These results are supplied for informational purposes only.
Prescribing decisions should be made based on the approved package insert in the country of prescription.

Sponsor: Sanofi **Drug substance(s):** Polyantibiotic-resistant *Bacillus clausii* spores	**Study Identifiers:** UTN Number: U1111-1223-4563 EudraCT Number: 2020-000970-10 **Study code:** LPS16140
Title of the study: Phase III, randomized, double blind, parallel groups, clinical trial to evaluate the efficacy and safety of *Bacillus clausii* versus placebo in the prevention of antibiotic associated diarrhea (AAD) in children	
Study center(s): This study was conducted at 18 centers that enrolled 335 and randomized 332 participants in 2 countries (8 centers in Turkey and 10 centers in Hungary).	
Study period: 20-Nov-2020 to 11-Jan-2022 Study Status: Terminated. The study was prematurely stopped, because the planned futility analysis showed an AAD rate below the futility boundary of 6.0% defined in the protocol.	
Phase of development: Phase 3	
Objectives: **Primary objectives** To evaluate the efficacy of *Bacillus clausii* versus placebo in prevention of AAD in children in an outpatient setting. **Secondary objectives** To evaluate the efficacy of *Bacillus clausii* versus placebo on prevention of abdominal signs/symptoms (other than diarrhea) associated with antibiotics such as abdominal swelling (belly tension), and vomiting. To evaluate the efficacy of *Bacillus clausii* versus placebo on prevention of infectious diarrhea (diagnosis based on positive tests). To evaluate the efficacy of *Bacillus clausii* versus placebo on prevention of hospitalization to manage diarrhea, or prevention of (intravenous [IV]) rehydration. To evaluate the safety of *Bacillus clausii*.	

Methodology:

This was a Phase III, double-blind, randomized, placebo-controlled, parallel-group, multicenter clinical trial, to assess the efficacy and the safety of *Bacillus clausii* 4 billion spores/day versus matching placebo for the prevention of AAD in pediatric outpatients. The randomization was stratified by the type of antibiotic prescription (broad spectrum beta-Lactam antibiotic; Macrolide) and for the breastfeeding status (yes; no; mixed). Participants had to be 3 months to 5 years of age inclusive.

Three visits were planned in this study:

- Visit 1, the baseline visit, was conducted to assess if the participant fulfilled all inclusion and exclusion criteria of the study. Eligible participants were randomly assigned to one of the two treatment groups (1:1 ratio) after they gave their written informed consent. All randomized participants were planned to receive the blinded study medication. Legal guardians/parents were given a diary in which abdominal signs and symptoms, number of stools per day, stool consistency, study medication intake, concomitant medication intake and adverse events (AEs) were recorded daily.
- Visit 2 was performed by phone as soon as possible after the last dose of antibiotic treatment (between Day 3 and Day 10, depending on antibiotic), but not later than 3 days after the end of antibiotic treatment.
- Visit 3, the end of study visit, was performed 2 weeks after the end of antibiotic treatment (between Day 17 to Day 24, depending on antibiotic treatment duration) within a visit window of 3 days. A questionnaire of satisfaction with study treatment was completed by the legal guardians/parents on. Study physicians also completed a questionnaire of satisfaction with study treatment at the end of the visit.

Number of participants:

Planned: 660

Enrolled/Randomized: 335/332

Evaluated:

Safety: 331

Diagnosis and criteria for inclusion:

Children aged 3 months to 5 years who had to start short-term (3-10 days) treatment with oral antibiotics (broad spectrum beta-lactam AB [amoxicillin-with or without clavulanic acid-, or second and third generation of cephalosporines], or macrolides [erythromycin, clarithromycin, roxithromycin and azithromycin]) were included in the study.

Participants were excluded if they were suffering from chronic or acute diarrheal disease, severe or persistent vomiting or other chronic GI problems, were immunodeficient or receiving immunosuppressive treatment, or had urinary infection or chronic diseases of endocrine, cardiovascular, renal or respiratory system. Critically ill or hospitalized or severely malnourished participants were also excluded from the study.

Study products

Investigational medicinal product(s): *Bacillus clausii*

Formulation/Form & composition: oral suspension, presented in a mini-bottle.

Route(s) of administration: oral

Dose regimen: *B. clausii* 4 billion spores/5 mL/day, one mini-bottle once a day (every day in the same time frame of the day) for the duration of antibiotic treatment and 7 days thereafter.

Investigational medicinal product(s): Placebo

Formulation/Form & composition: oral suspension, presented in a mini-bottle.

Route(s) of administration: oral

*D*ose regimen: once a day (every day in the same time frame of the day) for the duration of antibiotic treatment and 7 days thereafter.

Duration of treatment/participation:

Participants were treated for the duration of antibiotic treatment (3-10 days) plus 7 days thereafter. Including the follow-up visit, 7 days after the end of treatment, the duration of the study was 17-24 days.

Criteria for evaluation:

Primary endpoints

Percentage of participants with AAD during the study randomized period (ie, from randomization up to 2 weeks after the end of antibiotic therapy). Note: Antibiotic-associated diarrhea (AAD) defined as ≥3 loose or watery stools (Type 5, 6 or 7 on Bristol Stool Form Scale) per 24 hours for a minimum of ≥48 hours, occurring during and/or up to 2 weeks after the end of the antibiotic therapy.

Secondary endpoints

1) During the study randomized period:

- Percentage of participants with any of the abdominal signs/symptoms (other than diarrhea).
- Percentage of participants with abdominal swelling (belly tension).
- Percentage of participants with vomiting.

2)Percentage of participants with infectious diarrhea during the study randomized period. Infectious diarrhea defined as diarrhea having pathogen such as bacterium (ie, Salmonella spp. diarrhea, Shigella spp. diarrhea…), as virus (ie, rotaviral diarrhea, adenoviral diarrhea...), as parasite or as toxin (ie, Clostridium difficile diarrhea…)

3) Percentage of participant with hospitalization to manage the diarrhea, and/or intravenous rehydration during the study randomized period.

4) Incidence of adverse events throughout the study.

Statistical methods:

The primary efficacy variable, AAD response rate during the study randomized period (ie, from Randomization up to 2-weeks after the last intake antibiotic therapy), was to be analyzed on the ITT population using a logistic regression model adjusted on randomization strata (type of antibiotic prescription, breastfeeding status) and age. Intercurrent events were to be handled

following treatment policy strategy, with the exception of premature discontinuation of study intervention due to COVID-19 pandemic (hypothetical strategy: off-treatment data were to be set as missing).

The secondary endpoints were to be analyzed using the same statistical model and following the same intercurrent events handling strategy as for the primary endpoint.

All safety analyses were descriptive and were performed on the safety population. The TEAE period was defined as the time between the first administration of IMP and the last administration of the IMP + 24 hours.

Summary Results:

Demographic and other baseline characteristics:

In the safety population, the mean (SD) age in the *B. clausii* treatment group was 2.71 (1.31) years and in the placebo group it was 2.89 (1.21) years.

The majority of the patients (70.9% in the *B. clausii* group and 78.9% in the placebo group) were ≥2 years old, and there were slightly more male participants (53.3% in the *B. clausii* treatment group and 53.6% in the placebo group) than females.

Only one participant in each group showed signs of some dehydration.

In the *B. clausii* treatment group 4 (2.4%) participants were breastfed compared with 2 (1.2%) participants in the placebo group.

Most participants (107 [64.8%] in the *B. clausii* group and 106 [63.9%] in the placebo group received broad spectrum Beta-Lactam antibiotics, all others received macrolides. In both groups, the median (min, max) planned duration of antibiotics therapy was 6 (3, 10) days.

Efficacy Results:

The study was prematurely stopped, because the futility analysis showed a low global AAD rate of 3.6% (95% CI: 1.41; 5.79), which was below the futility boundary of 6.0% defined in the protocol. Consequently, none of the efficacy analyses originally planned were performed.

At study end, the rate did not change: overall, 12/332 (3.6%) participants were identified to have had at least one AAD episode.

Safety results:

The incidence of all treatment emergent adverse events (TEAEs) reported during the study was similar between the study intervention groups.

The numbers of participants with treatment emergent serious adverse events ([SAEs]; 1 in the *B. clausii* treatment group versus 0 in the placebo group), TEAEs that led to discontinuation (2 in the B. clausii treatment group versus 1 in the placebo group) and TEAEs assessed as related to treatment (5 in the *B. clausii* group versus 7 in the placebo group) were all comparable between the treatment groups.

The SAE (respiratory syncytial virus bronchiolitis) and all AEs that led to discontinuation (gastroenteritis and otitis media in the *B. clausii* group and urticaria in the placebo group) were assessed to be not related to IMP or to study procedures.

Two participants, one in the *B. clausii* group and one in the placebo group, experienced asymptomatic overdose.

Issue date: 8th Jun 2022