Multicenter double-blind placebo-controlled randomized clinical trial of efficacy and safety of Kolofort in the treatment of patients with functional dyspepsia

Phase IV

Sponsor	**OOO «NPF «MATERIA MEDICA HOLDING»**
Protocol number	**MMH-KOL-003**
Version date:	**January 19, 2017**
ClinicalTrials.gov Id:	**NCT03119766**

Protocol Summary

This document represents the protocol summary for the study on human subjects. The study will be carried out in accordance with ICH GCP, National Standard of the Russian Federation GOST 52379-2005 "Good Clinical Practice", Helsinki Declaration of World Medical Association, relevant requirements of the regulatory authorities as well as the study procedures.

Title of Study

Multicenter double-blind placebo-controlled randomized clinical study of efficacy and safety of Kolofort in the treatment of patients with functional dyspepsia.

Phase: IV

Sponsor: OOO «NPF «MATERIA MEDICA HOLDING», Moscow, Russia

Protocol No. MMH-KOL-003

Objective of the study

- To obtain additional data on efficacy and safety of Kolofort in the treatment of patients with functional dyspepsia.

Endpoints

Primary endpoint

1. Changes in severity of functional dyspepsia (FD) symptoms on the GIS (Gastrointestinal symptom score) after 8 weeks from the start of the study therapy.

Secondary endpoints

1. Percentage of patients with a decrease in the severity of FD symptoms on the GIS scale after 8 weeks from the start of the study therapy.
2. Change in the severity of the functional dyspepsia index NDI after 8 weeks from the start of the study therapy.
3. Changes in the quality of life of patients on the SF-36 scale after 8 weeks from the start of study therapy.
4. Percentage of patients terminating the study early due to lack of efficacy of the study therapy.[1]
5. Indicators of therapeutic and side effects values, efficacy index according to Clinical Global Impression (CGI-EI) scores after 8 weeks of treatment.

[1] Lack of efficacy of the study therapy is defined as retention or progression of the symptoms of functional dyspepsia resulting in prescription of the products for FD therapy (proton pump inhibitors, prokinetics, spasmolytics).

Safety assessment

- Occurrence and nature of adverse events (AE) during the treatment; AE severity, causal relationship to the study drug, and outcome.
- Changes in vital signs.

Study design

A multicenter double-blind placebo-controlled randomized clinical trial was planned to evaluate the efficacy and safety of the study treatment.

The study will enroll the patients of either gender aged 18-45 years old with verified diagnosis of "functional dyspepsia" according to Rome-IV criteria and intensity of dyspeptic symptoms ≥ 6 according to GIS (Gastrointestinal symptom score).

At the screening visit 1 (Visit 1, from -14 to -1 days), after signing patient information sheet (informed consent form) to participate in the clinical trial, complaints and medical history are collected, an objective examination is performed. The investigator evaluates intensity of dyspeptic symptoms according to GIS. The patient undergoes an abdomen ultrasound examination, esophagogastroduodenoscopy (EGDS)[2], and diagnostic tests for Helicobacter pylori (H. pylori) infection. In case of the previous use of proton pump inhibitors, prokinetics, antispasmodics, antacids, bismuth drugs the investigator evaluates the possibility of canceling these drugs at least 7 days before the patient is randomized. For women of reproductive age, a pregnancy test is performed.

On the day of randomization (Visit 2, Day 0) collection of complaints and objective examination are carried out. The investigator evaluates the results of laboratory and instrumental research methods, the severity of dyspepsia symptoms on the GIS scale, registers changes in concomitant therapy.

If a patient meets all inclusion criteria and does not have any exclusion criteria, he/she is randomized into one of two groups: Patients in Group 1 receive Kolofort for 8 weeks; Patients in Group 2 – Placebo on the study drug regimen. The patient completes the Nepean Dyspepsia Index (NDI) and Quality of Life (SF-36) questionnaires.

The patient's treatment lasts for 8 weeks, during which 3 visits to the research center are carried out. At Visit 3 (Week 2 ± 3 days), complaints are collected, an objective examination of the patient is performed. The investigator monitors the prescribed and concomitant therapy, evaluates the safety of the therapy and the degree of adherence to treatment (compliance). At visit 4 (week 4±3

[2] It is possible to use the results of an EGDS performed earlier in any medical facility, provided that the procedure was carried out no more than one month ago from the screening visit and that the patient has a corresponding video recording and conclusion.

days) and 5 (week 8±3 days) the investigator collects complaints, registers the physical examination data, monitors the prescribed and concomitant therapy, assesses the safety of the treatment and compliance. Questionnaires GIS, NDI are filled in.

Additionally, at Visit 5 the patient fills out the SF-36 scale, the investigator fills out the Clinical Global Impression-Efficacy Index (CGI-EI).

The patients will be allowed to take symptomatic therapy and medications for their co-morbidities during the study, except for the medicines listed in "Prohibited Concomitant Treatment".

Inclusion and exclusion criteria

Inclusion criteria

1. Patients of both genders aged 18-45 years old.
2. Diagnosis of functional dyspepsia established according to Rome-IV criteria (2016).
3. Severity of symptoms of dyspepsia ≥ 6 on the GIS scale.
4. Negative test result for H. pylori infection[3].
5. Availability of signed patient information sheet and informed consent form for participation in the clinical trial.
6. Patients who gave their consent to use reliable contraception during the study.

Exclusion criteria

1. Organic diseases of the gastrointestinal tract (gastroesophageal reflux disease (GERD), peptic ulcer, chronic pancreatitis, cholelithiasis, hepatosis, hepatitis, hepatic cirrhosis, etc.)[4]
2. Verified diagnosis of other functional GI diseases, i.e. biliary dyskinesia, irritable bowel syndrome, etc.
3. Discontinuation of proton pump inhibitors, prokinetics, antispasmodics, antacids, bismuth preparations less than 7 days before randomization.
4. H. pylori eradication within 2 months prior to enrollment.
5. Intestinal infection within 2 months prior to enrollment.
6. History/suspicion of oncology of any location.
7. Previously diagnosed cardiovascular diseases with functional class IV (according to the classification of the New-York Heart Association, 1964), hypothyroidism, diabetes mellitus, chronic renal disease C3-5, hepatic diseases with portal hypertension and/or signs of severe decompensation of function (> 6 points according to the Child-Pugh classification).

[3] Biopsy express test and/or fecal antigen and/or breath test.
[4] Enrollment of subjects with morphological signs of chronic gastritis without signs of atrophy, metaplasia, hemorrhages, erosions and ulcerative lesions of mucosa is allowed.

8. Any other severe comorbidity that, in the opinion of the investigator, may affect patient participation in the clinical trial.
9. Allergy/intolerance intolerances to any of the components of the study drugs.
10. Pregnancy, breast-feeding.
11. Patients who, from the investigator's point of view, will not comply with the observation requirements of the study or adhere to study drug dosing regimens.
12. Scheduled hospitalization during the study for any diagnostic or therapeutic procedures.
13. Use of drugs or alcohol (more than 2 alcohol units daily), presence of mental diseases.
14. Use of any medications specified in the "Prohibited Concomitant Treatment" within 1 month prior to inclusion in the study.
15. Participation in other clinical trials in the previous 3 months.
16. Patients who are related to any of the on-site research personnel directly involved in the conduct of the trial or are an immediate relative of the study investigator. 'Immediate relative' means husband, wife, parent, son, daughter, brother, or sister (regardless of whether they are natural or adopted).
17. Patients who work for MATERIA MEDICA HOLDING (i.e. the company's employees, temporary contract workers, appointed officials responsible for carrying out the research or immediate relatives of the aforementioned).

Criteria for Withdrawal or Termination

1. Screening failure.
2. Lack of study therapy efficacy[5].
3. Inability or refusal of patient to comply with the protocol requirements.
4. Necessity in medicines prohibited within the study.
5. An adverse event requiring cancellation of the study drug.
6. Incorrect inclusion of ineligible patient.
7. Pregnancy.
8. Desire of patient to complete the study ahead of schedule due to any reason.
9. Cases not specified by the protocol where the investigator decides that further participation may harm the patient.

Number of subjects

It is planned to enroll 370 subjects, among those at least 258 patients are expected to complete all procedures of the protocol (129 patients each in the Kolofort and Placebo groups).

[5] Lack of efficacy of the study therapy is defined as retention or progression of the symptoms of functional dyspepsia resulting in prescription of the products for FD therapy (proton pump inhibitors, prokinetics, antispasmodics).

Interim analysis

A blinded interim analysis is planned in order to specify the study parameters As a result of an interim analysis, it is possible to change the sample size, but only upward.

Treatment

Group 1

Name of the medicinal product: Kolofort

Active ingredient: affinity purified antibodies to human TNFα – 0.006 g*

Affinity purified antibodies to S100 brain-specific protein – 0.006 g*

Affinity purified antibodies to histamine – 0,006 g*

** applied onto lactose monohydrate as a mixture of three active aqueous-alcoholic dilutions of the substance diluted 100^{12}, 100^{30}, 100^{200} times, respectively*

Excipients: lactose monohydrate, microcrystalline cellulose, magnesium stearate

Method of administration: Tablet for oral use. Dose per administration: 2 tablets. 2 tablets twice daily (4 tablets per day). The tablets should be held in the mouth until complete dissolution, without meal.

Dosage form: Tablets

Description: Flat cylinder-shaped scored bevelled edge white to off-white tablets

Storage conditions: Store protected from light at temperature below 25°C. Keep out of the reach of children.

Group 2

Name of the medicinal product: Placebo

Active ingredient: NA

Excipients: lactose monohydrate, microcrystalline cellulose, magnesium stearate

Method of administration: Tablet for oral use. Dose per administration: 2 tablets. 2 tablets twice daily (4 tablets per day). The tablets should be held in the mouth until complete dissolution, without meal.

Dosage form: Tablets.

Description: Flat cylinder-shaped scored bevelled edge white to off-white tablets

Storage conditions: Store protected from light at temperature below 25°C. Keep out of the reach of children.

Treatment duration

Kolofort/Placebo treatment duration is 8 weeks.

Observation period

In total the patients will be monitored for 10 weeks (screening, randomization up to 2 weeks, treatment - 8 weeks).

Symptomatic (Standard) treatment

Patients are allowed to receive medications used to treat the underlying and concomitant diseases other than those representing exclusion criteria, except for the drugs specified in the section "Prohibited concomitant treatment".

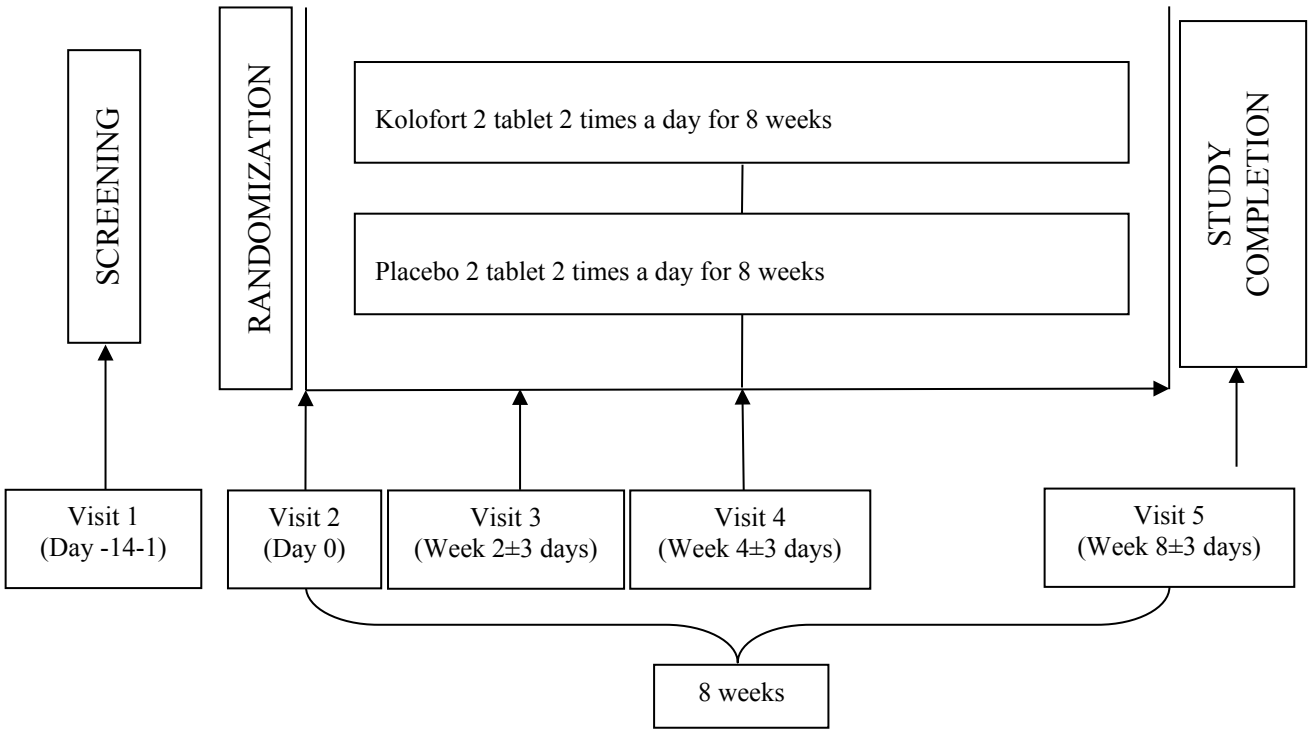
Prohibited concomitant therapy

Within 1 month prior to enrollment and throughout the study (after signing information sheet (informed consent form) and screening onset) the patients are not allowed to use any of the following medications (ATC group is specified in brackets):

1. Preparations used for conditions associated with acidity disorders (A02)
2. Preparations for treatment of functional gastrointestinal disorders (A03)[6]
3. Fenspiride (R03DX03)
4. Antihistamines (R06)
5. Steroidal and non-steroidal anti-inflammatory preparations (except for topical/inhalation corticosteroids)
6. Anti-inflammatory and antirheumatic preparations (M01) and analgesics (N02)
7. Antineoplastic preparations (L01) and antineoplastic hormones (L02)
8. Immunostimulants (L03)
9. Immunosuppressants (L04)
10. Immune sera and immunoglobulins (J06)
11. Vaccines (J07)
12. Preparations of calcium, iron, zinc, potassium
13. Antibacterials for systemic use (J01)
14. Proproten, Tenoten, Brizantin, Divaza, Ergoferon, Arthrofon, Rengalin, Prohistam
15. Homeopathic drug products
16. Preparations the use of which the patient has previously had allergic reactions.

Study design scheme

[6] Administration of a spasmolytic agent drotaverine by the subject is allowed at the total dose of no more than 640 mg (equivalent to 16 drotaverine tablets 40 mg or 8 drotaverine tablets 80 mg) throughout the study period.



Schedule of study procedures

Procedure/Visit	Screening	Randomization	Treatment Administration of Kolofort/Placebo		
	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5
	From day –14 to day -1	Day 0	Week 2±3 days	Week 4 ± 3 days	Week 8± 3 days
Informed consent	+				
Collection of complaints	+	+	+	+	+
Medical history	+				
Concomitant conditions and diseases	+	+			
Physical examination	+	+	+	+	+
Completing the GIS scale	+	+		+	+
Abdominal ultrasound	+				
EGDS	+				
H. pylori test	+				
Concomitant therapy	+	+	+	+	+
Necessity in concomitant therapy (for dyspepsia therapy)			+	+	
Pregnancy test	+				
Inclusion/exclusion criteria	+	+			
Randomization, prescription of the study therapy		+			
Completing the NDI Questionnaire		+		+	+
Evaluation of quality of life according to SF-36 scale		+			+
Study drug supply		+	+	+	

Study drug accountability and return, compliance assessment			+	+	+
Clinical Global Impression assessment (CGI-EI)					+
Evaluation of treatment safety		+	+	+	+

Statistical Analyses

Samples

Total set includes all the patients who have signed ICF. This sample will consider all adverse events throughout the study.

The sample including all subjects who received at least one dose of the study product to be used for ***analysis of the study treatment safety and tolerability*** (*Safety population*), as all adverse events identified after the study product administration will be recorded.

Full Analysis Set This sample will consist of all enrolled subjects, except for those who met at least one of the following criteria:

1) noncompliance with inclusion/non-inclusion criteria;
2) patient has not taken any dose of the study drug;
3) lack of any data on the patient after randomization.

This is the most appropriate set for the Intention-to-treat principle, so it will be used in the ***Intention-to-treat analysis (ITT-analysis) of efficacy of the study therapy.***

Per Protocol set. This sample includes all patients who completed the therapy as per the study protocol, without any missing visits or major protocol deviations. This set will be used for ***Per Protocol analysis (PP analysis) of efficacy of the study therapy.***

Mean value of the total set for the relevant day will be used to fill lacking/missing data.

Data treatment and all statistical calculations under the protocol will be carried out using SAS-9.4 statistical software.[7]

Evaluation of sample size

The sample size was assessed in accordance with the following rules and assumptions:

1. Statistical assumptions:
   1.1 the power of statistical tests "P=(1−β)" will be taken as being equal to 80% (the probability of correct rejection of the null hypothesis is 0.8)
   1.2 the probability of type 1 error 'α' is less than 5% (the probability of false acceptance of the alternative hypothesis is less than 0.05)
   1.3 statistical criteria of intergroup comparisons used are two-sided

[7] Holder of license: OOO "NPF "MATERIA MEDICA HOLDING", No. 70100045.

1.4 calculation of sample size is based on the assumptions on the expected effect declared in the primary efficacy criterion of the protocol

1.5 ratio between Kolofort and Placebo sample sizes is 1:1 (1 Kolofort subject per 1 Placebo subject)

1.6 statistical null and alternative hypotheses will be as follows:

$$\mathbf{H_0: \Delta\mu_к = \Delta\mu_п}$$

$$\mathbf{H_a: \Delta\mu_к \neq \Delta\mu_п},$$

where $\mathbf{\Delta\mu_K}$ - mean reduction (baseline – final score) total GIS score in Kolofort group

$\mathbf{\Delta\mu_п}$ – mean reduction (baseline - final score) of total GIS score in Placebo group

1.7 calculation of sample size will be made using the following formula:

$$n_1 = n_2 = \frac{2(z_{\alpha/2} + z_\beta)^2\sigma^2}{\epsilon^2}$$

where $\mathbf{n_1}$ and $\mathbf{n_2}$ are sample sizes in study drug and placebo groups, respectively

$\boldsymbol{\varepsilon}$ – expected difference in mean reduction of total score between Kolofort and Placebo groups

$\boldsymbol{\sigma}$ – standard deviation of reduction in total score (assessed based on bibliographic data)

$z_{\alpha/2}$ – tabular value of two-sided z-test for $\boldsymbol{\alpha}$

z_β – tabular value of one-sided z-test for $\boldsymbol{\beta}$

1.8 final sample size will be determined using the formula:

$$\mathbf{N_T = N_{PP} / (1 - К_в)},$$

where $\mathbf{N_T}$ – final sample size;

$\mathbf{N_{PP}}$ – result of calculation in cl. 1.7, i.e. scheduled number of patients completing the study per protocol

$\mathbf{К_в}$ – withdrawal coefficient.

2. Assumptions on expected clinical study effects:

2.1 The difference between reduction ("total score at baseline visit" – "total score at final visit") in total GIS score in Kolofort and Placebo groups will be at least **15%** from baseline value (**d=0.15**, calculated as the difference between group values of GIS reduction attributed to mean starting value).

2.2 The values of population variance of reduction in total GIS score and baseline mean total score were evaluated based on bibliographic review as follows:

$\mathbf{\varepsilon = d*(E - z_{(1-\alpha/2)}*S_0/n^{0,5})}$ is the lower limit of confidence interval for mean total GIS score for placebo group in the relevant study (conservative estimate)

d=0.15 – claimed effect size

E=12 – mean starting GIS score based on the source cited

S_0=4.7 – sample standard deviation at the first visit in Placebo group based on the source cited

$\sigma=S*((n-1)/\chi^2_{\alpha/2,(n-1)})^{0,5}$ is the upper limit of 95% confidence interval for population standard deviation of total score reduction (conservative estimate)

where **S** is sample standard deviation for the reduction in total GIST score between the first and last visits based on the source cited

S=4.3

n=157

$\chi^2_{\alpha/2,(n-1)}$ is the corresponding quintile of chi-square distribution

$\chi^2_{0.025,\,156}$=123.3

therefore, effect size and standard deviation estimates are equal to

ε=0.15*(12-1.96*4.7/12.52) = 1.69

$\sigma=4.3*(156/123.3)^{0.5}$ = 4.84.

Therefore, the sample size has been found to be **129** patients in each of the groups (Kolofort and Placebo) to estimate superiority of the study drug over placebo.

Taking into account that potential withdrawal of **30%** patients during the study for various reasons, at least **370** patients in each group will be required to sign informed consent, with **185** patients per group.

Statistical criteria

All statistical calculations will be made using two groups of statistical criteria:

- parametric - to evaluate continuous and interval random values;
- nonparametric – to obtain:
  - evaluations of equality/inequality of proportions of the subjects upon their comparison for various visits, during analysis of frequencies of the features compared
  - evaluation of continuous and interval random values in case of non-compliance with normal random distribution.

Parametric criteria

Prior to analysis using parametric statistics, data samples under comparison will be tested for normality (the Kolmogorov-Smirnov test).

The following parameters and approaches are to be used:

1. To evaluate differences in continuous variables in one group at two different visits - Student's test for paired samples.
2. To evaluate the over time dynamics of the compared values – analysis of variance (ANOVA) or covariance (ANCOVA) in the modification with repeated measures.
3. In case of multiple comparisons of the groups various corrections for multiplicity will be used, e.g. Dunnett, Tukey, Scheffe, Holm adapted test, etc.
4. In case of abnormal data distribution, approaches with the Generalized Linear Models and / or Mixed Linear Models will be used.
5. Selection of the type of distribution, specification of factor and covariance structures of the model will be made using fit-statistics such as AIC (Akaike information criterion).

The following SAS software procedures are supposed to be applied to the above listed tests and techniques:

- UNIVARIATE: normality check of the distributions under comparison;
- CORR, MEANS - calculation of descriptive statistics
- TTEST – Student's test with all modifications
- GLM – generalized linear models for analysis of time changes (ANOVA, ANCOVA)
- GENMOD – generalized linear models
- MIXED – mixed linear models.

Non-parametric criteria

Below are the main types of possible comparisons with relevant criteria:

1. To evaluate the over time dynamics in the parameters compared – Friedman test, nonparametric analogue of repeated measures analysis of variance.
2. For frequency analysis of contingency tables 2×2 – $\chi 2$ (if the frequency under comparison > 5) or exact Fisher's test (if one of the frequencies under comparison < 5).
3. Cochran-Mantel-Haenszel test (modified $\chi 2$ test for multiple comparisons) – to perform frequency analysis based on independent strata.
4. For frequency analysis of data on presence/absence of an event or outcome during repeated measurements (contingency tables with dependent strata) – survival analysis.

To perform the above-mentioned nonparametric statistical analysis the following SAS procedures are to be used:

- FREQ – Friedman test, $\chi 2$ test and/or exact Fisher's test; Cochran-Mantel-Haenszel test
- LIFETEST – survival analysis
- NPAR1WAY - Mann-Whitney test.

Safety parameters

Adverse events recorded during the study will be classified by severity, seriousness and analyzed if there is a relationship with the study drug.

Data presentation

Descriptive statistics will be provided for each study continuous/interval variable. Numerical data will be presented by mean, standard deviation, min and max values. Outliers will be analyzed individually. The data will be grouped by visits. The categorical variables will be presented as frequency tables by visits.