Istituto Giannina Gaslini

Study Title:

"EFFICACY OF ORAL SUCROSOMIAL IRON SUPPLEMENTATION IN CHILDREN WITH CELIAC DISEASE AND IRON DEFICIENCY OR ANEMIA: A DOUBLE-BLIND, RANDOMIZED, PLACEBO-CONTROLLED TRIAL."

Study Code	CD-GAS-FESUCR
EU Number	Not applicable (food supplement study)
Protocol Version	Version 1.1
Protocol Date	11-Mar-2025
Sponsor	U.O.C. Gastroenterologia Pediatrica ed Endoscopia Digestiva, Centro Regionale di Riferimento per la Malattia Celiaca IRCCS Istituto Giannina Gaslini Largo G. Gaslini 5, 16147, Genova Director of the U.O.C.: dr. Paolo Gandullia
Investigators	Dr. Marco Crocco U.O.C. Gastroenterologia Pediatrica ed Endoscopia Digestiva, Centro Regionale di Riferimento per la Malattia Celiaca IRCCS Istituto Giannina Gaslini Dr. Federica Malerba DINOGMI - Dipartimento di Neuroscienze, Riabilitazione, Oftalmologia, Genetica e Scienze Materno-Infantili, University of Genova IRCCS Istituto Giannina Gaslini.
Financial Supporter	Pharmanutra SpA Via Campodavela, 1 56122 Pisa (Italy)

Confidential Information

Coordinating Investigator's Signature Page

Study Title

"EFFICACY OF ORAL SUCROSOMIAL IRON SUPPLEMENTATION IN CHILDREN WITH CELIAC DISEASE AND IRON DEFICIENCY OR ANEMIA: A DOUBLE-BLIND, RANDOMIZED, PLACEBO-CONTROLLED TRIAL"

I have read and understood all pages of this clinical study protocol and appendices and I agree that they contain all information required to conduct this clinical study. I agree to oversee the study as outlined in the protocol and to comply with all terms and conditions set out therein. I will follow the principles of the Declaration of Helsinki and ICH-GCP and will work in accordance with local regulations.

I will support to the best of my knowledge the study team in pursuing the goal of protection of study patient and obtaining credible study data.

IRCCS "G. GASLINI"
U.O.C. Gastroenterologia Pediatrica
ed Endoscopia Digestiva
Dr. Marco Crocco
O.M. GE 16215

11/03/2025

(Date)

Dr. Marco Crocco
U.O.C. Gastroenterologia Pediatrica ed Endoscopia Digestiva, Centro Regionale di Riferimento per la Malattia Celiaca
IRCCS Istituto Giannina Gaslini

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Sponsor's	U.O.C. Gastroenterologia Pediatrica ed Endoscopia Digestiva, Centro Regionale di Riferimento per la Malattia Celiaca IRCCS Istituto Giannina Gaslini Largo G. Gaslini 5, 16147, Genova Director of the U.O.C.: dr. Paolo Gandullia
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List of abbreviations

ADR	Adverse Drug Reaction
AE(s)	Adverse Event(s)
ALT	Alanine Transaminase
AST	Aspartate Transaminase
BMI	Body Mass Index
CD	Celiac Disease
CRF	Case Report Form
DMC	Data Monitoring Committee
DMT1	Divalent Metal Transporter type 1
DSMB	Data Safety Monitoring Board
EC	Ethics Committee
ECOG	Eastern Cooperative Oncology Group
EMA	European Medicines Agency
ESPGHAN	European Society of Pediatric Gastroenterology Hepatology and Nutrition
FAS	Full Analysis Set
FT4	Thyroxine
G	Gram(s)
GCP	Good Clinical Practice
GFD	Gluten-Free Diet
GLP	Good Laboratory Practice
GMP	Good Manufacturing Practice
Hb	Hemoglobin
HDL	High Density Lipoprotein
HRQoL	Healthy-Related Quality of Life
ICH	International Conference on Harmonization
IEC	Independent Ethics Committee
ITT	Intent-To-Treat
Kg	Kilogram(s)
LDL	Low Density Lipoprotein
LPLV	Last Patient Last Visit
MCH	Mean Corpuscular Hemoglobin

MCHC	Mean Corpuscular Hemoglobin Concentration
MCV	Mean Corpuscular Volume
MedDRA	Medical Dictionary for Regulatory Activities
Mg	Milligram(s)
ml	Milliliter(s)
PI	Principal Investigator
PPAS	Per Protocol Analysis Set
PT	Preferred term
QoL	Quality of Life
SAE(s)	Serious Adverse Event(S)
SAF	Safety Analysis Set
SAP	Statistical Analysis Plan
SD	Standard Deviation
SOC	System Organ Class
SoC	Standard of Care
SOP	Standard Operating Procedures
TMF	Study Master File
TSH	Thyroid Stimulating Hormone
WHO	World Health Organization

Study synopsis

Study Title	"EFFICACY OF ORAL SUCROSOMIAL IRON SUPPLEMENTATION IN CHILDREN WITH CELIAC DISEASE AND IRON DEFICIENCY OR ANEMIA: A DOUBLE-BLIND, RANDOMIZED, PLACEBO-CONTROLLED TRIAL"
Study Number	CD-GAS-FESUCR
EU Number	Not applicable (food supplement study)
Sponsor	U.O.C. Gastroenterologia Pediatrica ed Endoscopia Digestiva, Centro Regionale di Riferimento per la Malattia Celiaca IRCCS Istituto Giannina Gaslini
Countries and Sites	1 recruiting clinical site in Italy
Indication	Iron deficiency/anemia due to iron deficiency in CD children and adolescents
Study Design	Randomized, parallel groups, double-blind, placebo-controlled study
Objectives	*Primary objective:* to evaluate the effects, in terms of time elapsed from the diagnosis of celiac disease for the normalization of iron reserves and hemoglobin, of oral supplementation with Sucrosomial® iron as an add-on to the GFD compared to GFD alone in pediatric patients of school age and adolescents with hypoferritinemia and/or iron deficiency anemia at onset. *Secondary objectives:* 1. to evaluate the effects of oral supplementation with Sucrosomial® iron, as an add-on to the GFD in pediatric patients with hypoferritinemia and/or iron deficiency anemia, at the onset of celiac disease on levels of chronic fatigue; 2. to evaluate the effects of oral supplementation with Sucrosomial® iron as an add-on to the GFD in pediatric patients with hypoferritinemia and/or iron deficiency anemia at the onset of celiac disease on patient's quality of life; 3. to evaluate the adherence to the GFD in patients with and without Sucrosomial® iron supplementation; 4. to evaluate the tolerability, in terms of gastrointestinal symptoms, of Sucrosomial® iron supplementation; 5. to evaluate the general safety of Sucrosomial® iron supplementation in pediatric patients with hypoferritinemia and/or iron deficiency anemia at the onset of the celiac disease. *Exploratory objectives:*

	1. to evaluate the effects of oral supplementation with Sucrosomial® iron as an add-on to the GFD in pediatric patients with hypoferritinemia and/or iron deficiency anemia at the onset of celiac disease on inflammatory biomarkers.
Endpoints	*Primary endpoint:* The evaluation of the time to change of blood parameters indicative for iron status normalization (Hb, MCV, MCH, MCHC, reticulocytes, ferritin, transferrin (sat%), iron, vitamin B12 and folic acid) from baseline, in the two treatment groups. *Secondary endpoints:* 1. the chronic fatigue change from baseline, in the two groups, through the pediatric questionnaire PedsQL Multidimensional Fatigue Scale; 2. the change from baseline, in the two treatment groups, of the score of Coeliac Disease Dutch Questionnaire (CDDUX) and Pediatric Quality of Life (PedsQL) 4.0 QoL; 3. the adherence to diet and to treatment through questions during visits and patient's diary data; 4. the change from baseline to each time point, in the two treatment groups, of the PedsQL™ 3.0 Gastrointestinal Symptoms Module score; 5. the adverse events evaluation. *Exploratory endpoints:* 1. the change in inflammatory biomarkers (see the flow-chart notes for details) from baseline to any time point, in the two treatment groups.
Treatment groups and study supplement	SiderAL® FORTE verum oral drops in addition to GFD. Matching PLACEBO oral drops in addition to GFD.
Study Duration	Total study duration (per patient) will be about 6 months; total treatment duration (per patient) will be 6 months.
Number of Patients	60 planned Two typologies of patients will be included: with hypoferritinemia and with anemia due to iron deficiency. The randomization process will be stratified, so that: - 15 patients with hypoferritinemia receive active treatment and 15 patients receive placebo; - 15 patients with anemia due to iron deficiency receive active treatment and 15 patients receive placebo. The age of patients will also be considered for the randomization (to assign the correct number of product bottles).
Target Study Population	Children and adolescents with celiac disease and iron deficiency or anemia due to iron deficiency.

Selection Criteria	*Inclusion Criteria:* 1. Diagnosis of CD according to the current European ESPGHAN guidelines (clinical or histological) with confirmed hypoferritinemia or iron deficiency anemia. 2. Age at diagnosis of CD between 8 and 18 years (inclusive). 3. Absence of oral martial supplementation in the 30 days before the diagnosis and intravenous martial supplementation in the 90 days prior to the diagnosis of CD. 4. Patients who have not already started GFD before diagnosis. 5. Exclusion of other causes of anemia. 6. Patients (and parents/legal guardian) able to understand and willing to participate in the study, with collaborative attitude. 7. Informed consent release by both parents/legal guardian. *Exclusion Criteria:* 1. Potential celiac disease. 2. Hb < 8 g/dL at screening or clinical visits 3. Other causes of anemia, hemoglobinopathies or coagulopathies. 4. Active bleeding or surgery or major trauma in the last 6 months. 5. Other inflammatory diseases, neoplasms or IgE mediated food allergies. 6. Syndromes or presence of vascular malformations. 7. Pregnant or lactating patients (based on self-certification by the parents and by the patient, where applicable). 8. Patients with known or suspected allergy or hypersensitivity to the study products or any of their excipients. 9. Taking oral iron-based medications in the 30 days prior to diagnosis and intravenous iron-based medications in the 90 days prior to diagnosis. 10. Use of other investigational drug(s) within 30 days before study entry or during the study. 11. Any other condition, illness or treatment that in the Investigator's opinion does not make the patient suitable for the study.
Schedule	See the flow-chart.

Sample size	**Study Parameters:** • Minimum clinically significant difference between two groups (delta): 10 weeks • Standard deviation (sigma): 8 weeks • Significance level (alpha): 0.05 • Statistical power (1-beta): 0.90 • Expected dropout rate: 5% • Type of statistical analysis: Independent samples t-test **Sample Size Calculation:** We will use the formula for calculating the sample size for an independent two-sample t-test: n = 2 * [(Z(1-alpha/2) + Z(1-beta)) * sigma / delta]^2 Inserting the values: n = 2 * [(1.96 + 1.28) * 8 / 10]^2 n ≈ 2 * (3.24 * 0.8)^2 n ≈ 2 * 6.63552 n ≈ 13.27104 Therefore, approximately 14 patients will need to be enrolled per group. **Dropout Rate Correction:** We must increase the sample size to compensate for the expected 5% dropout rate. Corrected sample size = n / (1 - dropout rate) Corrected sample size = 14 / 0.95 = 14.73684 Therefore, the corrected sample size is approximately 15 patients per group. Study plans to enroll 60 patients randomized in a 1:1 ratio (30 for the intervention arm and 30 for the placebo control group), stratified per pathology severity (iron deficiency/anemia due to iron deficiency).
Populations for analysis	*Full analysis set (FAS):* All patients of the SAF who have performed the baseline assessments and have at least one post-baseline assessment of any performance endpoint (primary or secondary). *Per-Protocol analysis set (PPAS):* All patients of the FAS who also meet all inclusion/exclusion criteria, having no major protocol deviation and who have completed the entire treatment protocol as originally planned and with high compliance (>80%) will be included in the analysis. *Safety analysis set (SAF):*

	All patients who took at least one dose of the study treatment.
Statistical Analysis – Efficacy endpoints	Continuous data will be summarized using means and standard deviations (SD) and medians and interquartile ranges for non-normally distributed data. Categorical variables will be presented as counts and percentages. Quantitative variables will be compared using the Student's t-test or ANOVA, and the Mann-Whitney U test or Kruskal-Wallis test for non-normally distributed data. Fisher's exact test or the chi-squared test will be employed for qualitative variables. Confidence intervals of 95% for rates will be calculated using the Wilson method. Bivariate and multivariate analyses will be performed to investigate potential relationships between variables. The significance level for statistical tests will be set at $p<0.05$. Additional statistical tests may be employed as needed to ensure accurate interpretation of the results.
Statistical Analysis – Safety endpoints	Adverse events will be coded using the Medical Dictionary for Regulatory Activities (MedDRA) to give a preferred term (PT) and a system/organ class term (SOC) for each event. The number of patients who experienced at least one AE, study product-related AE, serious AE, severe AE and the number of patients withdrawn due to AE will be summarized by treatment arm. Concomitant medications will be summarized by treatment using descriptive statistics. Physical examination data in each treatment group will be summarized in a frequency table (normal/abnormal) containing counts and percentages at any visit by system. Anthropometric parameters in each treatment group will be described by n, arithmetic mean, standard deviation, median, minimum and maximum.

Study Flow-Chart

Phase of the study	Screening / Baseline [a]	Phone call [f]	Follow-up	Phone call [f]	End of study
Visit number	**1**		**2**		**3**
Time (month)	**0**	**1&2**	**3**	**4&5**	**6**
Informed consent signature	X				
Inclusion/Exclusion criteria	X				
Patient randomization	X				
Demographic data collection	X				
Medical and surgical history collection/family history [b]	X				
Concomitant medication and therapies	X		X		X
Physical examination	X		X		X
Anthropometric measurements [c]	X		X		X
Food diary delivery to patient [g]	X		X		
Food diary collection from patient	X		X		X
Dietary assessment and GFD prescription (diet start)	X				
Diary delivery	X		X		
Diary collection			X		X
Check on GFD adherence		X	X	X	X
Study treatment dispensation to patient	X		X		
Study treatment return and accountability			X		X
Study treatment adherence check		X	X	X	X
Fatigue questionnaire	X		X		X
QoL questionnaire	X		X		X
Gastrointestinal symptoms questionnaire	X		X		X
Blood collection [d]	X		X		X
Stool specimen collection [e]	X		X		X
Adverse events assessment		X	X	X	X

(a) the screening-baseline visit will be conducted in a timeframe of about 1 week, in order to have the food diary compiled for 3 days (2 weekdays and 1 weekend day) before the patient receives the GFD regimen to follow.

(b) Including CD diagnosis data, presence of clinical symptoms/signs according to the Oslo International Classification objective evaluation of organs and systems, family history of CD or other autoimmune or haematological pathologies. Iron deficiency/anemia definition. Data obtained from esophagogastroduodenoscopy executed at diagnosis, will be collected too (macroscopic endoscopy data and histological data will be collected according to the Marsh-Oberhuber and Corazza classification).

(c) Including height, weight, BMI, waist and hip circumference.

(d) Blood sample collection for the analysis of the following parameters: Hb, MCV, MCH, MCHC, reticulocytes, ferritin, transferrin (sat%), iron, vitamin B12 and folic acid, lipid profile (total cholesterol, LDL, HDL, triglycerides), liver function (AST, ALT, gammaGT) and thyroid function (FT4, TSH), intestinal inflammatory biomarkers for celiac disease (antitransglutaminase IgA antibodies), cytokines profile IL-6, IL-10, alpha TNF, serum zonulin (about 3ml of blood will be obtained from the same test tube used for the dosage of IgA antitransglutaminase).

(e) Alpha 1 fecal antitrypsin and fecal calprotectin evaluation.

(f) Phone calls will be every month, to assess GFD adherence, treatment adherence and any safety.

(g) The food diary will be compiled for 3 days (2 weekdays and 1 weekend day) at screening, before the GFD start and then for 3 days just before the next visits (including always 2 weekdays and 1 weekend day).

1. BACKGROUND AND RATIONALE

1.1 Background

Celiac disease (CD) is a chronic autoimmune enteropathy, primarily involving the small intestine, triggered by the ingestion of gluten, a protein found in wheat, barley and rye, in genetically predisposed subjects (HLA DQ2/8) [1-2]. In people with celiac disease, the immune system mistakenly attacks the lining of the small intestine when gluten-containing foods are ingested, which can lead to damage and inflammation of the intestinal wall. This mechanism causes the alteration of the intestinal mucosa, characterized by atrophy of the villi (flattening) with excessive accumulation of pro-inflammatory cells, thus limiting the normal function of nutrient absorption in the intestinal tract. Symptoms of celiac disease can vary widely from person to person and may include abdominal pain, bloating, diarrhea, constipation, fatigue, anemia, weight loss, and rash. Celiac disease, if not treated, can lead to complications such as malnutrition, osteoporosis, and an increased risk of certain types of cancer [5].

CD in pediatric age affects approximately 1 in 70 children [3] and is, at the moment, the only autoimmune disease for which the triggering environmental factor (gluten) is well known and therefore represents an ideal model for studying intestinal malabsorption diseases.

Anemia is a condition frequently found in pediatric age and is characterized by hemoglobin (Hb) or hematocrit (Htc) values lower than 2 standard deviations (<-2 SD) compared to the mean values corrected for age and sex [4]. It is estimated that approximately 1 in 30 people affected by anemia is affected by celiac disease [5], and vice versa, approximately 1 in 4 patients at the onset of celiac disease presents anemia of varying degrees and in over half of the patients there is a celiac deficiency with hypoferritinemia in the absence of frank anemia [6].

In patients with celiac disease, the cause of anemia is most commonly secondary to iron malabsorption, but folic acid and/or vitamin B12 deficiency are also frequent. Anemia in these patients can also be caused by chronic dark intestinal bleeding or more commonly by the chronic inflammatory state due to the underlying pathology that does not allow the correct use of iron (sideroacrestic anemia) [7].

A strict gluten-free diet (GFD) is currently the only possible treatment for celiac disease. GFD is in fact able to resolve chronic inflammation, as well as restoring the normal trophism of the intestinal villi ("mucosal healing"), also resolving chronic malabsorption and therefore anemia [8].

The supplementation of deficient micronutrients, and in particular iron, is still highly debated and there are no specific guidelines regarding the indication, timing and methods of any micronutrient supplementation. A study conducted on 26 adult patients with celiac disease demonstrated that in 78% of subjects the anemia

resolved within 6 months with GFD alone and almost all patients achieved satisfactory Hb values within 12 months of starting GFD without supplementation [9].

However, specific guidelines do not yet exist at the moment, in particular as regards the pediatric age, also considering the impact that anemia can have on the psycho-physical development of the child.

The recent position paper of the European Society of Pediatric Gastroenterology Hepatology and Nutrition (ESPGHAN) for the management and follow-up of CD in pediatric age always recommends an evaluation of the blood count and the micronutrient level at the time of diagnosis of celiac disease and its monitoring during follow-up in case of deficiencies or anemia until their normalization [10]. In this "consensus" of experts, however, no clear indications are made regarding the need for routine supplementation of micronutrients and iron since there is currently no sufficient evidence in the literature on the actual benefit compared to GFD alone.

Young children (preschool age) with anemia due to iron, folic acid, or vitamin B12 deficiency should receive supplementation in addition to the no-diet diet, as improvement may take too long in these children at a critical period of brain development and rapid growth. An integration can also be considered for older children.

In all cases, the resolution of the anemia must always be confirmed, and in cases where the anemia persists despite a rigorous gluten-free diet (to always be checked) the other causes of iron deficiency anemia must also be excluded.

As regards iron deficiency alone in the absence of anemia, an attitude of watchful waiting is considered appropriate as long as there is an improvement in iron reserves in the absence of supplementation. However, it should be noted that the recommendations of the ESPGHAN Position Paper are based on the only prospective work available [11].

Oral martial therapy is safer for the patient and has a better cost/effectiveness ratio than parenteral therapy which should only be reserved in cases of severe and/or symptomatic anemia. However, even oral iron supplementation is not free from side effects, in particular poor patient tolerance for gastrointestinal symptoms is often reported, which can lead to poor compliance with therapy, and also abdominal pain and nausea.

Sucrosomial® iron formulations, in which iron pyrophosphate is covered by a matrix of phospholipids and sucrose esters of fatty acids, have reported excellent absorption and better gastrointestinal tolerability, such as less abdominal and epigastric pain, bloating and constipation in adult patients with CD subjected to oral supplementation [12].

Gastroresistance is given by the sucrester matrix, as demonstrated in vitro studies, which protects iron from the acidity of the pH of the stomach. This allows the Sucrosomial® iron to arrive intact in the intestinal mucosa, where it is absorbed by enterocytes as a vesicular structure, therefore a completely different mode of absorption compared to conventional iron which is instead absorbed by a specific membrane channel placed on the apical part of the enterocytes, called DMT1 (Divalent Metal Transporter type 1), responsible for the reduced absorption of iron and, consequently, of its common adverse effects (e.g. gastralgia).

1.2 Rationale

Based on previous positive experiences in adults, the present study is aimed to evaluate if Sucrosomial® iron supplementation in addition to GFD can be of benefit, compared to GFD alone, in children affected by hypoferritinemia and/or iron deficiency anemia caused by CD, at onset. The study, hopefully, will confirm that iron supplementation is to be recommended in the young patients affected by CD, to obtain a quick normalization of iron reserves and hemoglobin. The study will also evaluate the safety and tolerability of the oral administration of Sucrosomial® iron formulation.

2. STUDY OBJECTIVES AND ENDPOINTS

Primary objective and endpoint

The primary objective of this study is to evaluate the effects, in terms of time elapsed from the diagnosis of celiac disease for the normalization of iron reserves and hemoglobin, of oral supplementation with Sucrosomial® iron as an add-on to the GFD compared to GFD alone in pediatric patients of school age and adolescents with hypoferritinemia and/or iron deficiency anemia at onset.

The primary endpoint will consist in the evaluation of the time to change of blood parameters indicative for iron status normalization (Hb, MCV, MCH, MCHC, reticulocytes, ferritin, transferrin (sat%), iron, vitamin B12 and folic acid) from baseline, in the two treatment groups.

Secondary objectives and endpoints

Secondary objectives of this study are:

1. to evaluate the effects of oral supplementation with Sucrosomial® iron, as an add-on to the GFD in pediatric patients with hypoferritinemia and/or iron deficiency anemia, at the onset of celiac disease on levels of chronic fatigue;

2. to evaluate the effects of oral supplementation with Sucrosomial® iron as an add-on to the GFD in pediatric patients with hypoferritinemia and/or iron deficiency anemia at the onset of celiac disease on patient's quality of life;
3. to evaluate the adherence to the GFD in patients with and without Sucrosomial® iron supplementation;
4. to evaluate the tolerability, in terms of gastrointestinal symptoms, of Sucrosomial® iron supplementation;
5. to evaluate the general safety of Sucrosomial® iron supplementation in pediatric patients with hypoferritinemia and/or iron deficiency anemia at the onset of the celiac disease.

Secondary endpoints of the study are:

1. the chronic fatigue change will be measured through the pediatric questionnaire PedsQL Multidimensional Fatigue Scale. The questionnaire score change from baseline to will be evaluated in the two groups.
2. the change from baseline to each time point, in the two treatment groups, of the score of Coeliac Disease Dutch Questionnaire (CDDUX) and Pediatric Quality of Life (PedsQL) 4.0 QoL
3. the adherence to diet and to treatment through questions during visits and patient's diary data;
4. the change from baseline to each time point, in the two treatment groups, of the PedsQL™ 3.0 Gastrointestinal Symptoms Module score;
5. the adverse events evaluation.

Exploratory objectives and endpoints

Exploratory objective of this study is:

1. to evaluate the effects of oral supplementation with Sucrosomial® iron as an add-on to the GFD in pediatric patients with hypoferritinemia and/or iron deficiency anemia at the onset of celiac disease on inflammatory biomarkers.

Exploratory endpoint of the study is:

1. the change in inflammatory biomarkers (IL-6, IL-10, alpha TNF, serum zonulin) from baseline to any time point, in the two treatment groups.

3. INVESTIGATIONAL PLAN

3.1 Study Design

This is a pilot, randomized, placebo controlled, monocenter, investigator-initiated study with two parallel groups of patients.

The study plans to enroll 60 children and adolescents at the time of diagnosis of CD with hypoferritinemia or iron deficiency anemia. At the time of diagnosis, all patients will start GFD as required by clinical practice (the only therapy for celiac disease) and will be randomized in a 1:1 ratio to receive martial supplementation with Sucrosomial® iron or placebo, as add-on treatment.

The study outcomes will be assessed in a double-blinded fashion i.e. neither the study staff at clinical sites (Investigators, nurses, psychologists, pharmacist) nor the patient will be aware of the treatment assigned.

Patients will be randomly assigned to one of the following treatment groups:

- Sideral forte® verum drops for oral intake in addition to GFD.
- Sideral forte® matching placebo drops for oral intake in addition to GFD.

Randomization will be stratified in order to reach a balanced enrolment between patients with hypoferritinemia and patients with anemia due to iron deficiency. Patients will start the study treatment simultaneously with GFD start, after their eligibility is confirmed at Visit 1 (screening) and will continue for 6 months.

All patients will be treated with GFD that, so far, is considered the Standard of Care for CD. Based on this consideration, and taking into account that there is currently no alternative treatment whose efficacy is consolidated and recognized, the use of placebo in this clinical study seems to be justified.
All the activities foreseen in this study are aligned with the clinical practice for the diagnosis and follow-up of the celiac patient. In particular the laboratory examinations planned are those normally done for this typology of patient, with the only exception of the cytokines profile that will be an exploratory endpoint of the study.

3.2 Study Duration, development and End of Study Definition

Total study duration (per patient): about 6 months; total treatment duration (per patient): 6 months.
Potentially eligible patients will be identified during the CD diagnosis phase. New diagnosed patients will be tested to assess hypoferritinemia and/or iron deficiency anemia or anemia status. If confirmed the patient will be evaluated to check if he/she meets inclusion/exclusion criteria, after study explanation and informed

consent collection. Once eligibility is confirmed, the patient will be randomized to active treatment or placebo.

Two visits will be planned, after the first (screening/baseline), at 3 and 6 months. At months 1, 2, 4 and 5 the patient (parents/legal guardian) will be contacted by telephone to check the diet, the treatment adherence and to verify any safety problem since treatment start. The third visit (at month 6) will be the conclusive visit (the study treatment will end the evening before the visit, while the GFD will continue as standard of care).

The enrolment period is foreseen of about 9 months; therefore, the expected total study duration is about 15 months, from the first patient in date.

The start of the clinical study will be the first informed consent signature date and, unless premature interruption occurs, the end of the study will be the last patient last visit (LPLV).

3.3 Study Population

It is planned to enroll 60 children and adolescents who are diagnosed with CD and have hypoferritinemia or anemia due to iron deficiency. The enrolment should be balanced so that 30 patients for each typology are enrolled.

3.3.1 Inclusion Criteria

For inclusion in the study, all of the following inclusion criteria must be fulfilled:

1. Diagnosis of CD according to the current European ESPGHAN guidelines (clinical or histological) with confirmed hypoferritinemia or iron deficiency anemia.
2. Age at diagnosis of CD between 8 and 18 years (inclusive).
3. Absence of oral martial supplementation in the 30 days before the diagnosis and intravenous martial supplementation in the 90 days prior to the diagnosis of CD.
4. Patients who have not already started GFD before diagnosis.
5. Exclusion of other causes of anemia.
6. Patients (and parents/legal guardian) able to understand and willing to participate in the study, with collaborative attitude.
7. Informed consent release by both parents/legal guardian.

3.3.2 Exclusion Criteria

Patients will not be considered eligible for this study if they fulfil any of the following exclusion criteria:

1. Potential celiac disease.
2. Hb < 8 g/dL at screening
3. Other causes of anemia, hemoglobinopathies or coagulopathies.
4. Active bleeding or surgery or major trauma in the last 6 months.
5. Other inflammatory diseases, neoplasms or IgE mediated food allergies
6. Syndromes or presence of vascular malformations
7. Pregnant or lactating patients (based on self-certification by the parents and by the patient, where applicable)*
8. Patients with known or suspected allergy or hypersensitivity to the study products or any of their excipients.
9. Taking oral iron-based medications in the 30 days prior to diagnosis and intravenous iron-based medications in the 90 days prior to diagnosis.
10. Use of other investigational drug(s) within 30 days before study entry or during the study.
11. Any other condition, illness or treatment that in the Investigator's opinion does not make the patient suitable for the study.

*Self-certification of non-pregnancy status is considered sufficient given that the product under study is a safe and well-tolerated dietary supplement that has already been tested in pregnant women [21] and is prescribed to pregnant women for anemia prevention.

4. STUDY TREATMENT

4.1 Study Products

The iron supplementation will be done with SiderAL Forte® drops provided for this clinical study by the pharmaceutical company Pharmanutra S.p.A. based in Pisa. SiderAL Forte® is a nutritional supplement that contains Sucrosomial® Iron. In Sucrosomial® iron formulations, iron pyrophosphate is covered by a matrix of phospholipids and sucrose esters of fatty acids. The sucrester matrix guarantees high gastroresistance, protecting iron from the acidity of the pH of the stomach. This allows the Sucrosomial® iron to arrive intact in the intestinal mucosa, where it is absorbed by enterocytes as a vesicular structure. This mode of absorption assures excellent iron absorption and better gastrointestinal tolerability respect to other iron-based products. Iron oral administration may in fact cause side effects like diarrhea, constipation, nausea, vomiting, abdominal pain or discomfort, metallic aftertaste and discoloration of mucous membranes and stool. None of this adverse effect was reported so far with SiderAL Forte®, thanks to its particular formulation.

The placebo will be also provided by Pharmanutra S.p.A.

4.1.1 Preparation

The following products will be used in the study:

Active product:

Name: SiderAL® FORTE drops

Formulation: oral drops to be reconstituted. The pack contains 1 bottle of 30 ml and 1 stick of 2,5g.

Ingredients:

Stick Ingredients: Sideral® r.m. – Sucrosomial® Iron (iron pyrophosphate, sucrose esters of fatty acids, sunflower lecithin spray-dried on rice flour and tricalcium phosphate, pregelatinized rice starch), maltodextrins. **Gluten free.**

Bottle Ingredients: Demineralized water, sucrose, maltodextrins, preservatives: potassium sorbate, sodium benzoate; flavour; thickening agent: xantan gum; acidifying agent: citric acid; emusifier: sucrose esters of fatty acids. **Gluten free.**

Nutritional Information:

SiderAL® FORTE Average contents	**Daily dose (1 ml)**	**%NRV**
Iron	14mg	100%

Placebo:

Name: Matching Placebo

Formulation: oral drops to be reconstituted. The pack contains 1 bottle of 30 ml and 1 stick of 2,5g.

Ingredients:

Stick Ingredients: tricalcium phosphate, maltodextrins, caramel, E172. **Gluten free**.

Bottle Ingredients: Demineralized water, sucrose, maltodextrins, preservatives: potassium sorbate, sodium benzoate; flavour; thickening agent: xantan gum; acidifying agent: citric acid; emusifier: sucrose esters of fatty acids. **Gluten free**.

4.1.2 Packaging and Labelling

Packaging and labelling will be in accordance with applicable local regulatory requirements and applicable GMP Guidelines. All elements of the patient kits including the kit itself, the enclosed supplement boxes and the individual supplement sachets will have mandatory labelling for the clinical study.

Patient kits: each patient kit will be composed of a variable number of bottles of the supplement, depending on the patient age and on the severity of the baseline condition (and the iron need, consequently).

Patients' kits will be composed of:

- No. 7 bottles in the case of patient who will be prescribed to intake 1ml/day of SiderAL® FORTE drops (i.e. children with hypoferritinemia from 8 years until development [Tanner stage <=3]);
- No. 13 bottles in the case of patient who will be prescribed to intake 2ml/day of SiderAL® FORTE drops (i.e. children with hypoferritinemia from development [Tanner stage >3] up to 18 years until development and children with iron deficiency anemia from 8 years until development [Tanner stage <=3]);
- No. 20 bottles in the case of patient who will be prescribed to intake 3ml/day of SiderAL® FORTE drops (i.e. adolescents with iron deficiency anemia from development [Tanner stage >3] up to 18 years).

Kits will be prepared and labeled according to 4 randomization lists (see also paragraph 4.1.8) in order to:

- balance placebo and active allocation in the two categories of patients (iron deficiency/anemia) and
- dispense the right number of bottles to patients (more or fewer bottles will be given according to the patient's age).

The number of bottles provided in each kit will be in excess, with respect to the hypothetical need.

Bottle: bottles of SiderAL® Forte drops and placebo will be manufactured and packaged SIIT. – Via Canova 2/4 20090 Trezzano S/N (MI) – Italy. Study products will be then labeled by Pharmanutra Spa and supplied to clinical sites. There will be no detectable differences in the presentation of SiderAL® Forte drops and its placebo.

Dispensation: participants will receive (dispensed by hospital pharmacy) approximately half of the prescribed number of bottles (i.e. 4, 7 or 10 bottles) of active or placebo at first visit and will be asked to return both empty, partially used and unused bottles at the following Visit 2, for the purpose of accountability. At second visit they will receive the remaining bottles (i.e. 3, 6 and 10 bottles), enough to complete the treatment until Visit 3 (end of study). Any partially used bottle returned at Visit 2 will be kept by the patient and completely used up to Visit 3. At Visit 3 patients will return empty, partially used and unused bottles, as well.

4.1.3 Handling and Storage

The study product shall be carefully stored below 25°C before reconstitution, not exposed to direct sunlight in a safe area, separate from other drugs and products, on-site. After reconstitution patients will be instructed to keep the bottle in the fridge.

The investigator (or designee) shall maintain a record of the study product delivery to the study site and inventory at the study location. After study conclusion, all unused study product shall be destroyed or returned to Pharmanutra S.p.A. after written agreement.

4.1.4 Study Product Accountability

The Investigator is responsible for ensuring accountability of the study product, including reconciliation of study products and maintenance of records. Upon receipt of the study product, the Investigator (or designee) will check the contents and acknowledge receipt by signing and dating the accompanying documentation. Such documentation (original or copy) will be retained in the Investigator File.

The dispensing of the study product will be carefully recorded on the medical records and an accurate accounting will be available for verification.

Study product accountability records will include:

- Confirmation of study product receipt at the clinical site.
- The inventory at the site of study product received.
- The bottles delivery to each participant.
- The return to the Pharmanutra S.p.A. or alternative disposition of unused study product.

The Investigator should maintain records that adequately document:

- That participants were provided the doses specified by the clinical study protocol/amendment(s); and
- That all study products provided by the Sponsor were fully reconciled.

Unused study product must not be discarded or used for any purpose other than the present study. Study product that has been dispensed to a patient must not be re-dispensed to a different patient.

4.1.5 Treatment Groups

Patients will be randomly assigned to one of the following treatments:

- SiderAL® FORTE verum oral drops in addition to GFD.
- Matching PLACEBO oral drops in addition to GFD.

Since in the study two typologies of patients will be included, with hypoferritinemia and with anemia due to iron deficiency, the randomization process will be stratified, so that:

- 15 patients with hypoferritinemia receive active treatment and 15 patients receive placebo;
- 15 patients with anemia due to iron deficiency receive active treatment and 15 patients receive placebo.

All patients will receive the recommendations for GFD.

4.1.6 Administration of Study Treatment

For patients with hypoferritinemia:

- From 8 years until development (Tanner stage <=3): 1 ml/day of SiderAL® FORTE/PLACEBO oral drops, equal to 14 mg of iron element;
- From development (Tanner stage >3) up to 18 years: 2 ml/day of SiderAL® FORTE/PLACEBO oral drops, equal to 28 mg of iron element.

For patients with iron deficiency anemia:

- From 8 years until development (Tanner stage <=3): 2 ml/day of SiderAL® FORTE/PLACEBO oral drops, equal to 28 mg of iron element;
- From development (Tanner stage >3) up to 18 years: 3 ml/day of SiderAL® FORTE/PLACEBO oral drops, equal to 42 mg of iron element.

The intake will be recommended in a single administration in the evening before going to bed (as far away from dinner as possible).

4.1.7 Blinding / Emergency Unblinding

This is a double-blind study, designed so that neither the patient nor the clinical site personnel (Investigator, sub-Investigator, study nurse, psychologists, pharmacist) will know which treatment is being administered. The identity of the treatments cannot be revealed except in an emergency at the discretion of the Principal Investigator.

The Principal Investigator will receive a study treatment identification key in the form of a sealed envelope containing the kit number (i.e. Kit code A-004 or Kit code B-001, etc.) and the corresponding treatment. The envelope may be opened only in case of an emergency where the identification of the study treatment assigned to the patient needs to be disclosed. Once the code is broken for a patient, this patient will be withdrawn from the study, with the completion of the final study evaluation (Visit 3), indicating the specific reason for the patient withdrawal.

Treatment codes and contents will not be freely available to the Principal Investigator or personnel monitoring the study until study completion and the database is locked.

4.1.8 Method of Assigning Patients to Treatment Groups

Patients will be assigned to active or placebo groups based on 4 randomization lists, two lists for patients with iron deficiency (**List A** for age from 8 years until development and **List B** for age from development up to 18 years)and two lists for patients with anemia due to iron deficiency (**List C** for age from 8 years until development and **List D** for age from development up to 18 years). Study products will be provided to the Investigator already labeled. Labels will report a study product code consisting of progressive numbers and letters referring to the list (i.e. products from List A = iron deficiency and age "8 years – development" will be labeled as "A-001, A-002, A-003…. etc"; products from List D = anemia and age "development - 18 years" will be labeled as "D-001, D-002, D-003 ….etc."). Once eligibility is established (see inclusion/exclusion criteria) the Investigator will assign the first available number of study treatment based on the patient typology (iron deficiency or anemia combined with the age). The study products (active and placebo) will be indistinguishable, so the Investigator will be blinded and follow the randomization lists by simply assigning products in a numerical progressive manner and, of course, drawing from the correct treatment group (A-B-C-D) based on patient characteristics.

The assigned study product code will be recorded by Investigator on medical records and on the CRF.

4.1.9 Management of Study product Overdose

There are no reports of SiderAL® FORTE drops or other Sucrosomial® Iron based formulations overdose, and no serious adverse events have been reported.

4.1.10 Occupational Safety

No occupational health and safety issue has been reported.

4.2 Non-Investigational Treatments

4.2.1 Prior and Concomitant Medication

Any medications (other than those excluded by the clinical study protocol) that are considered necessary for the patients' welfare and will not interfere with the study product may be given at the Investigator's discretion.

The Investigator (or designee) will record all concomitant medications taken by the patient in the appropriate section of the CRF at Visit 1, and all subsequent visits.

Any additional concomitant therapy that becomes necessary during the study and any change to concomitant drugs must be recorded in the corresponding section of the CRF, noting the name, dose, duration and indication of each drug.

4.2.2 Prohibited Prior and Concomitant Medication

Other iron-based drugs or supplements than the one administered for the study will not be allowed from 30 days if orally administered and from 90 days if intravenous administered before screening and during the study. Use of other investigational drug(s) within 30 days before study entry or during the study is prohibited.

4.2.3 Pharmacological Interaction

So far, no interaction of the food supplement or its ingredients with other drugs or products is known or expected.

5 STUDY CONDUCT

5.1 Study Visit Schedule

See Study Flow-chart.

5.2 Study Procedures by Visit

Potentially eligible patients will be identified during the diagnosis process for CD. Those confirmed as potentially eligible will be approached immediately after the diagnosis confirmation, before the GFD starts. Each enrolled patient will attend 3 visits during 6 months of study. Every month the patient/parents/legal guardian will also be contacted by phone to check GFD and treatment adherence, as well as any safety problem.

Prior to performing any study assessment not part of the patient's routine medical care or collecting any data for the study, the Investigator will ensure that both the patient's parents or the legal guardian have provided written informed consent. Each patient for whom written consent is obtained will be assigned a three-digit code, consisting of the progressive number (i.e. 001, 002, 003 etc). All screened patients will receive a screening code irrespective of whether or not they are randomized. If a patient discontinues from the study at any time, the screening code will not be reused.

During this visit, the following assessments will be performed and data collected:

Visit 1, Month 0 (screening/baseline visit):

The screening visit will take about 1 week to be completed, especially for food diary compilation and stool sample collection, before the GFD is prescribed.

The patient will be provided with a diary to be compiled for 3 days and to be returned before randomization. Also, the patient will be provided with materials in instructions for stool sample collection, to be delivered to the clinical center before the randomization and GFD and treatment start.

- Informed consent signature
- demographic data collection (age, sex, race, development stage);
- evaluation of medical and surgical history, including CD diagnosis data, presence of clinical symptoms/signs according to the Oslo International Classification [13] objective evaluation of organs and systems, family history of CD or other autoimmune or haematological pathologies. Iron deficiency/anemia definition. Data obtained from esophagogastroduodenoscopy executed at diagnosis according to the clinical practice for CD screening, will be collected too (macroscopic endoscopy data and histological data will be collected according to the Marsh-Oberhuber [14] and Corazza [15] classification);
- physical examination, weight, height, BMI, waist and hip circumference;
- previous and concomitant treatments evaluation and recording;
- blood sample collection for assessment of Hb, MCV, MCH, MCHC, reticulocytes, ferritin, transferrin (sat%), iron, vitamin B12 and folic acid, lipid profile (total cholesterol, LDL, HDL, triglycerides), liver function (AST, ALT, gammaGT) and thyroid function (FT4, TSH), intestinal inflammatory biomarkers for celiac disease (antitransglutaminase IgA antibodies), cytokines profile (about 3mL of blood will be collected on the same test tube used for the dosage of IgA antitransglutaminase);
- stool specimen collection for analysis of alpha 1 fecal antitrypsin and fecal calprotectin;
- fatigue questionnaire administration;
- quality of life questionnaire administration;
- gastrointestinal symptoms questionnaire administration;
- screening food diary delivery and collection after 3 days compilation;
- inclusion/exclusion criteria check for eligibility;
- randomization;
- study product delivery and instructions. The treatment will start on the same day of the GFD start;
- GFD prescriptions;
- two diaries delivery after randomization, one to register every day the study product intake and a 3 days food diary where to record the food consumption when close to the following visit (visit 2).

Visit 2, Month 3 and Visit 3, Month 6 (follow-up visit and end of study visit):

- physical examination, weight, BMI, waist and hip circumference;
- concomitant treatments change and recording;
- blood sample collection for assessment of Hb, MCV, MCH, MCHC, reticulocytes, ferritin, transferrin (sat%), iron, vitamin B12 and folic acid, lipid profile (total cholesterol, LDL, HDL, triglycerides), liver function (AST, ALT, gammaGT) and thyroid function (FT4, TSH), intestinal inflammatory biomarkers for celiac disease (antitransglutaminase IgA antibodies), cytokines profile (about 3mL of blood will be collected on the same test tube used for the dosage of IgA antitransglutaminase);
- stool specimen collection for analysis of alpha 1 fecal antitrypsin and fecal calprotectin; delivery of materials for the following sample collection;
- diaries collection and new diaries delivery (delivery only at Visit 2), to register the daily study product intake and the food consumption in the 3 days before the following visits;
- fatigue questionnaire administration;
- quality of life questionnaire administration;
- gastrointestinal symptoms questionnaire administration;
- study product collection and new delivery (new delivery only at Visit 2). Check on treatment compliance;
- check on GFD prescriptions compliance;
- adverse events evaluation and recording.

Telephonic follow-up

Four telephone contacts will be done at months 1, 2, 4 and 5 to assess treatment and GFD prescriptions adherence and safety.

5.3 Compliance

Patient's compliance with the protocol will be assessed on the basis of the study product accountability, adherence to the diet prescribed and patients' attendance at each visit.

The patients' compliance to the treatment will be checked by the study staff by looking at the used and unused bottles returned by the patients at Visit 2 and 3 and through the daily diary. The patients' compliance to all the other the recommendations on diet will be checked by asking the patient/parents/legal guardian and a food diary will be always compiled for 3 days before each visit.

5.4 Definition of Completion

A patient will be defined as "completed" if he/she completes the study treatment and all three study visits. Termination at a different time point will be considered as discontinuation. Patients discontinued before Visit 2 will be replaced.

5.5 Discontinuation Criteria

Patients may be discontinued at any time from the study for any of the following reasons:

- Hb decreases of 10% at first timepoint (3 months)
- Hb < 8 g/dL
- An AE occurs that, in the opinion of the Investigator, makes it unsafe for the patient to continue in the study
- The patient is lost to follow-up
- The patient dies
- The patient/parents/legal guardian withdraw consent
- The Investigator, for any reason, terminates the entire study, or terminates the study for that patient; or the attending physician requests that the patient be withdrawn for any medical reason

If a patient is discontinued from the study by the Investigator, the Investigator will complete the Visit 3 on the eCRF. The Investigator should try to ascertain the reason(s) for withdrawal, while fully respecting the patient's rights. As written in the previous paragraph, patients who discontinue the study before Visit 2 (3 months) will be replaced.

6 METHODS OF ASSESSMENT

6.1 Supportive Effect Assessments

Iron status, nutritional status and inflammatory biomarkers status:

Three blood samples will be collected at baseline (Visit 1) and after 3 and 6 months of treatment (Visit 2 and 3) as for routinary assessments in the celiac patient. The following parameters will be analysed under the clinical practice of the site to evaluate the changes after the gluten free diet is in place: iron status (Hb, MCV, MCH, MCHC, reticulocytes, ferritin, transferrin (sat%), iron, vitamin B12 and folic acid), lipid profile (total cholesterol, LDL, HDL, triglycerides), liver function (AST, ALT, gammaGT) and thyroid function (FT4, TSH), intestinal inflammatory

biomarkers for celiac disease (antitransglutaminase IgA antibodies). Data of such examinations will be recorded in the CRF of the study.

An additional sample of 3mL will be obtained (on the same test tube used for the dosage of IgA antitransglutaminase) for cytokines profile that will be assessed as exploratory endpoint. IL-6, IL-10, alpha TNF and serum zonulin will be determined.

Stool specimens will be collected at visits for analysis of alpha 1 fecal antitrypsin and fecal calprotectin. Patients will be provided with materials for stool samples collection and with instructions for the collection (the sample shall be collected within 48 hours from the visit and stored in the fridge until delivery to site).

Blood and stool samples will be stored at -80°C and analysed locally by the site laboratory; residual samples will be kept for 10 years for future analyses.

Questionnaire for fatigue assessment: PedsQL Multidimensional Fatigue Scale questionnaire

The PedsQL™ Multidimensional Fatigue Scale was designed as a generic symptom-specific instrument to measure fatigue in patients with acute and chronic health conditions [16] as well as healthy school and community populations. It is an 18-items questionnaire: 6 items on General Fatigue, 6 items on Sleep/Rest Fatigue, and 6 items on Cognitive Fatigue domains.
Higher scores indicate better HRQoL (healthy-related quality of life).

Different versions of the questionnaire are existing, for different ranges of age. Within this study the version for children (8-12 years) and the version for adolescents (13-18 years) will be used.

Questionnaires for Quality of Life assessment: CDDUX (Coeliac Disease Dutch questionnaire and Pediatric Quality of Life (PedsQL) 4.0 questionnaires

CDDUX is a disease-specific, health-related, quality-of-life questionnaire for children aged from 8 to 18 years, with celiac disease [17]. The questionnaire is composed by 12 items on three subscales, focused on three different domains of possible daily experiences: “Diet” (six items), where the children are invited to express their feelings about compliance, restrictions of the GFD, and the lifelong aspects; “Communication” (three items), where the children are invited to express their feelings when talking about CD to others and when explaining the disease to others; “Having CD” (three items), where the children are invited to express their feelings when offered food containing gluten or what they think about food containing gluten. The answers are marked on a five-point Likert face scale. Each face corresponds to a score, increasing up to a value of 100. The HRQoL is considered as very bad for scores up to 20, while 21–40 is bad, 41–60 is neutral, 61–80 is good, and 81–100 is very good.

The PedsQL 4.0 Generic Core Scales [18] is a modular instrument for measuring HRQoL in children and adolescents aged between 2 and 18 years. The PedsQL 4.0 Generic Core Scales consists of 23 items applicable for healthy school and community populations, as well as pediatric populations with acute and chronic health conditions. Different versions of the questionnaire are existing, for different ranges of age. Within this study the version for children (8-12 years) and the version for adolescents (13-18 years) will be used. The questionnaire includes 4 multidimensional scales (physical functioning - 8 items, emotional functioning - 5 items, social functioning - 5 items, school functioning - 5 items) and 3 summary scores (total scale score - 23 items, physical health summary score - 8 items and psychosocial health summary score - 15 items). Higher scores indicate better HRQoL.

PedsQL 3.0 Gastrointestinal Symptoms Module questionnaire

The PedsQL™ Gastrointestinal Symptoms Scales is a specific module of the PedsQL™ Versions for Children (8-12 years of age) and Adolescents (13-18 years of age) will be used.

The PedsQL™ Gastrointestinal Symptoms Scales™ were designed as generic symptom-specific scales to measure gastrointestinal symptoms in patients with acute and chronic health conditions as well as healthy school and community populations [19]. The PedsQL™ Gastrointestinal Symptoms Scales encompass 10 individual scales: (1) Stomach Pain and Hurt Scale (6 items), (2) Stomach Discomfort When Eating Scale (5 items), (3) Food and Drink Limits Scale (6 items), (4) Trouble Swallowing Scale (3 items), (5) Heartburn and Reflux Scale (4 items), (6) Nausea and Vomiting Scale (4 items), (7) Gas and Bloating Scale (7 items), (8) Constipation Scale (14 items), (9) Blood in Poop Scale (2 items), and (10) Diarrhea Scale (7 items). The format, instructions, Likert response scale, and scoring method for the PedsQL™ Gastrointestinal Symptoms Scales are identical to the PedsQL™ 4.0 Generic Core Scales, with higher scores indicating better GI-specific HRQoL and hence lower symptoms.

Patient diary

A patient diary will be used at screening and before each visit to evaluate the patient's diet before and during the GFD. Then another diary will be used to register the study product intake during the whole study.

6.2 Safety Assessments

No evidence available at the time of the approval of this study protocol indicated that special warnings or precautions were appropriate, other than those noted in the provided SiderAL® FORTE drops Instruction for Use.

Additional safety information collected between Instruction for Use updates, if any, will be communicated in the form of Investigator Notifications. This information will be included in the patient informed consent and should be discussed with the patient during the study as needed.

The assessment of safety will be based on:

- Gastrointestinal symptoms recorded through specific questionnaire to be compiled at visits, as product tolerability
- Number and typology of AEs related to the study treatment, as reported by the patients at visits and during the phone calls.
- Physical examination and anthropometric parameters.

6.2.1 Definition

Adverse Event (AE)

As defined by the International Conference of Harmonization (ICH) Guideline for Good Clinical Practice an Adverse Event is:

"Any untoward medical occurrence in a patient or clinical investigation subject administered a food supplement product and 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, for example), symptom or disease temporally associated with the use of a food supplement product, whether or not considered related to the food supplement product".

Adverse events observed during all period of the study (since informed consent signature) are to be recorded.

Planned hospitalizations scheduled prior to the informed consent but performed during the study should not be considered (serious) AEs.

Signs and symptoms considered as lack of efficacy and occurring during the study will not be recorded on the AEs Section of the eCRF except on the condition that, in the Investigator's opinion, the signs or symptoms are caused by any reason different from lack of efficacy of study product or meet the definition of serious AE.

Clinically significant findings at screening are not considered an AE/SAE.

If a clinical significant finding recorded at screening worsens (in terms of severity or frequency) during the study, it must be recorded as an AE/SAE.

Adverse Reaction (AR)

It is an adverse event where there is a suspected relationship to the study product (according to Paragraph 6.2.2 definitions of “Possible”, “Probable”, “Definite”).

Serious Adverse Event (SAE)

A SAE is any experience that suggests a significant hazard, contraindication, side effect or precaution.
It is any AE that at any dose fulfills at least one of the following criteria:

- is fatal (results in death; NOTE: death is an outcome, not an event)
- is life-threatening (NOTE: the term "life-threatening" refers to an event in which the patient was at immediate risk of death at the time of the event; it does not refer to an event which could hypothetically have caused death if it had been more severe)
- requires in-patient hospitalization or prolongation of existing hospitalization;
- results in persistent or significant disability/incapacity;
- is a congenital anomaly/birth defect;
- is another “important medical event” (any important adverse events/reactions that is not immediately life-threatening or do not result in death or hospitalization but may jeopardize the subject or may require medically significant or requires intervention to prevent one or other of the outcomes listed above). Examples of such events include, but are not limited to, intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalization.

Note: The term sudden death should be used only when the cause is of a cardiac origin as per standard definition. The terms death and sudden death are clearly distinct and must not be used interchangeably. The terms "severe" and "serious" are not synonymous. Severity refers to the intensity of an adverse event; the event itself may be of relatively minor medical significance (such as severe headache without any further findings). Severity and seriousness need to be independently assessed for each adverse event recorded on the eCRF.

Unexpected Adverse Reaction

An Unexpected Adverse Reaction is any experience not previously reported (in nature, severity or incidence) for SiderAL® FORTE drops.

6.2.2 Classification of Adverse Events

The Investigator will classify AEs based on their intensity and relationship to study product.

Intensity

For this study, the intensity of an AE will be rated according to the following definitions:

Mild — Symptom barely noticeable to patient; does not influence performance or functioning. Prescription medication is not ordinarily needed for relief of symptom but may be given because of the personality of a patient.

Moderate — Symptom of a sufficient intensity to make a patient uncomfortable; performance of daily activities influenced; patient is able to continue the study; treatment for symptom may be needed.

Severe — Symptom causes severe discomfort. May be of such intensity that a patient cannot continue the study. Intensity may cause cessation of treatment with study product; treatment for symptom may be given and/or patient hospitalized.

Relationship to Product Under Investigation

For this study, an AE cause and effect relationship to study product will be classified by the Investigator as follows:

None: No relationship between the experience and the administration of the study product; related to other etiologies such as concomitant medications or patient's clinical state.

Unlikely: The current state of knowledge indicates that the relationship is unlikely.

Possible: A reaction that follows a plausible temporal sequence from administration of the study product and follows a known response pattern to the suspected study product. The reaction might also have been produced by the patient's clinical state or other modes of therapy administered to the patient.

Probable: A reaction that follows a plausible temporal sequence from administration of the study product and follows a known response pattern to the suspected study product. The reaction cannot be reasonably explained by the known characteristics of the patient's clinical state or other modes of therapy administered to the patient.

Definite: An AE, which is listed as a possible adverse reaction and cannot be reasonably explained by an alternative explanation, e.g., concomitant drug(s), concomitant disease(s).

6.2.3 Reporting Adverse Events

As a minimum, at each visit during the study, the Investigator or sub-Investigator will ask the patient whether she has experienced any health problems or symptoms since the last visit. An open style of questioning will be used to try and avoid the possibility of influencing the patient.
During the course of the study, the Investigator is requested to report on the eCRF all AEs (including SAEs), except those that are clearly symptoms of the underlying disease, the CD. The Investigator will be responsible for ensuring that the correct information concerning all AEs is entered on the appropriate eCRF forms. AEs shall be timely reported in the eCRF (within 3 days).
The reporting period for AEs is the period starting from the time of Informed Consent signature and lasting until the last study visit (Visit 3).

6.2.4 Reporting Serious Adverse Events

Serious Adverse Event Reporting to the CRO/Sponsor

All SAEs, occurring from the time of signing of the informed consent until the end of the study, except those certainly caused by the underlying celiac disease, must be reported immediately to the vigilance responsible of the site. In the case of SAE with potential relationship to the study product (active or placebo), the Investigator shall promptly inform also Pharmanutra S.p.A.

At the end of this period, all reportable unresolved SAEs will be documented on the eCRF as “ongoing”. All SAEs that might be correlated to the use of the study product should be followed until resolution or stabilization.

Information on the actual fax and phone numbers are provided in the Investigator file as well as on page 6 of this protocol.

The Investigators must contribute to the clarification of the cause(s) of the SAE and to the assessment of potential risks by providing any relevant information obtained or requested with respect to the case.

Reporting to Local Ethics Committees and Competent Authority

It is responsibility of the Investigator to inform the local Ethics Committee (EC) about SAEs and to notify (directly or through Pharmanutra S.p.A.) adverse reactions (serious and not serious) to the competent Phytovigilance System (www.vigierbe.it) in accordance with the current legislation [20].

7 STATISTICAL METHODS

This section summarizes the statistical principles and methods planned to analyse the data for this clinical study. The reference document is the ICH Topic E9 Statistical Principles for Clinical Studies: Note for Guidance on Statistical Principles in Clinical Studies.

All data collected in this study will be documented with the help of patient data listings and summary tables. Data listings and summary tables will be presented by treatment group for each variable.

7.1 Sample Size Determination

This is a pilot study, therefore no formal calculation of the sample size has been done. Sixty patients are planned, mainly based on the clinical site capacity of recruitment.

7.2 Definition of Study Populations for Analysis

Full analysis set (FAS):

All patients of the SAF who have performed the baseline assessments and have at least one post-baseline assessment of any performance endpoint (primary or secondary).

Per-Protocol analysis set (PPAS):

All patients of the FAS who also meet all inclusion/exclusion criteria, having no major protocol deviation and who have completed the entire treatment protocol as originally planned and with high compliance (>80%) will be included in the analysis.

Safety analysis set (SAF):

All patients who took at least one dose of the study treatment.

7.3 Statistical Analysis

Continuous data will be summarized using means and standard deviations (SD) and medians and interquartile ranges for non-normally distributed data. Categorical variables will be presented as counts and percentages. Quantitative variables will be compared using the Student's t-test or ANOVA, and the Mann-Whitney U test or Kruskal-Wallis test for non-normally distributed data. Fisher's exact test or the chi-squared test will be employed for qualitative variables. Confidence intervals of 95% for rates will be calculated using the Wilson method. Bivariate and multivariate analyses will be performed to investigate

potential relationships between variables. The significance level for statistical tests will be set at $p<0.05$. Additional statistical tests may be employed as needed to ensure accurate interpretation of the results.
Adverse events will be coded using the Medical Dictionary for Regulatory Activities (MedDRA) to give a preferred term (PT) and a system/organ class term (SOC) for each event. The number of patients who experienced at least one AE, study product-related AE, serious AE, severe AE and the number of patients withdrawn due to AE will be summarized by treatment arm.
Concomitant medications will be summarized by treatment using descriptive statistics.
Physical examination data in each treatment group will be summarized in a frequency table (normal/abnormal) containing counts and percentages at any visit by system.
Anthropometric parameters in each treatment group will be described by n, arithmetic mean, standard deviation, median, minimum and maximum.

7.4 Planned Interim Analysis(es)

No interim analysis is planned for this study.

8 DATA SAFETY MONITORING BOARD / DATA MONITORING COMMITTEE

No Data Safety Monitoring Board (DSMB) or Data Monitoring Committee (DMC) will be convened for the evaluation of safety data during the study.

9 ETHICAL AND REGULATORY ASPECTS

9.1 Laws and Regulations

This clinical study will be conducted in accordance with the principles contained in the Declaration of Helsinki October 2024, and in compliance with all international laws and regulations and national laws and regulations of the country in which the study is performed, as well as any applicable guidelines.

9.2 Patient's Information Sheet and Informed Consent Form

The Investigator is responsible for and will obtain informed consent from each patient's parents/legal guardian, in accordance with the ICH-GCP Guidelines and the current version of the Declaration of Helsinki. In case of parents, both of them must sign the informed consent.

The child/adolescent will be provided with and information sheet, appropriate for his/her age and will be duly informed by the Investigator about the study activities and the study product.

All the documents will previously have been approved by relevant Ethics Committees (ECs) and may further be updated as new important information becomes available that may affect patient's willingness to participate or continue in the study.

All patients invited to participate in the clinical study are entitled to make their voluntary decision based on all current available information provided to them and to parents/legal guardian by the Investigator/designee.

The patient and the parents/legal guardian must be made aware that he/she can refuse to join the study or withdraw the consent at any time without prejudicing further medical care and that he/she is covered by an indemnity insurance in the event of a study-related injury. Patients and parents/legal guardian must also know that their personal medical records may be reviewed in confidence by the representatives and by Regulatory Authorities and EC and that personal information will be collected and retained in a confidential database. Consent will always be given in writing after the patient and parents/legal guardian have had adequate time to review the information and ask questions, if need be.

9.3 Ethics Review and Authorization by Competent Authorities

Prior to the start of the study, the protocol, amendment(s), consent form, information sheet, and any written information to be provided to patients will be submitted to the IECs and a copy of the written approval will be provided to the Investigator. All documents to review during the study, including any modifications made to the protocol after receipt of EC approval, must also be submitted to the committee for approval prior to implementation, unless they cover administrative issues only. The Investigator must also provide periodic reports as required, and promptly report important safety information (i.e., SAEs) to the EC.

The above responsibilities will be performed in accordance with the applicable ICH-GCP Guidelines.

9.4 Protocol Amendments

Changes to the protocol may only be made by means of a written amendment, which has to be approved and signed by the authorized individuals of the Sponsor and by the Investigators.

Exhaustive justifications that motivate the amendment to the protocol should clearly be addressed in the document.

All substantial protocol amendments must be submitted to ECs for review and approval unless it covers administrative issues only. In this case the ECs will be notified of the amendment without the request to review and approve it.

9.5 Protocol Deviations

The Investigator is to conduct the study in accordance with the relevant, current protocol and will only deviate when necessary to protect the safety, rights and welfare of the patients. In the event that an isolated, unforeseen instance occurs resulting in a protocol deviation, the Investigator is to document this deviation and notify the EC as soon as possible. In no instance should this increase the patient's risk or affect the validity of the study.

9.6 Data Collection

During each patient's study visit, the study Investigator (or designee) will collect and report study data in the relevant patient's chart, documenting all significant observations.

Any contact with the patient, parents/legal guardian via telephone or other means that provides significant clinical information must be documented in the source data and will be promptly entered in the CRF.

An Electronic Case Report Form (e-CRF) will be used for recording patient's study data.

The Investigator will maintain a list of all persons authorized to make entries and/or corrections on the CRFs. Each authorized person will be provided with a user-specific ID protected by a renewable password. Data entries and corrections will be made only by the authorized persons. The e-CRF system will record date and time of any entry and /or correction and the user ID of the person making the entry/correction. The system will keep track of all old and new values (audit trail). It is the responsibility of the Investigator to ensure that the CRFs are properly and completely filled in. The CRFs must be completed for all patients who have been included in the study. The Investigator will review all CRFs and electronically sign and date them for each patient, verifying that the information is complete, true and correct. All fields on the CRF must be completed as applicable.

Patients will be provided with paper questionnaires. Such documents will be filled by the patient during the study, to record data concerning their fatigue status, quality of life and product tolerability.

It is responsibility of the Investigators to instruct the study participants on how to fill in questionnaires in a clear way and preferably in black ball-point pen. The questionnaires are anonymous, each subject is identified through the subject screening number. At the end of the study a copy of all questionnaires and

diaries will be stored at the Site. It is responsibility of the Investigators to correctly enter the data collected on the in the relevant sections on the e-CRF. Questionnaires are considered source data.

9.7 Study Documentation and Record Retention

The medical records of study patients will be retained in accordance with national legislation and in accordance with the maximum period of time permitted by the institution (e.g., hospital).

According to the Good Clinical Practice guidelines (ICH E6) essential documents are those documents which individually and collectively permit evaluation of the conduct of a study and the quality of the data produced. The Investigator must arrange for essential clinical study documents (including ISF and CRFs) other than subject's medical files, to be kept:

• for at least 10 years after completion or discontinuation of the study.

9.8 Confidentiality

Study documents and data will be kept appropriately to ensure their confidentiality.

9.9 Study Report and Publication Policy

The results of the clinical study will be documented in an integrated clinical study report according to ICH E3 Note for Guidance on Structure and Content of Clinical Study Reports and communicated to the EC.

The Investigator is free to use the data collected for world-wide scientific product documentation, and for publication.

9.10 Insurance

Being the study an Investigator-Initiated study, it will be responsibility of the Investigator to provide for specific insurance coverage, through the hospital.

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Appendix 1

<u>Classificazione anatomo-patologica</u> (Marsh-Oberhuber [14] and Corazza [15])

Marsh-Ober.	IELs Oberhuber*/Corazza	Cripte	Villi	Corazza
Tipo 0	<30-40	Normale	Normale	
Tipo 1	>40-30*/>25	Normale	Normale	**Grado A:** aumento dei linfociti intraepiteliali ma nessuna atrofia dei villi (normale architettura dei villi con o senza iperplasia delle cripte e ≥25 IEL/100 enterociti)
Tipo 2	>40-30*/>25	Ipertrofiche	Normale	
Tipo 3a	>40-30*/>25	Ipertrofiche	Lieve atrofia (parziale)	**Grado B1:** villi ancora presenti ma accorciati (rapporto villo-cripto <3:1, conta IEL di>25/100 Enterociti)
Tipo 3b	>40-30*/>25	Ipertrofiche	Marcata atrofia (subtotale)	
Tipo 3c	>40-30*/>25	Ipertrofiche	Assenti (totale)	**Grade B2:** atrofia villosa completa (mucosa completamente piatta e atrofica, senza villi osservabili e ≥25 IEL/100 enterociti)
Tipo 4	<30*-40	Normale	Totale	

IEL: linfocitosi intraepiteliale. *numero di linfociti intraepiteliali modificato come spiegato da Corazza [49]: linfociti intraepiteliali duodenali (30 per 100 enterociti), linfociti intraepiteliali digiunali (40 per 100 enterociti)