PROTEA study: a protocol for a randomised controlled trial evaluating the efficacy, immune effects and cost-effectiveness of oral bacterial lysate therapy to protect moderate–late preterm infants from respiratory tract infections and wheezing

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Shareable abstract (@ERSpublications)
We describe the study protocol of an RCT that evaluates the efficacy, immune effects and cost-effectiveness of oral bacterial lysate (OM-85) therapy to protect moderate–late preterm born infants from respiratory tract infections and wheezing. https://bit.ly/4jL8HuN

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Abstract

Infants, children and adults born moderate–late preterm (after 30–36 weeks of pregnancy) are at increased risk of respiratory infections, wheezing and lower lung function leading to increased medication use and hospitalisation. Risk factors frequently present in this population are, at least in part, associated with (lack of) exposure to microbes and subsequent perturbations in microbiome and immune system development. This manuscript presents the protocol of the double-blinded randomised placebo-controlled PROTEA trial, which will demonstrate whether treatment with immunomodulatory bacterial lysates (OM-85) can reduce lower respiratory tract infections and wheeze in the first year of life. The follow-up PROTEA-2 trial will identify possible carry-over effects of OM-85 treatment and investigate the clinical effect of continued treatment in the second year of life. Infants included are otherwise healthy infants born after 30–36 weeks of gestation, excluding those small for gestational age (<3rd percentile). They are recruited shortly after birth in 22 medical centres in the Netherlands. Participants will take OM-85 or placebo starting from 6–10 weeks of life till age 1 year (PROTEA study) or 2 years (PROTEA-2 study) and are closely monitored regarding respiratory health through e-applications. Biological samples, lung function measurements and detailed information on covariates will be collected at ages 2, 6, 12 and 24 months. Biological samples will aid in estimating the impact of bacterial lysate administration on immune cell composition, activation and maturation, vaccination responses, and microbiome diversity and maturation. Participant recruitment started in March 2022.

Introduction

~10% of all live births worldwide occur preterm, of which by far the largest part is attributed to moderate–late preterm birth [1]. Definitions used for moderate–late preterm birth slightly vary, but cover births from 30–32 weeks of gestation to 36–37 weeks of gestation. Moderate–late preterm born infants more often experience upper and lower respiratory tract infections (RTIs) and preschool wheeze compared to term born infants [2–4]. In the first year of life, the incidence of bronchiolitis is almost 50%, compared to 11–15% in term born infants [5, 6]. Consequently, moderate–late preterm born infants receive antibiotics,

bronchodilators and inhalation corticosteroids more commonly [2, 7]; furthermore, respiratory-related hospitalisation is inversely related to gestational age (GA) until the age of 18 years [8]. During hospitalisation moderate–late preterm born infants show a more severe course of disease, experience longer hospital stays, and more often receive invasive treatments such as mechanical ventilation [9]. Later in life, moderate–late preterm birth is a risk factor for asthma and COPD [2, 8, 10].

Spirometry and impulse oscillometry in moderate–late preterm born children show lower forced expiratory volume in 1s (FEV_1) and FEV_1/forced vital capacity and higher adjusted airway resistance, suggesting airway obstruction. Further, lower GA is associated with lower childhood forced expiratory flow at 75% of FVC, suggesting persistent reduction in small-airways patency [11, 12]. However, fractional exhaled nitric oxide levels were similar between term and preterm born children, suggesting an alternative mechanism to eosinophilic inflammation for airway obstruction [13].

Large-scale studies have shown a decrease in respiratory disease risk for every week increase in gestation length up to 40 weeks, highlighting the impact of preterm birth throughout the full range of pregnancy [4, 11]. Moderate–late preterm-born infants are underrepresented in interventional research, since they are often excluded from studies on both the general population and preterm born infants. This results in a large and poorly studied group of infants that harbours a large potential health gain.

Respiratory morbidity in preterm born infants and children is partly being ascribed to interruption of the saccular stage of lung organogenesis, resulting in immaturity at birth [14]. Since this is less pronounced after moderate–late preterm birth, attention is rising for microbiome and immune system immaturity. Some important risk factors for respiratory morbidity are lack of exclusive breastfeeding, antibiotic exposure, birth *via* caesarean section and early viral RTI. All are more frequent in preterm born infants and, at least in part, associated with exposure to microbes and subsequent development of the microbiome and immune system [15–17]. Reinforcement of these systems using bacterial lysates may help to reduce respiratory morbidity in moderate–late preterm born infants.

Bacterial lysates modulate the immune system and reduce respiratory morbidity [18, 19]. In children with recurrent RTIs, bacterial lysates reduce the frequency and duration of RTIs [18, 20, 21]. In children suffering from preschool wheeze or asthma, bacterial lysates reduce the incidence and duration of exacerbations [19, 22]. Bacterial lysates as a primary preventive agent is less well investigated. One randomised controlled trial in 59 infants at risk of asthma showed that OM-85 (oral bacterial lysate) treatment could reduce the incidence of severe lower RTI in early life [23]. Another large trial is currently ongoing to find support for this outcome (NCT02148796). In all studies till now, infants born preterm were excluded, despite the fact that they might benefit the most. The current manuscript will describe the PROTEA trial and (follow-up) PROTEA-2 trial, which address the described knowledge gaps.

Objectives

Our primary objective is to demonstrate whether OM-85 reduces lower RTI and wheezing in the first year of life in moderate–late preterm born infants. The primary objective of the follow-up study PROTEA-2 is to measure any carry-over effect of OM-85, and to investigate the clinical effect of continued treatment in the second year of life.

Secondary objectives are: 1) to investigate the impact of OM-85 on the developing immune system and microbiome: 2) to investigate the relation between efficacy of OM-85 and the immune system and microbiome; 3) to evaluate the effect of OM-85 on airway obstruction, measured by impedance pneumography; 4) to predict infants at risk and treatment success using system biology to reach personalised medicine; and 5) to evaluate cost-effectiveness and the effect on quality of life of OM-85 treatment.

Trial design

PROTEA is a double-blinded randomised (1:1 ratio) placebo-controlled trial comparing OM-85 treatment to placebo in the first year of life. Subsequently, informed consent will be obtained for PROTEA-2 in which participants who received OM-85 in the first year of life will be randomised to another year of OM-85 or placebo (1:1 ratio). Protocol details are described in the manuscript according to the SPIRIT statement; also see table 1 for administrative details.

Methods

Participants

Patients are recruited from 21 secondary care hospitals and one paediatric primary healthcare facility in the Netherlands. Infants are eligible when born at a GA of 30–36 weeks, and they are included 6–10 weeks

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TABLE 1 SPIRIT checklist administrative information: items 1–5d

Title	1	PROTEA study: a protocol for a randomised controlled trial evaluating the efficacy, immune effects and cost-effectiveness of oral bacterial lysate (OM-85) therapy to protect moderate–late preterm infants from respiratory tract infections and wheezing	
Trial registration	2a	EudraCT: 2020-005868-67 Clinical trials.gov: NCT05063149	
	2b	Date of registration in primary registry	7 December 2020
		Secondary identifying numbers	NL76165.100.20
		Source(s) of monetary or material support	Dutch Lung Foundation OMPharma, Switzerland (ISS and in kind) Ventica, Finland (in kind)
		Primary sponsor	Franciscus Gasthuis & Vlietland
		Secondary sponsor(s)	
		Contact for public queries	Dr Gerdien Tramper (g.tramper@franciscus.nl)
		Contact for scientific queries	Dr Gerdien Tramper (g.tramper@franciscus.nl)
		Public title	Protecting late–moderate preterm infants from respiratory tract infections and wheeze in their first year of life by using bacterial lysates (PROTEA)
		Scientific title	Protecting late–moderate preterm infants from respiratory tract infections and wheeze in their first year of life by using bacterial lysates (PROTEA)
		Countries of recruitment	The Netherlands
		Health condition(s) or problem (s) studies	Lower respiratory tract infections and wheezing in late–moderate preterm born infants
		Intervention(s)	Active comparator: OM-85 (Broncho-Vaxom) Placebo comparator: Matching capsules containing no active ingredient, base is corn starch
		Key inclusion and exclusion criteria	Inclusion criteria: Infants aged 6–10 weeks at randomisation born after a pregnancy of 30–36 weeks Exclusion criteria: parents unable to speak Dutch or English; underlying other severe respiratory disease such as broncho-pulmonary dysplasia, haemodynamic significant cardiac disease, immunodeficiency, severe failure to thrive, birth asphyxia with predicted poor neurological outcome and a syndrome or a serious congenital disorder; dysmaturity <P3 and/or weight <2.5 kg at the age of randomisation; maternal immunosuppression during pregnancy and/or breastfeeding which still could affect the child at the moment of randomisation; known allergic hypersensitivity to the active ingredient or any of the excipients of OM-85
		Study type	Double-blinded randomised placebo-controlled phase III medication trial
		Date of first enrolment	January 2022
		Target sample size	500
		Recruitment status	Recruiting
		Primary outcome(s)	Lower respiratory tract infections and wheezing episodes in the first years of life
		Key secondary outcome(s)	All respiratory tract infections (number, time to first and duration), healthcare and medication use, maturation of the immune system, development of the microbiome (gut and upper airways), distribution of viruses and lung function

Continued

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TABLE 1 Continued

Protocol version	3	Original first version accepted by medical ethical committee: Version 3, issue date: 7 April 2021 Protocol amendments: • Version 4, issue date 27 October 2022. Primary reason for amendment: addition of recruiting sites • Version 5, issue date 22 December 2022. Primary reason for amendment: remove exclusion criterion "Lower respiratory tract infection preceding randomisation" to optimise representativeness and generalisability of the study • Version 8 (Version 8 was the first accepted next version after Version 5. Versions 6 and 7 were adapted after feedback of the medical ethical committee). Issue date 2 August 2023. Primary reason for amendment: introduction of digital informed consent procedure and addition of more recruiting sites • Version 9, issue date 14 November 2023. Primary reason for amendment: addition of follow-up study PROTEA-2 Authors (of all versions): Dr G.A. Tramper-Stranders, paediatrician/clinical researcher Franciscus Gasthuis & Vlietland, Rotterdam Prof Dr Hermelijn H. Smits, immunologist, professor at Leiden University Medical Centre Prof Dr John Penders, metagenomic epidemiologist and medical microbiologist, professor at Maastricht University Medical Centre
Funding	4	Primarily supported by the Dutch Lung Foundation. Investigator initiated support from OM Pharma for laboratory measurements and IMP. Ventica lung function measurement devices are provided by Ventica. An educational grant from Franciscus Gasthuis & Vlietland is provided to finance PROTEA-2. The design, management, analysis, interpretation of the data and reporting of the study are entirely independent of the manufacturers of OM-85 and the Ventica devices
Roles and responsibilities	5a	G.A. Tramper-Stranders, H.H. Smits and J. Penders conceived of the study and designed the protocol. L. Duijts joined the study team later to strengthen epidemiological perspectives. G.A. Tramper-Stranders is principal investigator. Researching physician I.C. van Duuren carries out the implementation of the protocol and contributed to the protocol and the amendments. All authors contributed to refinement of the study protocol and approved the final manuscript
	5b	Trial sponsor: "Franciscus Gasthuis & Vlietland" Kleiweg 500 3045 PM Rotterdam Wetenschapsbureau@franciscus.nl 010 461 6161
	5c	The researchers are independent from the funders. Both funders and trial sponsor (named above) have no role in the study design, data analysis, interpretation of data or writing of the report. Trial sponsor is employer of G.A. Tramper-Stranders and I.C. van Duuren
	5d	Consortium members G.A. Tramper-Stranders, H.H. Smits and J. Penders initiated the design of the protocol together. Principal investigator (G.A. Tramper-Stranders) and researching physician (I.C. van Duuren) are responsible for: • Conduct of the PROTEA study • Organising consortium meetings • Keeping trial master file and reporting AE/SAE/SUSAR to the DSMB and the ethical committee• Data collection and sample collection J. Penders and H.H. Smits are responsible for: • Microbiological and immunological analyses on collected samples • Contribute to consortium meetings and protocol amendments L. Duijts is responsible for: • Advice on epidemiological subjects during data collection, analysis and interpretation

after birth. Their parents must speak Dutch or English and provide written informed consent. Infants are excluded from participation in case of underlying other severe respiratory disease such as broncho-pulmonary dysplasia, haemodynamic significant cardiac disease, immunodeficiency, severe failure to thrive, birth asphyxia with predicted poor neurological outcome, and a serious syndrome or congenital disorder. Other exclusion criteria are dysmaturity <P3 and/or weight <2.5 kg at the age of randomisation, maternal immunosuppression during pregnancy and/or breastfeeding which could affect the child at the moment of randomisation, and known allergic hypersensitivity to the active ingredient or any of the excipients of OM-85.

Interventions

The intervention group will receive 3.5 mg of OM-85 and the control group a matching placebo for 10 consecutive days per month from the age of 6–10 weeks to the age of 12 months. Timing of treatment enhancement is aimed at starting within the window of opportunity for immune development and before broad exposure to respiratory pathogens in the home situation [24, 25]. Timing is in line with the routine vaccinations, which start from 6 weeks after birth in the Netherlands. This is a well-accepted age to provide the immune system with stimulating agents. Next, from an ethical viewpoint, we do not want to start treatment during the vulnerable period for necrotising enterocolitis in preterm infants. Parents will retrieve the powder (either OM-85 or placebo) from capsules and mix it with water, breastmilk or formula. The suspension will be administered orally, preferably on an empty stomach. Parents are asked to use a spoon or syringe in order to obtain sublingual exposure, if possible. The use of OM-85 in infants has been proven safe in previous studies; common side-effects are mild gastrointestinal symptoms and rarely a rash occurs. Side-effects are experienced in both OM-85- and placebo-treated groups [20, 23]. The GCP-compliant RespiRecord app (https://respirecord.com) will provide parents with reminders on treatment days. Treatment adherence is tracked *via* self-reporting in the app and *via* IMP capsules return at the end of the study. All regular concomitant care or interventions are allowed during the trial.

Outcomes

Primary outcomes of PROTEA are as follows:

1. Total number of doctor diagnosed lower RTIs, which is defined as a parental report in the digital diary of a doctor's visit which led to the diagnosis of bronchitis, bronchiolitis or pneumonia, and/or a medical record describing a lower RTI. The reported diagnoses are verified with medical records from both hospitals and general practitioners, which are evaluated using the definition as described in table 2.
2. Total number of parental reported wheezing episodes, which is defined as a parental report in the digital diary of the symptom wheezing and/or the use of bronchodilators and/or a doctor's visit resulting in the diagnosis "baby-asthma/RTI accompanied by wheezing". Also this reported diagnosis is verified with medical records.

The primary outcome of PROTEA-2 is time to first lower respiratory episode (combined, either lower RTI or wheezing, defined as described for PROTEA) after 12 months of age. Clinical secondary outcomes in both PROTEA and PROTEA-2 are number of (lower and all) RTIs, time to first RTI, duration of RTI, incidence and severity of respiratory symptoms, healthcare use, medication use, safety, lung function, perceived health, daycare absenteeism and parental quality of life. Other secondary outcomes are the maturation of the immune system, infant vaccination titres at age 1 year, the development of the microbiome of the gut and the upper airways, and the presence and distribution of respiratory viruses.

Follow-up and participant timeline

Enrolment will be at the age of 6–10 weeks; two further visits are scheduled at ages 6 and 12 months including questionnaires, optional biosampling (targeting ∼50% of participants) and optional lung function

TABLE 2 Definition used to guide verification of the diagnosis "lower respiratory tract infection"

1	Clinical signs/symptoms (one or more of the following): coughing, shortness of breath/breathing difficulties, chest sounds, problems with feeding/drinking combined with lower respiratory tract signs AND
2	Physical examination (one or more of the following): tachypnoea (2–6 months >40·min^{-1}; 6–12 months >30·min^{-1}), dyspnoea (inter/subcostal chest retraction, nasal flaring); auscultatory crackles, crepitations, wheezing or partial absence of breathing sounds AND OPTIONAL
3	Additional examinations (not obligatory for the diagnosis): oxygen saturation decreased after nasal saline washing (<95%); abnormal chest radiograph

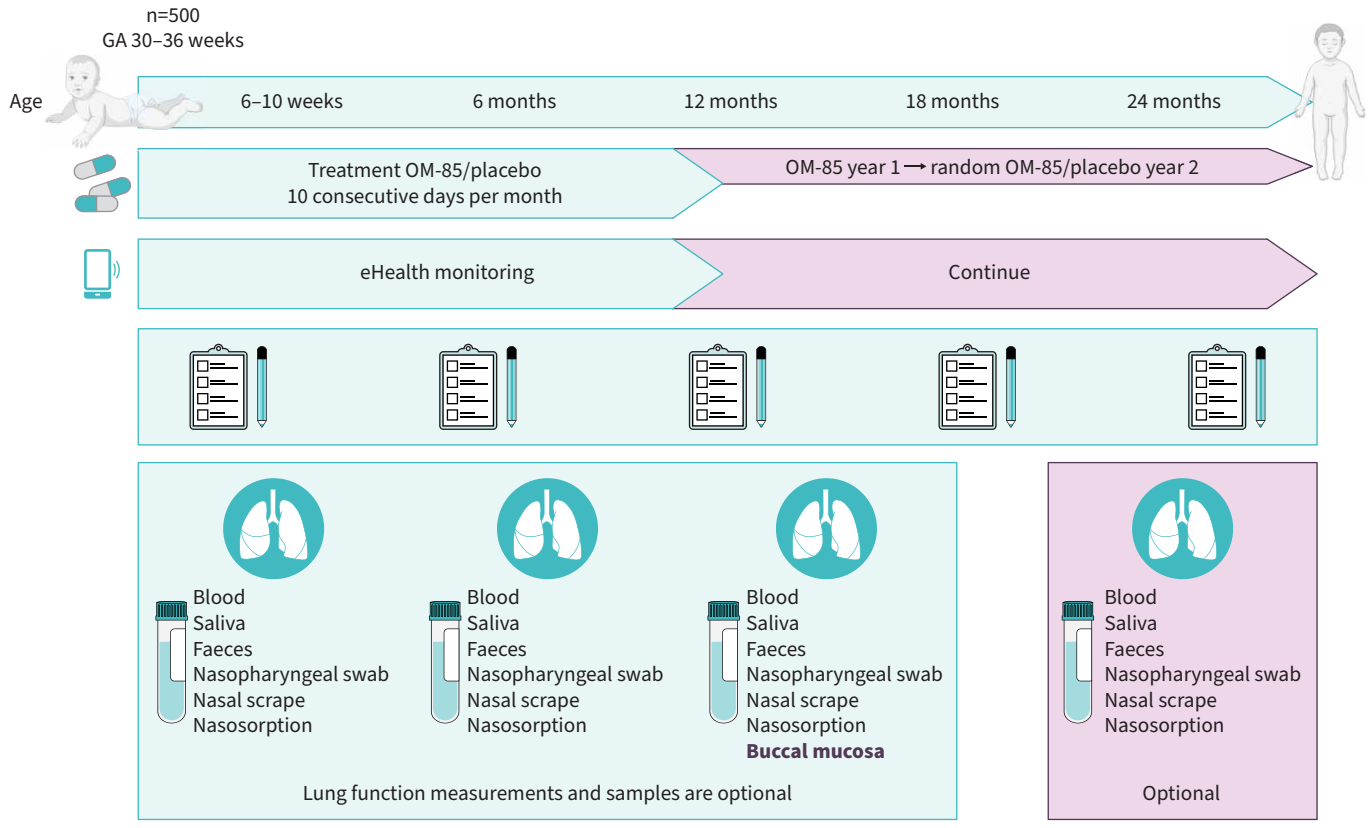

FIGURE 1 Participant timeline PROTEA trial. GA: gestational age.

measurement (targeting ~100 participants). During the trial, eHealth *via* the RespiRecord app will be used to closely monitor all participants. In case parents give consent for participation in PROTEA-2, one additional visit will be planned at age 24 months. For a visual overview, see figure 1.

Sample size

Previous studies showed that about 47% of moderate–late preterm infants experienced one or more lower RTI episodes in the first year of life [5, 26]. Studies in toddlers show a 30–40% reduction in total RTI and/or wheezing episodes after bacterial lysate treatment, compared to placebo. The OMPAC trial, which looked into the primary preventive effect of OM-85 in children at risk of asthma, showed a 26% decrease in infants experiencing severe lower RTI. Based on these data we assume a clinically relevant effect size of at least 25–30%. For a power of 80% using a two-sided alpha of 0.05 we need 227 patients per group to show an effect size of 27% in the primary outcome. To compensate for a possible ~10% putative dropouts before the primary end-point, we target 250 participants per group (total=500).

PROTEA-2 is largely explorative, as we do not know whether there is a carry-over effect of the first year treatment in the second year and how long this effect lasts. The primary end-point in PROTEA-2 is time to first lower respiratory event in the second year of life. Previous studies show that the prevalence of lower respiratory events among children aged 2 years is heterogeneous, but that time to lower respiratory event is 1.65 to 4 times larger in the bacterial lysate treatment group compared to the regular care group [23, 19]. Estimation of the treatment effect in year 2 will be conditional on having been under OM-85 during the first year of life. Therefore, assuming some carry-over effect, we conservatively assume the time to event (lower respiratory episode) to be 1.5 times larger in the 2 years old treatment group compared to 1 year old treatment group. We assume a median time to event in the control group of 100 days and in the treatment group of 150 days. For a power of 80% using a one-sided alpha of 0.025 and taking into account a dropout rate of 10% we need 103 patients per group (total=206).

Biosampling will be performed in 250 participants. This sample size will allow us to study immune cellular and inflammatory mediator analysis in 50–125 patients per arm and look into smaller subgroups based on treatment effect. For studying the effect of immunostimulants on vaccine responses, earlier studies included ∼70–135 children, of which only the largest sample showed significant differences [27, 28]. For microbiome analysis, large numbers are beneficial given the large inter-individual variation and many factors that impact early life microbiome composition. Previous microbiome studies showed that sample sizes ∼200 seem to be sufficient [29, 30]. Altogether, we assume 250 participants is a reasonable amount to reach meaningful analyses and allowing the study group to select specific subgroups for detailed analyses without unnecessary bothering of the infants.

Lung function measurements will be performed in 100 participants. This is largely explorative, since this technique has not earlier been applied to preterm born infants. However, one study comparing tidal breathing flow-volume loops between late preterm and term infants showed interesting results in a group of 100 infants [31].

Recruitment practices

Candidates are screened and shortly informed about the study by the local paediatrician during hospitalisation for prematurity or follow-up visits. Parents receive a short leaflet with links to the website and a video providing an overview of the trial (healthcare-animations powered by informed). After a few days parents report whether they want to be further informed by the central study team or not. The central study team provides complete oral and written information about the study through a phone call and the subject information sheet. Another phone call is scheduled a couple of days later to answer questions after which parents are asked to decide on participation. The central study team signs digital or paper informed consent (depending on parents' preference), prescribes trial medication and carries out study visits. Per site, between 60 and 120 candidates will be born every year. We assume a conservative inclusion rate of ∼10–20%, which might lead to ∼250 participants per year provided that all recruitment sites are active.

Assignment of interventions

Participants will be randomly assigned to either OM-85 or placebo treatment with a 1:1 allocation, as per a computer-generated randomisation schedule using permuted blocks of random sizes. A list of sequential codes was supplied by the sponsor. A box with corresponding code is requested from the pharmacy for each new participant. The treatment allocation corresponding to the code is unknown for everyone involved in conduct of the study and for the participants. A sealed envelope with the treatment allocation per code is present at the pharmacy to be used only: 1) when crucial for a medical emergency; 2) when the participant will continue with PROTEA-2 and the database concerning year 1 is locked for this specific participant; and 3) at trial completion after the last patient visit.

Stratification is applied according to GA, separating moderate preterm born infants (GA at birth 30 to 33 weeks) and late preterm born infants (GA at birth 33 to 36 weeks). In case of twins, both twins will receive the same intervention in order to avoid mixing up of the products.

For PROTEA-2, participants in the active treatment arm will again be randomised over OM-85 and placebo by stratified block randomisation with ratio 1:1 using the same method. Randomisation will be stratified for lower respiratory episodes (<2 and $\geqslant 2$) in the first year of life and for GA (moderate and late preterm).

Data collection and management

Clinical data

Parents record their child's health using the RespiRecord app during the first 2 years of life. Information is collected weekly on any symptoms, doctor visits, doctor diagnoses, hospitalisations, medication usage and medical procedures. During an episode of respiratory symptoms, parents will fill in a diary that reports on symptoms (runny or stuffy nose, cough, shortness of breath, wheezing, stridor, noisy breathing/rattling chest, throat ache, earache, apnoea, snoring, fever, loss of appetite, vomiting), symptom severity (mild, moderate, severe), provided medication and, in case of a doctor's visit, the doctor's diagnosis (common cold, ear infection, throat infection, croup, whooping cough, bronchiolitis, bronchitis, pneumonia, (viral) wheezing/(baby)asthma). Parents are instructed (and weekly reminded) to start and stop diaries in the RespiRecord app at onset and resolution of respiratory symptoms. Diagnoses and treatments reported by parents will be verified with information retrieved from medical records. The study team is automatically alerted when a weekly or daily questionnaire is not filled in within 3 days after appearing, after which the

study team contacts the parents. If parents do not provide consent for using the RespiRecord app in the second year of life, monitoring will be through questionnaires at ages 18 and 24 months.

Parents receive a questionnaire regarding respiratory symptoms (based on ISAAC [32]), risk and preventive factors for respiratory morbidity, environmental exposures (based on ISAAC [32]), quality of life (CarerQoL [33]), loss of productivity (iPCQ [34]) and medical consumption (IMCQ [35]) at study onset and at ages 6, 12, 18 and 24 months (see table 3).

In case a participant ceases IMP treatment, parents are invited to continue follow-up (except for sample collection and lung function measurement), in order to allow for intention-to-treat analysis.

All data are entered and stored electronically by the central study team or by parents directly when filling in a digital questionnaire. All data are coded. Modifications to data are tracked, documented and accounted for. The type of activity that an individual user may undertake is regulated by the privileges associated with his/her account. Data will be stored for a period of 15 years after completion of the study. Metadata consisting of a list of codes and associated meaning are available. A complete backup of the primary database is performed twice daily and access to study data is restricted.

Lung function measurements are performed at ages 2, 6 and 12 months in the first 100 participants whose parents give consent. In case of participation in PROTEA-2 one additional measurement will be performed at age 24 months. Ventica devices will be used, applying impedance pneumography to obtain the expiratory variability index and the ratio of time to reach peak tidal expiratory flow to total expiratory time (TPEF/TE) from tidal breathing. Reduced expiratory variability and TPEF/TE are associated with bronchial obstruction [36, 37].

Biological data

Biological samples will be collected during the study visits from the first 250 participants whose parents give consent for this. These samples will be used to evaluate the impact of bacterial lysate administration on immune cell composition, activation and maturation and on microbiome diversity and maturation. Cellular immune phenotype will be determined in blood and nasal cells by mass cytometry. Single cell transcriptome data will be obtained from peripheral blood mononuclear cells (PBMCs). Whole blood stimulation assays will be applied with OM-85, LPS, poly(I:C) and CL-97 (to mimic viral stimulation) for 24 h, and supernatant will be collected to measure a multiplex panel of cytokines and chemokines by cytokine bead array. Total secretory IgA in saliva will be analysed by ELISA. Multiplex cytokine and chemokine analysis will be performed on the nasal lining fluid. Microbiota composition will be analysed in nasopharyngeal swabs and faeces through metagenomic DNA isolation followed by 16S rRNA amplicon gene sequencing. Serum specific IgE and vaccination titres are assessed from serum taken at age 12 months. Buccal mucosa/saliva will be collected at age 12 months to allow future genetic testing in relation to the primary outcome and responsiveness to the treatment. For an overview of all collected data, see table 3.

Data analysis

The primary outcome will be compared between the two groups by Poisson or negative binomial regression analysis depending on the distribution of the data. The data will be analysed for the intention-to-treat group (including all participants as randomised, provided they received IMP at least once and reported on respiratory outcomes at least once) and per-protocol group. Pre-specified analyses will be performed for the following subgroups:

- Moderate and late preterms (GA 30–33 weeks and 33–36 weeks)
- Infants with parental atopy
- Infants with and without anti-respiratory syncytial virus prophylaxis

Covariate adjustment will be applied in case covariates are not evenly distributed among the groups, such as delivery mode, feeding type and day care attendance. See supplementary material 1 for all recorded covariates. Estimates, confidence intervals and p-values will be presented. For all tests we will use a two-sided p-value with alpha $\leqslant 0.05$ level of significance.

The primary outcome of PROTEA-2 will be compared with survival curves and hazard ratios by the Fleming–Harrington weighted log-rank test, since it is assumed that the proportional hazards will not be identical in time. Covariate adjustment will be applied equal to PROTEA-1. Further, a recurrent event model will be used to compare lower respiratory episodes between groups for the complete study period taking into account that one event increases the risk for a subsequent event.

TABLE 3 Overview of collected data for PROTEA and PROTEA-2 trials

Collected data	Method	Time point
Clinical outcomes		
Respiratory symptoms (runny or stuffy nose, cough, shortness of breath, wheezing, stridor, noisy breathing/rattling chest, throat ache, earache, apnoea, snoring, fever, loss of appetite, vomiting) plus severity (mild, moderate, severe)	Diaries in RespiRecord app and extra questions in questionnaires (per symptom, the frequency in the last 6 months is asked: no, seldom, occasionally, regularly, very often, mostly, always)	Continuous and at 6 and 12 months
Number of episodes of respiratory symptoms in the last 6 months (runny or stuffy nose, cough, shortness of breath, wheezing, stridor, noisy breathing/rattling chest, throat ache, earache, apnoea, snoring, fever, loss of appetite, vomiting)	Questionnaire (aimed at gathering information about participants that quit filling in diaries in year 2)	18 and 24 months
Wheezing, dry cough and asthma according to ISAAC questionnaires	Questionnaire	6, 12, 18, 24 months
Any other symptoms	Weekly journals in RespiRecord app	Continuous
Doctor visits (including diagnosis and prescribed treatment) and hospitalisations	Weekly and daily journals in RespiRecord app, plus verified with medical record	Continuous
Medication use	Weekly and daily journals in RespiRecord app	Continuous
Adverse events potentially related to investigated product	Questionnaires and interview. Also, all possible symptoms are recorded in the weekly journals in RespiRecord app	6, 12, 18 and 24 months plus weekly
Expiratory variability index	Measured with Ventica recorder	Study onset, 6, 12 and 24 months
Covariates		
Demographic details (sex, ethnicity, parental age, parental education)	Baseline questionnaire	Study onset
Medical history (gestational age at birth, birthweight, mode of delivery, antenatal steroids, surfactant therapy, method and duration of respiratory support, lower respiratory tract infections, antibiotic exposure during labour and in early life)	Medical record	Study onset
Growth (weight and height)	Measured or from medical record	During study visits
Vaccination status (including RSV prophylaxis, maternal pertussis and maternal influenza vaccines)	Questionnaire	Study onset, 6, 12 and 18 months
Smoke exposure (in pregnancy and early life)	Questionnaire	Study onset and 12 months
Day care attendance	Questionnaire	Study onset and 12 months
Other children in the household	Questionnaire	Study onset and 12 months
Living environment	Questionnaire	Study onset
Exposure to animals (pets and farm animals)	Questionnaire	Study onset
Familial respiratory disease and atopy	Questionnaire	Study onset
Feeding type (duration of breastfeeding and start of solids)	Questionnaire	Study onset, 6, 12 and 18 months
Cost-effectiveness information		
Quality of life	CarerQoL [33]	6, 12, 18 and 24 months
Loss of productivity	iPCQ [34]	6, 12, 18 and 24 months
Medical consumption	IMCQ [35]	6, 12, 18 and 24 months
Immunological and microbiological data		
Cellular immune phenotyping data	Venous blood and nasal scrape	2, 6, 12 and 24 months
Single cell transcriptome data from peripheral blood mononuclear cells	Venous blood	2, 6, 12 and 24 months
Whole blood stimulation essay data	Venous blood	2, 6, 12 and 24 months
Serum specific IgE titres	Venous blood	12 months
Vaccine response (titres)	Venous blood	12 months
Total secretory IgA	Saliva	2, 6, 12 and 24 months
Genetic testing (only with specific consent)	Saliva/buccal mucosa	12 or 24 months
Multiplex cytokine and chemokine analysis	Nasal lining fluid	2, 6, 12 and 24 months
Microbiota composition and development	Nasopharyngeal swabs and faeces	2, 6, 12 and 24 months
Viruses and specific bacteria (*Mycoplasma*; *Bordetella*) per PCR	Nasopharyngeal swab	During lower respiratory tract infections

ISAAC: International Study of Asthma and Allergies in Childhood; RSV: respiratory syncytial virus; iPCQ: iMTA (Institute for Medical Technology Assessment) Productivity Cost Questionnaire; iMCQ: iMTA Medical Consumption Questionnaire.

The frequency of primary outcomes during 0–12, 0–24 and 12–24 months of age will be compared by negative binomial regression. We will compare the following groups: 1) 2-year OM-85; 2) 1-year OM-85 1-year placebo; 3) 1-year placebo 1-year control group (never OM-85). Adjusting for covariates will be included in the regression model.

Secondary outcomes are compared by appropriate statistics. The role of covariates, and subsequently adapted statistical analyses, will be considered. A table with all secondary outcomes compared between groups can be found in supplementary material 1.

Missing data can result from unfinished questionnaires. This is being minimised by automatically generated alerts to the study team. Missing data is anticipated to be completely at random and will be handled using single stochastic imputation when appropriate.

Monitoring and safety

An independent monitor will check the study is in line with Good Clinical Practice. Adverse events are collected during study visits and *via* the RespiRecord app. (S)AEs and SUSARs will be reported as required by law. Hospitalisation due to RTI or wheezing are expected and will not be reported according to serious adverse event (SAE) guidelines, but only listed in regular progress reports.

The Data Safety Monitoring Board will examine safety parameters every 4 months and evaluate progress of the trial through at least one interim analysis after 250 participants have reached the age of 1 year. If not yet broken for participation in PROTEA-2, blinding will be kept for the primary investigators, parents and doctors. The study will be terminated prematurely in case of:

1. interim analysis showing serious adverse events to occur more than two times more frequently in the treatment group and at least 30 SAEs have occurred in this group (we expect hospitalisation in at least 6% of patients in our population);
2. new toxicological findings related to the study investigations that might affect the benefit-to-risk ratio leading to an unacceptable risk.

Informed consent is obtained by trained research physicians. Parents of candidates will be provided with oral and written information as explained under “recruitment practices”.

Patient and public involvement

Volunteers from the patient advisory board of pulmonary medicine, lung foundation and Care4Neo were involved in the development of the study design and the information for parents. For the design of PROTEA-2, parents who participated in the PROTEA study were involved also.

Ethics and dissemination

This protocol is approved by the Medical Research Ethics Committees United (MEC-U) Nieuwegein, the Netherlands and the national central committee on research involving human subjects (CCMO). Protocol modifications need approval from both organisations and will subsequently be communicated to all recruiting sites and if relevant, to the participants. We will follow the ethical principles of the Declaration of Helsinki. The study is registered at ClinicalTrials.gov with number NCT05063149. Results will be published in peer-reviewed journals and presented at international conferences.

Conclusion

The main objective of this randomised controlled trial is to determine the effectiveness of OM-85 in reducing lower RTIs and wheeze in the first 2 years of life in moderate–late preterm born infants. Using OM-85 as a primary preventive agent is a new concept and to our knowledge has only been investigated in a small group of children. Not only will this trial deliver a significant addition to the knowledge about the clinical effects of OM-85, but it will also give a unique insight into the impact of OM-85 on the developing microbiome and immune system of moderate–late preterm born infants. The extensive follow-up and sampling of the participants will deliver a large data set with the possibility to increase knowledge on respiratory health in moderate–late preterm infants and predict infants at risk and treatment success, helping us to achieve personalised medicine.

Data availability: All coded individual participant data (including data dictionaries) will be available after publication of the data in separate articles, immediately following the publication without an end date. Additionally, the study protocol and statistical analysis plan will be available. The data are available for investigators whose proposed use of the data has been approved by the consortium that owns the data. Proposals

should be directed to g.tramper@franciscus.nl. After approval by the consortium and signing of a data access agreement, access to the online repository will be provided.

Provenance: Submitted article, peer reviewed.

This clinical trial is prospectively registered with ClinicalTrials.gov as NCT05063149.

Ethics statement: The study protocol is approved by the Medical Research Ethics Committees United Nieuwegein, the Netherlands, and the national central committee on research involving human subjects (CCMO). Protocol modifications need approval from both instances and will subsequently be communicated to all recruiting sites and in case it affects the participants also to their parents. The study will follow the ethical principles of the Declaration of Helsinki. Results will be published in peer-reviewed journals and presented at international conferences.

Conflict of interest: I.C. van Duuren has nothing to disclose. H.H. Smits reports support for the present study from Dutch Lung Foundation; grants from Netherlands Organisation for Scientific Research and OM Pharma; support for attending meetings from OM Pharma; leadership role with Netherlands Respiratory Society (President and past president), Dutch Society for Immunology (Treasurer) and the Foundation for Asthma Prevention (Board member). L. Duijts reports grants from European Union's Horizon 2020 research and innovation program (ENDOMIX, number 101136566; ATHLETE, number 874583; EUCAN-Connect, number 824989), Asthma Control Foundation (Stichting Asthma Bestrijding), Foundation Vrienden van Sophia (Corona study, number OTH20-0), ZonMW, Co-KIDS Studies (numbers 10150062010006 and 1043036222001); payment or honoraria for lectures, presentations, manuscript writing or educational events from Astra Zeneca, British Thoracic Society, Karolinska Institutet, University of Copenhagen, Universitat Pompeu Fabra, Barcelona Institute for Global Health, ZonMW; and is a member of the Scientific Committee of VENI. J. Penders reports grants from The Protea Study (funded by Lung Foundation Netherlands), Maag, Lever, Darmstichting and Dutch Research Agenda (NWA); and is a Scientific Advisory Board member of the EU project INITIALISE and the MSCA ITN TranSYS consortium. G.A. Tramper-Stranders reports grants from the Dutch Lung Foundation, ZonMw, Asthma Foundation, Franciscus Foundation, OM Pharma, Revenio Research and AstraZeneca (all investigator-sponsored studies); and honoraria for lectures and presentations from OM Pharma (presentation during EAACI events).

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