PROTOCOL SYNOPSIS

Study Title	Randomized Clinical Trial to Evaluate the Efficacy of Bifidobacterium BB-12® in the Treatment of Infantile Colic
Protocol no.	PSC-DS BIGI
Sponsor	SOFAR S.p.A.
Name of finished product	Bifidobacterium BB-12®
Investigators and study centers	Prof. Roberto Berni Canani Dipartimento di Scienze Mediche Traslazionali – Sezione Pediatrica Laboratorio Europeo per lo Studio delle Malattie Indotte da Alimenti Università degli Studi di Napoli "Federico II" Via S. Pansini 5, Napoli, Italy
Objectives	The primary objective of this study was to evaluate the proportion of successful treatments, defined as a 50% or greater reduction in the average daily duration of crying episodes after 28 days of treatment. The secondary objectives were to evaluate the following variables: • Decrease from baseline in the number of crying episodes and in the persistence of colics at the end of the study (day 28). • Decrease in the number of regurgitation and vomiting episodes. • Number of respiratory, gastrointestinal, cutaneous and urinary infections during the study. • Number of bowel movements and consistency of fecal mass.
Methodology	This was a randomized, double-blind, placebo-controlled clinical study. The study was conducted together with five family pediatricians practicing in the city of Naples. The pediatricians were asked to refer potentially eligible patients and to provide support, if necessary, to their parents. The objectives and procedures of the study were thoroughly presented to each pediatrician during a preliminary meeting held at the single trial center. During the study, the center was at the disposal of the pediatricians in case of need or clarification. The Investigator was responsible for enrolling and evaluating the patients, as well as for completing the case report forms (CRF). After the patient's parents had provided written informed consent, and once all eligibility criteria had been met, patients were required to follow a one-week pre-enrollment period during which fermented milk and nutritional supplements containing probiotics were prohibited. If after this period the diagnosis of infantile colic was confirmed, the patient was randomized in a 1:1 ratio to receive one of the two following products: • *Bifidobacterium animalis* subsp. *lactis*, BB-12®: 6 drops/day (which guaranteed 1 billion live cells) for 28 consecutive days. • Placebo oil drops: 6 drops/day for 28 consecutive days. The patient's parents were provided with a diary and were instructed on how to complete it with data concerning the daily number and duration of crying episodes, the consistency of their baby's feces (according to the Bristol scale), and the daily number of bowel movements. Data was collected from these diaries at each weekly visit. Each patient was to undergo a total of 6 visits over a 5-week period.
Number of patients	The study population was made up of 80 children who met eligibility criteria.
Diagnosis and main criteria for inclusion	***Inclusion criteria*** 1. Exclusively breastfed babies of both sexes ≤ 7 weeks of age. 2. Diagnosis of infantile colic according to Rome III Criteria.

	3. Written informed consent of parents. ***Exclusion criteria*** 1. Weight at birth < 2500 g. 2. Gestational age < 37 weeks. 3. Apgar score at 5 minutes < 7. 4. Use of baby formulas. 5. Stunted growth/weight loss (< 100 g/week from birth to last reported weight). 6. Neurological diseases. 7. Confirmed or suspected food allergy. 8. Gastroesophageal reflux disease. 9. Intake of substances that alter intestinal microbiota (probiotics, prebiotics and antibiotics, gastric antacids) in the 2 weeks prior to enrollment. 10. Fever and/or infectious diseases in the 2 weeks prior to enrollment. 11. Current systemic infections. 12. History of congenital infection. 13. Chronic intestinal disease (cystic fibrosis or other forms of primary pancreatic insufficiency). 14. Primary or secondary gastrointestinal malformations (esophageal atresia, intestinal atresia, short bowel syndrome, malrotation). 15. Metabolic disease. 16. Genetic diseases and chromosomal abnormalities. 17. Primary or secondary immunodeficiency. 18. Insufficient reliability or presence of conditions that make the patient's compliance with the protocol unlikely. 19. Previous participation in this study.
Test product, dose and mode of administration	• Investigational treatment: dietary supplement containing Bifidobacterium (BB-12®) as active ingredient. Each 8 ml bottle with dropper contained 1 billion CFU per 6 drops. Dosage: 6 drops a day for 28 days. OR • Placebo indistinguishable from the investigational product in packaging, color, weight, smell and taste. Dosage: 6 drops a day for 28 days.
Criteria for evaluation	***Primary efficacy variable*** The primary efficacy variable was the proportion of successful treatments, defined as a 50% or greater reduction in the average daily duration of crying episodes after 28 days of treatment. ***Secondary efficacy variables*** • Decrease from baseline in the number of crying episodes and in the persistence of colics at the end of the study (day 28) • Decrease in the number of regurgitation and vomiting episodes • Number of respiratory, gastrointestinal, cutaneous and urinary infections during the study • Number of bowel movements • Consistency of fecal mass using the Bristol scale • Effects of BB-12 on intestinal microbiota • Variations of the intestinal production of innate immunity peptides (calprotectin, β defensin type 2, LL37) and short-chain fatty acids (butyrate e propionate) ***Safety variables*** Safety was assessed based on the results of physical exams and on the incidence of adverse events (AE).
Statistical methods	The following analysis sets were defined: • Per Protocol Set (PP): all randomized patients who completed the study without any significant protocol violation. • Intention to Treat Set (ITT): all randomized patients who received at least one dose of study treatment. • Safety Set: all randomized patients who received at least one dose of study treatment. Patients were analyzed according to the treatment received.

	A patient who came back for Visit T2 was considered as having received at least one dose of study treatment. The primary efficacy analysis was performed on the ITT population, and on the PP population as supportive. The secondary efficacy analysis was performed on the ITT population only. The safety analysis was performed on the Safety population. ***Primary variable*** Mean daily crying duration is described for each week by means of descriptive statistics for continuous data and was calculated on non-missing values; observations with values equal to zero were included in the computation. Mean change from baseline (mean of Week 1) was computed for each week. The treatment success rate was evaluated in terms of the reduction of crying duration, comparing mean daily duration of the last Week (from T4 to T5) with that of Week 1 (from T0 to T1). Success was defined as a reduction ≥ 50%. The proportion of patients with successful outcomes in the two groups is summarized using descriptive statistics for categorical data and analyzed by means of a Chi-Square test.
Date and version	English version 1.0, 4 December 2019 – based on Protocol version 3, 16 May 2016 (document in Italian language)