Official Title:	**Multicenter, Double-blind, Randomized, Placebo-controlled, Parallel-group Efficacy and Safety Study of LRG-002 Hard capsules (Lek d.d., Slovenia), Used in the Prophylaxis of Antibiotic-associated Diarrhea in Adults**
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Statistical Analysis Plan

Sponsor	Sandoz JSC
Protocol title	Multicenter, double-blind, randomized, placebo-controlled, parallel-group study of the efficacy and safety of LRG-002, hard capsules (Lek d.d., Slovenia), in the prophylaxis of antibiotic-associated diarrhea in adults
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Approved by

Full name	Position	Signature	Date
[redacted]	[redacted]	*/Signature/*	*March 22, 2021*
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Table of contents

List of abbreviations and definitions of terms

AAD	Antibiotic-associated diarrhea
AB	Antibiotics
AB-therapy	Antibiotic therapy
ARLD	Alcohol-related liver disease
BP	Blood pressure
GA	Gastric adenocarcinoma
ALT	Alanine aminotransferase
JSC	Joint-stock company
ASH	Alcoholic steatohepatitis
AST	Aspartate aminotransferase
ATP	Adenosine triphosphate
ATPase	Adenosine triphosphatase
ATC	Anatomical Therapeutic Chemical (classification system)
ROS	Reactive oxygen species
DS	Dietary supplement
BSS	Bristol Stool Scale
IM	Intramuscular
IV	Intravenous
ULN	Upper limit of normal
HIV	Human immunodeficiency virus
IBD	Inflammatory bowel disease
WMA	World Medical Association
IUS	Intrauterine system
IUD	Intrauterine device
RSC	Russian Society of Cardiology
WHO	World Health Organization
HPLC	High-performance liquid chromatography
g	Gram
GOST	State standard
GC	Gas chromatography

d.d.	"d.d." or "delniška družba," Slovenian, public limited company
DBP	Diastolic blood pressure
CI	Confidence interval
DMH	Dimethylhydrazine
DNA	Deoxyribonucleic acid
TD	Traveler's diarrhea
ADI	Acceptable daily intake
GI tract	Gastrointestinal tract
CJSC	Closed joint-stock company
EU	European Union
IAAD	Idiopathic antibiotic-associated diarrhea
CF	Case form
IL	Interleukin
PI	Prescribing information
Full name	First Name, Patronymic, Last Name
eCRF	Electronic case record form
ELISA	Enzyme-linked immunosorbent assay
IFN	Interferon
IFN-γ	Interferon gamma
CRO	Contract research organization
kg	Kilogram(s)
NDI	Numbers of days of illness
CFU	Colony-forming units
CPS	Capsular polysaccharides
CRC	Colorectal cancer
L	liter(s)
LGG	Lactobacillus rhamnosus GG
LD50	Half-lethal dose
LPS	Lipopolysaccharides
CF	Cystic fibrosis
mg	Milligrams

MDA	Malondialdehyde
MoH	Ministry of health
min	Minutes
ICD	International Classification of Diseases
µg	Micrograms
mL	Milliliters
mm	Millimeters
PBMC	Peripheral blood mononuclear cells
INN	International nonproprietary name
MNNG	Methylnitronitrosoguanidine
MIC	Minimum inhibitory concentration
NPE	Number of polychromatophilic erythrocytes
NAFLD	Non-alcoholic fatty liver disease
e.g.	For example
NEC	Necrotizing enterocolitis
AR	Adverse reaction
IEC	Independent Ethics Committee
AE	Adverse event
AWD	Acute watery diarrhea
AGE	Acute gastroenteritis
SCS	Spent culture supernatant
LLC	Limited liability company
RR	Relative risk
ARD	Acute respiratory disease
ICU	Intensive care unit
FEV	Forced expiratory volume
OR	Odds ratio
PVC	Polyvinyl chloride
TJ	Tight junctions
PCTFE	Polychlorotrifluoroethylene
PCA	Pyrrole-2-carboxylic acid
PCR	Polymerase chain reaction

times/day	Times a day
WG	Working group
RIGT	Radiation-induced gastrointestinal toxicity
RCS	Randomized clinical studies
BC	Breast cancer
RMSAH	The Russian Medical Society on Arterial Hypertension
RNA	Ribonucleic acid
ORS	Oral rehydration solution
RD	Risk difference
MA	Marketing authorization
RF	Russian Federation
SBP	Systolic blood pressure
SAR	Serious adverse reaction
SAE	Serious adverse event
et al.	And other authors
SOP	Standard operating procedure
ESR	Erythrocyte sedimentation rate
MD	Mean difference
IBS	Irritable bowel syndrome
day	day
i.e.	That is
TCID50	Tissue culture infectious dose
TEER	Transepithelial electrical resistance
FAP	Functional abdominal pain
FD	Functional dyspepsia
FL	Federal law
Full name	First name, Patronymic, Last name
TNF	Tumor necrosis factor
FSH	Follicle-stimulating hormone
BU	Bread unit
CIGT	Chemotherapy-induced gastrointestinal toxicity

h	Hours
NNT	Number needed to treat
RR	Respiration rate
HR	Heart rate
ECG	Electrocardiography, electrocardiogram
EPS	Exopolysaccharides
AFB	Acid-fast bacilli
AGRICOLA	Agricultural Online Access
AMED	Allied and Complementary Medicine Database
ATCC	ATCC 53103, strain L. rhamnosus
AUC	Area under the curve
$AUC_{0-\infty}$	Concentration–time curve from time zero to infinity
BALB	A laboratory-bred strain of albino house mouse widely used in clinical studies
BG2FO	One of the strains of Lactobacillus spp.
C	Degree Celsius
C. difficile	*Clostridium difficile*
C57BJ	Inbred strain of laboratory mice widely used as models of human diseases
C57BL	Inbred strain of laboratory mice widely used as models of human diseases
CaH	Calcium hydride
CBA	Inbred strain of laboratory mice
CCL C-C	Motif Chemokine Ligand 11: chemokine 11 containing a C-C motif

CD	Cluster of differentiation
CD62p	Markers of platelet activation
CdAD	Clostridium difficile-associated diarrhea
CdD	Clostridium difficile-associated diarrhea
CHCC	One of the strains of Lactobacillus spp.
CHO	Chinese hamster ovary
CINAHL	Cumulative Index to Nursing and Allied Health Literature
CMPG 5153	Mutant L. rhamnosus GG strain
CP	Organophosphate pesticides parathion and chlorpyrifos
CTM	Clinical Trial Manager
CXCL	Interleukin-8
CYP	Cytochrome P450
CYP2E	Cytochrome P450
GCP	Good clinical practice
DN-114001	One of the strains of Lactobacillus casei
DOI	Digital object (article) identifier
E. cloacae	*Escherichia cloacae*
E. coli	*Escherichia coli*
EFSA	European Food Safety Agency
EMBASE	Biomedical and pharmacological bibliographic database
EPEC	Enteropathogenic *Escherichia coli*
ERK	Protein kinase signaling pathway
ESPGHAN	European Society of Pediatric Gastroenterology, Hepatology, and Nutrition
et al.	And others
ETEC	Enterotoxigenic E. coli
F_0F_1-	Structural complex of ATP synthase

FAO	Food and Agriculture Organization of the United Nations
FDA	Food and Drug Administration
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
GR	One of the L. rhamnosus strains
GRAS	Generally Regarded As Safe – FDA
GSH	*Glutathione*
H. pylori	*Helicobacter pylori*
HCI	Hydrochloric acid
HIF	Hypoxia-inducible factors
HIV	Human immunodeficiency viruses
HNOO 1-DR20	One of the probiotic Lactobacillus rhamnosus
HN001	One of the L. rhamnosus strains
HN019	One of the L. rhamnosus strains
HNOO	One of the L. rhamnosus strains
HNO1	One of the L. rhamnosus strains
ICH	The International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use
ITT	Intention-To-Treat
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IkB	Inhibitory protein
JAMA	Journal of the American Medical Association
L.	Lactobacillus
LCR	Lactobacillus casei subsp. rhamnosus strain
LGG	One of the L. rhamnosus strains
LGR	One of the L. rhamnosus strains

LPPV	Local Person for Pharmacovigilance
LPXTG	Binding protein
MANTIS	Manual, Alternative, and Natural Therapy Index System
MAMP	Microbe-associated molecular patterns
MAPK	Mitogen-activated protein kinase
MedDRA	The Medical Dictionary for Drug Regulatory Affairs
MRS	Liquid medium for the isolation of lactobacilli, according to the prescription recommended by Man, Rogosa, and Sharpe as well as according to ISO standards
mTg	Murine thyroglobulin
NNT	Number needed to treat
OTA	Ochratoxin A
PECD62p	Marker of platelet activation
PJS	Propionibacterium freudenreichii ssp. shermanii JS
PP	Per-Protocol
PROSAFE	Collection of probiotic and human strains
PRR	*Pattern recognition receptor*
QPS	Qualified Presumption of Safety
RC	One of the L. rhamnosus strains
RR	Risk ratio
S. boulardii	*Saccharomyces boulardii*
SF68	Enterococcus faecium SF68
spp.	Species
subsp.	Subspecies

SUSAR	Suspected Unexpected Serious Adverse Reaction
TGF	*Transforming growth factor*
TLR	Toll-like receptor
TOXLINE	Toxicology Literature Online
TY21A	*Salmonella typhimurium*
ToxFILE	Bibliographic database of biochemical, pharmacological, physiological, and toxicological effects of drugs and other chemicals
VRE	Vancomycin-resistant enterococci
ZO-1	Zonula occludens-1

1. Introduction

This clinical study is conducted to investigate the efficacy and safety of the investigational medicinal product LRG-002, capsules, versus placebo to LRG-002, capsules, against the background of standard antibiotic therapy in patients with acute respiratory disease for the prevention of AAD. Based on the results of this clinical study of the medicinal product, the state registration (authorization) of LRG-002, capsules, is planned.

Statistical analysis for this study will be carried out using the R programming language, version 3.7.

2. Study objectives and design

2.1. Study objective

The objective of this study is to investigate the efficacy and safety of using the investigational medicinal product LRG-002, capsules (Lek d.d., Slovenia), versus placebo in addition to standard antibiotic therapy in patients with acute respiratory diseases (ARD) as an auxiliary therapy for the prevention of AAD.

2.2. Study design

A prospective, multicenter, double-blind, randomized, parallel-group clinical study.

Patients will be included by applying stratified randomization in two strata:

- Age < 50 years
- Age ≥ 50 years

Each treatment group will be balanced as 1:1 in each stratum.

In this study, neither the investigator nor the patient shall be aware of whether the investigational medicinal product or placebo is being administered to the patient.

The investigational medicinal product LRG-002 is used according to the following scheme: a single dose of 1 capsule for oral administration with a meal and a little water; the medicinal product will be taken orally 2 times a day for 14 days.

Placebo is administered under the following scheme: a single dose of 1 capsule for oral administration with a meal and a little water; the medicinal product will be taken orally 2 times a day for 14 days.

If the prescription is received in the afternoon, the protocol allows only one capsule to be taken on Day 1. The interval between taking the oral antibiotic and the investigational medicinal product shall be at least 3 hours.

The study design is depicted in Figure 1.

2.2.1. Study design flowchart

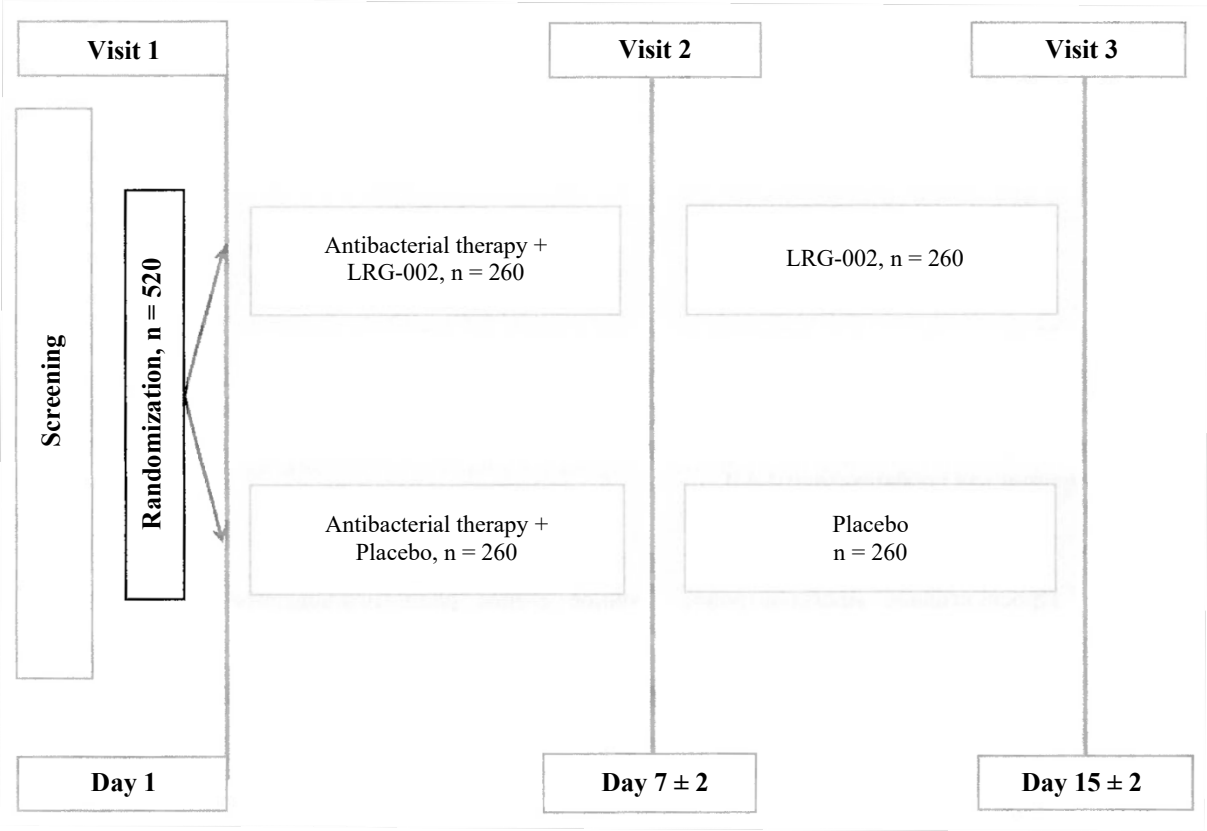

Figure 1. Study design flowchart

2.2.2. Study duration

The duration of the study will be no more than 17 days for patients.

1) Screening/randomization/initiation of therapy: Day 1
2) Interim observation visit: Day 7 ± 2
3) Final visit: Day 15 ± 2
4) Total enrollment duration: about 8 months

2.2.3. Study stages

It is allowed to conduct visits in the form of outpatient visits by a doctor and a nurse at the patient's home.

Screening/randomization/therapy initiation (Visit 1)

Visit 1 is scheduled for Day 1. At this visit, the doctor evaluates the patient's eligibility for the study. If non-inclusion criteria are not identified, randomization is carried out, the patient is given the investigational/reference product, a laboratory kit for collecting stool samples with appropriate instructions, a patient diary, and training on filling out the patient diary (Appendix 3).

Procedures at Visit 1:

Visit 1 (Day 1)

- Collection of demographic data (year of birth, sex, age);
- Measurement of weight and height;
- Anamnesis collection;
- Registration of concomitant therapy (including other medications, dietary supplements, and the use of medical devices);
- Collection of complaints, actualization of anamnesis;
- Physical examination;
- Assessment of vital signs (heart rate, respiration rate, blood pressure, temperature);
- Hematology;
- Biochemistry;
- Express test for the qualitative detection of immunoglobulin G (IgG) and immunoglobulin M (IgM) antibodies to COVID-19;
- Urine pregnancy test;
- 12-lead ECG;
- Evaluation of inclusion/non-inclusion criteria;
- Randomization;
- Dispensing of an investigational medicinal product (IMP)/ placebo;
- Issuance of a patient diary, mercury thermometer, and appropriate instructions;
- Issuance of a laboratory kit with the necessary consumables for collecting stool samples;

- Evaluation of adverse events.

Visit 2 (Day 7 ± 2)

Procedures performed at Visit 2 in each treatment group:

- Collection of complaints, actualization of anamnesis;
- Records on the concomitant therapy;
- Weight measurement;
- Physical examination;
- Measurement of vital signs;
- Hematology;
- Biochemistry;
- Adverse events registration;
- Evaluation of exclusion criteria;
- Issuance of a laboratory kit with the necessary consumables for collecting stool samples (if necessary);
- Compliance evaluation (diary completion, administration of the investigational medicinal products);
- Assessment of diarrhea based on patient diary entries and face-to-face interviews in relation to antibiotic use (AAD or non-AAD) according to the investigator's opinion (in the absence of stool analysis results at the time of the visit, the assessment is carried out after the investigator receives the results from the laboratory).

Treatment completion visit (Visit 3, Day 15 ± 2)

Visit 3 is conducted on Day 15 ± 2; the patient must visit the trial site in the fasted state and bring the filled-in diary and unused medicinal product.

- Collection of complaints, actualization of anamnesis;
- Body weight measurement;
- Records on the concomitant therapy;
- Physical examination;
- Measurement of vital signs;
- Adverse events registration;
- Evaluation of exclusion criteria;

- Compliance evaluation (diary completion, administration of the investigational medicinal products);
- Hematology;
- Biochemistry;
- 12-lead ECG;
- Assessment of diarrhea based on patient diary entries and face-to-face interviews for classification as AAD according to the doctor's opinion (in the absence of stool analysis results at the time of the visit, the assessment is carried out after the investigator receives the results from the laboratory);
- Return of the unused investigational medicinal product;
- Return of the unused laboratory stool collection kit;
- Return of the patient's diary.

Unscheduled visits

The unscheduled visits will be conducted when necessary, e.g., in case of the index disease deterioration, AEs, or intolerability of IMPs (LRG-002 or placebo).

Every unscheduled visit, irrespective of its cause, must include the procedures listed below with completion of the relevant eCRF pages (Unscheduled visit):

- Physical examination;
- Measurement of vital signs (blood pressure, heart rate, respiration rate, body temperature);
- 12-lead ECG;
- Evaluation of adverse events;
- Evaluation of the concomitant therapy.

In case of indications, any of the study procedures may be additionally performed upon the decision of the Principal Investigator.

Taking into account the frequency of visits, unscheduled visits may be conducted on the same days as scheduled ones but at a different time (e.g., in case of worsening, the patient may visit the trial site in the afternoon).

Procedures for planned clinical study visits are the same in each treatment group during each study period.

Early study termination

The Sponsor has the right to terminate the study, and the Investigator has the right to stop recruiting patients at any time. In the event of early closure of the site/study, all completed and unused CRFs (including unused pages of partially completed CRFs) and other documentation (with the exception of documentation that must be kept at the site) must be returned to the Sponsor. Study materials can be destroyed only with the consent of the Sponsor.

2.3. Sample size

Calculation of the sample size is based on the following works:

1. Efficacy of probiotics in prevention of acute diarrhea: a meta-analysis of masked, randomized, placebo-controlled trials. Sazawal S, Hiremath G, Dhingra U, Malik P, Deb S, Black RE. Lancet Infect Dis. 2006 Jun; 6(6):374-82. Review. PMID: 16728323. DOI:10.1016/S1473- 3099(06)70495-9.
2. Chow S, Shao J, Wang H. Sample Size Calculations in Clinical Research. 2nd Ed. Chapman & Hall/CRC Biostatistics Series, 2008.
3. McFarland LV. Meta-analysis of probiotics for the prevention of antibiotic associated diarrhea and the treatment of Clostridium difficile disease. Am J Gastroenterol. 2006 Apr;101(4):812-22.
4. Videlock EJ, Cremonini F. Meta-analysis: probiotics in antibiotic-associated diarrhoea. Aliment Pharmacol Ther. 2012 Jun;35(12): 1355-69. doi: 10.1111/j.1365-2036.2012.05104.x. Epub 2012 Apr 24.
5. H. Szajewska, M. Kolodziej. Systematic review with meta-analysis: Lactobacillus rhamnosus GG in the prevention of antibiotic-associated diarrhoea in children and adults. AP&T, Volume 42, Issue 10, November 2015, Pages 1149–1157.

The primary endpoint is an assessment of the incidence of AAD from Day 1 to the last day of the course of LRG-002 use vs. placebo in ARD patients receiving standard antimicrobial therapy. The primary efficacy criterion will be considered met if the odds ratio for AAD between the two groups is statistically significantly greater than 1 (i.e., the incidence of AAD in the LRG-002 group is lower than in the placebo group).

A test based on the odds ratio was used to calculate the sample size.

The following data were obtained from Figure 2 in a meta-analysis [1] (only data for AAD were used):

Using only data for AAD studies in adults from the cited publication, the rate for the control group is 0.24 and for the treatment group 0.14.

Using only data for LGG studies in adults and children, the rate for the control group is 0.26 and for the treatment group 0.15.

In meta-analysis [14], figures from Table 1 give a rate of 0.196 for a treatment group and 0.29 for a placebo group (only studies for adults with LGG were taken into consideration).

In meta-analysis [15], figures from Table 2 give a rate of 0.188 for a treatment group and 0.269 for a placebo group (only studies for adults with LGG were taken into consideration).

The consideration of AAD studies for LGG from [16], concerning infections in adults (including H. pylori), gives 0.14 for a probiotic group and 0.22 for a control group.

Combining all the strains, we obtain an incidence of 0.26 for the placebo and 0.15 for the probiotic group.

The following indicators were used for calculations:

1. The difference of absolute risks of AAD in the placebo group and the treatment group is expected to be positive.

The incidence of AAD = 0.26 is assumed for the placebo and 0.15 for the treatment group.

Possibility of type I error: 0.05

Power of the study is 80%, which corresponds to the type II error: 0.20.

Statistical hypothesis is the evidence of superior efficacy:

$$H0: OR = 1$$
$$H1: OR \neq 1,$$

where OR is the odds ratio between both groups.

The ratio between the treatment and placebo group sizes is 1:1.

Amount of the patient in each group could be calculated using the following formula [2]:

$$n_T = n_T = \left(\frac{Z_{1-\alpha/2} + Z_\beta}{p_T - p_R} \right)^2 \left[p_T (1 - p_T) + p_R (1 - p_R) \right] = 208$$

Thus, the minimum number of patients required for statistical analysis is 416 (208 patients per group). Taking into account a 20% withdrawal rate, one needs to randomize 520 subjects (260 patients per group).

3. General analysis values

3.1. Clinical and statistical significance

All the statistical tests within this study will be performed at 95% confidence level (threshold value *p* to confirm statistical significance is less than 0.05). The two-sided statistical criteria will be used for all other studied parameters.

3.2. Procedure for missing, unanalyzable, and questionable data

During the monitoring visits to the study sites, the clinical study specialists (monitors) authorized by the Sponsor will analyze CRFs for data completeness. In the absence of data on CRFs and the availability of relevant information in the primary documentation, questions for investigators and regulations to address inconsistencies will be formulated. At the database check, the expert in statistics authorized by the Sponsor, the Data Control and Processing managers will analyze the study results for questionable, missing, and unanalyzable data, which might be the basis for the questions for investigators. If possible, the investigators will eliminate the errors identified in CRFs and inform the Principal Investigator and authorized representatives of the Sponsor hereof. In case the identified data errors cannot be eliminated after the completion of the patients' participation in the study, the analysis of the resulting parameter sensitivity to questionable data will be conducted in the statistical analysis. Information on missing, questionable, and unanalyzable data will be summarized in the final clinical study report. A method of filling in the missing data based on maximum likelihood estimation (MLE) for the primary endpoint will be applied if needed. The analysis of the rest of endpoint types and other parameters will be performed only on the basis of the available information without filling in the missing data in view of the short duration of the clinical study.

4. Study participants

4.1. Patient distribution

It will be a double-blind, randomized study.
Patients will be included by applying stratified randomization in two strata:
Age < 50 years
Age ≥ 50 years
Each treatment group will be balanced as 1:1 in each stratum.

Stratified randomization is a two-stage procedure in which patients who enter a clinical study are first grouped into strata. Within each stratum, patients are then assigned to treatment according to separate randomization schedules. The randomization module of EDC system assigns patient unique randomization code based on a treatment group and stratum. Randomization schedules will be hidden from medical personnel and all other persons involved in the study (monitors, data management, biometry, sponsor, etc.), except for the specialists responsible for IMP labeling and emergency unblinding.

4.2. Study population

4.2.1. Safety population

The safety population (safety): All randomized subjects who received at least one dose of the investigational/reference product and have completed at least one visit aimed at the evaluation of safety parameters (i.e., at least Visit 1). As distinct from ITT population, the safety population will be analyzed depending on actually received treatment (not only prescribed) (in case of difference between the prescribed and received therapies).

4.2.2. ITT population receiving treatment

The intention-to-treat (ITT) population: All randomized subjects who received at least one dose of the investigational medicinal product/placebo and have completed at least one visit aimed at the evaluation of efficacy parameters (i.e., at least all the procedures of Visit 1).

4.2.3. Per protocol set

The per protocol (PP) population: All randomized subjects who completed participation in the study in accordance with the protocol (have completed the prescribed period of treatment and follow-up without significant deviations from the protocol).

5. Demographics and baseline characteristics

All the data obtained in the groups before the study therapy initiation (demographic, laboratory, instrumental, and physical examinations data, vital signs, etc.) will be compared to determine the groups' comparability for analysis.

For quantitative characteristics, it is planned to calculate the following statistics:

1) Quantity
2) Number of missing values
3) Median
4) 95% CI for the mean
5) Standard deviation
6) Median
7) Minimum
8) Maximum
9) First quartile
10) Third quartile
11) Interquartile range

For categorical variables, it is planned to calculate the following characteristics:

1) Absolute number by the group
2) Percentage relative to groups

3) Percentage of visits by group

Quantitative variables will be checked for compliance with the normal distribution using the Shapiro–Wilk test.

To compare quantitative data distributed according to the normal distribution, it is planned to use parametric criteria: Student's t-test for dependent/independent samples, analysis of variance (ANOVA) for repeated measurements to compare more than two stages.

To compare quantitative data distributed according to a principle other than normal distribution, it is planned to use standard nonparametric tests: Mann–Whitney U test, Wilcoxon T test for dependent samples, and Friedman test for dependent samples.

Comparison of the frequencies of indicators between treatment groups will be carried out using Pearson's chi-square test if the number of points in each cell of the crosstab is ≥ 5, or Fisher's exact test if at least one of the crosstab cells contains < 5 points.

In the event that any of the initial data reveal the incomparability of the study groups (statistically significant differences in demographic and other initial data between the groups), an analysis of the parameters of efficacy and safety will be additionally carried out together with the primary planned analysis using multivariate statistics (ANCOVA or logistic regression depending on the type of parameter under study), adjusted for the initial indicator(s), according to which the groups initially differed.

6. Concomitant therapy

6.1. Permitted concomitant therapy

Oral beta-lactam antibiotic (for example, amoxicillin, amoxicillin + clavulanic acid, cefixime, etc.) (see Background Antibacterial Therapy) as per inclusion criterion No. 3, in the same dosage regimen as at the study initiation.

Medicinal products for the treatment of comorbidities that were used prior to registration without violating any non-inclusion criteria. Female patients that take oral contraceptives may continue taking them during the study.

Standard symptomatic treatment agents used to treat acute respiratory infections (in case of medical need), including anilides, triazole derivatives, with the exception of medicinal products that can have a laxative effect (for example, throat lozenges with sugar substitutes).

Agents for relieving symptoms of acute diarrhea (in case of medical need), including electrolyte solutions, with the exception of sorbents.

The prescription of therapy for acute diarrhea and symptomatic therapy for acute respiratory infections is possible only by the decision of the investigator.

6.2. Prohibited concomitant therapy

1. Systemic glucocorticosteroids within the last 8 weeks prior to and during the study;
2. Any antibacterial agents in the last 3 months before the study and during the study, with the exception of antibacterial medicinal products provided for in inclusion criterion No. 3 during the study;
3. The use of any proton pump inhibitors within 3 months prior to the study;
4. Other probiotics (bacteria or yeast, medicinal products, food supplements, fermented products) and Broncho-munal® within 2 weeks prior to and during the study;

Any therapy (including medicinal products, medical devices, and dietary supplements) that may affect stool consistency, in the opinion of the investigator, shall not be used within 14 days prior to Visit 1 (with the exception of the medicinal products specified in items 3 and 4 of the Permitted Concomitant Therapy section).

Pharmacotherapeutic groups	**Active substance**
Systemic glucocorticosteroids	Hydrocortisone, cortisone, prednisolone, methylprednisolone, prednisone, triamcinolone, dexamethasone, betamethasone and their analogs, and/or combinations and derivatives
Antibacterial agents	Aminoglycosides, amphenicols, ansamycins, glycopeptides, carbapenems, lincosamides, macrolides and azalides, penicillins,

	tetracyclines, cephalosporins – as monotherapy and/or combinations
Probiotics, incl. dietary supplements	Lactobacillus acidophilus, kefir fungi, Bifidobacterium bifidum, Escherichia coli, lactobacillus, lysozyme, Enterococcus faecium, Bifidobacterium longum, Bifidobacterium animalis, lactulose, Lactobacillus plantarum, Broncho-munal® – as monotherapy and as part of a medicinal product/ dietary supplement in any combination
Adsorbents	Activated carbon, aluminum oxide, attapulgite, colloidal silicon dioxide, lactulose, hydrolytic lignin, povidone, polymethylsiloxane polyhydrate, dioctahedral smectite – as monotherapy and as part of a medicinal product/ dietary supplement in any combination
Calcium products	Calcium glubionate, calcium lactobionate, calcium carbonate, calcium lactogluconate – as monotherapy and as part of a medicinal product/ dietary supplement in any combination
Antidiarrheal medicinal products	Attapulgite, Bergenia rhizoma, Bacillus subtilis, Bifidobacterium bifidum, Snakeweed rhizoma, burnet rhizomes with roots, loperamide + simethicone, loperamide, plantain oval seed coat, Racecadotril, Dioctahedral smectite, fruits of bird cherry, fruits of common blueberries – as monotherapy and as part of a medicinal product/ dietary supplement in any combination

Myotropic antispasmodics	Bendazole, metamizole, papaverine, drotaverine, theobromine, bencyclan, darifenacin, dicycloverine, codeine, camilofin, mecloxamine, propyphenazone, ergotamine, mebeverine, oxybutynin, otilonium bromide, platyphylline, pinaverium bromide, trimebutine as well as herbal antispasmodics – as monotherapy and as part of a medicinal product/ dietary supplement in any combination
Antiemetics and medicinal products affecting gastrointestinal motility	Pyridostigmine, neostigmine, itopride, cisapride, aprepitant, bromopride, granisetron, dimenhydrinate, domperidone, levomenthol solution in menthyl isovalerate, meclozine, moxastine, ondansetron, palonosetron, perphenazine, thiethylperazine, trifluoperazine, tropisetron, fosaprepitant as well as herbal medicinal products – as monotherapy and as part of a medicinal product/ dietary supplement in any combination

7. Efficacy analysis

Analysis of efficacy endpoints will be carried out in accordance with Section V of the Recommendations of the Board of the Eurasian Economic Commission *On Guidelines on the Principles of Applying Biostatistics in Clinical Trials of Medicines* No. 19 dated November 3, 2020. The main analysis tool will be the *R* programming language, version 3.7.

7.1. Primary endpoint analysis

Assessment of the incidence of AAD from Day 1 to the last day of the course of LRG-002 use vs. placebo in ARD patients receiving standard antimicrobial therapy. The primary efficacy criterion will be considered met if the odds ratio for AAD between the two groups is statistically significantly greater than 1 (i.e., the incidence of AAD in the LRG-002 group is lower than in the placebo group).

The primary efficacy endpoint will be analyzed using a fixed-factor logistic regression model for the treatment group and age group. The treatment effect will be displayed using the adjusted odds ratio and the corresponding two-sided 95% confidence interval.

PCT will be calculated using the following set of functions of the R language:

- `glm` – building a logistic regression model;
- `summary` – displays the regression coefficients with an indication of the statistical significance;
- `confint` – displays confidence intervals for regression coefficients.

An example of a planned way of using these functions is shown in Figure 2. In the figure, the *Outcome* variable encodes the outcome (AAD – yes/no), *Treatment* encodes belonging to a particular study group, the *Age* variable encodes the age category (over or under 50 years old). The *p*-value is highlighted in red, the two-sided confidence interval is highlighted in blue, according to which the decision on reaching the PCT will be made.

```
> model_out <- glm(Outcome ~ Treatment + Age, data = df, family = "binomial")
> summary (model_out)
Call:
glm(formula = Outcome ~ Treatment + Age, family = "binomial", data = df)
Deviance Residuals:
   Min      1Q     Median      3Q      Max
-0.3327  -0.3327  -0.1635  -0.1635  2.9402

Coefficients:
             Estimate   Std. Error z value   Pr(>|z|)
(Intercept)   -4.3090      0.5949   -7.243   4.4e-13***
Treatment2     1.4427      0.6520    2.213     0.0269*
Ageage >=50   -0.5368      0.6558   -0.819     0.4130
```

```
Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

(Dispersion parameter for binomial family taken to be 1)

   Null deviance: 135.35 on 509 degrees of freedom Residual deviance: 128.52 on
507 degrees of freedom
AIC: 134.52

Number of Fisher Scoring iterations: 7

> confint(model_out)
Waiting for profiling to be done...
             2.5%       97.5%

(Intercept) -5.7259968 -3.3150721

Treatment2  0.2808742  2.9311963

Age >=50    -2.0306660 0.6338301
```

Figure 2. Running functions for calculating PCT

7.2. Analysis of secondary endpoints

The whole secondary efficiency analysis will be considered investigational, therefore multiplicity correction will not be performed; for test results, no formal capacity calculation will be provided.

Secondary efficiency points are classified into three groups:

1. Points characterizing the likelihood of a particular clinically significant event:

- The occurrence rate of any diarrhea in the treatment group as compared to the placebo group;
- The occurrence rate of C. difficile-associated AAD in the treatment group as compared to the placebo group;
- The incidence of AAD not associated with C. difficile in the treatment group as compared to the placebo group;

- Frequency of gastrointestinal symptoms, including nausea, vomiting, flatulence, abdominal pain, and decreased appetite (according to the diary data), in the treatment group as compared to the placebo group;
- Number (share) of patients using standard symptomatic therapy to relieve symptoms of acute diarrhea in the treatment group as compared to the placebo group;
- The hospitalization rate in the treatment group as compared to the placebo group.

For these types of BKT, contingency tables 2×2 will be generated where the rows will show the groups, the columns will show the facts of the presence or absence of a particular event, the elements of the table are the patients included in the analysis. The contingency table data will be analyzed using Fisher's test (using the `fisher.test` function) or the chi-square test (using the `chisq.test` function) depending on the number of observations per cell (< 5 or ≥ 5).

2. Points characterizing the severity (or duration) of a particular clinical manifestation:

- Occurrence of bowel movements per day (according to the diary data) in the treatment group as compared to the placebo group;
- Duration of AAD (the time from the onset of AAD to the normalization of stool form according to BSS (types 1, 2, 3, and 4) and the presence of normal stool within 48 hours)) in the treatment group as compared to the placebo group;
- Duration of any diarrhea (time from diarrhea onset to normalization of stool form according to BSS (type 1, 2, 3, and 4) and presence of normal stool for 48 hours) in the treatment group as compared to the placebo group;
- Severity of the gastrointestinal symptoms, including nausea, vomiting, flatulence, abdominal pain, and decreased appetite (according to the diary data);
- A number of days of using symptomatic therapy to relieve symptoms of acute diarrhea in the treatment group as compared to the placebo group.

Due to the obvious difference between the distribution of these indicators from the normal (they have a discrete distribution), the nonparametric Mann–Whitney–Wilcoxon test implemented in the `wilcox.test` function will be used for intergroup comparison.

3. Other secondary endpoints:

- Weight change at Visit 3 compared with Visit 1 in the placebo group as compared to the treatment group. This BKT can have a distribution close to normal. Accordingly, at the first stage, the normality of the distribution will be assessed (using the Shapiro–Wilk test implemented in the shapiro.test function), at the second stage, either the t-test (t.test function) or the Mann–Whitney–Wilcoxon test (wilcox.test function) will be used.
- Stool consistency change (according to Bristol Stool Scale) (according to the diary) in the treatment group as compared to the placebo group; any individual changes in stool consistency will be classified as improved/unchanged/worsened, and the difference between the groups will be analyzed using chi-square criterion (chisq.test function).

8. Safety analysis

Safety parameters will be evaluated at every planned or unscheduled visit.

The safety assessment will be based on the following indicators (between Visits 1 and 3) compared between the investigational medicinal product and placebo:

- Changes in the vital signs over time and the incidence of pathological abnormalities revealed during the physical examination will be summarized by the group; a comparison will be made between the groups using appropriate tests for quantitative and qualitative data.

The data will be checked for compliance with the normal distribution law using the Shapiro–Wilk test.

Intergroup comparison of secondary endpoints related to quantitative scales will be performed using Student's t-test for independent samples if the data correspond to the normal distribution law; otherwise, the Mann–Whitney U test will be applied to independent samples.

Intergroup comparisons of secondary endpoints related to categorical scales will be performed using Pearson's chi-square test if the number of points in each cell of the crosstab is ≥ 5, or Fisher's exact test if at least one of the crosstab cells contains < 5 points.

The dynamics will be assessed using ANOVA methods with repeated measurements if the data correspond to the normal distribution, otherwise, the Friedman test will be applied.

8.1. Adverse events

Safety assessment will also be based on the following parameters (between Visits 1 and 3) compared between the investigational medicinal product and placebo:

Incidence of adverse events (AEs) and/or serious adverse events (SAEs) in the treatment groups including:

- Overall AE rate as compared to placebo;
- AE rate according to the results of physical examinations during each visit compared with placebo;
- AE incidence according to the assessment of the vital signs (HR, BP, BT, RR) at each visit as compared to placebo;
- AE incidence according to the ECG results on Day 15 (± 2) in comparison with placebo;
- AE incidence according to the results of laboratory examinations on Days 7 (± 2) and 15 (± 2) as compared to placebo;
- AE incidence according to the results of daily self-examination of the state of health recorded in Self-Observation Diary.

The study doctor will review the entries in the Self-Observation Diary. If the study doctor believes that the information in the Diary complies with the AE criteria, they will ask the patient about this event and document all information about the AE (including the causality assessment).

The AEs will be described according to the scheme given below:

- AE description
- Severity of the adverse event (meeting the severity criteria)
- AE severity
- AE duration
- Relation to the IMP
- Measures taken in relation to the investigational medicinal product
- Measures taken in relation to the patient

- Outcome

Adverse events will be coded according to the MedDRA

Methods of descriptive statistics will be used to represent the results. A comparison of the incidence of new AE cases in the study groups will be performed using the Fisher's exact test or the chi-square test, depending on the number of proposed observations in one cell (< 5 or ≥ 5), and a comparison of the severity (and the possible causality between the administered medicinal product and AE) will be conducted using the Cochran–Armitage test for linear trends for ordered categorical data.

8.2. Other safety endpoints

No other safety endpoints are foreseen in this study.

9. Exploratory analysis

In the course of this study, exploratory analysis is not envisaged.

10. Interim analysis

Interim data analysis is not planned for this study.

Appendix 1. Table layouts

Table No. 1. Example of a statistical table for a quantitative variable

Visit	Group	Parameter	Variable name
Visit number	Group 1	N	
		Missed	
		Mean	
		95% CI	
		SD	
		Median	
		Min	
		Max	
		Q1	
		Q3	
		IQR	
	Group 2	N	
		Missed	

Visit	Group	Parameter	Variable name
		Mean	
		95% CI	
		SD	
		Median	
		Min	
		Max	
		Q1	
		Q3	
		IQR	

Table No. 2. Example of a group comparison table for a quantitative variable

Visit	No.	No.	No.	No.
Variable	variable name			

Shapiro–Wilk test, p-value	Group 1				
	Group 2				
Student's t-test, p-value					
Mann–Whitney test, p-value					

Table No. 3. Example of a repeated comparison table for a quantitative variable

Variable	**Group**	**Visit**	**Friedman test/ ANOVA for repeated measurements**	**Wilcoxon test/ Student's t-test for repeated measurements**
variable name	Group 1	Comparison of visits		
		No. vs. No.		

Variable	Group	Visit	Friedman test/ ANOVA for repeated measurements	Wilcoxon test/ Student's t-test for repeated measurements
		No. vs. No.		
		No. vs. No.		
		No. vs. No.		
		No. vs. No.		
		No. vs. No.		
	Group 2	Comparison of visits		
		No. vs. No.		
		No. vs. No.		
		No. vs. No.		
		No. vs. No.		

Variable	Group	Visit	Friedman test/ ANOVA for repeated measurements	Wilcoxon test/ Student's t-test for repeated measurements
		No. vs. No.		
		No. vs. No.		

Table No. 4. Example of a statistical table for a categorical variable

Variable	Visit	Group	Variable value	Amount	% by group	% by groups for visit	Fisher's test, p-value
variable name	No.	Group 1	value				
			value				
		Group 2	value				
			value				

Table No. 5. Example of a table of adverse events for calculating the number of all adverse events

Adverse events	Group	Amount	% of the number of AEs in the group	% of the total number of AEs	Binomial test, p-value
All adverse events	Group 1				
	Group 2				
SOC	Group 1				
	Group 2				
PT	Group 1				
	Group 2				

Table No. 6. Example of a table of adverse events for calculating the number of adverse events in the context

Adverse events	Group	variable name	Amount	% of the number of AEs in the group	% of the total number of AEs	Fisher's test, p-value
	Group 1	value				
		value				

Adverse events	Group	Variable name	Amount	% of the number of AEs in the group	% of the total number of AEs	Fisher's test, p-value
All adverse events		value				
	Group 2	value				
		value				
		value				
SOC	Group 1	value				
		value				
		value				
	Group 2	value				
		value				
		value				
PT	Group 1	value				
		value				
		value				
	Group 2	value				
		value				

Adverse events	Group	variable name	Amount	% of the number of AEs in the group	% of the total number of AEs	Fisher's test, p-value
		value				