International multicenter double-blind placebo-controlled randomized parallel-group clinical trial of Anaferon for children efficacy in prevention of influenza and other acute respiratory viral infections in children during the peaks of seasonal morbidity

Phase IV

Sponsor	**OOO «NPF «MATERIA MEDICA HOLDING»**
Protocol number	**MMH-AD-008**
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ClinicalTrials.gov Id:	**NCT03301155**

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

International multicenter double-blind placebo-controlled randomized parallel-group clinical trial of Anaferon for children efficacy in prevention of influenza and other acute respiratory viral infections in children during the peaks of seasonal morbidity.

Phase: IV
Sponsor: OOO "NPF "Materia Medica Holding", Moscow, Russia
Protocol No. MMH-AD-008

Objective of the study

- To obtain additional data on the efficacy and safety of 12-week course of therapy with Anaferon for children for prevention of influenza and other acute respiratory viral infections in children during the peaks of seasonal morbidity.

Endpoints

Primary endpoint

1. Time from taking the first dose of the study drug to the onset of influenza/ARVI symptoms.

Secondary endpoints

1. Percentage of children not falling ill with influenza or another ARVI during 4, 8, or 12-week preventive course (based on medical records).
2. Percentage of children with the symptoms of a respiratory or ear-nose-throat bacterial infection requiring antibacterial therapy within 12-week preventive course (based on medical records).
3. Percentage of children hospitalized for influenza/ARVI or their complications within 12-week preventive course (based on medical records).

Safety assessment

- Presence and type of adverse events, their severity, relation to investigational drug, outcomes.

Study design

Design: an international, multicenter, double-blind, placebo-controlled, randomized study in parallel groups.

The study will enroll children of both gender from 1 month to 6 years old. Children can participate in the study regardless of the frequency of previous ARVI. A potential study participant should not be in the incubation (if known), prodromal, acute /subacute periods of any infectious disease (except for the recovery period). Schedule for enrollment of participants: during the period of a seasonal rise in influenza/ARVI incidence in the Russian Federation and the Republic of Uzbekistan. Screening and randomization of participants will be subject to availability of official information on the incidence of influenza/ARVI in the relevant study region.

The doctor makes the first visit to the participant in the medical center or at home. After parent/adoptive parent signs the information sheet (informed consent form), the doctor evaluates the possibility of the participant being included in the trial. If the child meets all the inclusion criteria and does not have all non-inclusion criteria, then he/she is included in the study, the doctor fills in the Clinical Research Form. At visit 1 (Day 1), the participant is randomized into one of two groups: the 1st group participants will take Anaferon for children according to the preventive regimen for 12 weeks; the 2nd group participants will take Placebo according to the regimen of Anaferon for 12 weeks.

In total, the study participant will be observed for 12 weeks (screening and randomization up to 1 day, preventive treatment for 12 weeks).

During the observation period at 4 (Visit 2), 8 (Visit 3), and 12 (Visit 4) weeks, three visits are planned.

Visits 2 (Week 4 ± 3 days) and 3 (Week 8 ± 3 days) are conducted in the form of a telephone survey of parents/adoptive parents about the participant's health status, presence/absence of symptoms of influenza/ARVI, possible use of antibacterial drugs and/or hospitalization during the course preventive therapy.

Visit 4 (Week 12 ± 3 days) is carried out at home or in a medical center; the doctor collects complaints, examines the participant, registers concomitant therapy, assesses the compliance of the therapy.

If in the period from 2 to 12 weeks a participant falls ill with influenza/ARVI[1], then he prematurely completes participation in the study (as having reached the primary endpoint). A participant is considered fallen ill with influenza/ARVI if the doctor identifies the following symptoms: febrile/subfebrile body temperature, presence at least one flu-like nonspecific symptom (decreased activity/impaired behavior/weakness; headache; chills) and at least one respiratory symptom (runny nose; nasal stuffiness; hoarseness/husky voice; sore throat; cough). In this case, the doctor makes an unscheduled visit (at home or in a medical center), which is final. During the visit, the doctor carries out the procedures of Visit 4. A nasopharyngeal swab is taken to identify the most common pathogens of influenza/ARVI[2].

If ARVI/influenza occurs within the first seven days from the onset of preventive therapy, the disease will not be recorded as an adverse event and will not be taken into account to evaluate the efficacy of the study drug, since an early manifestation of influenza/ARVI may indicate that the participant was included in the study when he/she was in the incubation period of an infectious disease.

During the study, concomitant therapy for underlying chronic conditions, as well as routine vaccination of the participant, are allowed, with the exception of the drugs indicated in the section “Prohibited Concomitant Treatment”.

Inclusion and exclusion criteria

Inclusion criteria

1. Children of either gender aged from 1 month to 6 years old[3].
2. The absence of clinical symptoms of any infectious disease, but not earlier than 14 days from its onset.
3. Seasonal rise in ARVI incidence, confirmed by official information.
4. An information sheet (Informed Consent form) for the subject participation in the clinical trial signed by one parent/adopter of the patient.

Exclusion criteria

1. Acute or subacute period of infectious disease of any etiology (viral, bacterial, fungal, etc.) and localization (including upper and lower respiratory tract infection, meningitis, sepsis, otitis media, urinary tract infection, intestinal infection, etc.).

[1] The symptoms associated with teething or vaccination (post-vaccination reactions and complications) are not considered as manifestations of influenza/ARVI.

[2] PCR-based diagnosis with the AmpliSens Reagent Kit to identify the most common pathogens of ARVI and influenza, 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.

[3] The study participants are aged 1 month to 6 years 11 months and 29 days.

2. History of (verified previously) or current suspected conditions such as:
   - primary or secondary immunodeficiency[4];
   - bronchopulmonary dysplasia, primary ciliary dyskinesia, cystic fibrosis, other chronic pulmonary diseases;
   - malformations of the respiratory and ENT organs (ear, throat, mouth, tongue, larynx, trachea, neck and salivary and thyroid glands, etc.);
   - immunopathological diseases (including Marshall syndrome, Behcet's syndrome, Kawasaki disease, etc.);
   - hematological diseases (including agranulocytosis, leukemia);
   - oncologic conditions.
3. Exacerbated or decompensated of chronic diseases affecting the participant's ability to participate in the clinical trial.
4. Malabsorption syndrome, including congenital or acquired lactase or another disaccharide deficiency, galactosemia.
5. Allergy/intolerance to any component of the study drug.
6. Course administration of the drug products specified in the section ‘Prohibited concomitant medications’ within 2 weeks prior to enrollment.
7. Children whose parents/adopter parents will fail to comply with the observation requirements of the trial or with the intake regimen of the study drug, from the investigator's point of view,
8. Participation in other clinical trials within 3 month prior to enrollment in the study.
9. The patient's parent/adopter parent is a member of the research team of the investigational site directly involved in the study or a close relative of an investigator. Close relatives are defined as husband/wife, parents, children, brothers (sisters) regardless of whether they are biological or adopted.
10. The patient's parent/adopter parent works for OOO "NPF "MATERIA MEDICA HOLDING" (i.e., the company's employee, part-time employee under contract or appointed official in charge of the trial, or their immediate family).

Discontinuation criteria

1. Failure or refusal of the child or his/her parents/adopters to comply with the protocol.

[4] Primary and secondary immunodeficiencies, regardless of etiology, are characterized by persistent immunodeficiency. Transient immunomodulation do not represent immunodeficiency. Children with recurrent respiratory infections have transient immunomodulation. High rates of infectious diseases during the first years of life should not be considered as a pathology; this condition is not attributable to immunodeficiency and does not exhibit negative effect on immunity formation. Such subjects are eligible.
Subjects with immunodeficiency need complex therapy using the products forbidden within the study.

2. The necessity to use medications not permitted within the study.
3. An adverse event requiring discontinuation of the investigational product.
4. The desire of the child or parent/adoptive parent to complete the study ahead of schedule for any reason.
5. Cases not specified by the protocol, when the investigator decides that the further participation may harm the child.
6. Incorrect inclusion of ineligible child.
7. The patient did not pass the screening procedure.
8. The participant got influenza/ARVI.

Number of subjects

1036 participants of both genders aged 1 month to 6 years old (518 participants in each group) are planned to be included and randomized in the clinical trial. It is estimated that approximately 880 children (440 participants in each group) will complete participation in the study in accordance with the protocol.

Interim analysis

Since the recruitment of participants covers more than one epidemic season, a blind intermediate analysis is carried out in each season (without revealing the participant's membership in one or another experimental group) to possibly clarify a priori research parameters.

Based on the results of a blind interim analysis, a significant difference between the base population frequency of the events studied and the planned one will be revealed, the sample size of the study is reassessed.

Treatment

Group 1

Name of the medicinal product: Anaferon for children

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

** applied to lactose monohydrate as water ethanol mixture containing no more than 10^{-16} ng/g of active substance*

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

Method of administration: Tablet for oral use. One tablet per intake, once daily (approximately at the same time).

The product is administered outside a meal (in the interval between meals or 15 min prior to meal or fluid intake), the tablets should be held in mouth until complete dissolution. For young

children (aged 1 month to 3 years old), the tablet is recommended to be dissolved in a small amount (1 tablespoon) of drinking water of room temperature.

Dosage form: Tablets.

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

Storage conditions: Store at temperature 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 for children scheme.

Dosage form: Tablets.

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

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

Treatment duration

Anaferon for children/Placebo treatment duration is 12 weeks.

Observation period

According to the protocol, the child's participation in the study lasts 12 weeks, including screening and randomization (the first day) and the period of study therapy (12 weeks).

Basic therapy

During the entire study period, the participant can receive therapy for the underlying and concomitant diseases that are not criteria for non-inclusion.

Prohibited concomitant therapy

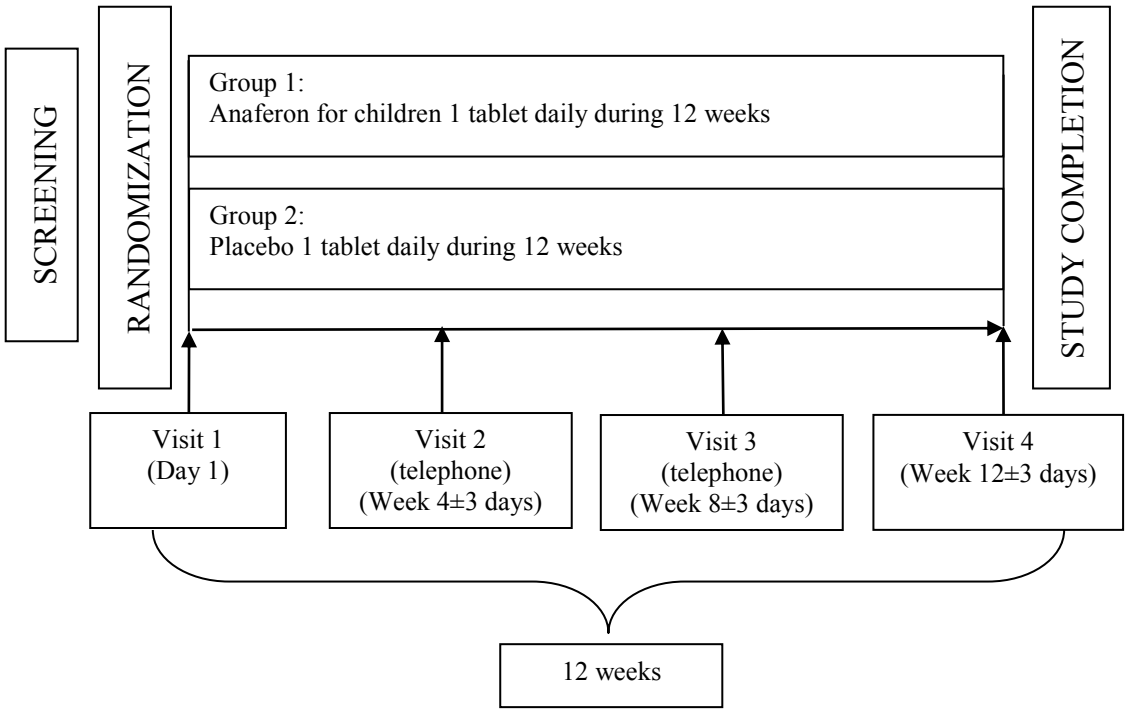
Two weeks prior to enrollment and during the study the following drugs are prohibited:

1. Antivirals (J05), except for Anaferon for Children prescribed in this trial.
2. Immunostimulants (L03), including:
    - interferon inducers (acridonacetic acid, meglumine acridoacetate/cycloferon®, umifenovir/arbidol®, kagocel®, tilorone/amixin®, lavomax®, tilaxin®, polyadenylic acid + polyuridylic acid/poludan®, oxodihydroacridinylacetate odium/neovir®, ribonucleate sodium/ridostin, etc, deoxyribonucleate sodium/derinat® etc.)
    - interferons
    - bacterial immunomodulators (including ribomunyl®, IRS-19, imudon®, broncho-munl® and etc.)

- pidotimod/imunorix
- interleukins
- synthetic immunostimulants (levamisole, alpha-glutamyl-triptophan/thymogen, etc.)
- thymus hormone-containing drugs
- herbal immunostimulants (immunal®, etc.)

3. Immunosuppressants (L04).
4. Antineoplastic agents (L01) and hormones (L02).
5. Immune sera and immunoglobulins (J06).
6. Fenspiride (R03DX03), omalizumab (R03DX05).
7. Systemic corticoids (H02AB).
8. Homeopathic drugs.
9. Drugs known to cause allergic reactions in the patient.

Study design scheme



Schedule of study procedures

Procedure/visit	Visit 1 (Day 1)	Visit 2 «phone» (Week 4±3 days)	Visit 3 «phone» (Week 8±3 days)	Visit 4 (Week 12±3 days)
Obtaining signed informed consent	+			

form (ICF)				
Eligibility assessment	+			
Recording concomitant medications	+	+	+	+
Randomization and prescription of study therapy	+			
Study drug supply	+			
Drug accountability and return, assessment of treatment compliance				+
Evaluation of treatment safety	+	+	+	+
Telephone survey		+	+	
Collection of nasopharyngeal swabs for PCR diagnosis*		** Performed in case the child falls ill with influenza/ARVI during unscheduled (additional) visit with Visit 4 procedures*		

Statistical Analysis

Samples

Statistical analysis will be carried out in the following sets:

Total set includes all participants whose parents/adoptive parents signed the Informed Consent Form.

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

Full Analysis Set consists of all enrolled and randomized patients, except for those who met at least one of the following criteria:

1) failure to meet inclusion/non-inclusion criteria;
2) subject failing to take any dose of the study drug;
3) absence of any data on the subject after randomization.

This is the best set for the Intention-to-treat method, so it will be used in the Intention-to-treat efficacy analysis of the test therapy.

Per Protocol set. This sample includes all subjects who completed the therapy as per the study protocol, without any missing visits or major protocol deviations. This set will be used in the Per Protocol efficacy analysis of the test therapy.

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)' is 80% (the probability of correct rejection of the null hypothesis is 0.8)

1.2 the probability of type 1 error 'a' is less that 5% (the probability of false acceptance of the alternative hypothesis is less than 0.05);

1.3 statistical criteria of intergroup comparisons will be two-sided;

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

1.5 ratio between Anaferon for children and placebo sample sizes is 1:1 (one Anaferon for children subject: one placebo subject).

1.6 statistical hypothesis will be as follows:

null and alternative hypothesis on Anaferon for children superiority using the dosing scheme specified:

$$\mathbf{H_0: T_1 - T_2 \leq \Delta}$$

$$\mathbf{H_1: T_1 - T_2 > \Delta},$$

in terms of Cox proportional hazard model,

$$\mathbf{H_0: \ln(T_1/T_2) \leq \delta}$$

$$\mathbf{H_1: \ln(T_1/T_2) > \delta},$$

where $\mathbf{T_1}$ – median influenza/ARVI time in Anaferon for children group;

$\mathbf{T_2}$ – median influenza/ARVI time in placebo group;

$\mathbf{\Delta}$, $\mathbf{\delta}$ –minimum clinically relevant effect.

1.7 Therefore, sample size will be calculated taking into account

a. Assumption on expected effects declared in the main efficacy criterion;

1.8 calculation of sample size for two-sided test for the final analysis will be made using the formula:

$$n = \frac{(z_{\alpha/2} + z_{\beta})^2}{(b - \delta)^2 p_1 p_2 d}$$

where **n** – **total** sample size of the product and placebo, $\mathbf{p_1}$ – proportion of subjects from the total sample size in Anaferon for children group, $\mathbf{p_2}$ – proportion of subjects from the total sample size in placebo group, **p1=p2=0.5**); **b** – effect size (calculated as $\mathbf{b = \ln(T_1/T_2)}$. $\mathbf{\delta}$ – minimum clinically relevant effect $\mathbf{\delta=\ln(1+\Delta/T_2)}$; $\mathbf{z_{\alpha/2}}$ and $\mathbf{z_{\beta}}$ – relevant tabular values of z-test; **d** – expected proportion of subjects reaching primary endpoint during the study.

1.9 Final sample size will be determined using the formula:

$$\mathbf{N_F = N_{PP} / (1 - K_B)},$$

where $\mathbf{N_F}$ – final sample size; $\mathbf{N_{PP}}$ – result of calculation in cl. 1.8, i.e. scheduled number of children completing the study per protocol; $\mathbf{K_B}$ – withdrawal coefficient.

2. Assumptions on expected clinical study effects.

It was initially suggested that in Anaferon for children group proportion of subjects falling ill with ARVI/influenza in 12 weeks (84 days) after the treatment onset will not exceed 65%, in placebo group will be at least 80% (conservative estimate based on the study), therefore the difference between the study arms will be less than 15%. Minimum clinically relevant difference between the groups will be 5%.

However, due to essential inability to obtain complete data on all the disease cases in all the subjects enrolled during the study (data censored, right) and necessity to determine typical time to the disease development, we will reformulate the condition on effects in the assumption of exponentially distributed disease cases:

$$\mathbf{P_i=1-\exp(-\lambda_i T),}$$

where P_i – proportion of ill subjects in I group, λ_i – risk of disease in I group, T – follow-up time.

Therefore, evaluation of risk for experimental groups is defined as

$$\mathbf{\lambda_i = \ln(1-P_i)/(-T),}$$

median time to the event

$$\mathbf{T_{1/2,i}=\ln(2)/\lambda_i}$$

At that the expected median time to the disease in placebo group is **36** days, minimum clinically relevant difference is **6** days (therefore, minimum median time for superiority is **42** days). The scheduled effect is equal to **19** days (median time to the disease in Anaferon for children group is **55** days).

Thus, the intended effect in terms of Cox model will be $\mathbf{b= \ln(56/36) = \ln(1.53) = 0.425}$, minimum clinical effect will be $\mathbf{\delta=\ln(42/36)=0.154}$. Based on the assumptions on the effects we will also take the presumed total proportion of subjects falling ill during the study as equal to $\mathbf{d=0.725}$. Therefore, sufficient number of influenza/ARVI cases required to reject null hypothesis is **437**, the total required number of subjects (PP) is **604 (accurate value 602.75).**

As the subject recruitment covers more than one epidemic season, **blind interim analysis** will be made in each season to assess nuisance parameters of the study.

According to the above mentioned statistical terms and assumptions, sample size of each group for the final analysis will be **302** subjects for each group (PP set is **604** in total).

Given potential withdrawal of at least **15%** of the subjects at the screening and during the study for various reasons, at least **355** subjects should be enrolled in each group (**710** subjects in total, see 1.9).

Interim analysis

The analysis is made in accordance with point 2 without unblinding the treatment group.

Statistical criteria

All statistical calculations will be made using the following groups of statistical criteria:

- parametric – to assess continuous and interval accidental values;
- non-parametric – to obtain:
    - evaluations on equality/inequality of proportions of subjects compared for various visits,
    - analysis of frequencies in features compared,
      and
    - evaluation of continuous and interval accidental values in case of violation of requirement on normal sample distribution.

Parametric criteria will be checked for normality of the samples compared (Kolmogorov-Smirnov test).

The following *parametric methods and approaches* are suggested:

1. To evaluate differences in continuous variables obtained in one group at two different visits – Student t-test for paired samples.
2. For evaluation of time changes in parameters under comparison – analysis of variance (ANOVA) or covariance (ANCOVA) analysis in a modification with repeated measurements (Repeated Measures).
3. In the case of multiple comparisons of groups among themselves, various corrections for multiplicity will be applied (Dunnett, Tukey, Scheffe, adapted Holm criterion, etc).
4. For more complex data structures, approaches will be used with the construction of Generalized Linear Models and / or Mixed Linear Models.
5. Selection of the type of distribution, refinement of the factor and covariance structures of the model is carried out using fit statistics, such as AIC (Akaike Information Criterion).

To carry out the statistical analysis and approaches specified above the following SAS procedures are suggested:

- UNIVARIATE – check for normalcy of distributions compared
- CORR, MEANS – calculation of descriptive statistics
- TTEST – Student t-test with all modifications
- GLM – analysis of general linear models for studying temporal dynamics (ANOVA, ANCOVA)
- GENMOD – analysis of generalized linear models
- MIXED – mixed linear model analysis
- PHREG – survival analysis.

Nonparametric criteria:

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

1. For evaluation of time changes in parameters under comparison, the Friedman test, a nonparametric analogue of variance analysis with repeated measurements.
2. For frequency analysis of contingency tables 2×2 – χ^2 test (if the frequencies compared exceed 5) of exact Fisher's test (if one of the frequencies compared is < 5).
3. For frequency analysis of contingency tables with multiple comparisons – Cochran-Mantel-Haenszel test (modification of χ^2 test for multiple comparisons).
4. For the frequency analysis of data on the presence / absence of an event or outcome during repeated measurements (contingency 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 – 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 product.

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. Comparisons suggesting statistical conclusion will have the relevant confidence intervals. Outliers will be analyzed individually. The data will be grouped by visits. Categorical variables will be presented as frequency tables by visits.

SAS-9.4[5] software will be used for statistical analysis including various parametric and nonparametric tests.

[5] Licensee: LLC NPF Materia Medica Holding, №70100045.