

**International multicenter double-blind placebo-controlled
randomized parallel group clinical trial of efficacy of
Anaferon for children in the treatment of influenza and
acute respiratory viral infections in children**

Phase IV

Sponsor	OOO «NPF «MATERIA MEDICA HOLDING»
Protocol number	MMH-AD-004
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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 efficacy of Anaferon for children in the treatment of influenza and acute respiratory viral infections in children.

Phase: IV

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

Protocol No. MMH-AD-004

Study purposes

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

Endpoints

Primary endpoint

1. Average illness duration (until recovery¹ or significant improvement²).

Secondary endpoints

1. Percentage of patients with recovery¹/improvement² in health on days 2, 3, 4 and 5 of observation treatment (according to the patient's diary), on days 3 and 5 of therapy (according to physician's objective examination).
2. Changes in fever (changes in temperature on days 2, 3, 4 and 5 of observation treatment).
3. Percentage of patients with normal body temperature ($\leq 37.0^{\circ}\text{C}$) on days 2, 3, 4 or 5 of observation treatment.
4. Severity of clinical manifestations of influenza/ARVI in scores on days 3 and 5 of observation treatment – according to physician's objective examination; on days 2-6 of observation – according to patient's diary.
5. Assessment of the Severity of Influenza Virus / ARVI Using the "Area Under the Curve" for an Overall Symptom Assessment (based on the results of area under curve for total

¹ **Recovery criteria:** temperature $\leq 37.0^{\circ}\text{C}$ + lack of symptoms within 24 hours.

² **Improvement criteria:** temperature $\leq 37.2^{\circ}\text{C}$ + total symptom score ≤ 2 .

symptom score³ and nasal/throat/chest score on days 1, 3, 5 and 7 of observation – according to physician’s objective examination; on days 1-7 of observation – according to patient’s diary).

6. Number of intakes of antipyretic drugs according to indications on days 1, 2, 3, 4 and 5 of therapy.
7. Changes in viral load⁴ during the treatment and follow-up periods.
8. Percentage of patients with exacerbation of the disease course (complications requiring antibiotic drugs or hospitalization).

Safety assessment

- Presence and nature of adverse events during therapy, their relationship with the product and other characteristics.

Study design

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

The study will enroll outpatients of both genders aged 3-12 years with clinical signs of influenza/ARVI including fever $\geq 38.0^{\circ}\text{C}$ seeking for medical advice within the first day after the disease debut. The first visit shall be made at the subject’s home. After signing patient information sheet (informed consent form) by the parent/adoptive parent for the subject’s participation in the study his/her medical history will be collected, objective examination will be performed and concomitant therapy will be recorded. Nasal swab for rapid influenza diagnostics will be collected. In case of positive rapid test the physician will take nasal and oropharyngeal swabs (nasopharyngeal swab) for further PCR diagnosis of influenza A and/or B to determine the viral load. In case of negative result of rapid test the physician will take nasopharyngeal swab for further PCR to detect other respiratory viruses.

If the subject meets inclusion criteria and has no exclusion criteria at visit 1 (Day 1) he/she shall be enrolled and randomized into one of the two groups: group 1 will receive Anaferon for children for 5 days; group 2 – placebo according to Anaferon for children for 5 days. Parent/adoptive parent will receive a diary to record axillary temperature of the subject on a daily basis in the morning and evening (temperature is measured with a standard non-mercuric

³ **Total symptom score** is calculated based on intensity of each influenza symptom at subsequent statistical treatment by the experts of OOO “NPF “Materia Medica Holding”.

⁴ Viral load will be evaluated in subjects with positive rapid test for influenza A and B. Viral RNA levels [influenza A/B] \log_{10} copies/mL in nasal and oral swabs will be determined by real-time RT-PCR.

thermometer Geratherm Classic) as well as intensity of the disease symptoms. Training on the diary filling will be performed for parents/adoptive parents.

The subject will be followed for 14 days (screening, randomization – 1 day, treatment – 5 days, follow-up – 1 day; delayed phone “visit” – day 14).

During the treatment and follow-up periods the subject/physician shall make 4 visits, the fifth phone “visit” is stipulated additionally: 1) physician/subject visits – on days 1, 3, 5 and 7 (Visits 1, 2, 3 and 4) – at medical facility or at home; 2) phone “visit” (Visit 5) – on day 14.

At visits 2-4 the physician shall record objective examination findings, collect nasopharyngeal swabs for further PCR diagnosis in subjects with positive rapid test (to determine viral load and its reduction against therapy), monitor the prescribed and concomitant therapy, revise the patient’s diary. Phone “visit” will be made to inquire on the subject’s condition, presence/absence of secondary bacterial/viral complications, administration of antibacterial drugs.

Symptomatic therapy and therapy of co-morbidities is allowed except for the products specified in section “Prohibited concomitant therapy”.

Inclusion and exclusion criteria

Inclusion criteria

1. Subjects of both sexes aged 3-12 years inclusively.
2. Diagnosis of influenza/ARVI according to physician’s examination: body temperature $\geq 38.0^{\circ}\text{C}$ at the time of examination + symptom score ≥ 4 (at least 1 systemic symptom ≥ 2 and 1 nasal/throat/chest symptom ≥ 2 or several symptoms ≥ 1 score).
3. The first 24 hours from the beginning of manifestations of influenza/ARVI.
4. The possibility to start therapy within 24 hours from the first symptoms of ARVI.
5. Availability of patient information sheet (informed consent form) for parents/adoptive parents for participation in the study signed by one of the parents/adoptive parents.

Exclusion criteria

1. Suspected pneumonia, bacterial infection or severe disease requiring antibacterial products (including sulfanilamides) starting from the first day of the disease.
2. Clinical symptoms of severe influenza/ARVI⁵ requiring hospitalization.

⁵ Severe influenza/ARVI criteria (RF MoH, 2009; WHO 2012; CDC 2009-2013): severe intoxication syndrome (toxic shock III, vomiting, dehydration symptoms, reduced urine output/anuria, restlessness, cry or abrupt weakness up to full adynamia, tachypnea, tachycardia not correlating with temperature, seizures, meningism symptoms), impairment of consciousness, hemorrhagic syndrome (epistaxis, skin and mucosa petechiae), hemodynamic instability, expressed obstructive disorders (stenosing laryngotracheitis, bronchial obstruction syndrome), acute respiratory failure, primary and secondary pneumonia. Risk factors for severe influenza/ARVI and complications: chronic cardiovascular, respiratory diseases, diabetes mellitus, immunodeficiencies, oncology [2, 13, 22, 23, 35, 36].

3. Suspected initial manifestations of the diseases having the symptoms similar to the ones of influenza/ARVI (other infectious diseases, influenza-like syndrome at debut of systemic connective tissue diseases, oncohematological and other diseases).
4. History of primary and secondary immunodeficiencies: a) lymphoid system immunodeficiencies (T-cell and/or B-cell, immunodeficiencies with predominant antibody deficiency); b) phagocytic deficiencies; c) complement factor deficiency; d) combined immunodeficiencies including AIDS secondary to HIV-infection; toxic, autoimmune, infectious, radiation panleukopenic syndrome; systemic lymphocytopenic syndrome; polyclonal lymphocytic activation syndrome; postsplenectomic syndrome; congenital asplenia; immune complex pathological syndrome associated with infectious, autoimmune and allergic diseases.
5. Medical history of sarcoidosis.
6. Oncology.
7. Exacerbation or decompensation of chronic diseases affecting ability to participate in the clinical study.
8. Medical history of polyvalent allergy.
9. Allergy/intolerance to any of the components of medications used in the treatment.
10. Malabsorption syndrome, including congenital or acquired lactase or other disaccharidase deficiency, galactosemia.
11. Intake of medicines listed in the section “Prohibited concomitant therapy” within 1 month prior to the inclusion in the study.
12. Drug addiction, alcohol consumption at more than 2 alcohol units⁶ per day by the subject's parents/adoptive parents.
13. Mental diseases of the subject, parents/adoptive parents.
14. Subjects whose parents/adoptive parents, according to the investigator's point of view, will not follow the observation requirements during the study or study product dosing regimen.
15. Participation in other clinical studies within 3 months prior to the inclusion in the study.
16. Parent/adoptive parent of the subject is related to the investigator team of medical facility directly involved in the study or is a close relative of the investigator. Close relatives include spouse, parents, children or brothers (sisters) regardless of whether they are biological or adoptive ones.
17. Parent/adoptive parent of the subject is working in OOO “NPF “Materia Medica Holding”, i.e. is the company official, temporary contract worker or an appointed official responsible for the study or their close relatives.

⁶ 1 alcohol unit – 0.33 L light beer/150 mL of unfortified wine, 40 mL of liquors.

Discontinuation criteria

1. Inability or refusal of the subject, his/her parents/adoptive parents to compliance the protocol.
2. Necessity in the medical products not allowed within the study.
3. Development of adverse event requiring the product discontinuation.
4. Subject's or parents'/adoptive parents' wish to terminate the study early due to lack of therapeutic efficacy or any other reason.
5. Cases not specified by the protocol when, according to the investigator, further subject's participation is dangerous.
6. Enrollment of ineligible subject.

Number of subjects

Signed informed consent form is supposed to be obtained from 972 subjects (of them 772 – in the Russian Federation, 100 – in Republic of Belarus and 100 – in Ukraine); this would allow to complete all protocol procedures by at least 508 subjects (n=254 in Anaferon for children and Placebo groups).

Interim analysis

An interim statistical analysis is scheduled within the study. The interim analysis will be based on the findings from examinations and therapy of the protocol-set number of subjects who completed the study. Based on the proposed effect, the minimum required Per Protocol (PP) sample size will be 254 subjects (n=127 in Anaferon for children and Placebo groups).

Treatment

Group 1

Name: Anaferon for children

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

** applied to lactose monohydrate as water-alcohol mixture containing no more than 10^{-16} ng/g of the active form of the active ingredient*

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

Dosage: Per os. One tablet per intake (hold in mouth until complete dissolution – regardless of meal). The first 2 hours – every 30 minutes, then 3 additional doses with equal intervals. On days 2-5 – on tablet 3 times a day.

Dosage form: Orodispersible tablets

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

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

Group 2

Name: Placebo

Active ingredient: NA

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

Dosage: Placebo using Anaferon for children scheme

Dosage form: Orodispersible tablets

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

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

Treatment period

Anaferon for children/Placebo treatment period is 5 days.

Observation period

Overall the subject is observed for 14 days (screening, randomization, treatment initiation – day 1, study therapy period – 5 days, follow-up – 1 day, phone visit – day 14).

Basic therapy

Throughout the study the subject may have symptomatic therapy of influenza/ARVI based on the accepted treatment standards including expectorants, mucolytics, vasoconstrictive nasal drops, where necessary – detoxifying therapy, as indicated* – approved antipyretics**, in case of bacterial complications of influenza/ARVI – antibacterial drugs except for antiviral, immunomodulating and other products specified in section “Prohibited concomitant therapy”.

* Indications for antipyretics products [11, 14, 34]:

Body temperature > 38.5°C;

Body temperature 38.0°C against concomitant chronic pulmonary, cardiac, nervous system diseases.

In case the antipyretic product was prescribed and given to the child by parents/adoptive parents on their own (without physician’s prescription) with no indications, the subject will not be withdrawn. Parents/adoptive parents should record temperature values in patient’s diary prior to the product administration, its name and dose.

** The products allowed as antipyretics (ATC group is specified in brackets):

1. Paracetamol (N02BE01).

2. Ibuprofen (M01AE01).

3. Metamizole sodium (N02BB02) – on prescription only (for emergency assistance in case of hyperthermia uncontrolled by paracetamol/ibuprofen able, parenterally).

The product will be chosen by the investigator who will issue the antipyretic product to the subject at Visit 1.

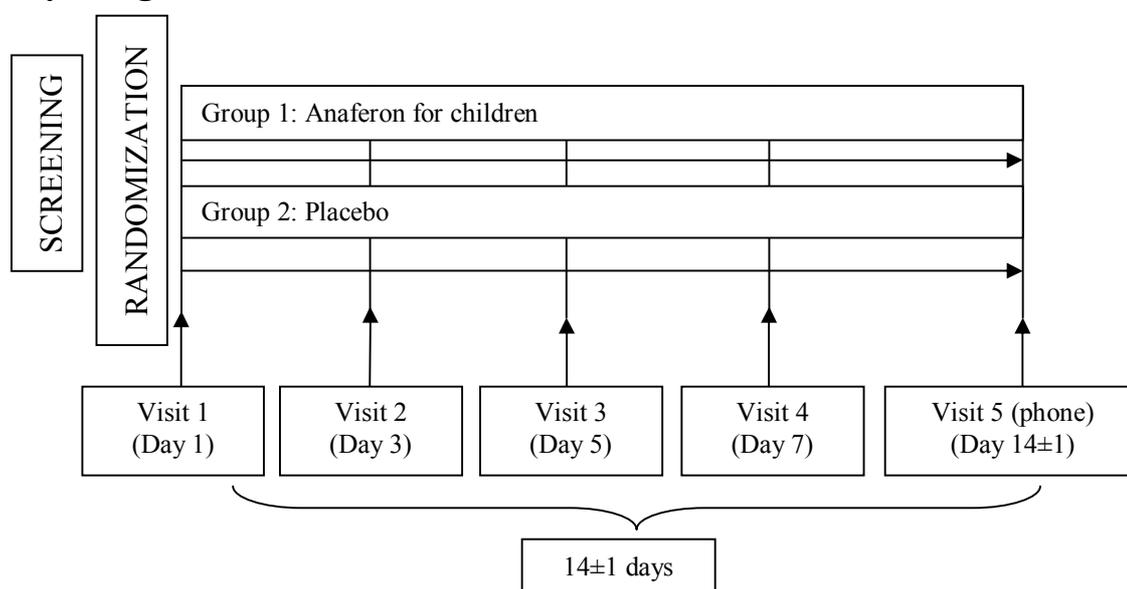
Prohibited concomitant therapy

One month prior to enrollment as well as throughout the study (from signing patient information sheet (informed consent form) and screening initiation) the following products are not allowed (ATC group is specified in brackets):

1. Antiviral products (J05) except for Anaferon for children prescribed within the study.
2. Immune sera and immunoglobulins (J06).
3. Vaccines (J07).
4. Antitumour products (L01) and antitumour hormonal products (L02).
5. Immunostimulants (L03) including:
 - Colony-stimulating factors (L03AA) – filgrastim, molgramostim, lenograstim, pegfilgrastim.
 - Interferons (L03AB) – interferon-alpha, interferon-gamma, interferon alpha-2a, interferon alpha-2b, interferon alpha-n1, interferon beta-1a, interferon beta-1b, peginterferon alpha-2b, peginterferon alpha-2a.
 - Interleukins (L03AC) – aldesleukin.
 - Other immunostimulants (L03AX) – BCG-vaccine; pidotimod; glatiramer acetate.
 - Interferon inducers (including acridonacetic acid, meglumine acridonacetate, polyadenyl and polyuridyl acid complex, methylphenylthiomethyl-dimethylaninomethyl-hydroxybromindole carboxylic acid ethyl ether, oxodihydroacridinylacetate sodium, cagocel, tilorone).
 - Products containing thymus hormones (alpha- thymosin, alpha-glutamyl- tryptophane, thymus extract, thymoptin).
 - B-activin.
 - Synthetic immunostimulants (including levamisole, alpha-glutamyl- tryptophane).
 - Bacterial immunomodulators (including ribomuyil, sodium ribonucleate, deoxyribonucleate sodium, IRS-19, imudon, etc.).
6. Immunodepressants (L04).
7. Products for which allergic reactions have been recorded previously.
8. Products forbidden as antipyretic products:
 - Butylpyrazolidones (M01AA) including phenylbutazone.

- Acetic acid (M01AB) derivatives including indomethacin, diclofenac, ketorolac, aceclofenac.
- Oxicams (M01AC) including piroxicams, meloxicam, tenoxicam, lornoxicam.
- Propionic acid (M01AE) derivatives.
- Coxibs (M01AH).
- Niflumic acid (M01AX02).
- Acetylsalicylic acid (N02BA01) including in combination with other products.
- Pyrazolones (N02BB) including combinations with other products (except for metamizole sodium – on prescription).

Study design scheme



Schedule of study procedures

Procedure/Visit	Visit 1 (Day 1)	Visit 2 (Day 3)	Visit 3 (Day 5)	Visit 4 (Day 7)	Visit 5 (Day 14±1)
Obtaining signed informed consent form (ICF)	+				
Collection of complaints	+	+	+	+	
Collection of medical history	+				
Objective examination	+	+	+	+	
Recording influenza/ARVI symptoms	+	+	+	+	
Inclusion/exclusion criteria assessment	+				
Recording concomitant therapy	+	+	+	+	
Rapid test for influenza viruses in nasal swabs	+				
Obtaining nasal and oropharyngeal	+	+	+	+	

swabs for PCR diagnosis of influenza (in case of positive result of rapid test for influenza)					
Obtaining nasal and oropharyngeal swabs for PCR diagnosis of other respiratory viruses (in case of negative result of rapid test for influenza)	+				
Randomization and prescription of the study therapy	+				
Issue of the study product	+				
Issue of antipyretic product	+				
Accounting and return of the study product, determination of compliance				+	
Issue of patient's diary	+				
Revision of correctness of diary filling		+	+	+	
Diary return				+	
Therapy safety assessment		+	+	+	+
Phone contact					+

Statistical Analyses

Samples

Statistical analysis will be carried out in the following sets:

Total set – all enrolled subjects whose parents/adoptive parents had signed ICF. This set will take into account all adverse events and related conditions during the study including prior to the study therapy initiation.

Total set of the subjects receiving at least one dose of the study product will be used for safety and tolerability analysis (Safety population), as all adverse events recorded in the subject after administration of the product will be taken into account.

Full Analysis Set. This set includes all enrolled subjects excluding those having at least one of the following events:

- 1) non-compliance with inclusion/exclusion criteria;
- 2) the subject did not receive a single dose of the study product;
- 3) lack of any data on the subject after prescription of the study product

This set most fully conforming to Intention-to-treat principle will be used for Intention-to-treat analysis (ITT-analysis) of the study product efficacy.

Per Protocol set. This set includes all subjects receiving per protocol therapy in full, completing all scheduled visits and having no major deviations from the protocol. This set will be used for Per Protocol analysis (PP-analysis) of the study therapy efficacy.

Last Observation Carried Forward (LOCF) will be used for filling missing values.

Evaluation of sample size

Evaluation of sample size was based on the following rules and assumptions:

1. Statistical provisions.
 - 1.1 Power of statistical criteria " $P = (1 - \beta)$ " is considered to be equal to 80% (probability of correct rejection of null hypothesis is 0.8).
 - 1.2 Probability of error of the first kind " α " is admitted to be $< 5\%$ (probability of erroneous acceptance of alternative hypothesis is < 0.05).
 - 1.3 The statistical criteria used are two-sided.
 - 1.4 Calculation of total sample size was based on the assumptions on expected effects on primary endpoint claimed in the protocol.
 - 1.5 Effect of interim statistical analyses on probability of error of the first kind is regulated at each point (interim analysis, final analysis) using O'Brien-Fleming boundary.

2. Assumptions on expected clinical study effects.

It is assumed that the difference between average disease duration (until recovery or significant improvement) in Anaferon for children and Placebo groups will be less than 0.5 days, while standard deviation in both groups will not exceed 2 days.

Taking into account that statistical analysis will be performed twice – at interim analysis and at final analysis – based on the assumed effect the minimum required size of each group (product and placebo) will be as follows:

Number of subjects	Interim analysis	Final analysis
Number of subjects in both groups	254	508
including		
- number of subjects in Anaferon group	127	254
- number of subjects in Placebo group	127	254

Given withdrawal at screening, including due to negative rapid influenza test (the ratio is 1 subject with positive test/5-6 subjects with influenza-like symptoms and negative influenza test) and during the study, the final sample size will be 972 subjects to be enrolled.

This calculation is made using the formula: $N_T = N_{PP} / (1 - C_w)$, where N_T – final sample size; N_{PP} – scheduled number of subjects completing the study per protocol ($N_{PP}=508$); C_w – withdrawal coefficient ($C_w=0.4774$).

Interim analysis

Interim analysis of *Particular Per Protocol (pPP)* is scheduled within the study.

Purposes of interim analysis:

- Ability to adjust sample size in case in minor deviation of the effect achieved from the effect specified at baseline;
- Ability of early discontinuation of the study in case of major deviation⁷ of the effect achieved from the effect specified at baseline.

Within the protocol one interim analysis point has been scheduled.

Interim analysis is scheduled for the time when at least 127 subjects in each group complete the study, i.e. receive per protocol therapy in full, undergo all scheduled visits and have no major protocol deviations.

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:

⁷ Early study termination is possible both on safety reasons in case of lack of efficacy and after the required power of statistical efficacy conclusions is achieved.

- 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;
 - interim analysis procedures.

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

The following *parametric methods and approaches* are suggested:

1. To evaluate various continuous variables obtained in two different (independent) groups – Student’s test for independent samples.
2. To evaluate differences in continuous variables obtained in one group at two different visits – Student’s test for paired samples.
3. For multiple assessment at different visits – Bonferroni’s test (Student’s test modification for multiple comparisons).
4. For comparison of several sample means with baseline (control) – Dunnett’s test (Student’s test modification for multiple comparisons with control).
5. For evaluation of time changes in parameters under comparison – analysis of variance (ANOVA, MANOVA) in modification with repeated measures.

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’s test with all modifications;
- GLM, GENMOD, GLIMMIX – analysis of variance (ANOVA/ANCOVA, MANOVA/MANCOVA).

Nonparametric criteria:

1. For evaluation of differences in continuous variables obtained in two various (independent) groups – Mann-Whitney test;
2. For evaluation of time changes in parameters under comparison – one-way non-parametric analysis of variance (ANOVA) in modification with repeated measures;
3. 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);

4. For frequency analysis of contingency tables with multiple comparisons – Cochran-Mantel-Haenszel test (modification of χ^2 test for multiple comparisons).

Non-parametric analysis will be made using SAS procedures:

- FREQ – χ^2 test and/or exact Fisher's test; Cochran-Mantel-Haenszel test test;
- NPAR1WAY – Mann-Whitney test.

5. Terms for early termination of the study will be established by O'Brien-Fleming boundary.

Interim analysis procedures:

Interim analysis will be made using SAS procedure SEQTEST.

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-9.3 software will be used for statistical analysis including various parametric and nonparametric tests.