Multicentre Double-blind Placebo-controlled Parallel Group Randomized Clinical Trial of Efficacy and Safety of Anaferon in the Treatment of Acute Respiratory Viral Infections

Phase IV

Sponsor	**OOO «NPF «MATERIA MEDICA HOLDING»**
Protocol number	**MMH-AN-005**
Version date:	**May 28, 2018**
ClinicalTrials.gov Id:	**NCT03707912**

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

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Phase: IV

Sponsor: OOO "NPF "Materia Medica Holding", Moscow, Russia

Protocol No. MMH-AN-005

Objective of the study

- To obtain additional data on the efficacy and safety of Anaferon in the treatment of acute respiratory viral infections (ARVI).

Endpoints

Primary endpoint

1. Time to resolution of ARVI symptoms[1] (clinically diagnosed and/or PCR-confirmed).

Secondary endpoints

1. ARVI severity (clinically diagnosed and/or PCR-confirmed); based on the area under the curve (AUC) data for the Total Severity (TS) score[2] on days 1-6 of the observation).
2. Percentage of patients with resolution of ARVI symptoms (clinically diagnosed and/or PCR-confirmed).
3. Time to resolution of ARVI symptoms (PCR-confirmed).
4. Percentage of patients with resolution of ARVI symptoms (PCR-confirmed).
5. Dosing frequency of antipyretics on days 1-3 of therapy.
6. The percentage of patients requiring administration of antibiotics during 4-7 days of the observation

[1] Time from enrollment to symptom resolution. **Criteria of resolution of ARVI symptoms:** body/axillary temperature ≤37.3^{0}C for 24 hours (without subsequent increase within the observation period) + absence/presence of general ARVI symptoms with ≤2 point of Total Severity (TS) score.

[2] **Total Severity (TS)** index will be calculated from the rating scores obtained for each of the symptoms of ARVI (body temperature, non-specific/flu-like symptoms, and nasal/throat and chest symptoms); the subsequent data analysis will be performed by Materia Medica Holding. For TS index, absolute body temperatures (in degrees Celsius) will be converted to arbitrary units (or scores) using the following scale: ≤37.3^{0}C=0 points; 37.4-38.0^{0}C=1 point; 38.1-39.0^{0}C=2 points; ≥39.1^{0}C=3 points.

Safety assessment

- Adverse events (AEs) during the treatment, AEs severity and relationship to the study drug, and AEs outcomes.
- Changes in vital signs during the treatment.

Study design

Study design: multicenter, double-blind, placebo-controlled, parallel-group randomized trial.

The study will enroll patients of either gender aged 18-70 years old with clinical manifestations of ARVI within the first day after the onset of the disease. Signed information sheet for patient will be obtained from all participants prior to the screening procedures. Medical history, concomitant medication, thermometry, patient examination by a doctor, assessment of ARVI symptoms severity will be performed at screening visit.

The nasopharyngeal swabs will be performed for Real-time reverse transcription polymerase chain reaction (PCR) assay[3] to confirm viral etiology of ARVI and to verify respiratory viruses prior to the therapy.

If a patient meets all inclusion criteria and does not have any exclusion criteria, at Visit 1 (Day 1) he/she will be randomized into one of two groups: the 1st group patients will take Anaferon according to the dosage regimen until the end of the study; the 2nd group patients will take Placebo according to Anaferon dosage regimen until the end of the study.

The patients will be provided with a patient diary (paper or electronic) where daily they will record axillary body temperature (using a Geratherm Classic thermometer) and each ARVI symptom severity twice a day (in the morning and in the evening). In addition, antipyretic administration (if applicable) as well as any possible worsening of the patient's condition (if applicable, for safety evaluation/AEs documentation) will also be recorded in a patient diary. An investigator will provide the instructions on filling out the diary and will help the patient to make first records of ARVI symptom severity and body temperature in the diary.

Patients are observed up for 7 days (screening, randomization - 1 day, study therapy - 5 days, follow-up period - 2 days). During treatment and follow-up period two visits are scheduled (at home or at the study site) on days 5 (Visit 2) and day 7 (Visit 3). At Visits 2 and 3, the investigator will carry out physical examination, record dynamics of ARVI symptoms and concomitant therapy and check patient diaries.

[3] Real-time reverse transcription polymerase chain reaction (PCR) assay is carried out using the AmpliSens kit to detect the most common ARVI and influenza agents, including (1) Influenza A virus; (2) Influenza B virus; (3) Influenza A (H1N1)pdm, similar to A/California/4/2009; (4) Human metapneumovirus; (5) Human respiratory syncytial virus; (6) Human rhinovirus; (7) Human adenovirus; (8) Human bocavirus; (9) Human parainfluenza virus 1; (10) Human parainfluenza virus 2; (11) Human parainfluenza virus 3; (12) Human parainfluenza virus 4; (13) Human coronavirus OC43; (14) Human coronavirus 229E; (15) Human coronavirus HKU1; (16) Human coronavirus NL63.

Treatment compliance will be evaluated at Visit 3.

During the study, symptomatic therapy and therapy for underlying chronic conditions are allowed with the exception of the drugs indicated in the section "Prohibited Concomitant Treatment".

Inclusion and exclusion criteria

Inclusion criteria

1. Patients of either gender aged 18-70 years.
2. Diagnosis of ARVI based on medical examination: axillary temperature ≥ 37.8°C at examination + non-specific/flu-like symptom score ≥4, nasal/throat/chest symptom score ≥2.
3. The first 24 hours after ARVI4 onset.
4. Seasonal rise in ARVI incidence.
5. Patients giving their consent to use reliable contraception during the study.
6. Signed patient information sheet (informed consent form).

Exclusion criteria

1. Suspected pneumonia, bacterial infection (including otitis media, sinusitis, urinary tract infection, meningitis, sepsis, etc.) requiring administration of antibacterial drugs from the first day of disease.
2. Suspected initial manifestations of diseases with symptoms similar to ARVI at onset (other infectious diseases, flu-like syndrome at the onset of systemic diseases of connective tissue, oncohaematological and other diseases).
3. Clinical symptoms of severe influenza/ARVI requiring hospitalization[5].
4. Subjects requiring concurrent antiviral products forbidden by the study.
5. Medical history of primary and secondary immunodeficiency.
6. Oncologic conditions /suspected oncologic conditions.
7. Aggravation or decompensation of chronic diseases affecting a patient's ability to participate in the clinical trial.
8. Impaired glucose tolerance, diabetes mellitus.
9. Malabsorption syndrome, including congenital or acquired lactase or other disaccharidase deficiency, galactosemia.
10. Allergy/ hypersensitivity to any component of the study drug.

[4] The time of ARVI onset is the time of manifestation of fever or febrile illness (axillary temperature ≥37.4^{0}C, oral or tympanic temperature ≥37.6^{0}C, or rectal temperature ≥38.0^{0}C).

[5] Severe influenza/ARVI criteria (WHO 2011; CDC 2017): shock syndrome III, crucial deterioration in the general condition, decreased activity or lightheadedness up to loss of strength, severe tachycardia/bradycardia, tachypnoea, hypo-/hyperventilation, circulation disorder, peripheral cyanosis, prolonged capillary nail refill time, vomiting, dehydration symptoms, decreased urine formation/anuria, convulsions, meningism), hemorrhage (epistaxis, petechiae on the skin or in mucous membranes), haemodynamic instability, severe obstructive airway disorders (constrictive laryngotracheitis, bronchial obstruction syndrome), acute respiratory failure, primary and secondary pneumonia. Risk factors for severe influenza/ARVI and complications: chronic diseases, including cardiovascular diseases, respiratory diseases, diabetes mellitus, immunosuppression, and cancer.

11. Pregnancy, breast-feeding; childbirth less than 3 months prior to the inclusion in the trial, unwillingness to use contraceptive methods during the trial.
12. Consumption of narcotics, alcohol > 2 alcohol units per day, mental diseases.
13. Course administration of the drug products specified in the section “Prohibited Concomitant Therapy” within two weeks prior to inclusion in the study.
14. Patients who will not fulfill the requirements during the study or follow the order of administration of the studied drug products, from the Investigator's point of view.
15. Participation in other clinical trials for 3 months prior to enrollment in this study.
16. Patients who are related to any of the on-site research personnel directly involved in the study or are an immediate relative of the 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 OOO “NPF “Materia Medica Holding” (i.e. the company's employees, temporary contract workers, designated officials responsible for carrying out the research or any immediate relatives of the aforementioned).

Criteria for Withdrawal or Termination

1. Patient failed screening procedure.
2. Inability or refusal of patient to comply the Protocol requirements.
3. Deviation from the schedule of visits 2 and 3 for more than 1 day.
4. Necessity in medicines prohibited within the study.
5. An adverse event requiring study drug cancellation.
6. Desire of patient to complete the study ahead of schedule due to inefficacy of therapy or any other reason.
7. Pregnancy.
8. Cases not specified by the protocol where the investigator decides that further participation may harm the patient.
9. Incorrect inclusion of ineligible patient.

Number of subjects

It is planned to include total of 204 patients (102 patients in Anaferon and Placebo groups).

Interim analysis

The protocol does not schedule unblinded interim analyses. At the sponsor’s request blinded interim analysis may be carried out to specify population parameters and potential further specification of sample size (increase only).

Treatment

Group 1

Name of the medicinal product: Anaferon

Active ingredient: affinity purified antibodies to human interferon gamma – 0.003 g*

** applied onto lactose monohydrate as a water-alcohol mixture containing no more than 10-15ng/g active form of the active ingredient.*

Excipients: Lactose monohydrate – 0.267 g, microcrystalline cellulose – 0.03 g, magnesium stearate – 0.003 g.

Method of administration: Tablet for oral use. Single dose – 1 tablet, to be held in the mouth until complete dissolution outside of meal (between meals or 15-30 minutes before meals). Dosing scheme. One dose every 30 minutes for the first 2 hours, followed by three more doses spaced regularly during the rest of the day. From day 2 to 5: 1 tablet taken 3 times daily.

Dosage form: Tablets.

Description: White to off-white, round, flat, scored on one side and beveled tablets.

Storage conditions: Store below 25ºC. Keep out of the reach of children.

Group 2

Name of the medicinal product: Placebo

Active ingredient: NA

Excipients: Lactose monohydrate – 0.267 g, microcrystalline cellulose – 0.03 g, magnesium stearate – 0.003 g.

Method of administration: Placebo using Anaferon scheme.

Dosage form: Tablets.

Description: White to off-white, round, flat, scored on one side and beveled tablets.

Storage conditions: Store below 25ºC. Keep out of the reach of children.

Treatment duration

Anaferon/Placebo treatment duration is 5 days.

Observation period

In total, the patient is observed up for 7 days (screening and randomization up to 1 day, therapy for 5 days, follow-up from 5 to 7 day).

Symptomatic (Standard) treatment

Throughout the study, patients can receive symptomatic flu/ARVI therapy based on the approved standards of care.

Indications for prescription of antipyretics:

Increased body temperature > 39°C in subjects without complications and co-morbidities and > 38°C in subjects with co-morbidities (congestive heart failure, hepatic, renal diseases; history of convulsive syndrome).

If antipyretic was taken by the patient on his own (without doctor recommendation) with no indications the patient is not excluded from the study. The patient should record the values of thermometry before taking the drug, its name and dose.

Drug product allowed for use as antipyretic (the ATC group is indicated in brackets):

- Ibuprofen (M01AE01).

The antipyretic ibuprofen (Nurofen®, 200 mg tablets, Reckitt Benckiser Healthcare International Ltd.) will be provided by the Sponsor for all the participants of the study.

The investigator will be responsible for explaination dosing rules for concomitant therapy to the patient and issue the product based on the indications.

Prohibited concomitant therapy

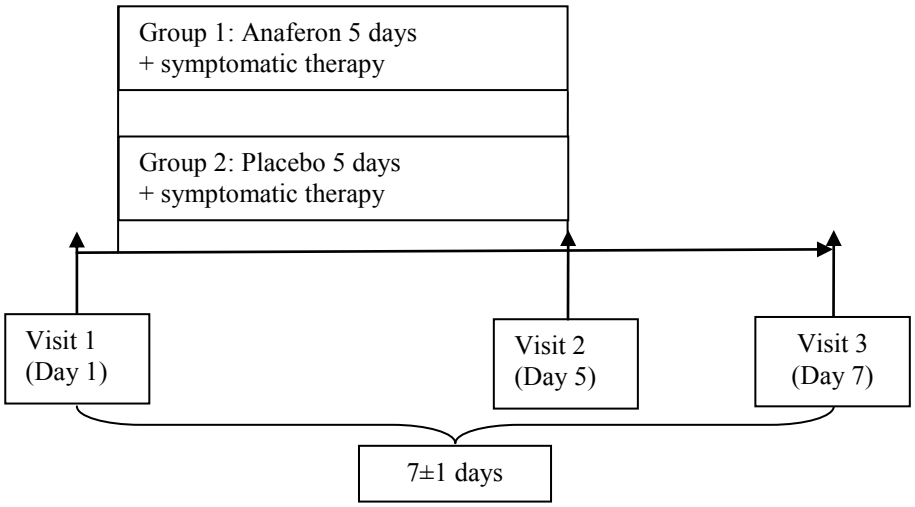
Two weeks before inclusion in the study*, as well as during the study (from signing of the information sheet for patient /informed consent form) it is prohibited to administer the following drug products (their ATC group is indicated in brackets):

1. Antivirals (J05), except for Anaferon prescribed within this study.
2. Antimicrobials and antiseptics for local treatment of oral diseases (A01AB).
3. Medications for the treatment of throat diseases (R02A).
4. Immunostimulants (L03), including:
    - interferon inducers (acridonoacetic acid, meglumine acridone acetate/cycloferon®, umifenovir/arbidol®, kagocel®, tiloron/amixin®, polyadenyl acid + polyuridylic acid/poludan®, sodium oxodihydroacridinyl acetate/neovir®, lavomax®, tilaxin®, etc.)
    - interferons;
    - bacterial immunomodulators (including ribomunyl®, sodium ribonucleate/ridostin, etc., sodium deoxyribonucleate/derinat®, etc., IRS-19, imudon®, broncho-munal®, etc.).
    - pidotimod/immunorix;
    - interleukins;
    - synthetic immunostimulants (levamisole, alpha-glutamyl-tryptophan/thymogen, etc.);
    - drug products with thymus hormones.
5. Non-steroidal anti-inflammatory drugs (M01, except for ibuprofen).

6. Analgesics and antipyretics (N02A).
7. Combination preparations for relief of URI symptoms.
8. Homeopathic preparations.
9. Biologically active food supplements (BAFS).
10. Systemic (oral or parenteral) corticosteroids.
11. Immunosuppressants (L04).
12. Antineoplastic agents (L01) and combined (with hormones) antineoplastic endocrine therapy (L02).
13. Immune sera and immunoglobulins (J06).
14. Vaccines (J07).
15. Drugs that previously caused hypersensitivity/ allergic reactions in patient.

**Patients were allowed to take a single dose of prohibited medications (except for 10-14) prior to inclusion, if the interval between the medication intake and randomization was more than 12 hours.*

Study design scheme



Schedule of study procedures

Procedure/visit	Visit 1 (Day 1)	Visit 2 (Day 5)	Visit 3 (Day 7±1)
Informed consent	+		
Registration of a study subject in the IVRS and assignment of patient ID	+		
Collection of complaints	+	+	+
Medical history	+		
Physical examination	+	+	+
ARVI symptoms registration	+	+	+
Patient Diary	+	+	+
Pregnancy test	+		
Concomitant therapy	+	+	+

Eligibility assessment	+		
Nasopharyngeal swabs for PCR	+		
Randomization and prescription of study drug	+		
Study drug supply	+		
Concomitant medication supply	+		
Study drug accountability and return			+
Treatment compliance			+
Evaluation of treatment safety	+	+	+
Visit completion	+	+	+
Study completion			+

Statistical Analyses

Samples

Total set: all patients who have signed ICF. This sample will consider all the recorded AEs, including those occurred prior to the study therapy.

Safety population: all patients who received at least one dose of the study drug. This sample will be used for ***analysis of the study treatment safety and tolerability***, as all adverse events identified after the study drug administration will be recorded. The AEs registered in patients of the *Total set* sample from the moment of signing the Informed Consent Form, but before the test drug administration will not be taken into account when analyzing the study therapy safety.

Full Analysis Set. This sample includes all enrolled patients, except for those who met at least one of the following events:

1) non-compliance with inclusion / exclusion criteria;
2) the patient did not take a single dose of the study drug;
3) lack of any data about the patient after administration of the study drug.

This sample, the most consistent with the "Intention-to-treat" principle**,** will be used for the ***Intention-to-treat analysis (ITT analysis)*** of the study therapy efficacy.

Per protocol set. This sample includes all patients who have received the complex therapy under the Protocol, completed all the scheduled visits and had no significant deviations from the Protocol. This sample will be used for the ***Per Protocol analysis (PP- analysis)*** of the study therapy efficacy.

Evaluation of sample size

The sample size was assessed on the basis of the following rules and assumptions:

1. Statistical provisions.
   1.1 the power of the statistical tests "P = (1 - β)" is assumed to be 80% (the probability of correct rejection of the null hypothesis is 0.8);

1.2 the probability of a type I error "α" is allowed to be less than 5% (the probability of the erroneous acceptance of an alternative hypothesis is less than 0.05);

1.3 the statistical criteria used are 2-sided;

1.4 the calculation of the sample size is based on the assumptions about the expected effects, declared in the primary efficacy endpoint;

1.5 the ratio between the sample sizes of the Product and Placebo groups is 1:1 (1 Product patient - 1 Placebo patient);

1.6 the significance level for type I error is distributed between primary and secondary endpoints: 0.04 for the primary and 0.01 for the secondary (0.04, 0.01)

1.7 statistical hypotheses: null and alternative hypotheses about the superiority of the study drug over placebo using the applied dosing regimen:

primary test:

$$\mathbf{H_0: M_1\text{-}M_2 = 0}$$

$$\mathbf{H_a: M_1\text{-}M_2 \neq 0}$$

where M1 – the median time to resolution of all symptoms in the Product group;

M2 – the median time to resolution of all symptoms in the Placebo group.

The sample size is calculated for the Wilcoxon non-parametric test in compliance with the O'Brien-Castelloe approximation.

Programme code[6]:

```
proc power;
twosamplewilcoxon
vardist("Trt") = normal(5.18,1.36)
vardist("Pl") = normal(5.84,1.28)
variables = "Trt" | "Pl"
nbins=5000
alpha=0.04
npergroup = .
power = 0.8;
run;
```

Additional test:

Null and alternative hypotheses:

$$\mathbf{H_0: AUC_1\text{-}AUC_2 = 0}$$

$$\mathbf{H_a: AUC_1\text{-}AUC_2 \neq 0}$$

where $\mathbf{AUC_1}$ – the area under curve in the Product group,

[6] Performed using the SAS software. Assessment of the parameters for distributions – refer to p.2

$\mathbf{AUC_2}$ – the area under curve in the Placebo group;

the sample size for statistical tests was calculated using the following formula:

$$\mathbf{n_1 = kn_2}$$

$$\mathbf{n_2} = \frac{\left(z_{\alpha/2} + z_{\beta}\right)^2 * (1 + 1/k) * \sigma^2}{\varepsilon^2}$$

where $\mathbf{n_1}$, $\mathbf{n_2}$ – the sample size of the study drug and the placebo groups respectively;

$\varepsilon = AUC_1 - AUC_2$ – the expected difference in the severity AUC between the Product and Placebo groups;

k – the ratio of choice between Product and Placebo (1 to 1);

σ – the standard deviation of the AUC;

$z_{\alpha/2}$ – a tabulated two-tailed z-test for **α**;

z_{β} – a tabulated one-tailed z-test for **β**.

1.8 final sample size will be determined using the formula:

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

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

$\mathbf{N_{PP}}$ – the value calculated in section 1.8, i.e. the expected number of the subjects completing the study per protocol;

$\mathbf{C_w}$ – withdrawal coefficient.

2. Assumptions about the expected effects of the clinical study.

Time to resolution of symptoms in the Product group is 5.18 days, time to resolution of symptoms in the Product group is 5.84 days.

A priori estimates for tested parameter variables in the groups

***Primary endpoint (efficacy criterion)**:*

Time to resolution of all symptoms of a clinically diagnosed or PCR-confirmed URI (according to a patient diary).

Placebo group:

Time to resolution of symptoms was estimated on the basis of the previous studies.

T_{pl}=*(<max(N_t(2.5,1.47), N_{intox}(5.8,1.32)>)*[7]

Where <…> stands for averaging operation, N(x,s) – normally distributed random variable with the mean x and standard deviation s. Indices: t – fever, intox – intoxication symptoms.

[7] Based on the clinical trial "A study to assess the therapeutic efficacy and safety of Anaferon against influenza and other upper respiratory tract infections in adults". Duration distributions for symptom domains are considered independent. 95% confidence interval is taken for the corresponding standard deviations.

The distribution of the maximum of a number of samples of N normal distributions corresponds to the Gumbel type I distribution[8]

for calculation of the sample size, this empirical distribution is approximated by the normal distribution with characteristics m=5.84,σ=1.28

Study drug group:

T_{tr}*=(<max(N(2.6,1.77), N(5,1.47))>)*

Where <…> stands for averaging operation, N(x,s) – normally distributed random variable with the mean x and standard deviation s. Indices: t – fever, intox – intoxication symptoms, cat - catarrhal symptoms.

corresponds to the Gumbel type I distribution

for calculation of the sample size, this empirical distribution is approximated by the normal distribution with characteristics m=5.17, σ=1.36.

Therefore, the sample size has been found to be (**71**) patients in each of the groups (Product and Placebo) to estimate superiority of the study drug over placebo.

Given potential withdrawal of at least 30% subjects (**C_w=0.3**) during the study for various reasons, at least **204** subjects will be required to sign informed consent, **102** per group (see. 1.8).

Statistical criteria

All the statistical calculations will be performed using two groups of statistical criteria:

- parametric – to obtain effective evaluations for parameters of random values, if the relevant conditions of applicability of methods/models are not violated (e.g. sphericity, normality, proportionality of risks, etc.);
- non-parametric – in any other cases.

Parametric criteria

The application of parametric criteria will be accompanied by a check for normality of the compared samples (Kolmogorov-Smirnov test).

The following parameters and approaches are to be used:

1. To evaluate the differences of continuous variables obtained in one group at two different visits – Student's test for matched samples.
2. To evaluate the temporal dynamics of the compared indicators – analysis of variance (ANOVA) or covariance (ANCOVA) in the modification with repeated measures.
3. In case of multiple comparisons between the groups will apply a variety of corrections for multiplicity (Dunnett), (Tukey), (Scheffe), Holm adaptive test, etc.

[8] On the distribution of the maximum of N independent normal random variables: IID and INID cases. SDSSU Multidisciplinary Research Journal Vol. 1 No. 2, 2013

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, clarification of the factor and covariance structures of the model is carried out with fit statistics such as AIC (Akaike information criterion).

To perform the above-mentioned statistical tests and techniques, it is assumed that the following SAS procedures are used:

- UNIVARIATE – check for normality of the compared distributions;
- CORR, MEANS – calculation of descriptive statistics;
- TTEST – Student t-test with all the modifications;
- GLM – analysis of Generalized Linear Models for studying temporal dynamics (ANOVA, ANCOVA);
- GENMOD – analysis of Generalized Linear Models.
  MIXED – analysis of Generalized Linear Models.

Non-parametric criteria

Below, there are the main types of possible comparisons with the respective criteria:

1. To evaluate time changes in the parameters compared – Friedman test, nonparametric analogue of repeated measures analysis of variance.
2. For the frequency analysis of 2×2 cross tables – χ^2-test (if the compared frequencies are greater than 5) or Fisher exact test (if one of the compared frequencies is less than 5).
3. For the frequency analysis of cross tables with independent strata – Cochran–Mantel–Haenszel test (modification of the χ^2-test for multiple comparisons).
4. For the frequency analysis of data on the presence / absence of an event or outcome during repeated measures (cross tables with dependent strata) – survival analysis.

To perform the above-mentioned non-parametric statistical analysis options, it is assumed that the following SAS procedures are used:

- FREQ – Friedman test, χ^2-test and / or Fisher exact test; Cochran–Mantel–Haenszel test
- LIFETEST, PHREG – survival analysis
- NPAR1WAY – Mann-Whitney U-test.

Safety parameters

Adverse events recorded during the study will be grouped into frequency tables by severity, seriousness and 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. Relevant confidence intervals will also be provided for the data suggesting statistical conclusion. Extreme values (outliers) will be analyzed additionally. The data will be grouped by visits. The categorical variables will be presented as frequency tables by visits.