the investigational product and resulting in:

- Death.
- Life-threatening.
- In-patient hospitalization or prolongation of existing hospitalization.
- Persistent or significant disability/incapacity.
- Congenital anomaly/birth defect.

- Other event(s) considered important medical event.

Examples of other events which could be considered medically important and reported as serious are: intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization; or development of drug dependency or drug abuse.

Clarifications:

- "Occurring at any dose" does not imply that the patient is receiving investigational product.
- Life-threatening means that the patient was, in the view of the Investigator, at immediate risk of death from the event as it occurred. This definition does not include an event that, had it occurred in a more severe form, might have caused death.
- Hospitalization for elective treatment of a pre-existing condition that did not worsen during the study is not considered an SAE.
- Complications that occur during hospitalization are AEs. If a complication prolongs hospitalization, the event is a SAE.
- "In-patient" hospitalization means the patient has been formally admitted to a hospital for medical reasons. This may or may not be overnight. It does not include presentation at a casualty or emergency room.
- With regard to important medical events, medical and scientific judgement should be used in deciding whether prompt reporting is appropriate in this situation.

An unexpected adverse event is an AE whose nature, severity or outcome is not consistent with the information given into the reference document as defined in the study protocol.

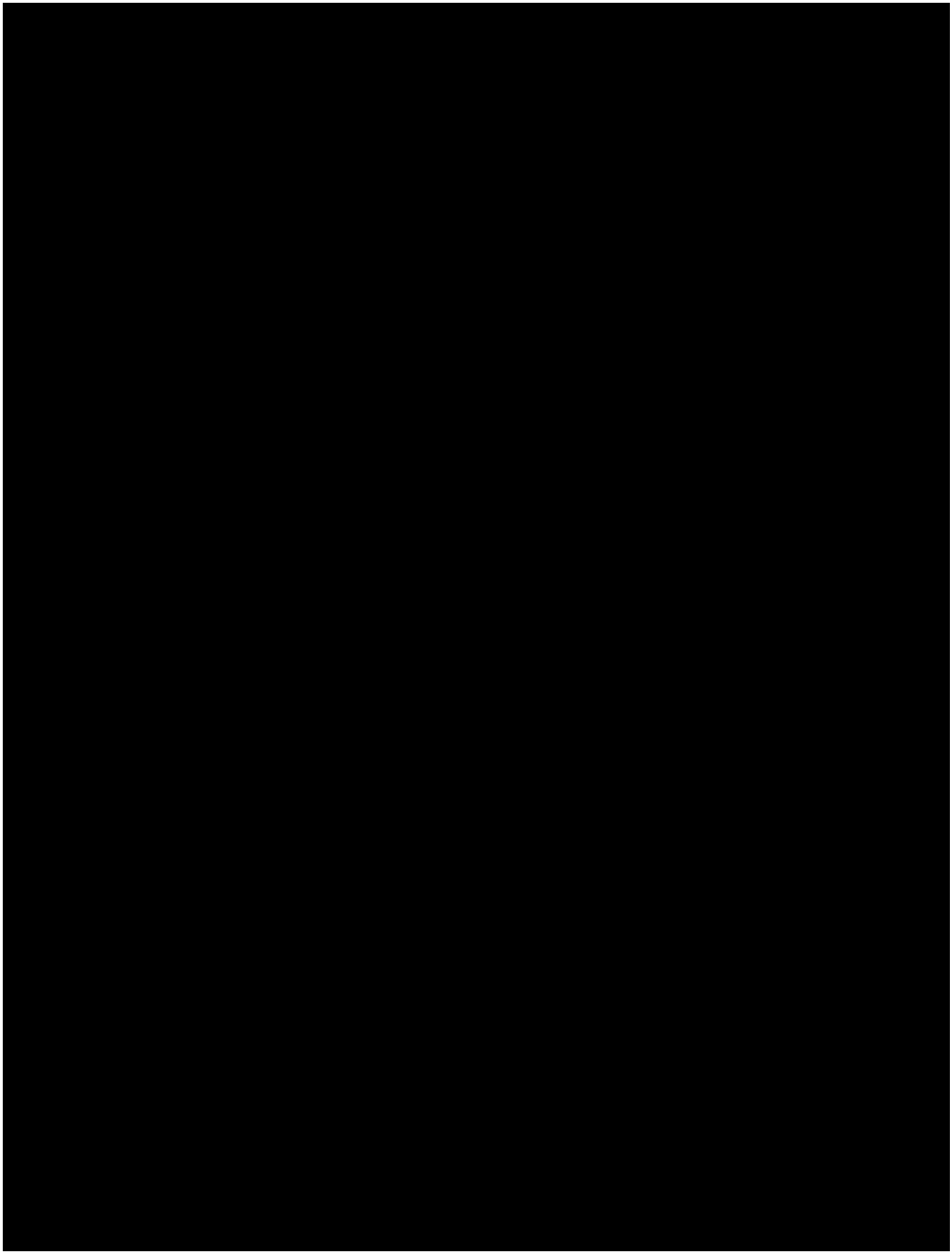
Expected serious adverse reactions should be considered always unexpected if:

- ➢ have an unexpected outcome (e.g., a fatal outcome).
- ➢ have an increase in the rate of occurrence, which is judged to be clinically important.

Expectedness evaluation is performed by the Sponsor based on Reference Safety Information (RSI).

[redacted]

[redacted]



[redacted]

15.1.8 Prompt Reporting of SAE to the Sponsor

Any serious adverse event occurring during the study, once the Investigator determines that the event(s) meet the protocol definition of a SAE, regardless whether related or not to the investigational drug, must be reported immediately (i.e. within 24 hours) to the Sponsor (or its designee).

Information about all SAEs will be reported using the SAE tool of the eCRF. In case of technical difficulties, SAE notification can be carried out filling paper SAE Report form and it must be mailed or faxed using the following contact details:

E-mail: [redacted]

or

Fax: [redacted]

One "SAE Report form" should be used for each SAE. However, if at the time of initial reporting, multiple SAEs are present that are temporally and/or clinically related, they may be reported on the same "SAE form" preferably as a diagnosis.
The SAE form must be written in easily readable reading way. It must be completed as thoroughly as possible with all available details of the event, signed by the Investigator (or appropriately qualified designee), and reported immediately to the Sponsor (within 24 hours from first awareness of the event).
If the Investigator does not have all information regarding the event, he/she will not wait to receive additional information before compiling the form and notifying the event to the Sponsor, although the following minimal information is required:

The following minimal information is required as initial report:

- Name, affiliation, telephone and fax number of the reporting Investigator
- Investigational product(s)
- Study code
- Study Centre number
- Patient identification number, sex and age (years)
- Last investigational drug administration
- Description of the adverse event,
- Measures taken if any
- Causal relationship by the investigator

The Investigator will always provide his/her assessment of causality at the time of the initial report.

The additional information as soon as available will be recorded in a separate SAE form completed in every part that will be sent as a follow-up report.

If follow-up information obtained subsequently leads the investigator to change the assessment of causality, the SAE form may be appropriately amended, signed and dated, and resubmitted to the Sponsor as follow up report.

SAEs will be followed-up until resolution or the patient is lost to follow up. SAEs still present at the end of the study period and for the subsequent 30 days, must be followed until an outcome is determined or the patient is lost to follow up.

If a patient dies during participation in the study or during a recognized follow-up period, the Investigator should send to the Sponsor, together with SAE Form, any other available post-mortem information, including autopsy and histopathology.

The Sponsor must be informed of any code breaking (see Section 11.5 Unblinding of randomization

in case of emergency).

In accordance with local IEC requirements, the Investigator must also notify IEC of any SAEs according the guidelines of the Ethics Committee.

15.1.9 Regulatory Reporting Requirements for SAEs

The Sponsor has a legal responsibility to notify both the local regulatory authority and other regulatory agencies about the safety of a drug under clinical investigation.

Prompt notification of SAEs by the Investigator to the appropriate project contact for SAE receipt is essential so that legal obligations and ethical responsibilities towards the safety of other patients are met.

The Investigator must promptly report all SAEs to the Sponsor or his designee, in accordance with the procedures detailed in section 15.1.8 Prompt Reporting of SAE to the Sponsor.

The Investigator, or responsible person according to local requirements, must comply with the applicable local regulatory requirements related to the reporting of SAEs to regulatory authorities and the IEC.

15.1.10 Post study AEs and SAEs

A post-study AE/SAE is defined as any event that occurs outside the AE/SAE detection period as defined previously.

Investigators are not obliged to actively seek AEs or SAEs after the clinical study termination. However, if the Investigator becomes aware of any SAE, including death, at any time after a patient has been discharged from the study and he/she considers the event reasonably related to the Investigational Product, the Investigator should promptly notify the Sponsor, according to the process explained in section 15.1.8 Prompt Reporting of SAE to the Sponsor.

Serious and non-serious AEs will be recorded from the ICF signature to the Final/Early Termination Visit regardless of whether the participant is on treatment.

SAEs will be recorded and reported as appropriate and followed-up until resolution or stabilization. Non-serious AEs will be recorded as appropriate and followed-up until Final/Early Termination Visit.

15.2 WARNINGS AND PRECAUTIONS

Clinical trial exclusion criteria must be followed.

16 STATISTICAL PLAN AND DETERMINATION OF SAMPLE SIZE

16.1 DETERMINATION OF SAMPLE SIZE

The sample size is based on the main analysis (t-test) of the primary endpoint that consists of the differences between velusetrag and placebo within each paired treatment cycle (2 per subject).

A total of 16 subject [redacted] will be randomized leading to 32 pairs. Accounting for 25% dropouts/missing pairs, 18 pairs should be available for the primary analysis on the subgroup of subjects with history of benefit from [redacted] or naïve to [redacted], and 24 pairs should be available for the analysis on the overall population

With a two-sided significance level of 5%, the planned sample size will lead to power levels above 80% to detect effect size (ES) above 0.7 (see table 16.1 below).

Table 16.1 Power for different values of effect size and number of pairs

Power for different values of Effect Size* and number of pairs			
Number of pairs	ES: 0.6	ES: 0.7	ES: 0.8
18	67%	80%	89%
24	80%	91%	96%
28	86%	94%	98%
32	91%	97%	99%

** More than medium to moderate effect size (Cohen, 1988)*

Estimates obtained using SAS PROC POWER

If based on a blinded review of the data, the rate of missing pairs is found to be higher the sample size might be increased by the Sponsor.

16.2 DEFINITION OF STUDY POPULATIONS

Study populations definitions are provided below. The numbers of subjects in each population and reasons for exclusion will the summarized.

All decisions on populations will be taken during the Data Review Meeting and will be detailed in the relevant documents.

16.2.1 Screened Population

The Screened Population is defined as the set of all subjects who provided informed consent.

A screening failure is defined as a subject who has not been randomized to treatment.

16.2.2 Safety Set

The Safety Set (SS) is defined as the set of subjects treated (i.e.: having received at least one [redacted] of investigational product).

Analysis on the SS will be performed according to the actual treatment received.

16.2.3 Full Analysis Set (FAS)

The Full Analysis Set (FAS) is defined as the set of all subjects randomized and treated.

The Modified-Full Analysis Set 1 (mFAS1) is defined as the set of all subjects responder/naïve to [redacted] randomized and treated who reported data on the primary endpoint at least once during a velusetrag treatment period and at least once during a placebo treatment period.

[redacted]

Following the intent-to-treat principle analysis on the FAS, mFAS1 [redacted] will be performed according to the treatment and stratum assigned at randomization.

16.2.4 Per Protocol Set (PPS)

The Per Protocol Set (PPS) is defined as the set of all subjects in the mFAS1 who fulfil the study protocol requirements in terms of compliance to treatment and collection of primary efficacy data and with no major deviations that may affect study results.

Examples of deviations are:

1. Intake of study treatment other than the one assigned by the IWRS
2. Lack of compliance overall defined by percent compliance (number of [redacted] taken divided number of planned [redacted] *100) below 80%.

In addition, if within a period, the compliance is below 80%, the primary endpoint data of this period will be excluded from the analysis. E.g., if compliance data are 100%, 100%, 60%, 60%, the subject is included in the PPS (overall compliance at 80%) but the observations of the third and fourth period are excluded.

The complete list of deviations will be defined in a specific protocol deviations handling plan and a blind data review meeting will occur prior to unblinding. The decisions for exclusion of subjects from the PPS will be taken in a blinded manner and documented.

Subjects receiving a study treatment different from the randomized one will be excluded from the PPS. Subjects randomized to a wrong stratification factor level will not be excluded from the PPS and will be analyzed according to the actual stratum (not the randomized one).

16.2.5 Disposition, Demographic and Other Baseline Variables

The number of subjects screened, failed screening (and reason for screening failure), randomized, treated and completing the study treatment periods (and reason for not completing) will be presented in frequency tables by treatment sequence and stratum.

The number of subjects in each analysis population (SS, FAS, mFAS1, [redacted] and PPS) will be summarized by treatment sequence and stratum.

All major protocol violations will be presented as a frequency table by treatment sequence.

Baseline values are the last recorded values collected prior to treatment initiation.

Subject demographics, medical history and disease characteristics and others baseline characteristics measured before randomization will be summarized descriptively by treatment sequence on the FAS. Selected summaries will be done by treatment sequence and stratification factors (details to be provided prospectively in the SAP).

Selected baseline data will be also summarized on the mFAS1 and PPS.

16.3 STATISTICAL AND ANALYTICAL PLANS

A detailed Statistical Analysis Plan (SAP) analysis plan will be developed and approved prior to unblinding.

Descriptive statistics per sequence, visit/period, and stratum will be presented as number of observations, number of missing observations, mean, standard deviation, median, minimum and maximum and 25th and 75th percentiles for continuous variables; frequency distribution (n, %) for categorical variables.

Graphical visualization for each subject overtime, including all assessments, will be used for the evaluation of efficacy and safety.

All efficacy analyses have the objective to show the superiority of velusetrag over placebo. All statistical analysis will be two-sided at a nominal level of 5%. Confidence intervals will be set at the 95% level.

Analysis of the primary efficacy endpoints will be performed on the mFAS1, [redacted] FAS and PPS. Results on the mFAS1 will be considered primary. For secondary endpoints, analysis will be performed on selected populations. Details will be provided in the SAP.

Safety analysis based on SS will be presented by actual treatment received in each period.

Most recent version of appropriate coding dictionaries will be used.

SAS software (Version 9.3 or subsequent) will be used for all statistical analyses.

16.3.1 Primary Efficacy Endpoints and Statistical Model of Analysis

The primary endpoint is the change in weekly global gastrointestinal symptoms average index score from pre-treatment to the end of each treatment period.

The average weekly global gastrointestinal symptoms index score is obtained by averaging the scores for each of the 4 symptoms assessed weekly: abdominal pain, bloating, nausea and vomiting. The use of the average of the 4 symptom scores to obtain a global score is similar to the scoring methodology applied for largely used patient questionnaires such as PAGI-SYM (Patient Assessment Gastrointestinal Symptoms) and ANMS-GCSI-DD (American Neurogastroenterology and Motility Society – Gastroparesis Cardinal Symptom Index – Daily Diary).

Rating of symptoms (i.e. abdominal pain, bloating, nausea, vomiting) is using a recall period of 7 days and a Likert scale with the following categories:

0 – Absent

1 - Mild (not influencing usual activities)

2 - Moderate (diverting from, but not urging modification of, usual activities)

3 - Severe (influencing usual activities markedly enough to urge modifications)

4 - Extremely severe (precluding daily activities)

The average score ranges thus between 0 and 4 with lower scores representing a better health for the subject. [redacted]

The primary analysis is performed on the mFAS1. [redacted]

[redacted]

A stratified t-test will be used to analyze the treatment effect. For the primary analysis on the mFAS1, missing pairs will not be imputed (each subject contributing to at least one pair). [redacted]

[redacted]

[redacted]

16.3.2 Secondary Efficacy Endpoints and Statistical Model of Analysis

Continuous efficacy endpoints will be summarized and analyzed in the same way as the primary endpoint. In case of deviation from normality, non-parametric method will be considered. Binary outcome will be compared between treatments with an odds ratio and confidence interval using logistic model and Cochran-Mantel-Haenszel test. Poisson modelling will be used for count data. Full details will be provided in the SAP.

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

16.3.4 Statistical Analysis of Adverse Events

Adverse events starting on or after the first intake of investigational product are considered treatment emergent AEs (TEAEs). Verbatim terms for AEs will be coded using the most current version of MedDRA coding.

[redacted]

- [redacted]
- [redacted]
- [redacted]
- [redacted]

An overview of the number and percentage of subjects with at least one TEAEs listed above will also be produced.

In addition, events will be classified as velusetrag emergent if the last treatment taken before the start of the adverse event was velusetrag, or placebo emergent if the last treatment taken before the start of the adverse event was placebo. All summaries described above will be done according to this classification.

16.3.5 Handling of Missing and Incomplete Data

Primary endpoint

Missing pairs (treatment difference velusetrag minus placebo) can occur when at least one of the following is missing:

- Pre-treatment velusetrag value missing
- End of treatment velusetrag value missing
- Pre-treatment placebo value missing
- End of treatment placebo value missing

If the pre-treatment value is missing for a period, the average of the values of other subjects starting this period will be used.

If, within a period, intermediate values (from pre-treatment value up to the end of a period) are available, the last observation carried forward will be used to impute the end of treatment value. ESV will be considered as SOT-2 or SOT-3 or SOT-4, as appropriate.

The above approach will be used for the FAS and as sensitivity analysis for mFAS1, PPS [redacted]

Other endpoints:

Missing continuous efficacy endpoints will be using the same approach as the primary endpoint.

For binary outcomes, missing data will be considered as failures.

Details will be provided in the SAP.

16.4 Additionally Planned Statistical Analyses

16.4.1 Interim Analyses and Data Monitoring Committee

No interim analysis data monitoring committee have been planned.

16.4.2 Multicenter study

Up to 5 centers will be initiated to enroll 16 subjects. Given the limited number of subjects expected per center, the effect of center will not be included in a statistical analysis model, nor in subgroup analysis.

16.4.3 Examination of Subgroups

The analysis of the primary endpoint will also be done by stratum (if sample size allows).

17 DOCUMENTATION, RECORD ACCESS AND ARCHIVING

17.1 DOCUMENTATION OF ESSENTIAL DOCUMENTS/SUPPLEMENTS AT STUDY CENTRE DURING THE TRIAL

An “Investigator Study File” will be established at the study center at the beginning of the trial. The investigator/institution must maintain the trial documents as specified in the Guideline for Essential Documents for the Conduct of a Clinical Trial (ICH E6 (R2) - EMA/CHMP/ICH/135/1995) and the applicable regulatory requirement(s).

17.2 SCREENING/ENROLLMENT LOG

The date of screening of all subjects fulfilling the inclusion requirements before any study related action is taken will be documented on the “Screening/Enrolment Log”.

The Investigator will document that the subject satisfies the inclusion and exclusion criteria, and therefore if the subject is enrolled or is screening failure.

The screening list must not permit the identification of subjects. The original list will be handed to the sponsor, and a copy will be archived in the "Investigator's Study File"

With this list the investigator documents the relationship between the general patient population in the specific indication and the study population.

17.3 DOCUMENTATION OF SUBJECTS’ PARTICIPATION

The investigator must record all subject identification data (full name, initials, date of birth, screening number, randomization number, hospital admission-number and date of admission [if relevant], study termination date) for all subjects who have given informed consent - whether the subject has received any investigational product(s) or not - in the "Confidential Subject Identification List". The subject identification list must allow the definite identification of subjects who take part in this study. The subject identification list is kept by the investigator in his “Investigator Study File” and archived according to the requirements of the applicable national/international regulations, in particular, in compliance with the EU Regulation 2016/679.

The investigator should inform the subject's General Practitioner of the subject's participation in the trial, if the subject agrees.

The clinical study personnel at the clinical center will provide the subject personal Information to the home nursing service to arrange home visits and to the travel agency for accommodation and travel services when the subjects need to attend clinic visits.

17.4 DATA PROTECTION

Personal data are securely stored to prevent unauthorized access, disclosure, dissemination, alteration or loss of information and unauthorized personal data processing. Access to personal information is restricted so that only personnel who are required to access personal data as part of their job role can do so. All personnel who access personal information are bound by a duty of confidentiality.

Technical arrangements surrounding the electronic storage and use of data are as follows:

Computers storing electronic personal data are protected by antivirus software and the network on which computers are linked are protected by industry grade firewalls

Electronic access of data is limited according to user roles

- All data are stored on password protected computers

Organizational arrangements are as follows:

Manual files of personal data are stored within locked cabinets that can only be accessed by authorized personnel

- Data security and/or confidentiality provisions are utilized in agreements with third parties

- Internal audit and compliance functions provide regulatory oversight

The identification data of the volunteers will be replaced in the records with appropriate codes; therefore they will be pseudonymized and cannot be traced back to the individual except by the investigator, who will be responsible for keeping the decoder key safely from unauthorized access.

The Clinical Centre and the Sponsor will be acting as Data Controller in respect of the personal data of the study subjects collected in connection with the study, and shall act in accordance with the relevant data protection laws in relation to the collection and processing of those personal data.

The study subjects' pseudonymized personal data shall be collected and processed for the purposes of the study and may also be added to research databases and used in the future by the Sponsor and its affiliates for certain additional clinical research, for product regulation and safety reporting purposes and for ensuring compliance with legal requirements. The study subjects' pseudonymized personal data may be processed for such purposes by other parties including: the Sponsor's affiliates and licensing partners, its business partners, regulatory agencies and other health authorities, and ECs. The Sponsor is a company that resides in Italy and is subject to current European regulations also for what concerns the Data Protection.

Therefore, the Sponsor, as Data Controller shall give the subject a Privacy Notice, informing on how the data will be processed in compliance with article 13 of the European Regulation 2016/679.

The information provided shall include:

A. the identity and the contact details of the controller and, where applicable, of the controller's representative.
B. the contact details of the data protection officer, where applicable.
C. where the processing is based on point (a) of Article 6(1) or point (a) of Article 9(2), the existence of the right to withdraw consent at any time, without affecting the lawfulness of processing based on consent before its withdrawal.
D. the purposes of the processing for which the personal data are intended as well as the legal basis for the processing.
E. the recipients or categories of recipients of the personal data, if any.
F. where applicable, the fact that the controller intends to transfer personal data to a third country or international organization and the existence or absence of an adequacy decision by the Commission, or in the case of transfers referred to in Article 46 or 47, or the second subparagraph of Article 49(1), reference to the appropriate or suitable safeguards and the means by which to obtain a copy of them or where they have been made available.
G. the period for which the personal data will be stored, or if that is not possible, the criteria used to determine that period.
H. the existence of the right to request from the controller access to and rectification or erasure of personal data or restriction of processing concerning the data subject or to object to processing as well as the right to data portability.
I. the existence of the right to withdraw consent at any time, without affecting the lawfulness of processing based on consent before its withdrawal.
J. the right to lodge a complaint with a supervisory authority.

17.5 PERSONAL DATA BREACH

Each incident and violation of personal data must be communicated to the Sponsor within 48 hours at the latest and, in any case, without undue delay after becoming aware of a personal data breach.

Notification must contain at least the following information:

- a description of the nature of the data breach including the following elements:
- the categories and the approximate number of data subjects involved.
- the categories and the approximate number of personal data involved.
- the data breach impact level.
- the safety measures implemented.
- the name and contact details of the Data Protection Officer or other contact point from which further information may be obtained.
- the name of the Data Protection Officer or appropriate privacy office of any sub-responsible parties involved in the processing that can be contact for further information.
- a description of the measures adopted and/or the measures that intends to adopt to remedy the data breach, including, where it is appropriate, measures to mitigate the possible negative consequences.
- a description of the probable consequences of the personal data breach.
- any further information necessary to notify the competent control Authority of the breach.

If it is not possible to provide all this information simultaneously, the information may be communicated at a later moment, without undue delay.

Every party shall be obliged to notify any case of data breach, regardless of its degree of severity. Any assessment of the seriousness of a possible data breach is assessed exclusively by the sponsor.

In any case, every party ensures the maximum collaboration in order to examine all the necessary and useful aspects to identify the causes and the consequences of the breach, also in terms of impact on adverse events. Once the reasons for the breach have been defined, the parties shall implement as quickly as possible all physical and/or logical and/or organizational security measures, designed to prevent the occurrence of a new breach of the same type as that occurred.

17.6 SOURCE DOCUMENTS

Source documents consist of inpatient hospital charts, clinic notes, outpatient records, original test results, laboratory data, worksheets, drug accountability records, consent forms, subject's diaries, etc. Source documents must be available for review and inspection during on-site monitoring of the study by the Sponsor, its designees, IRB/IEC, and/or appropriate regulatory authorities.

17.7 SUBJECTS' RECORDS

Critical data collected for this clinical study will be verified against the source documents. This will include critical entries in subject Case Report Forms (CRFs) and laboratory data.

All required study data must be entered in the e-CRF created for the study. The investigator shall ensure that all data from participant visits are promptly entered into the eCRFs in accordance with the specific instructions given. The investigator must sign each e-CRF to verify the integrity of the data recorded.

It is the Investigator's responsibility to ensure that all relevant data are recorded in the subject's medical file, for example medical history/concomitant diseases, date of study enrollment, visit dates, results of examinations and AEs. In case of home visits performed by a nurse, all the collected data will be reported by the study nurse on a specific study dedicated home visit chart; this chart will be delivered as soon as possible to the study site in order to be entered in the e-CRF.

The investigator must maintain source documents, such as laboratory reports, endoscopy or radiology reports, X-rays, ECGs, consultation reports, and complete medical history and physical examination reports. All information in the e-CRF must be traceable to the source documents in the participant's file.

The investigator(s)/institution(s) will permit trial-related monitoring, audits, IRB/IEC reviews, and regulatory inspections, and provide direct access to source data/documents.

If an electronic data system is used to collect source data at investigational site, this system must be a validated system and the monitor should have a dedicated access authorizing him/her to read the medical records containing the study data only. If this is not the case, the investigator must print-out, sign and date the subjects' data for data verification purposes at definite times (monitoring, audits and inspections). The investigator must assure in writing that the data on the printouts are identical to the electronic data and are complete.

These printouts are to be archived in the "Investigator's Study file".

17.8 Case Report Forms

An e-CRF is used to record clinical trial data and is an integral part of the trial and subsequent reports. They must reflect subject's status at each phase during the trial.

All information requested on the e-CRF should be entered. If one is not available or is not applicable, this must be indicated. A User Manual with detailed instructions about e-CRF filling in will be provided to each Investigator and a specific training will be performed.

Subjects must not be identified on the e-CRF by name, but by subject's identification number. The e-CRF is specifically designed to record the data required by this protocol.

The eCRFs system will foresee an audit trail allowing the tracking of all the changes and corrections performed to the eCRFs, with the indication of date and author of entry and of correction.

17.9 Data Management

The contract research organization (CRO) Data Management will identify and implement the most effective data acquisition and management strategy for the clinical trial protocol and deliver datasets which support the protocol objectives. Subject's data will be entered into a defined eCRFs and then combined with data provided by other sources (e.g., electronic Subject Diary, Central Lab, central ECG Lab, etc.). Clinical data management will be performed in accordance with CRO standards and data cleaning procedures with the objective of removing errors and inconsistencies in the data, which would otherwise impact on the analysis and reporting objectives, or the credibility of the Clinical Study Report. Adverse events, Medical History and concomitant medication terms will be coded using validated dictionaries such as MedDRA and Who-Drug.

This study will conform to SDTM standards and Analysis Data Model (ADaM) and will be fully Clinical Data Interchange Standards Consortium (CDISC) compliant adhering to the latest CDISC standards including all associated documentation to aid review such as the study data reviewers guide, the analysis data reviewers guide, metadata and Define.xml for both SDTM and ADaM.

17.10 Database Processing

Clinical data will be captured using a study specific e-CRF using a validated and Code of Federal Regulations (CFR) Part 11 compliant Electronic Data Capture (EDC) system. Sites will receive training and have access to the study specific e-CRF completion guidelines.

Pre-defined data validation checks will be run within the e-CRF as the data are entered and submitted by authorized site staff. The resulting data queries will be reviewed by the clinical site and resolved.

An electronic audit trail of all changes made to the e-CRF will be kept within the EDC system. This

audit trail identifies the user making the change and date and time of change.

At the end of the study, each site will receive their subject's data in an electronic readable format (i.e., PDF format) burned on an adequate media (e.g., CD or DVD).

17.11 Archiving Requirements for Sponsor and Investigator

Essential documents (as defined in the Guideline for Good Clinical practice E6 (R2) EMA/CHMP/ICH/135/1995) must be retained until at least 2 years after the last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region or at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational product. These documents must be retained for a longer period however if required by the applicable regulatory requirement(s) or if required by Alfasigma S.p.A. The subject identification list must be retained according to the requirements of the applicable national/international regulations after the completion, or discontinuation, of the study. Alfasigma S.p.A will notify the Investigator(s)/Institution(s) in writing when the trial related records are no longer needed. If an investigator moves, withdraws from a trial or retires, the responsibility for maintaining the records may be transferred to another investigator who accepts this responsibility. Notice of this transfer must be given to and agreed upon by Alfasigma S.p.A.

18 PROJECT MANAGEMENT

18.1 Investigator Information and Training

The Investigators and essential support staff will be trained by the Sponsor or its designee with regards to the International Council on Harmonization (ICH) GCPs and all aspects of protocol application and study management. It is the responsibility of the Investigator to train ancillary study staff and to document such training.

18.2 Quality Assurance and Quality Control

The sponsor will implement and maintain quality assurance and quality control systems with written Standard Operating Procedures (SOPs) in accordance with the Guidelines for Good Clinical Practice E6 (R2) (EMA/CHMP/ICH/135/1995).

18.2.1 Audit and Supervision of the Study

The study may be audited by the Sponsor or its designee. If such an audit occurs, the Investigator agrees to allow the auditor direct access to all relevant documents and to allocate his/her time and his/her staff to the auditor to discuss findings on any relevant issue. In the event that on-site auditing visits cannot occur, alternative measures (e.g., remote audits) may be considered, as allowed by local regulations and according to the relevant SOPs.

An independent representative for quality assurance will ensure quality by auditing the conformity of protocol, monitoring data handling and archiving (trial master file) with the Guidelines for Good Clinical Practice E6 (R2) (EMA/CHMP/ICH/135/1995), national drug law(s) and SOP(s).

In addition, regulatory agencies may conduct a regulatory inspection of this study. If such an inspection occurs, the Investigator agrees to allow the inspector direct access to all relevant documents and to allocate his time and his staff to the inspector to discuss findings and any relevant issue. Inspections may be performed by regulatory authorities.

18.2.2 Monitoring

This study will be monitored by the Sponsor or its designee, in accordance with the Guidelines for Good Clinical Practice E6 (R2) and the "Regulation (EU) 2016/679". By signing this protocol, the Investigator agrees to periodic, on-site monitoring of all appropriate study documentation.

The monitors will establish contact between the investigator and the Sponsor.

The monitors will evaluate the competence of each study center and inform the sponsor of any problems relating to the facilities and technical staff. During the study the monitors will check that informed consent was obtained from all subjects, that the data are recorded correctly and completely, the Investigator providing direct access to source data/documents for data verification, and that the investigator complies with the protocol (and any amendments), GCPs, and all applicable regulatory requirements.

Study monitors will perform ongoing source data verification to confirm that data entered into the e-CRF by authorized site personnel are accurate, complete, and verifiable from source documents; that the safety and rights of subjects are being protected; and that the study is being conducted in accordance with the currently approved protocol and any other study agreements, ICH GCP, and all applicable regulatory requirements. The investigator shall permit the site assigned Monitor to review study data as frequently as deemed necessary to ensure that data are being recorded in an adequate manner and that protocol adherence is satisfactory.

The investigator shall access medical records for the Monitor in order that entries in the e-CRF may be verified. In case of restrictions to on-site monitoring visits due to SARS-CoV2 pandemic, remote monitoring activities will be implemented in order to remotely check the correspondence of the data inputted in the e-CRF throughout the sharing of Pseudonymized copy of the source data, protecting the subjects' privacy.

The investigator may not recruit subjects into the study until all regulatory approvals are in place, the site initiation visit has been made by a sponsor/CRO monitor (in which a detailed review of the protocol and all study related documents is performed and all the site staff has been properly trained) and finally the site receive the official activation.

Monitoring details describing strategy (e.g., risk-based initiatives in operations and quality such as Risk Management and Mitigation Strategies and Analytical Risk-Based Monitoring, assessment of the impact of the envisaged processing operations on the protection of personal data), methods, responsibilities and requirements, including handling of non-compliance issues and monitoring techniques (central, remote, or on-site monitoring) are provided in the Monitoring Plan.

18.3 TRIAL STEERING COMMITTEE

For the present study, a trial steering committee (TSC) was established, in order to provide scientific input for the study design. The TSC has the responsibility to approve the study protocol and any amendments, monitor and supervise the trial towards its overall objectives. The TSC will support the sponsor in maintaining adequate the quality standards, by raising the attention on any specific repeated operational or safety issue occurring during the study conduct.

18.4 DATA MONITORING COMMITTEE

No data monitoring committee has been planned for this study.

18.5 AMENDMENTS TO THE PROTOCOL

Modifications of the signed protocol are only possible by protocol amendments with the agreement of all responsible persons.

The investigator should not implement any deviation from, or changes to the protocol without agreement by Alfasigma S.p.A. and prior review and documented approval/favorable opinion from the IRB/IEC of an amendment, except where necessary to eliminate an immediate hazard(s) to trial subjects, or when the change(s) involves only logistical or administrative aspects of the trial.

Protocol amendments must be submitted to the appropriate regulatory authorities.

Alfasigma S.p.A. and the Investigator/Institution must have approval/favorable opinion from the IRB/IEC for any amendment to the protocol.

Any protocol amendment must be distributed to those who received the original protocol and be

appended to it.

18.6 Closure or Discontinuation of Study and Site

Upon completion of the study, the following activities, when applicable, must be conducted by the monitor in conjunction with the Investigator, as appropriate:

- Return of all study data to the Sponsor or its designee
- Data clarifications and/or resolutions
- Accounting, reconciliation, and final disposition of used and unused investigational product
- Review of site study records for completeness.

18.7 Premature Discontinuation of the Study in a Trial Site

The study site can be discontinued at the request of the Sponsor, the Investigator, or regulatory authorities.

Conditions that may warrant discontinuation of the study site include, but are not limited to the following:

- The center cannot include an adequate number of subjects within the planned time.
- Serious and/or persistent non-compliance with the protocol.
- Careless or premeditated false documentation in the CRFs.
- Inadequate co-operation with the Sponsor.
- Non-compliance with GCP, SOPs or regulatory requirements.
- The investigator asks to discontinue the trial.
- The submission of knowingly false information from the research facility to regulatory authorities

If the trial is prematurely terminated or suspended for any reason, the subjects will be informed promptly, appropriate therapy and follow-up will be assured and where required the relevant regulatory authorities will be informed. The IRB/IEC will be promptly informed and provided with a detailed written explanation.

18.8 Premature Discontinuation of the Whole Study

If the trial is prematurely terminated or suspended, the Sponsor will promptly inform the Investigators/Institutions, and the regulatory authorities with reasons. A written explanation will be promptly sent to the IRB/IEC by the Sponsor or the Investigator/Institution, as specified in the regulatory requirements.

Conditions that may result in the termination of the study or part thereof include, but are not limited to the following:

1. The principal Investigator and/or the Sponsor feel that the number and/or severity of AEs justify discontinuation of the study.
2. The Sponsor considers the applied doses of the investigational product to be no longer relevant.
3. Data not known before become available and raise concern about the safety of the investigational product so that continuation would pose potential risks to the subjects.

Premature termination of the study must be mutually agreed upon by the Coordinating Investigator and the Sponsor and must be documented. However, study results must be reported according to the requirements outlined in this protocol as far as applicable.

If the study is prematurely discontinued, all study data must be returned to the Sponsor or its designee. In addition, the site must conduct final disposition of all unused investigational products in accordance with Sponsor's procedures for the study. Study termination and follow-up will be performed in compliance with the conditions set forth in ICH GCPs.

Financial compensation to Investigators and/or Institution will be in accordance with the agreement established between the Investigator and the Sponsor.

18.9 INSURANCE FOR SUBJECTS

Alfasigma S.p.A will arrange insurance for all study subjects. The following guidelines must be taken into account:

- Any serious adverse event which may be correlated or not to investigational product or diagnostic procedures during the trial must be reported to the sponsor or the CRO immediately.
- The insurance company is entitled to ask all doctors involved in the treatment of a subject, other insurance companies and the national insurance for information which might help to clarify the cause of injury. Before enrolment in the study a subject must be informed of insurance against trial-related injuries and who to contact for compensation.

The terms of the insurance will be included in the Investigator's Study File.

18.10 DISCLOSURE OF ALL INFORMATION AND RESULTS

By signing the protocol, the investigator agrees to keep confidential all information and results concerning the study and the investigational product, until the data are published.

18.11 PUBLICATION POLICY

The Sponsor declare its intent to publish the results of the study, after completion of the regulatory Report.

The personal data especially sensitive data regarding the clinical trial, will be only disseminated in strictly anonymous form.

The Sponsor will work with the protocol development team and will identify a lead author for the manuscript development. The authors for the manuscript will be determined by the amount of effort and participation each Investigator contributes towards the study design, the study conduct, as well as the analysis of study results.

All of the parties agree to provide the other with the text sufficiently in advance to allow

examination, prior to submission to a scientific journal.

It is understood by the Investigator that the information developed in the clinical study may be disclosed, as required, to other clinical Investigators, and regulatory authorities.

In the case of a multicenter trial an investigator cannot publish the study results relevant to his/her single center separately, unless global results have already been published.

18.12 OWNERSHIP

All data and records provided by the Sponsor or generated during the study (other than a subject's medical records) and all inventions discovered in the course of conducting the study are the property of the Sponsor. If a written contract for the conduct of the study which includes ownership provisions inconsistent with this statement is executed, then contract's ownership provisions shall apply rather than this statement.

18.13 CONTRACTS, FINANCES

In addition to the protocol trial-related duties, functions and financial aspects must be specified in a

separate contract between Alfasigma S.p.A., the investigator and any other parties involved in the clinical trial.

19 Reporting

After completion of the study an "integrated" full report will be prepared (according to the ICH Harmonized Tripartite Guideline Topic E3 “Structure and Content of Clinical Study Reports”).

20 REFERENCE LIST

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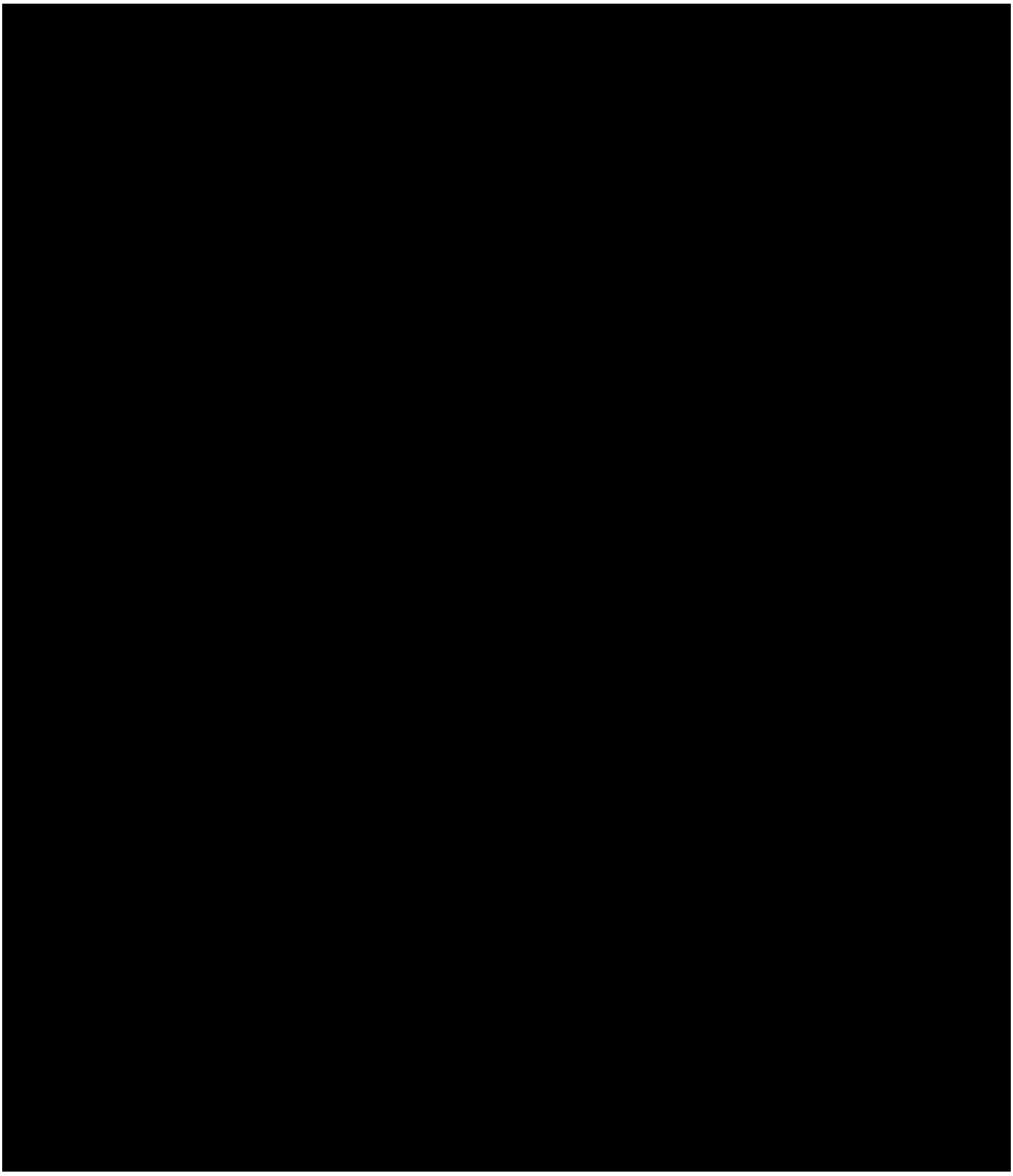
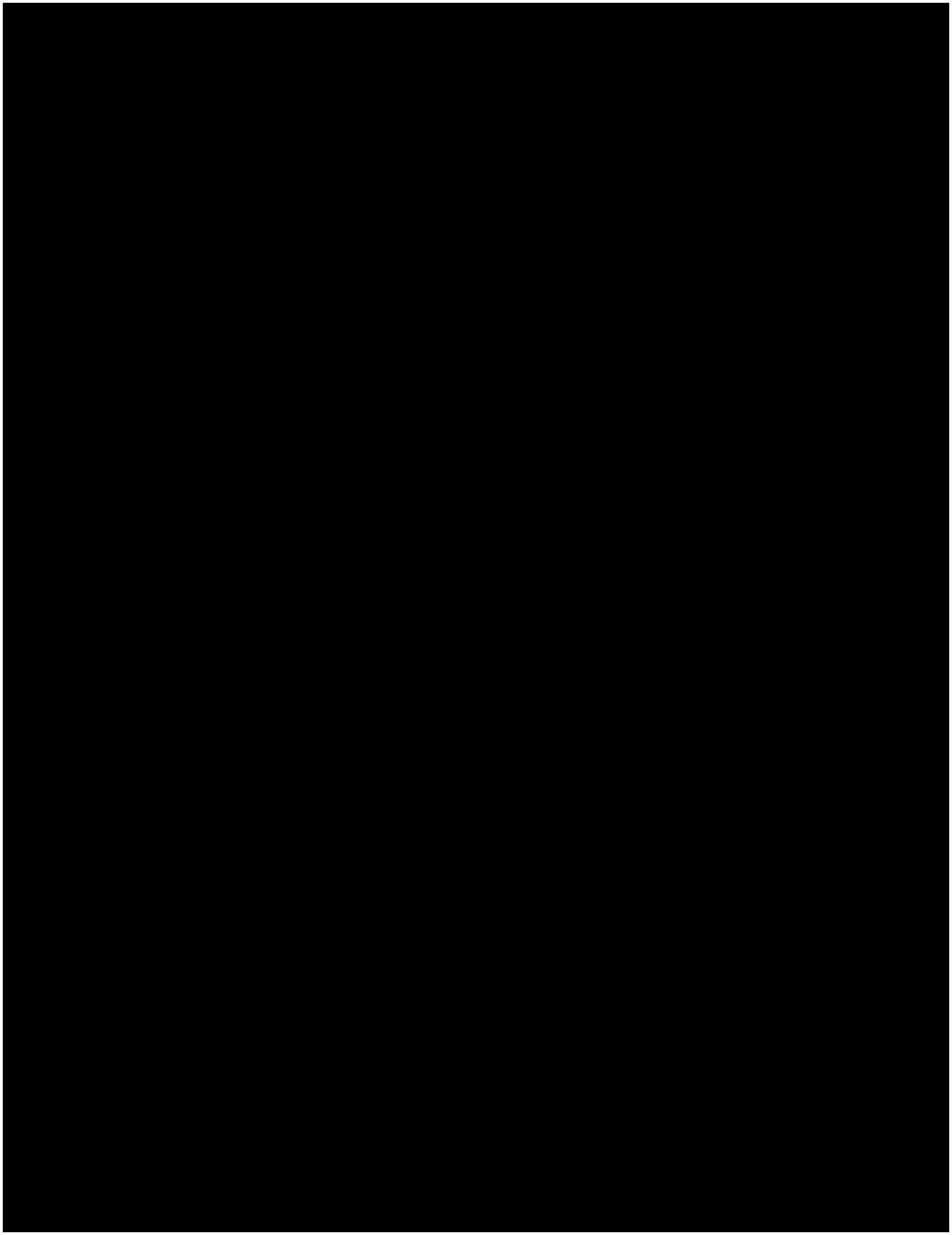
21 APPENDICES

21.2 APPENDIX 2: SUBJECT'S ELECTRONIC (E)-DIARY





21.3 APPENDIX 3: "ARTIFICIAL FOOD NEED" (AFN) SCALE FOR CIPO PATIENTS

DocuSign

Certificate Of Completion

Envelope Id: EB22B13B32D74B7AB6447979BDCE06AF
Status: Completed
Subject: Please DocuSign: VE-CIP2001_2021 protocol_final 1.0_24 may 2021.pdf
Source Envelope:
Document Pages: 94
Signatures: 7
Certificate Pages: 3
Initials: 0
AutoNav: Enabled
EnvelopeId Stamping: Disabled
Time Zone: (UTC+01:00) Amsterdam, Berlin, Bern, Rome, Stockholm, Vienna

Envelope Originator:
[redacted]
Via Ragazzi del '99 n. 5
Bologna, Bologna 40137
[redacted]
IP Address: [redacted]

Record Tracking

Status: Original
27-05-2021 | 14:41

Holder: [redacted]

Location: DocuSign

Signer Events	Signature	Timestamp
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Signer Events	Signature	Timestamp
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In Person Signer Events	**Signature**	**Timestamp**
Editor Delivery Events	**Status**	**Timestamp**
Agent Delivery Events	**Status**	**Timestamp**
Intermediary Delivery Events	**Status**	**Timestamp**
Certified Delivery Events	**Status**	**Timestamp**
Carbon Copy Events	**Status**	**Timestamp**
Witness Events	**Signature**	**Timestamp**
Notary Events	**Signature**	**Timestamp**
Envelope Summary Events	**Status**	**Timestamps**
Envelope Sent	Hashed/Encrypted	27-05-2021 | 14:58
Certified Delivered	Security Checked	27-05-2021 | 14:59
Signing Complete	Security Checked	27-05-2021 | 15:00
Completed	Security Checked	28-05-2021 | 09:30
Payment Events	**Status**	**Timestamps**