STUDY PROTOCOL **Effect of Vaxoral® (OM-85) on frequency of upper respiratory tract infections and size of adenoid tissue in preschool children with adenoid hypertrophy**	
Study Product(s)	Vaxoral® (OM-85)
Indication	Recurrent RTIs, adenoid hypertrophy
Sponsor	Dr Sami Ulus Maternity and Children Research and Training Hospital, Department of Pediatric Allergy and Immunology, Ankara, TURKEY
Anticipated Countries	Turkey
Introduction	OM-85 significantly reduces RTIs in children. This effect was proved by many clinical studies and meta-analyses[1]. A Cochrane meta-analysis first published in 2006 and updated recently (Del-Rio-Navarro 2012) showed that immunostimulants (IS) could reduce acute RTIs (ARTIs) by almost 39% when compared to placebo. Among the different IS, OM-85 showed the most robust evidence with 4 trials of "A quality" according to the Cochrane grading criteria. Pooling six OM-85 studies, the Cochrane review reported a mean number of ARTIs reduction by -1.20 [95% CI: -1.75, -0.66] and a percentage difference in ARTIs by -35.9% [95% CI: -49.46, -22.35] compared to placebo[1]. Adenoid hypertrophy (AH) is one of the most important respiratory disease in preschool children[2]. In normal conditions adenoid tissue enlarges up to 5 years and become smaller afterwards. But in some children who have recurrent URTIs, it keeps growing and this can be associated with complications[2]. AH may cause recurrent respiratory infections and each infection contributes to enlargement of adenoid tissue thus promoting a vicious cycle. Additionally enlarged adenoids are known to be reservoir for microbes and cause of recurrent or long lasting RTIs[3]. AH is associated with chronic cough, recurrent and chronic

	sinusitis, recurrent tonsillitis, recurrent otitis media with effusion, recurrent other respiratory problems such as, nasal obstruction and sleep disturbances, sleep apneas[2-3]. Eventually, AH causes loss of appetite and growth delay; it is often associated with misuse or over use of antibiotics and often eventually requires surgery. It decreases quality of life both in children and parents and it represents a burden not only for families but also for HCS and society due to increased health cost[4]. In one study which investigated the structural and immunological aspects of tonsils and adenoids of 105 children (54 males and 51 females, aged between 4 and 18 years) who were affected by chronic inflammatory hypertrophy of palatine tonsils and adenoids which had not responded to previous medical treatments and who underwent adenotonsillectomy because of recurrent inflammatory episodes with fever, it was demonstrated that deficit in the activation of the immune system could be represented by the small quantity of mRNAs for interleukin-2 (IL-2) and interleukin-4 (IL-4) detected in our population, suggesting a defective activation of Th1 and Th2 lymphocytes[5]. Clinical research question: Can OM-85 reduce the recurrence of infections in children with AH by stimulating the immunological response of the host and therefore, as a consequence reduce the size of adenoid tissue in children with adenoid hypertrophy? Can this prevent further complications as surgery need? **References:** 1. Del-Rio-Navarro BE et al. Immunostimulants for preventing respiratory tract infection in children (Review) Evid.-Based Child Health (A Cochrane Review Journal).2012;7(2): 629–717. 2. Zautner AE. Adenotonsillar disease. Recent Pat Inflamm Alergy Drug Discov. 2012 May;6 (2):121-9. 3. Battistini A, Siepe F, Marvasi R. The tonsils and adenoids as a site of infection and the cause of obstruction. Pediatr Med Chir. 1998 Jul-Aug; 20(4):237-47.

	4. Ericsson E, Lundeborg I, Hultcrantz E. Child behavior and quality of life before and after tonsillotomy versus tonsillectomy. Int J Pediatr Otorhinolaryngol. 2009 Sep;73 (9):1254-62. 5. Passali D, et al. Structural and ımmunological characteristics of chronically ınflamed adenotonsillar tissue in childhood. Clinical and Diagnostic Laboratory Immunology 2004; 11 (6): 1154–1157
Rationale	OM-85 is an oral bacterial lysate of 21 different strains of 8 species and sub-species of the most common respiratory tract pathogens that has been administered in millions of children above 1 year of age. OM-85 stimulates the innate immune system by improving alveolar macrophage activity in the BAL fluid of patients with airway disease, increasing dendritic cells activation and increasing antibodies in the lung of patients with airway diseases. OM-85 stimulates Th1 immunity and inhibits Th2 immunity[1]. Its clinical efficacy in reducing the rate of RRTIs in children is supported by several well-designed and conducted clinical studies and it has showed a good benefit risk profile, unchanged in nature and frequency over more than 30 years of clinical paediatric use. A recent meta-analysis by Schaad et al. 2010[2] of 8 double-blind placebo-controlled trials conducted in children aged 1-12 years affected by recurrent infective episodes (≥ 3 RTIs in 6 months) has shown that the administration of OM-85 significantly reduced the mean number of RTIs by 35% vs placebo and that a lower proportion of treated patients (26.2%) experienced RRTIs. This effect was more evident in patients with the highest number of infectious in the year prior to study entry[2]. The majority of studies used a children posology of 1 daily dose (3.5mg) for 10 days repeated for 3 consecutive months followed by 3 months or 9 months follow-up. In one study 2 cycles (10 days/3 months) at a 6-months intervals were used. An effective and well tolerated prevention of RRTIs with OM-85 could result not only in a positive effect on children's quality of life

	but also in economic benefit for patients' families and societies as recently reported in a pharmacoeconomic study by Zaniolo et al. 2013[3] and Ravasio et al. 2015[4]. Because OM-85 significantly reduces RTIs in children, it is expected to be able to reduce the adenoid tissue size as a consequence of fewer inflammatory RTIs. **References:** 1. De Benedetto F, at al. Prevention of respiratory tract infections with bacterial lysate OM-85 Broncho-munal in children and adults: a state of the art. Multidiscip Respir Med. 2013 May 22;8(1):33 2. Schaad UB. OM-85 BV, an immunostimulant in pediatric recurrent respiratory tract infections: a systematic review. World J Pediatr 2010; 6(1): 5-12. 3. Zaniolo O et al. Costo/efficacia della prevenzione di infezioni alle prime vie aeree mediante un estratto batterico immunostimolante aspecifico (OM-85). Farmaeconomia e percorsi terapeutici 2013; 3(6):169-184 4. Ravasio R. Economic analysis of the immunostimulant OM-85 for the prevention of paediatric recurrent upper respiratory tract infections. Global & Regional Health Technology Assessment 2015; 0(0): 00-00. DOI: 10.5301/GRHTA.5000200.
Objectives	**Primary Objective:** • Reduction of number of RTIs within 12 months **Secondary Objective(s):** • Reduction in duration of RTIs • Reduction of antibiotic use • Reduction of absenteeism (missed school days or missed days of work for parents) • Adenoid and tonsil health survey

	(described in appendix 1) • Reduction of the size of adenoid tissue (described in appendix 3) • Reduction of surgery need
Design, incl. Treatment	This is a randomised, double-blind, placebo-controlled, parallel group, phase IV study. First group will receive OM-85 (10-10-10 standard treatment regimen) Second group will receive matching placebo at the same posology (10-10-10). A second cure of treatment will be given 6 months after inclusion. Patients will be recruited from 01 September 2017 to 01 March 2018. The trial will begin in September 2017 and will be completed in March 2019. By this way every patient will be studied over all seasons (1 year study).
Inclusion Criteria	• Children (age: 2-6 years) • Who experienced recurrent RTIs (at least 3 episodes in 6 months before the inclusion) • Who have symptoms of AH (snoring; mouth breathing awake; mouth breathing asleep; nasal congestion; hyponasal voice; chronic nasal discharge; daytime drowsiness, or hyperactivity; restless sleep; sleep apnoea <15 sec; night cough; and poor oral intake/weight loss) based on the symptoms score questionnaire (described in appendix 1).
Exclusion Criteria	• Atopy • Gastroesophageal reflux • Immune deficiency • Asthma or allergic rhinitis • Premature delivery • Anatomic alterations of the respiratory tract; chronic respiratory

	diseases (tuberculosis and cystic fibrosis); autoimmune disease; liver • Kidney failure; malnutrition; cancer • Treatment with inhaled or systemic corticosteroids within the previous month • Treatment with immunosuppressants, immunostimulants, gamma globulins, or anticonvulsive drugs within the previous 6 months.
Endpoints	**Primary Endpoint:** Cumulative number of RTIs over the 12 months. **Secondary Endpoints:** • Duration in days of RTIs • Antibiotic use (n. of cycles and days) • Missed school/work days • Adenoid and tonsil survey (described in appendix 1 of the original protocol) • Size of adenoid tissue over the 12 months according to radiographic and flexible nasopharyngoscopic evaluation (described in appendix 3 of original protocol). • Surgery need • AEs
Procedures	Patients will be recruited from 01 September 2017 to 01 March 2018. The trial will begin in 01 September 2017 and will be completed 01 March 2019. Children with the ages of 2-6 years who have recurrent RTIs and AH will be evaluated for the eligibility for the study. When eligible for the study, children's all demographic characteristics will be recorded (Table 1). Eligible children will be evaluated by ear-nose-throat (ENT) specialist and flexible laryngoscopy and radiographic evaluation of adenoid size will be performed in order to measure the adenoid size. All children will be evaluated by an allergy specialist. Complete blood count, serum IgG, IgA, IgE, IgM will be measured.

	Skin prick test will be performed with common allergens. All children will be randomized into two study arms (active treatment and placebo). OM-85 3.5 mg capsule or placebo will be given according to the standard treatment schedule (10-10-10) for the three consecutive months. All patients will receive 2 cures of treatment at the beginning and 6 Months after inclusion in the study. Every patients will be followed-up for 12 months. Patients (and care giver/parents) will be given a symptom score form (Table 2) in order to record all nasal symptoms, signs and duration of RTIs, antibiotic use, days of absenteeism from school and from work for family members, monthly. All patients will be asked to come to the clinic when they have symptoms of RTI in order to diagnose the type of RTIs. Then they will be asked to complete the infection form during the acute RTIs (Table 3). Every 3 months they will fill in the Tonsil and Adenoid Health Status Survey (Table 4). Every 3 months patients will be asked to come to the clinic and all their records will be collected and they will be evaluated by the ENT specialist for the adenoid tissue size. All these parameters will be recorded in specific forms by the investigator (Table 5). Patients will be allowed to use nasal corticosteroids, antihistaminic drugs and flu vaccine during the study period and all these study medications will be recorded. At the end of the study (12 months), immunoglobulin levels will be measured to compare them with basal level.

Sample Size	A total of 68 patients will be randomized in the study in a 1:1 ratio to Active or Placebo groups. **Active group:** 34 patients **Placebo group:** 34 patients
N^{o} Sites	**Number of study centers: 1** **Study center:** Dr Sami Ulus Maternity and Children Research and Training Hospital, Department of Pediatric Allergy and Immunology, Ankara, TURKEY. **Study Coordinator and Principal Investigator:** **Serap Ozmen** M.D. Associate Prof. of Pediatrics and Pediatric Allergy, Training staff on Pediatric Allergy **Other Investigators:** **Ilknur Bostanci** M.D. Professor of Pediatrics & Subspecialist on Pediatric Allergy and Pediatric Pulmonology Chief of Pediatric Allergy and Immunology Department **Vedat Tas** M.D. Otolaryngologist **Emin Cakmakci** M.D. Radiologist
Statistical Methods	**Statistical method:** All analyses will performed with a commercially available software program (SPSS Statistical Software, version 11.5; SPSS, Inc, Chicago,Ill). The Shapiro-Wilks test will use to evaluate normality of the distributions collected. When variables were normally distributed, they were expressed as means (SDs); otherwise, they will express as medians and interquartile ranges (25th-75th percentiles). The χ^2 test will use for categorical variables and expressed as observation counts (in percentages). An ANOVA (or Mann-Whitney U depending on the normality of data) statistics will be used to compare means between both treatment groups for continuous variables. Bonferroni multiple comparison test will use to compared paired intervals (0-3, 0-6 months). All P values are 2-tailed; a P

	value of less than .05 are considered statistically significant. **Sample Size:** In one study OM-85 group showed 3 less RTIs compared with placebo over 12 months period. A sample size of 54 randomized patients (27 per group) will provide at least 80% power to detect a mean difference of at least 2.0 RTIs between the two groups assuming a SD of 2.55 and assuming a drop-rate of 20% after 6 months, total of 68 patients (34 per group) will recruit to the study.[1] **Reference:** 1. María Dolores Gutiérrez-Tarango and Arturo Berber. Safety and Efficacy of Two Courses of OM-85 BV in the Prevention of Respiratory Tract Infections in Children During 12 Months. CHEST 2001; 119:1742–1748
Planned Timelines	Planned date first patient consented/enrolled/observed: 01 September 2017 Planned date last patient consented/ enrolled/ observed: 01 March 2018 Planned date last patient last observation/ treatment: 01 March 2019 Planned date CSR (Clinical study report) available: June 2019

1. Visit	2. Visit	3. Visit	4. Visit	5. Visit	6. Visit
Randomitazion of the patients	After randomization treatment plan (OM-85 capsules or placebo given in the first 10 days of each month for 3 months)	Control at the end of 3rd month of treatment (20 days after the last medicine/drug or placebo)	Control after 3 months of last drug or placebo intake and Given the product by the previous randomization again for 3 months	At the end of the second 3-month treatment (20 days after the last drug)	Control after 3 months of the last drug or placebo intake (20 days after the last medicine/drug or placebo)
• Patients will be assessed for type of respiratory infection and additional treatment by researchers (pediatric allergy specialists and/or ENT specialists) in the Pediatric Allergy and Immunology outpatient clinic when symptoms of respiratory tract infection is present.					

- Parents will be asked to fill in the relevant form [Symptom Score Form] during this acute respiratory tract infection.
- Every 3 months, parents will be filled.QoLQ "Tonsil and Adenoid Health Status Survey".

Appendix 1. Study Forms

Table 1. Demographic charecteristics

PATİENT FOLLOW-UP FORM	
Date	
Patient Number	
Treatment group (Randomisation code)	
Name Surname	
Birth date	
Gender	
Smoking at home	
Family history of asthma	
Family history of atopy	
DPT	
IgG	
IgA	
IgE	
IgM	
FİRST VİSİT	
RTIs in previous 12 months	
RTIs in previous 6 months	
Number of hospitalisation in previous 12 months	
Total days of hospitalisation in previous 12 months	
Total days of absenteisim from school	
Total days of absenteisim from work for mother	
Total days of absenteisim from work for father	
Antibiotic use in previous 12 months	
• **Number of box**	
• **Total number of days**	
Symptome score	

QoLQ (Score)	
Adenoid size	
• **Radiological** (AC/SP ratio)	
• **Nasopharingoscope** (perceived percent obstruction of the choana)	

Table 2. Symptomes score (will be completed for each month)

Symptomes	none (0 point)	rarely (1 point)	frequently (2 points),	constantly (3 points)
Snoring				
mouth breathing awake				
mouth breathing asleep				
nasal congestion				
hyponasal voice				
chronic nasal discharge				
daytime drowsiness, or hyperactivitiy				
restless sleep				
sleep apnea <15 sec				
night cough				
poor oral intake/weight loss				

Scores from eleven symptoms were added together to provide a total symptom score of 33.

1- Sclafani AP, Ginsburg J, Shah MK, Dolitsky JN. Treatment of symptomatic chronic adenotonsillar hypertrophy with amoxicillin/ clavulanate potassium: short and long-term results. Pediatrics 1998; 101: 675-81.
2- Demain JG, Goetz DW. Pediatric adenoidal hypertrophy and nasal airway obstruction: reduction with aqueous nasal beclamethasone. Pediatrics 1995; 95:355-64.

Table 3. Infection form (will be completed for each infection)

Type of RTI	
Duration of RTI	
Antibiotic consumption (days)	
Days of absenteism from school	
Days of absenteism from work for family members	

Table 4. Tonsil and Adenoid Health Status Survey (It will be completed by the parents in every 3 months)

TONSIL AND ADENOID SURVEY

We are conducting a study on children with tonsil or adenoid problems, and we are interested in the type of health problems that your child is having related to his (her) tonsils and adenoids. There are no correct or incorrect answers, and your opinion is most important to us. The survey is completely voluntary and will take about 3 minutes to complete.

Please answer these questions remembering the **past 6 months** for your child. The questions relate to the problems many children have related to their tonsils and adenoids. If a certain question is not a problem for your child, please circle "0" (not a problem) for that question. Please do not skip questions or leave any questions unanswered.

Over the past 6 months, how much of a problem were the following conditions for your child?

Please circle the most correct response

	Not a problem	*very mild problem*	*moderate problem*	*fairly bad problem*	*severe problem*	
1. Snoring loudly during sleep	0	1	2	3	4	1
2. Strep throat infections	0	1	2	3	4	2
3. Many trips to a doctor's office	0	1	2	3	4	3
4. Taking antibiotics for more than 3 weeks straight	0	1	2	3	4	4
5. Taking antibiotics over and over for less than 2 weeks at a time	0	1	2	3	4	5
6. Many calls to a doctor's office	0	1	2	3	4	6
7. Irregular or stopped breathing, also known as apnea, during sleep	0	1	2	3	4	7
8. Repeated short-term (or acute) infections of the tonsils that last less than 2 weeks	0	1	2	3	4	8
9. Constant, or chronic, infection of the tonsils that last more than 2 weeks	0	1	2	3	4	9
10. The cost of medical care and prescriptions	0	1	2	3	4	10
11. Breathing through the mouth during the day	0	1	2	3	4	11
12. Your child not growing or gaining weight as expected	0	1	2	3	4	12
13. Noisy breathing during the day	0	1	2	3	4	13
14. Problems with poor appetite or poor eating habits	0	1	2	3	4	14
15. Behavior problems at home or at school, or poor school grades or reports	0	1	2	3	4	15

Table 5. Follow-up form

Visit (3-6-9-12 months)	**Date:**					
Number of RTIs in previous 3 months:						
• Type	**AOM**	**AS**	**AT**	**AN**	**AC**	**P**

• Number						
• Duration (days)						
Antibiotic use in previous 3 months						
• Type						
• Number (box)						
• Duration (days)						
Hospitalisation:						
• Number						
• Duration (days)						
Symptome score						
QoLQ (Score)						
Adenoid size						
• **Radiological (AC/SP ratio)**						
• **Nasopharingoscope (**perceived percent obstruction of the choana**)**						

Appendix 2. Definition of infections:

Acute nasopharengitis (Common cold); was defined when nasal stuffiness or drainage, sore or scratchy throat, sneezing, hoarseness, cough, with or without fever and headache were present which resolved in 7-10 days.

Simasek M, Blandino DA. Treatment of the common cold. Am Fam Physician 2007;75: 515-20, 522.

Sinusitis; was defined when persistent symptoms of URTI (rhinoviral illness) were present without improvement after 10 days or a worsening of symptoms after 5 to 7 days with purulent nasal discharge, nasal congestion, postnasal drip, cough, and continued unwell state with or without fever, irritability, lethargy and facial pain

American Academy of Pediatrics Subcommittee on Management of Sinusitis and Committee on Quality Improvement. Clinical practice guideline: Management of sinusitis. Pediatrics 2001;108:798.

Acute otitis; was defined when acute onset of earache with erythema and limited mobility of the tympanic membrane determined by pneumatic otoscopy was present.

Tonsillitis; was defined when inflammation of one or both tonsils associated with a catarrhal exudate over the tonsil or the discharge of caseous or suppurative material from the tonsillar crypts was present

Viral croup; was defined when hoarseness, stridor and the characteristic “croupy” or barking cough developed after a viral prodrom by 12 to 72 hours of low-grade fever and coryza and resolved within one week.

Knutson D, Aring A. Viral Croup. Am Fam Physician 2004;69:535-40,541-2.

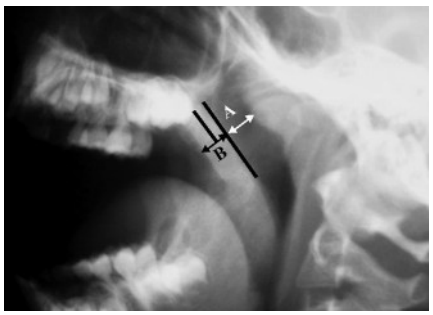
Pneumonia; was defined when fever, cyanosis, and more than one of the following signs of respiratory distress: tachypnea, cough, nasal flaring, retractions, rales, and decreased breath sounds were present with abnormal chest X-ray. (5,12,13). Pneumonia was diagnosed when tachypnea occured in a patient younger than two years with a temperature higher than 38°C temperature. Children without fever or symptoms of respiratory distress didn’t have the diagnosis of pneumonia.

Ostapchuk M, Roberts Dm, Haddy R. Community-Acquired Pneumonia in Infants and Children. Am Fam Physician 2004;70: 899-908.

Respiratory infections were also considered as mild or severe according to validated published criteria. Mild ARTIs involved the acute nasopharyngitis (common cold), tonsillitis or viral croup. Severe ARTIs involved the sinusitis, acute otitis or pneumoniae. Antibiotics were prescribed in the case of tonsillitis, otitis, sinusitis or lower ARTI.

Bardin PG, Fraenkel DJ, Sanderson G, Dorward M, Lau LCK, Johnston SL et al. Amplified rhinovirus colds in atopic subjects. Clin Exp Allergy 1994;24: 457-464.

Appendix 3. Radiographic evaluation of adenoid size:



Radiographic evaluation of adenoid size by the method of Cohen and Konak (1). Thickness (mm) of “Airway column” (A) and “Soft palate” (B) is measured and A/B ratio is calculated in order to evaluate nasopharyngial airway patency.

Lateral neck radiographs will taken and interpreted by the method of Cohen and Konak by a blinded radiologis (1). According to this method, the thickness of the soft palate (SP) in its superior anterior part and the airway column (AC) immediately posterior to it will measured and AC/SP ratio was calculated. The measurement will done about 1 cm below the upper end of the soft palate in children > 3 years of age and half a centimetre in younger children. A radiographic example of this method is demonstrated in Figure 1.

Degree of obstruction was graded as follows:

AC/SP ≥1 (grade 0 or no obstruction),

AC/SP= 0.50-0.99 (grade 1 or mild obstruction),
AC/SP= 0.01-0.49 (grade 2 or severe obstruction),
AC/SP= 0 (grade 3 or total obstruction).

Cohen D, Konak S. The evaluation of radiographs of the nasopharynx. Clin Otolarngol 1985; 10: 73-8.

Flexible nasopharyngoscope:

Topical anesthesia will use in the form of a mixture of lidocaine/ neosynephrine spray that will applied by pressurized nozzle prior to endoscopic examination. The patient will be seated upright in the examination chair without restraint during the exam. A standard 4.0 mm flexible nasopharyngoscope with attached fiberoptic light will be used to perform the nasopharyngoscopy. The data will be recorded as a perceived percent obstruction of the choana by the adenoid pad, as seen through the endoscope.

Lertsburapa K, SchroederJW, Sullivan c. Assessment of adenoid size: A comparison of lateral radiographic measurements, radiologist assessment, and nasal endoscopyInternational Journal of Pediatric Otorhinolaryngology 74 (2010) 1281–1285