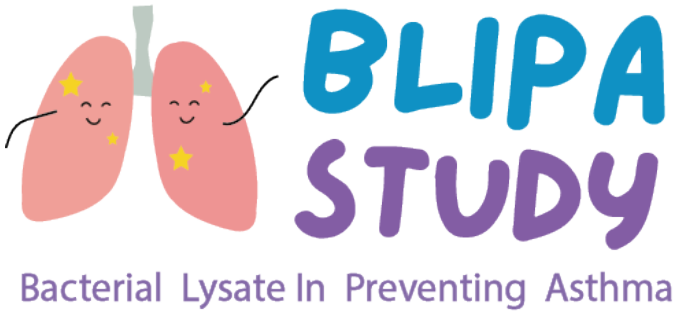
Queen Mary University of London

BLIPA STUDY
Bacterial Lysate In Preventing Asthma

NHS Barts Health NHS Trust



JRMO Research Protocol for MHRA Regulated Studies

Full Title	Oral bacterial lysate to prevent persistent wheeze in infants after severe bronchiolitis; a randomised placebo-controlled trial
Short Title	BLIPA - Bacterial Lysate in Preventing Asthma
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Glossary of terms and abbreviations

AE	Adverse Event
AR	Adverse Reaction
ASR	Annual Safety Report
BV	Broncho-Vaxom
CA	Competent Authority
Child	An individual who takes part in this clinical trial
CI	Chief Investigator
CRF	Case Report Form
CRO	Contract Research Organisation
CTA	Clinical Trial Authorisation
CTCAE	Common Terminology Criteria for Adverse Events
CTIMP	Clinical Trial of Investigational Medicinal Product
CTN	Clinical Trials Network
DC	Dendritic Cells
DMC	Data Monitoring Committee
EC	European Commission
EMA	European Medicines Agency
EU	European Union
EUCTD	European Clinical Trials Directive
EudraCT	European Clinical Trials Database
EudraVIGILANCE	European database for Pharmacovigilance
FPFV	First Patient First Visit
GAfREC	Governance Arrangements for NHS Research Ethics
GCP	Good Clinical Practice

GMP	Good Manufacturing Practice
IB	Investigator Brochure
ICF	Informed Consent Form
IMP	Investigational Medicinal Product
IMPD	Investigational Medicinal Product Dossier
ISF	Investigator Site File
ISRCTN	International Standard Randomised
JMRO	Joint Management Research Office
LPLV	Last Patient Last Visit
MA	Marketing Authorisation
Main REC	Main Research Ethics Committee
MHRA	Medicines and Healthcare products Regulatory Agency
MoA	Mechanism of Action
MS	Member State
NHS R&D	National Health Service Research & Development
PCTU	Pragmatic Clinical Trials Unit (Queen Mary)
PI	Principle Investigator
PIS	Participant Information Sheet
PPE	Personal Protective Equipment
QA	Quality Assurance
QC	Quality Control
QP	Qualified Person for release of trial drug
Queen Mary	Queen Mary University of London
RCT	Randomised Control Trial
REC	Research Ethics Committee
SAR	Serious Adverse Reaction
SAE	Serious Adverse Event
SDV	Source Document Verification
SOP	Standard Operating Procedures
SmPC	Summary of Product Characteristics
SSA	Site Specific Assessment
SSAR	Suspected Serious Adverse Reaction
SUSAR	Suspected Unexpected Serious Adverse Reaction
TMG	Trial Management Group
TSC	Trial Steering Committee
URTI	Upper Respiratory Tract Infection

Signature page

Chief Investigator Agreement

The study as detailed within this research protocol will be conducted in accordance with the principles of Good Clinical Practice, the UK Policy Framework for Health and Social Care Research, the Declaration of Helsinki, and the current regulatory requirements, including the Medicines for Human Use (Clinical Trials) Regulations 2004 (UK S.I. 2004/1031) and all subsequent amendments. I delegate responsibility for the statistical analysis and oversight to a qualified statistician (see declaration below).

Chief Investigator name: **Professor Jonathan Grigg**

Signature:

Date: **17/08/2023**

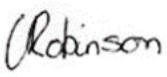
Statistician's Agreement

with the principles of Good Clinical Practice, the UK Policy Framework for Health and Social Care Research, the Declaration of Helsinki, and the current regulatory requirements, including the Medicines for Human Use (Clinical Trials) Regulations 2004 (UK S.I. 2004/1031) and all subsequent amendments, and ICH E9 - Statistical principles for Clinical Trials and ICH E10 - Choice of Control Groups.

I take responsibility for ensuring the statistical work in this protocol is accurate, and for the statistical analysis and oversight of this study.

Statistician's name: **Dr Clare Robinson**

Signature:



Date: **17/08/2023**

Principal Investigator Agreement Page

The clinical study as detailed within this research protocol **(Version 5.0, dated 17 Aug 2023)**, or any subsequent amendments, involves the use of an investigational medicinal product and will be conducted in accordance with the Research Governance Framework for Health & Social Care (2005), the World Medical Association Declaration of Helsinki (1996), Principles of ICH-GCP, and the current regulatory requirements, as detailed in the Medicines for Human Use (Clinical Trials) Regulations 2004 (UK S.I. 2004/1031) and any subsequent amendments of the clinical trial regulations.

Principal Investigator Name: _____

Please insert the Principal Investigator's Name.

Principal Investigator Site: _____

Please insert the Principal Investigator's Site.

Signature and Date:

Synopsis

Full title	BLIPA - Oral bacterial lysate to prevent persistent wheeze in infants after severe bronchiolitis; a randomised placebo-controlled trial
Short title and / or acronym	Bacterial Lysate In Preventing Asthma
Sponsor	Queen Mary University of London
MHRA Risk level	Type B - Study IMP is licensed in the EU, not the UK
Phase of the trial	IIb
Medical condition or disease under investigation	Pre-school wheeze
Study design and methodology	Multi-centre, randomised, double-blind, placebo-controlled trial with two arms (1:1)
Planned number of participants	894
Objectives	i) To test whether oral BV prevents parent-reported healthcare professional-confirmed wheeze in children after severe bronchiolitis. ii) To understand the effect of oral BV treatment on markers of atopy in blood.
Inclusion and exclusion criteria	**Inclusion criteria** 1. Parent/Guardian able to provide written informed consent. 2. Within 6 weeks of discharge from hospital for bronchiolitis 3. Child is aged 3-12 months at the time of consent to study 4. A diagnosis of Bronchiolitis requiring a hospital admission (defined as more than 4 hours in hospital) 5. Contactable for regular follow up by the research team **Exclusion criteria** 1. Any previous and separate hospital admission (more than 7 days ago) for bronchiolitis (defined as an admission to hospital requiring medical intervention, such as oxygen supplementation or medication to treat wheeze) 2. More than one episode of healthcare professional-diagnosed wheeze prior to index bronchiolitis episode 3. Premature gestational age less than 37 weeks

	4. Any severe chronic condition such as cystic fibrosis, sickle cell disease, severe developmental delay, immunodeficiency, or anything that has a significant impact on the respiratory tract (such as need for non-invasive ventilation) or increases vulnerability to respiratory tract infections. 5. History of clinically significant neonatal disease (e.g. neonatal pneumonia, congenital lung abnormality, neonatal chronic lung disease) 6. Genetic conditions that affect the immune system (e.g. Down's syndrome/Trisomy 21) 7. Current regular oral montelukast or inhaled corticosteroid therapy or inhaled salbutamol therapy 8. Current regular treatment with immunomodulatory drugs (e.g oral steroids) 9. Known allergy or previous intolerance to study medication. 10. Enrolment in another clinical trial of a medicinal product is excluded. Non-CTIMP study participation is allowed. 11. Sibling of a BLIPA participant (of the same household or family)
Investigational Medicinal Product(s)	Oral Broncho-Vaxom (3.5mg) administered daily for 10 days per month for 24 months
Treatment duration	24 months
Follow up duration	24 months
End of Trial definition	EOT is defined as three months after the Last Participant Last Visit (LPLV).

1 Introduction

1.1 BACKGROUND

1.1.1 The problem to be addressed

In the UK, asthma is a major non communicable disease, with no ways to prevent its development (primary prevention)[1]. The lifetime prevalence of doctor-diagnosed asthma in the UK is 16%, and the lifetime prevalence of patient-reported asthma symptoms is 30%[2]. This results in major costs to the NHS – with annual costs of over £1 billion[2]. In children, allergic asthma is usually diagnosed from 6 years onwards and tends to persist into adult life[3]. In children 1 to 5 years of age (i.e. the preschool age range), episodes of viral-triggered wheeze are common but, unlike asthma in later childhood, over 75% of cases resolve by school age[4]. However, a group of infants at much higher risk of developing preschool wheeze that will continue as school-age asthma, are those admitted to hospital with viral bronchiolitis in the first year of life. Infants develop bronchiolitis when they are infected for the first time with either the respiratory syncytial virus (RSV), or other respiratory viruses such as rhinovirus.

1.1.2 Bronchiolitis

Overall, 33% of all UK infants will develop clinical bronchiolitis, and 3% of all UK infants will require admission to hospital for this disease. The long-term consequences of severe bronchiolitis have been extensively reported with consistent evidence that infants admitted to hospital with bronchiolitis are at greatly increased risk of subsequent asthma. First, Dumas et al, in a study of infants admitted to hospital with bronchiolitis, found that by 3 years of age, 27% had developed recurrent episodes of wheeze requiring doctor-prescribed therapy [5]. Second, Valkonen et al reported that 3 years after hospital admission for infant bronchiolitis, 23% of children were being prescribed asthma medications [6]. Third, Bacharier et al followed up infants with bronchiolitis for up to 6 years and found that by 24 months of age 35% of infants who had severe bronchiolitis had developed doctor-diagnosed preschool wheeze or asthma (personal communication), and that by 7 years of age 47% had doctor-diagnosed active school-age asthma[7].

1.2 RATIONALE FOR STUDY DESIGN

1.2.1 The use of Bacterial Lysates in preventing infections

Recent epidemiological studies strongly suggest that exposure of infants to high levels of microbial products, or increased diversity of infants' intestinal microbiota, or a combination of both, prevents wheezing illnesses[8]. For example, exposure of infants to microbial products in dust in traditional farms protects against the development of preschool wheeze [9, 10]. One way of exposing infants to high levels of microbial products that already exists as a medicine is oral lysates of respiratory bacteria[11]. Several bacterial lysate preparations are used in continental Europe to prevent recurrent respiratory tract infections in children. But to date there are no published studies on whether oral bacterial lysates prevent asthma.

In clinical studies, oral bacterial lysates have shown some efficacy for both the prevention of respiratory infections and treating established viral-triggered preschool wheeze. The most widely used bacterial lysate in these studies in children, and thus with the most clinical and mechanistic evidence, is Broncho Vaxom (BV; also called OM-85 BV). This is a lyophilised and lysed extract of 21

respiratory bacterial strains from 5 pathogenic genera. For its effect on recurrent infections, a systematic meta-analysis of 8 clinical trials found that 32% of children treated with oral BV had recurrent respiratory tract infections compared with 58% in placebo-treated children[12].

For children with established preschool wheeze, Razi et al, reported that treatment with oral BV (3.5 mg) for 10 days per month for a total of 3 months in preschool children (1 to 6 years), reduced the mean incidence of wheezing attacks over a 12 month period by 38% compared with placebo (mean difference in attacks 2.1 BV vs. 5.7 Placebo) [13]. And more recently, Sly et al in a placebo-controlled RCT of oral BV (3.5 mg) given over 2 consecutive winter seasons to healthy infants with a parental history of asthma and allergies, reported that oral BV treatment increased the time to the first episode of severe lower respiratory tract illness (442 vs. 85 days) [14]. Sly et al speculated that there would be greater effect if oral BV was given not just during the winter period [14].

To date, BV at its licenced dose for the first 10 days of each month is the most widely used in respiratory studies. Continuous dosing has also been used. For example, Bodemer et al, reported that continuous oral dosing with BV (3.5 mg) for 9 months in infants and children with atopic dermatitis reduced the number of skin flares compared with placebo (HR 0.8, 95%CI 0.69 to 0.98), without significant side effects [15].

Although clinical studies suggest an effect of BV on children's innate immunity, specific mechanisms of action have not been elucidated. However, animal models suggest that oral BV treatment modulates T and dendritic cell function in the airway.

- Navarro et al in a mouse model, reported that oral BV treatment suppresses airway inflammation via increasing Foxp3+ Tregs [16].

- Strickland et al in a rat model, reported that oral BV reduces allergic airway inflammation by selectively recruiting CD4+CD25+ Foxp 3+ Tregs into the airway. This capacity of oral BV to recruit Tregs into the airway was also found in naïve, unsensitised, animals [17].

- Mincham et al reported that oral BV given to pregnant mice reduces the susceptibility of their offspring to allergic airway inflammatory disease via expansion of airway Tregs [18].

- Esposito et al reported that BV reduces rhinovirus infection of airway cells and corrects Th1/Th2 imbalance; the former a trigger of wheeze episodes, the latter the substrate for allergic asthma [19]

One potential marker of the complex interconnected changes that BV on the immune system is airway and gut microbiota. Airway and gut microbiota may not only passively reflect a healthy immune system, but also may directly influence immune development. For example, Raedler et al speculated that the beneficial effect of living on traditional farms on wheeze development is due to exposure to microbial products, which by preventing colonisation of the lower airways with harmful bacteria, supports "healthy" maturation of innate and adaptive immunity [20]. The figure from Raedler et al illustrates how mechanistically complex the development of wheeze is, and the potential role of BV (lower centre panel) [20].

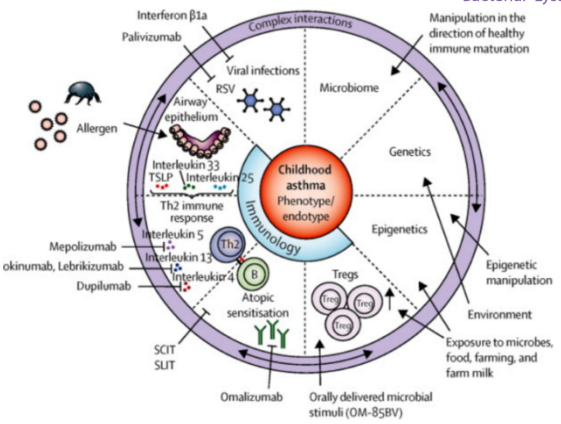


Figure 1: Schematic overview of selected determining factors on childhood asthma phenotypes and endotypes

Determining factors in childhood asthma phenotypes and endotypes are presented as a pie chart. Close interactions in both directions are shown in the surrounding purple circle. Potential therapeutic interventions are depicted in black lines (——I) and potential future preventive strategies are depicted by black arrows. RSV=respiratory syncytial virus. SCIT/SLIT=subcutaneous/sublingual immunotherapy. TSLP=thymic stromal lymphopoietin. Treg=regulatory T cell. [20]

Beneficial effects of BV on asthma development, in the model above, may either be modulated by direct effects on gut immune cells that migrate to the airway, or reduced susceptibility to viral infections, or by modulating gut microbiome/immune cell interactions, or by some combination. The effects of viral infection on the microbiome were reported by Teo et al in a 5 year longitudinal study of the nasopharyngeal microbiome of 244 infants, that viral infections shift the nasopharyngeal microbiome towards dominance by a small range of pathogenic bacteria [21]. Teo et al also found that the combination of colonisation with pathogenic bacteria and allergic sensitisation was associated with the development of persistent wheeze at school age[21]. Further evidence for a role of colonisation with pathogenic bacteria in wheeze development was provided by Bisgaard et al, who reported that airway colonisation with S. pneumoniae, H. influenzae, or M. catarrhalis in infancy is associated with an increased risk of subsequent development of recurrent preschool wheeze [34].

Thus mechanistic studies during BV therapy should include its effect on overgrowth of potential bacterial pathogens in the airway and gut, and T and dendritic cell development.

Of the nine BV studies registered in the ClinicalTrials.gov, four are for paediatric asthma (one is the published study by Razi et al) [13]. The PrecISE study (NCT04129931), plans to enrol from November 2019 onwards, 800 adults and children with severe asthma. One arm of PrecISE will assess the efficacy of BV or placebo 7 mg orally continuously once a day for 4 months. The other arms are medium chain triglycerides, Clazkizumab, Imatinib, Cavosonstat and placebo. The NIH-funded ORBEX trial (NCT02148796) is currently recruiting to a target of 1,076 healthy, asymptomatic, children aged 6 months to 1.5 years of age, with either parental history of asthma, or doctor-diagnosed atopic dermatitis. Infants in ORBEX are being randomised to receive either oral BV or placebo for 10 days per month for 2 years. The primary outcome is the “time to the occurrence of the first wheezing episode in the third observation year” with a study completion date of late 2024. ORBEX, unlike BLIPA, specifically excludes infants with a history of bronchiolitis.

1.3 ASSESSMENT AND MANAGEMENT OF RISK

1.3.1 Benefits

The clinical efficacy and mechanism of BV in potential reduction of asthma in children that have presented with wheeze in early life.

To understand the effect of this treatment of the immune cells in the body that are key in the development of asthma.

1.3.2 Risks

The safety of oral BV in both adults and children is well established. The ORBEX trial's website states that BV has been used safely by more than 43 million children worldwide. A systematic review of trials of BV in older children by Steurer-Stey *et al* identified 13 studies including 2,721 children, and found no evidence of significant BV side effects [22]. In a review of the use of bacterial lysates, Kearney *et al* concluded that they were "safe and that there were no reports of serious adverse reactions or mortality resulting from bacterial lysate therapy in children or adults", and no adverse effects are associated with vaccine delivery of influenza virus and concurrent bacterial lysate therapy [23].

The European Medicines Agency (EMA) has concluded that there is "some evidence of effectiveness of these medicines for prevention of respiratory tract infections" and that the "safety profile is in line with what is expected for this type of product". (https://www.ema.europa.eu/en/news/bacterial-lysate-medicines-respiratory-conditions-be-used-only-prevention-recurrent-infections).

Most recently, Esposito *et al* reported a randomised, placebo-controlled, double-blind, single-centre study, to assess the efficacy of oral BV in reducing the number of new respiratory tract infection episodes in children 1 to 6 years of age with a history of recurrent infections [24]. The researchers found that the number of RTIs in children who experienced at least one RTI was 50% lower among those receiving BV for 3 months than placebo. Rossi *et al* in a mouse model, reported that pre-treatment with oral BV reduces influenza viral load in the lung 5 days post infection, and improves survival from *S. pneumoniae* infection [25].

1.3.3 Summary

Overall, oral BV is safe in infants and there is proof of concept evidence to support its evaluation in an asthma prevention trial. Infants at increased risk of developing wheeze revealed by bronchiolitis represent an ideal population for wheeze prevention. This approach is supported by the US National Heart Lung and Blood Institute's opinion that immune modulation to prevent wheeze should be performed in high risk infants and that this evaluation should include BV [26], and the conclusion of Jartti *et al* that severe bronchiolitis defines a group of infants ideally suited for asthma prevention studies [27].

1.3.4 Dosage, Regimen and route

The safety of BV has been assessed in trials in infants and young children. Bodemer et al gave 3.5 mg BV or placebo once a day continuously for 9 months to children 6 months to 7 years of age with 67% of the BV group < 3 years of age [15]. There were 5 adverse events possibly related to BV (cough and diarrhoea) and 2 related to placebo (diarrhoea). Sly et al, gave oral BV 3.5 mg or placebo once a day for 10 days per month to infants 3 to 9 months of age over 2 separate winter seasons, and concluded that BV was safe and tolerable with no difference in adverse events between the groups [14]. Using a different bacteria lysate (Pro-Symbioflor), Lau et al, gave newborns 10 drops of lysate 3 times a day continuously for 7 months [28]. There were 73 adverse events reported in the active and 74 events in the placebo groups, none considered to be related to the study medication. The ongoing ORBEX study (described above) has not found safety concerns with 3.5 mg BV given once a day for 10 days per month to infants 5 to 17 months of age.

This trial is categorised as:

Type B = Somewhat higher than the risk of standard medical care, (studies are those testing authorised medicinal products according to treatment regimens outside the marketing authorization).

1.3.5 Future or ancillary studies

The trial will not assess the effect of BV on asthma at 7 years of age (i.e. school age). However, wheeze between 2.5 and 3.5 years of age is a clinically important and valid outcome, since Bacharier et al [7] found that the majority of infants who were hospitalised for bronchiolitis and subsequently developed wheeze at 2 to 3 years of age, developed doctor-diagnosed asthma at school age. BLIPA will also consent and seek approval for possible future contact at school age for data related to children's wheeze from GP records at ages 6 to 8 (subject to future funding).

Optional blood, stool and nasal samples will be collected for future mechanistic studies into the relationship between bronchiolitis, wheezing, and Broncho Vaxom.

1.3.6 Covid-19 considerations

The trial design minimises any additional visits to high-risk environments for COVID-19 exposure. The sampling is non-aerosol generating so does not pose a risk to the trial staff or participants. All trial staff will wear appropriate PPE.

1.3.7 BLIPA collaboration UK and Australia

The BLIPA study will combine the results of two multi-centre, randomised trials with similar but separate protocols: BLIPA-UK, with recruitment in London, Southampton, Edinburgh, and Aberdeen and BLIPA-Australia, with recruitment in Brisbane, Gold Coast, Melbourne, Darwin and Sydney.

BLIPA-UK is funded in the UK by the NIHR. BLIPA-Australia is funded in Australia by the ICTC initiative. ICTC supports Australian researchers to conduct clinical trial research in collaboration with international researchers.

In this collaboration, both trials are working towards a combined sample size of 894 participants. This will involve merging the data collected by both trials, each captured in a separate database and randomisation systems managed by the respective trial's lead site. The details of the collaboration are described in a collaboration document (available on request).

Maintaining aligned documentation will be essential to ensure a successful collaboration and maintain scientific integrity. Prof Jonathan Grigg and Prof Anne Chang will have primary responsibility for the development and alignment of the trial design, protocols and other documentation. Key documents such as the protocol, patient facing information, and case report forms will be jointly developed (with sufficient review and amendments) with each country having context specific documentation.

Data merging will occur only if a set of conditions are satisfied to ensure scientific quality and data integrity standards are met.

This protocol describes the study design and procedures for BLIPA-UK only.

2 Trial objectives

2.1 Research Question

In children hospitalised with bronchiolitis, does oral BV given for 10 days per month for a total of 24 months, prevent parent-reported, healthcare professional-confirmed, wheeze between 19 and 24 months post initiation of IMP/placebo.

The mechanistic hypothesis to be tested is that oral BV reduces the risk of wheeze after bronchiolitis by modulating T cell and DC maturation and altering the gut and airway microbiota.

2.2 Primary objective(s)

2.2.1 Primary clinical objective

To establish whether there is superiority of oral BV over placebo in the prevention of parent-reported, healthcare professional-confirmed, persistent wheeze between 19 and 24 months post initiation of IMP/placebo, after a hospital admission for severe bronchiolitis.

2.2.2 Hypothesis

- **Efficacy:** Oral BV reduces incidence of parent-reported, healthcare professional-confirmed wheeze between 19 and 24 months post initiation of IMP/placebo, after a hospital admission for bronchiolitis.
- **Clinical safety:** Treatment with oral BV for 10 days per month, for a total of 24 months, is a safe and effective way of preventing parent-reported, healthcare professional-confirmed wheeze after severe infantile bronchiolitis.

2.3 Secondary objective(s)

1. To establish whether there is a difference in children between treatment with BV granules or placebo in clinical outcome measures associated with wheeze and atopy between 19-24 months post initiation of IMP or placebo.
2. To assess the safety and tolerability of oral BV.

2.4 Exploratory objective(s)

2.4.1 Exploratory Objectives

1. Impact of BV on parent-reported clinical outcome measures associated with wheeze and atopy across the whole 24 months.
2. To understand patterns in compliance across treatment groups and the effect of compliance on the treatment effect.
3. Parents and healthcare professionals' self-assessment of blinding.

Optional blood samples will be collected;

To assess markers of atopy in blood (to include full blood count, total IgE and IgE to specific aeroallergens)

2.5 ENDPOINTS

2.5.1 Primary endpoint

Occurrence of parent-reported, healthcare professional-confirmed wheeze between 19 and 24 months after initiation of IMP or placebo (data collected at 24 months). As defined by the following process;

1. Parent or guardian reports wheeze in one of the monthly contacts (months 19-24), at the final contact (24 months post initiation of IMP or placebo) or a parent-initiated contact (months 19-24) with research staff. Wheeze episode is defined as an episode of wheezing that lasts at least one day with a sign of increased work of breathing such as shortness of breath, cough, or chest retraction or with any combination of these additional symptoms.
2. This wheeze is then confirmed by primary care records by one or more of the following after 24 months;
    a. Prescription for more than one salbutamol inhaler coded in primary care records between 19 to 24 months after initiation of IMP or placebo.
    b. Active wheeze code in primary care records between 19 to 24 months after initiation of IMP or placebo.
    c. Asthma diagnosis code in primary care records between 19 to 24 months after initiation of IMP or placebo.

Both parent report of wheeze and confirmation by primary care records must occur for a child to be classified as meeting the primary endpoint.

2.5.2 Secondary endpoint(s)

1. Impact of BV on clinical outcome measures associated with wheeze and atopy between 19-24 months:

- Prescription for more than one salbutamol inhaler
- Active wheeze diagnosis on primary care record
- Asthma diagnosis on primary care record
- Parental report of wheeze episode - time in days to first episode of wheeze from initiation of IMP or placebo.
- Number of unscheduled medical attendances for wheeze
- Number of hospital admissions for wheeze
- Number of days admitted to hospital for wheeze
- Number of unscheduled medical attendances for any lower respiratory symptoms
- Number of courses of systemic corticosteroids (within the 24 months post initiation of IMP or placebo)

- Number of courses of oral corticosteroids for wheeze
- Number of courses of antibiotics
- Prescription of regular oral montelukast (yes/no)
- Eczema (yes/no)
- Eczema confirmed by U.K. Working Party's Diagnostic Criteria for Atopic Dermatitis.

2. To assess the safety and tolerability of oral BV:
   - Number of AEs/SAEs/SUSARs across 0-24 months and 19-24 months.

2.5.3 Exploratory Endpoints

1. Impact of BV on parent reported clinical outcome measures associated with wheeze and atopy across the whole 24 months:

- Time in days to first episode of wheeze from initiation of IMP or placebo
- Number of unscheduled medical attendances for wheeze
- Number of hospital admissions for wheeze
- Number of days admitted to hospital for wheeze
- Number of unscheduled medical attendances for any lower respiratory symptoms
- Number of courses of systemic corticosteroids (within the 24 months post initiation of IMP or placebo)
- Number of courses of oral corticosteroids for wheeze
- Number of courses of antibiotics
- Prescription of regular oral montelukast (yes/no)
- Eczema (yes/no)
- Other atopy related factors: breastfeeding (yes/no), food allergies, pet exposure, smoke exposure

2. To understand patterns in compliance across treatment groups and the effect of compliance on the treatment effect.
- Compliance with medication assessed from returned treatment diaries completed by parents and guardians.
- The effect of oral BV on parent-reported, healthcare-professional confirmed wheeze in the stratum of patients that have met compliance as defined as 80% of capsules taken across the whole 24 months.

3. Parents and healthcare professionals' self-assessment of blinding:
- Parent prediction: BV or placebo

- Healthcare professional prediction: BV or placebo

4. Two optional blood samples collected at trial entry, 12 months and 24 months (end of the treatment period):

- Blood serum total and specific IgE to aeroallergens, and blood eosinophil count

2.6 OBJECTIVES AND ENDPOINTS SUMMARY

Primary Objective	**Primary Endpoint**
To establish whether there is superiority of oral BV over placebo in the prevention of parent-reported, healthcare professional-confirmed, persistent wheeze between 19 and 24 months post initiation of IMP/placebo, after a hospital admission for severe bronchiolitis.	Occurrence of parent-reported, healthcare professional-confirmed wheeze between 19 and 24 months after initiation of IMP or placebo.
Secondary Objective	**Secondary Endpoint**

To establish whether there is a difference in children between treatment with BV granules or placebo in clinical outcome measures associated with wheeze and atopy between 19-24 months post initiation of IMP or placebo.	Prescription for more than one salbutamol inhaler Active wheeze diagnosis on primary care record Asthma diagnosis on primary care record Parental report of wheeze episode - time in days to first episode of wheeze from initiation of IMP or placebo Number of unscheduled medical attendances for wheeze Number of hospital admissions for wheeze Number of days admitted to hospital for wheeze Number of unscheduled medical attendances for any lower respiratory symptoms Number of courses of systemic corticosteroids (within the 24 months post initiation of IMP or placebo) Number of courses of oral corticosteroids for wheeze Number of courses of antibiotics Prescription of regular oral montelukast (yes/no) Eczema (yes/no) Eczema confirmed by U.K. Working Party's Diagnostic Criteria for Atopic Dermatitis.
Safety and tolerability	Number of AE/SAE/SUSAR events across 0-24 months and 19-24 months
Exploratory Objective	**Exploratory Endpoint**
To explore whether there is a difference in children between treatment with BV granules or placebo in parent-reported clinical outcome measures between 0-24 months.	Time in days to first episode of wheeze from initiation of IMP or placebo Number of unscheduled medical attendances for wheeze Number of hospital admissions for wheeze Number of days admitted to hospital for wheeze Number of unscheduled medical attendances for any lower respiratory symptoms Number of courses of systemic corticosteroids (within the 24 months post initiation of IMP or placebo) Number of courses of oral corticosteroids for wheeze Number of courses of antibiotics Prescription of regular oral montelukast (yes/no)

	Eczema (yes/no) Other atopy related factors: Breastfeeding (Yes/no), food allergies, pet exposure, smoke exposure
To understand patterns in compliance across treatment groups and the effect of compliance on the treatment effect.	Compliance with medication assessed from monthly questionnaires completed by parents and guardians. The effect of oral BV on parent-reported, healthcare-professional confirmed wheeze in the stratum of patients that have met compliance as defined as 80% of capsules taken across the whole 24 months.
Parents and healthcare professionals' self-assessment of blinding	Parent prediction: BV or placebo Healthcare professional prediction: BV or placebo
Markers of atopy and BV function	Blood serum total and specific IgE and blood eosinophil count

2.7 STUDY DESIGN

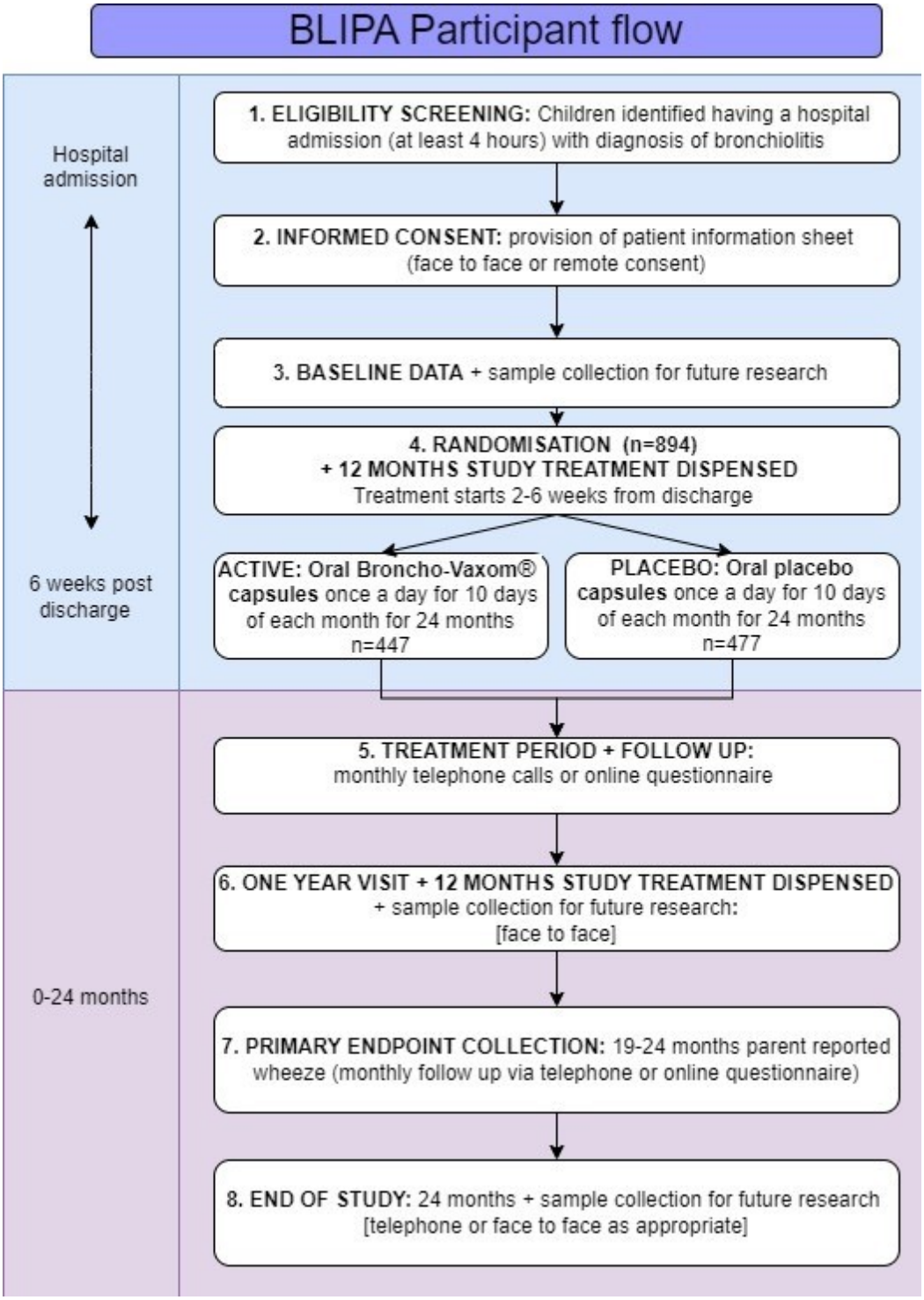
BLIPA-UK will be a multi-site, randomised in a 1:1 allocation, double-blind, placebo-controlled trial of oral BV with recruitment at sites in England and Scotland. The total study duration is 54 months. BLIPA-Australia is separate trial conducted in parallel in Australia, this trial is described separately in the BLIPA-Australia Protocol.

BLIPA-Australia will have 5 sites and BLIPA-UK will have 5 or more NHS sites in the UK with additional support from Participant Identification Centres (PICs). PICs are NHS/HSC organisations that identify potential research participants. They are not research sites and will not be treated in the same way as research sites. PICs will not recruit participants for the study but will refer potential participants to the co-ordinating recruiting research site(s).

The primary clinical objective is to recruit a population of eligible participants, to randomise them to oral Broncho Vaxom (3.5mg) or placebo, to be taken daily for 10 days a month over 24 months, follow up for 24 months and compare primary and secondary outcomes between trial arms. Parents or guardians of children, clinicians involved in their care and trial staff will be blinded to the treatment arm. Recruitment will be for at least 18 months and children's outcomes will be assessed for 24 months following initiation of IMP or placebo.

Within six weeks of hospital discharge following admission for bronchiolitis, parents or guardians can consent to their child partaking in the study, baseline data is collected, the child is randomised, and the IMP or placebo is initiated (12 months' supply). From the point of treatment initiation, children are followed up for 24 months, the same length as the treatment period. There will be at least one scheduled face to face visit at 12 months to dispense a further year's supply of IMP or placebo.

Figure 2: Flow chart illustrating the participant flow for the total duration of the study.



2.8 STUDY SETTING

This is a multi-site study (please contact the Trial Manager for a full list of sites) which will take place within NHS hospitals. All sites will identify children from those admitted (including paediatric and A&E departments) or referred to the hospital.

3 Patient Evaluability and Replacement

3.1 Target Accrual

A total recruitment of 894 children over the recruitment period across the UK and Australia. In the UK the target is 774 but this may increase if needed.

3.2 Participant identification and recruitment

In BLIPA-UK, children will be identified and recruited at five or more NHS sites in the UK. Children will be identified by the treating clinician or research team at each site from hospital admissions (including paediatric and A&E departments) or by screening admission records and databases. Optional study posters can be used in appropriate spaces in hospital paediatric departments to raise awareness of the study.

PIC sites will support patient identification, these sites will approach appropriate patients while in hospital or after discharge and confirm agreement for the recruiting site research team to contact the patients directly. No recruitment activity will take place at PIC sites. Patient contact details will then be provided to the recruiting site's research team of the co-ordinating NHS Trust using a secure data transfer or encryption method. All referrals and patient data transfer(s) via email must be made using NHS.net.

4 Informed consent procedures

Consent will be gained from the legal guardian or carer with parental responsibility by an appropriately trained and experienced qualified staff member.

Informed consent will be obtained prior to the child undergoing procedures that are specifically for the purposes of the study and are outside standard, routine care at participating sites. This includes collection of identifiable participant data.

The Principal Investigator (PI) has overall responsibility for the informed consent of parent or guardian at their site and will ensure that any person delegated responsibility to participate in the informed consent process is duly authorised, trained, and competent to participate according to the ethically approved protocol, principles of Good Clinical Practice (GCP), and Declaration of Helsinki. If delegation of consent occurs, then details will be provided in the site delegation log.

The right of a parent or guardian to refuse participation without giving reasons will be respected. The parent or guardian will remain free to withdraw at any time from the study without giving reasons and without prejudicing their further treatment and will be provided with a contact point where they may obtain further information about the study. Where re-consent is required (for example if new Reference Safety Information becomes available during the study, or following an amendment that affects the child, or new information needs to be provided to a parent or guardian) it is the responsibility of the PI to ensure this is done in a timely manner and prior to the next dose of IMP (where applicable).

4.1 WRITING, READING, AND TRANSLATION CONSIDERATIONS

All patient facing documents will be in UK English. For a child's parent or guardian who cannot read or write, a witness will be allowed to sign and/or date the consent form on behalf of the parent or guardian. For parents or guardians who are not fluent in English, the interpreter service present in each hospital/trust will be utilised, a face-to-face or telephone interpreter may be used. A member of the child's family may be present; however, they cannot act as an official interpreter with regards to obtaining informed consent.

4.2 CONSENTING PROCESS

The parents or guardians of all potential participants will be initially screened and approached by a member of the direct care team which if in line with local site policy and agreement may include the research team. Each parent approached will be given a BLIPA leaflet with information about bronchiolitis, asthma risk and the study's purpose. A member of the study team will approach them either during admission or after admission to hospital at an appropriate and convenient time to provide the patient information sheet and gauge the parent or guardian interest in the study.

If a potential participant is not approached about the BLIPA study in the hospital, a Patient approach letter with the leaflet enclosed can be sent to the parent or guardian.

Informed consent can be face to face or via telephone. Face to face consent will be the primary route of consent and will be highly encouraged to support rapport with parents. Telephone consent is available to parents who are unable to visit face to face and in case of social distancing measures, self-isolation and shielding based on current government guidelines.

The study will be explained to the parent or guardian and the PIS provided. The families will then be given ample time to consider the study (~24 hours is recommended) and ask all questions and consider taking part in the study. The discussion and consent process will be documented in the child's hospital records and the Investigator site file.

The PI is responsible for ensuring that all vulnerable parents or guardians and their children are protected and participate voluntarily in an environment free from coercion or undue influence.

To obtain consent remotely, potential participating parents and guardians will be offered a telephone or video call (following local site policy) to enable participants to go through the PIS and ICF with the PI or their designate. In order to obtain the signed ICF, a member of the local research team and a witness will call the participating parent or guardian following the BLIPA Telephone Consent Script and SOP. A copy of the signed consent form will be given to the parent via post or at the next hospital visit as appropriate.

We plan to follow up the children in BLIPA at age 6-8 years to study longer term outcomes. This will be a separate long-term follow up study and not part of the BLIPA CTIMP.

4.2.1 Additional consent provisions for collection and use of biological specimens in ancillary and future studies

The consent for the additional samples will be taken at the same time as the study consent, in the same consent form. Consent for the additional samples will not affect participation in the main study.

4.2.2 Additional consent provisions for collection and use of participant data in ancillary studies

Consent will be obtained for potential participation in future or ancillary studies related to the current study. This will not impact upon participation in the original study.

5 Participant allocation

Prior to randomising, documentation by the investigator that the clinical records were checked for all inclusion and exclusion criteria is required.

A log of children who are screened but not randomised will be documented. For CONSORT reporting, those not randomised should have the reason for not undergoing randomisation recorded.

6 Participant eligibility criteria

6.1 INCLUSION CRITERIA

1. Parent/Guardian able to provide written informed consent
2. Within 6 weeks of discharge from hospital following admission for bronchiolitis
3. Child is aged 3-12 months at the time of consent to study
4. A diagnosis of Bronchiolitis requiring a hospital admission (defined as more than 4 hours in hospital)
5. Contactable for regular follow up by the research team

6.2 EXCLUSION CRITERIA

1. Any previous and separate (more than 7 days ago) hospital admission for bronchiolitis (defined as an admission to hospital requiring medical intervention, such as oxygen supplementation or medication to treat wheeze).
2. More than one episode of healthcare professional-diagnosed wheeze prior to index bronchiolitis episode
3. Premature gestational age less than 37 weeks
4. Any severe chronic condition such as cystic fibrosis, sickle cell disease, severe developmental delay, immunodeficiency, or anything that has a significant impact on the respiratory tract (such as need for non-invasive ventilation) or increases vulnerability to respiratory tract infections.
5. History of clinically significant neonatal disease (e.g. neonatal pneumonia, congenital lung abnormality, neonatal chronic lung disease)
6. Genetic conditions that affect the immune system (e.g. Down's syndrome/Trisomy 21)
7. Current regular oral montelukast or inhaled corticosteroid therapy or inhaled salbutamol therapy
8. Current regular treatment with immunomodulatory drugs (e.g oral steroids)
9. Known allergy or previous intolerance to study medication.
10. Enrolment in another clinical trial of a medicinal product. Non-CTIMP study participation is allowed.
11. Sibling of a BLIPA participant (of the same household or family)

7 Study Schedule

7.1 SCHEDULE OF TREATMENT FOR EACH VISIT

Stage	Details of stage	Type of visit
1. Eligibility	Eligibility screening and provision of PIS and information regarding study.	a. In hospital - during initial bronchiolitis episode admission b. Remote contact c. In hospital – post discharge at clinical research facility d. Home visit
2. Consent	Informed consent is provided by the child's parent or guardian.	a. In hospital - during initial bronchiolitis episode admission b. Remote contact c. In hospital – post discharge at clinical research facility d. Home visit
3. Baseline data collection	Baseline data may also be reported by the child's parent or guardian or is collected by a member of the site research team.	a. Remote contact b. In hospital – post discharge at clinical research facility c. Home visit
4. Baseline sample collection 2- 6 weeks	Baseline samples are collected by a member of the site research team.	a. In hospital – post discharge at clinical research facility b. Home visit
5. Randomisation	Randomisation carried out by a member of the site research team.	a. Remote contact b. In hospital – post discharge at clinical research facility c. Home visit
6. Provision of IMP or placebo	Provision of oral BV or placebo alongside explanation of its use and prescription details. Discussion of start date to initiate IMP or placebo.	a. Remote contact – via pre-existing courier system at some sites b. In hospital – post discharge at clinical research facility c. Home visit
7. Monthly contact	Monthly after recruitment parents or guardians will be contacted and asked to complete the monthly questionnaire (a.k.a survey).	a. Remote contact
8. One year visit	At the one-year time point, parents and guardians will complete the monthly questionnaire as usual. The second 12 months supply of IMP/placebo will be dispensed, and blood, stool and nasal swabs taken if consent is obtained Parents or guardians return treatment diary (if used) and any	a. In hospital – post discharge at clinical research facility b. Home visit

	unused IMP from the first year's supply.	
9. End of study visit	The final questionnaire is completed. Optional samples collected. Parents or guardians complete and return treatment diary (if used) and any unused IMP from the first year's supply.	b. Remote visit (if samples are not required) c. In hospital – post discharge at clinical research facility d. Home visit
10. Parent initiated contact (Unscheduled visit)	Parents or guardians are encouraged to report wheeze and any SAEs to the research team as soon as possible via telephone. Any AEs can be reported via text or email.	a. Remote contact
*Stages 2-6 may be combined into one visit where suitable.		

7.2 RANDOMISATION METHOD

Randomisation will be performed by an authorised member of the research team at the site using the web-based randomisation service, developed and managed by Sealed Envelope (https://www.sealedenvelope.com/). Eligibility and consent will be verified (see Sections 4-6) before each child is randomised. Children will be allocated into two arms (oral BV and placebo) in a 1:1 ratio.

Randomisation will be stratified by site and parental asthma and the lists generated using random blocks of size 4 and 6. Site was chosen as a stratification factor to allow for potential differences in patient outcomes across sites, due to the characteristics of participants in different geographical locations or the characteristics of the sites themselves. Parental asthma was chosen as a stratification factor since it is a known risk factor for the development of recurrent wheeze [29].

7.3 RANDOMISATION PROCEDURE

After eligibility has been confirmed children will be randomised (within 6 weeks of hospital discharge). The baseline visit will take place prior to randomisation. Pharmacists at each site will be blinded.

7.4 COHORT ALLOCATION / SEQUENTIAL ALLOCATION

N/A

7.5 BLINDING

Parents or guardians will be blind to treatment allocation, as will the entire site study team involved in the management of the study, including the CI, PIs, sub-investigators, study nurses, and site coordinators. Trial Steering Committee (TSC) members will remain blind. The progress and safety of the study will be assessed by the Data Monitoring Committee (DMC). The DMC will therefore not be blinded. The main study trial manager (PCTU) and trial monitors will be blinded. The Sponsor's JRMO (including the JRMO monitor(s) will unblind themselves to specific patients in order to report SUSARs to the MHRA. The study pharmacist will be blinded, and the study pharmacy file will contain blinded

documents, please see pharmacy manual. The treatment allocation list will be available from the PCTU or their designate (Sealed Envelope™) on request should the need arise.

7.6 EMERGENCY UNBLINDING

To ensure patient safety the PI/authorised delegate may break the blind in emergency situations (where knowledge of the treatment is essential to the medical management of the child) without the need to obtain Sponsor or CI approval. A 24-hour online un-blinding service is available. A delegated member of the PI's team will be able to log on to the Sealed Envelope emergency on-line code break service and obtain the unblinding information. An on-call out of hours cover will be in place at any given time to ensure the unblinding service can be carried out. The code-break procedure should be made known to the on-call paediatric service at the trial site, in case of an out-of-hours emergency in relation to a study participant. However, wherever possible, it is advised that the CI/delegate will be contacted prior to un-blinding, to assess whether un-blinding may be avoided.

On receipt of the treatment allocation details the PI and/or treating health care professional will continue to deal with the participant's medical emergency as appropriate.

The PI will document the breaking of the code and the reasons for doing so on the Electronic Case Report Form (eCRF), in the site file and in the source documents/medical notes. If the PI was not aware of the un-blinding, the sub-investigator/delegate will notify the PI as soon as possible following the code break detailing the necessity of the code break. If the code-break takes place outside office hours, the PI will inform the trial manager, CI, Sponsor and the DMC as soon as possible.

If a child's randomised option is un-blinded to a specific physician but the child remains on treatment and in the study, that physician should no longer be able to assess the child in the study. The decision as to whether or not children whose allocation has been un-blinded will remain in the study will be made by mutual agreement after discussion between the Sponsor and the CI, on a case-by-case basis. The CI will ensure that the un-blinding is documented at the end of the study in any final study report and/or statistical report.

7.7 SCHEDULE OF ASSESSMENTS

		Enrolment	Treatment	One-year Visit	Treatment	Early Withdrawal	End of Study
	Visit No.	1*	2-12	13	14-24		25
	Month	0-1	1-11	12	13-23		24
	Eligibility screening	x					
	Informed consent	x					
	Informed consent for future research	x					
	Inclusion/Exclusion Criteria	x					
	Medical history and demographics (baseline data collection)	x					
	Randomisation	x					
	Monthly questionnaires		x	X	x		x
Optional samples	Blood (markers of atopy)	x		X			x
	Blood (future research sample)	x		X			x
	Nasal (future research sample)	x		X			x
	Stool (future research sample)	x		x			x
	End of Study Questionnaires						x
	Concomitant Medications	x	x	x	x		x
	Adverse events	x	x	x	x	x	x
IMP/ Placebo	Treatment diary/IMP accountability		x	x	x		x
	IMP dispensing	x		x			
	Treatment		x	x	x		x
	Information and guidance to parents on IMP	x		x			

*All the actions under enrolment are likely to be completed over a month period as a child can only have their baseline samples and IMP initiated at 2-6 weeks.

7.8 STUDY ASSESSMENTS

7.8.1 Baseline Visit

At the baseline visit the study will be explained again to the parents or guardians and consent checked. Blood, stool and Nasal samples will be taken for those with valid consent and the initial questionnaire will then be completed. The initial questionnaire will include demographics, family history of asthma and atopy, birth and medical history. A £20 gift voucher will be given to each parent or guardian to reimburse the families time and as a thank you for their willingness to participate in BLIPA.

7.9.2 Randomisation

The randomisation data will be collected and entered into the online randomisation system.

7.9.3 IMP Provision

The study medication will be provided to the family with detailed instructions on how and when to administer. The study medication starts between 2- 6 weeks post hospital discharge. Parents or guardians will be given information on signs and symptoms to look out for and the monthly contact questionnaire will be explained alongside a symptom diary.

7.8.4 Monthly Contact

Parents or guardians will be contacted monthly by either phone call, text or email. They will be provided with the questionnaire to complete, either online or over the phone with a member of the site research team, which will gather data on medication use and also on any unscheduled medical attendances, or wheeze episodes. All episodes of wheeze (episode of wheeze lasting more than 24 hours) will be reported. Adverse events will be collected on a monthly basis.

7.8.5 One Year Visit

Parents or guardians will complete their monthly contact questionnaire as normal. A face to face visit will be arranged to dispense the second 12 months supply of IMP, answer any questions the parent or guardian may have, and to collect blood, stool and Nasal swab samples for those with valid consent.

7.8.6 End of Study visit

A final questionnaire will be completed. Optional blood, stool and Nasal swab samples will be collected for those who have valid consent.

7.8.7 Laboratory Assessments

In participants with consent for the additional samples.

- Blood samples will be analysed for:
  - Serum total and specific IgE to aeroallergens, and blood eosinophil count
- Whole blood samples for future research
- Stool and Nasal swab samples for future research

7.9 FOLLOW UP PROCEDURES

Parents or guardians will be offered the option of consenting to be contacted again by the site research team at 72-96 months (subject to funding) for either a pre-planned follow up for parent (or guardian)-confirmed school-age asthma, or access to primary care records for a database search for healthcare professional-diagnosed asthma, asthma medication prescriptions or a combination of both.

8 Participant, Study, and Site discontinuation

Children may discontinue study treatment for the following reasons and a record made at the next monthly contact:

- At the request of the child's parent or guardian
- Adverse Event/ Serious Adverse Event: that has resulted from treatment administration where the Investigator considers that it would not be safe for the child to continue treatment, e.g. anaphylaxis. A serious adverse event that is not considered clinically related to the drug intervention will not be a criterion for withdrawal. It may be necessary to temporarily stop treatment for some expected Serious Adverse Events, e.g. injection site reaction, but treatment will be recommenced when clinically indicated and safe to do so.
- Eligibility violation e.g. contraindication of IMP or participating in another trial of an investigational medicinal product
- Allergic reaction to IMP
- If the investigator considers that a child's health will be compromised due to adverse events or concomitant illness that develop after entering the study.
- Sponsor terminated study

8.1 WITHDRAWAL PROCEDURE

The site PI may decide to withdraw a child from the study treatment at any time if an AE, intercurrent illness, or other medical condition suggests that continued treatment would not be in the best interests of the child. In this case the parent or guardian and child should continue with other aspects of the trial, including follow-up contacts, unless withdrawal from all aspects of the trial is judged by the PI to be in the child's best interests. Children who are withdrawn for clinical reasons will be referred to an appropriate clinical team for follow-up.

Parents and guardians will be informed that they have the right to withdraw from the trial at any time for any reason, without prejudice to their child's medical care. If a parent or guardian permanently discontinues the trial intervention, they and their child will be invited to continue to attend trial visits if possible, to allow for collection of key outcome and safety data.

The decision to withdraw from further trial procedures will be documented on the subsequent monthly contact form and an early withdrawal form will be completed, and in the medical notes. If a participant is withdrawn from all aspects of the trial, any data collected to date will still inform the

intention-to-treat (ITT) analysis, parents and guardians will be informed of this during the consent process. Once withdrawn from the trial, infants will not be able to re-enter.

Parents or guardians who have discontinued the trial intervention and/or have withdrawn their child from the trial will not be replaced, as the sample size allows for potential loss to follow-up.

9 Laboratories and samples

9.1 Central laboratories

There are no central laboratories involved in the trial.

9.2 Local laboratories

Each site's own NHS UKAS accredited pathology laboratories will carry out the following test for markers of atopy where possible.

- FBC (full blood count)
  Total IgE and specific IgE to aeroallergens

9.3 Sample collection, labelling, and logging

All protocol samples will be collected at the time-points specified in Section 7 and the Schedule of events in Section 7.9. Blood samples will be taken by delegated trained study staff; study staff will provide parents or guardians with appropriate containers for the provision of stool samples at visits, nasal swabs will be taken by delegated trained study staff.

All samples will be obtained, labelled, processed and transferred to the designated laboratory by delegated members of the study team. A sample log will be kept at site and centrally. No existing samples will be used in this trial.

All samples going to the HTA approved storage area will be pseudo-anonymised using the trial participant identifier number only. If results are shared outside of the study team, i.e. with funders, collaborators or in presentations and/or publications, the results will be fully anonymised.

Further detail on sample types, volume, types of tubes and containers, labelling, processing, and storage may be found in the study laboratory manual.

9.4 Sample transfer, chain of custody, and accountability

All samples will be processed and shipped under refrigerated condition to the HTA approved storage area.

9.5 Sample analysis procedures

Blood samples will be processed within the local laboratories.

Additional samples for future research will not be analysed for the purposes of this research study.

9.6 Sample Storage Procedures

Blood samples will be collected locally and stored at -80, they will be transported in batches to the Blizard Institute for final storage.

Stool and nasal swabs will be stored refrigerated and transferred to the relevant HTA approved storage area in batches throughout the study.

9.7 Sample and result recording and reporting

All samples obtained during the course of the trial will be recorded in a sample log maintained as part of the ISF at each trial site. Copies of any paperwork regarding samples sent to the central laboratories will also be maintained in the ISF for each child. The minimum recorded information will be participant ID, visit number, date and time of sample collection, type of sample (e.g. blood, stool, nasal swab), visit number and any processing performed and storage conditions.

9.8 Sample Management at End of study

All relevant material will be stored in an HTA approved storage area until analysis in future studies.

10 Study medication

10.1 Name and description of Investigational Medicinal Product(s) (IMP)

The IMP in this study is Broncho-Vaxom (OM-85) 3.5mg granules and matching placebo.

Broncho-Vaxom is a lyophilised bacterial lysate compound designed to be used in the prophylaxis of recurrent respiratory tract infections. The brand name is Broncho-Vaxom. Broncho-Vaxom 3.5mg granules and matching placebo granules are collectively referred to as study drug when detailing blinded study procedures. They are granules contained within a hard bovine gelatine capsule.

The capsules will be opened and the granules will be administered according to the schedule outlined in this protocol, 3.5mg daily for 10 days a month for a 24 month period.

Placebo capsules will be of identical shape, colour, and size. The placebo capsules consist of the following excipients: modified corn starch, magnesium stearate and mannitol.

10.2 Legal status of IMP

Broncho-Vaxom is not currently licensed for use in the UK however is licensed in the EU. Broncho-Vaxom is an EMA authorised medicine for the prevention of recurrent respiratory infections, with the exception of pneumonia.

The trial will be carried out under a Clinical Trial Authorisation (CTA). The drug is therefore only to be used by the named investigators, for the children specified in this protocol, and within the trial.

10.3 IMP Manufacturer(s) and supply arrangements

Manufacturing, capsule and granule pack production of both the IMP and placebo will be performed by OM Pharma. Secondary packaging and labelling of both IMP and placebo will be via OM Pharma. The release of the study drug will be done by OM Pharma and Thermo Fisher. Batches of study drug

will be released and shipped to pharmacies across the UK trial centres on receipt of a request by the agreed mechanism, as detailed in the pharmacy manual. Study drug will be shipped by courier to the sites.

A Certificate of Release will be provided for labelled IMP (active and placebo combined in one Certificate of Release) by the Qualified Person (QP). Details of the exact content of each shipment may be found in the Technical Agreement.

The manufacturer is responsible for labelling and QP release of study drug for use in the trial; this will be performed by OM Pharma. Study drug will be labelled in accordance with GMP annex 13.

Site pharmacy will acknowledge receipt of each shipment by the agreed mechanism outlined in the pharmacy manual. Study drug will be stored in the clinical trials pharmacy at each site until it is dispensed. The standard temperature to store and monitor transportation of the study drug is at controlled room temperature, 15°C to 25°C. Furthermore, stability studies by OM Pharma on BV capsules have shown the IMP can be used from 15°C to as high as 40°C without any impact to the quality of the product.

Storage conditions, including the minimum and maximum temperatures will be monitored on a daily basis (Monday to Friday only) and according to temperature logging policy at the local site. Further details may be found in the study pharmacy manual.

Broncho-Vaxom capsules dispensed to the parent or guardian for take-home dosing should remain in the granule packaging i.e within the capsule and stored as noted above until the point of use.

If a participant is unable to collect the trial IMP at the site, the IMP can be delivered to the participant's home. The IMP will be collected from the site and delivered to the trial participant's home by temperature-controlled courier service. The child's parent or guardian must consent to providing their contact details for shipping purposes and this must be documented in the medical notes. Delivery of the IMP to the participant's parent / guardian and confirmation that the IMP is not damaged should be confirmed in writing (by text/ email) or by telephone call to the site.

Comprehensive instructions will be provided to the parent or guardian (for both IMP home delivery or collection from site) in order to ensure compliance with dosing and storage procedures.

10.4 Summary of Product Characteristics

The IB for Broncho-Vaxom is being used for the BLIPA trial and will be updated annually (and/or as required) by OM Pharma.

10.5 Packaging and labelling of IMP(s), placebo(s)

The manufacturer is responsible for labelling and QP release of study drug for use in the trial; this will be performed by OM Pharma. Study drug will be labelled in accordance with GMP annex 13.

10.6 Accountability

The PI (or delegate) is responsible for ensuring IMP accountability, including reconciliation of drugs and maintenance of records.

• Upon receipt of IMP, the responsible person will check for accurate delivery and acknowledge receipt by signing or initialling and dating the appropriate documentation and returning it to the location specified. A copy will be archived for the Investigator Site File.

• IMP dispensing will be recorded on the appropriate drug accountability forms so that accurate records will be available for verification at each monitoring visit.

• Trial site IMP accountability records will include the following:

• Confirmation of IMP receipt, in good condition and in the defined temperature range.

• The inventory of IMP provided for the clinical trial.

• The use of each dose by each subject.

• The disposition (including return, if applicable) of any unused IMP.

• Dates, quantities, batch numbers, box numbers, expiry dates, and the individual participant ID numbers.

The Investigator site should maintain records, which adequately document that subjects were provided the doses specified in this protocol, and all IMPs provided were fully reconciled. Unused IMP must not be discarded or used for any purpose other than the present trial. No IMP that is dispensed to a subject may be re-dispensed to a different trial participant.

10.7 Assessment of compliance

Parents or guardians will self-report their child's adherence by keeping a treatment diary (or equivalent recording method) and reporting the number of doses taken in the monthly questionnaire. An individual child is said to be compliant if they take at least 80% of the number of IMP capsules. Non-compliance will prompt a reminder to support improving compliance going forwards. Reasons for missed doses will be collected from parents or guardians and an assessment will be made as to whether the reasoning constitutes non-compliance.

If a child is deemed non-compliant, the child will remain in the trial and followed up by the trial team at intervals via text, telephone and email to provide additional support. The site research team will monitor the completion of the monthly questionnaire to identify non-responsive participants. We understand that there can be a lot of changes in families with a young child(ren) so we will make efforts to support and empower parents to participate. Our study will have an open door approach to allow parents and guardians to resume participation after a non-responsive period.

Participants will be reminded of the dosing schedule at the monthly questionnaire. Parents or guardians will be provided with a treatment diary "treatment medication record sheet" for their optional use. This will act as both a reminder for them to take the treatment on a given day and will ask them to tick off that this was indeed done to record compliance.

10.8 Drug storage

Study drug will be stored in the clinical trials pharmacy at each site until it is dispensed. The standard temperature to store the study drug is at controlled room temperature, 15°C to 25°C. Furthermore, stability studies by OM Pharma on BV capsules at 30°C (for 5 years) and 40°C (for 6 months) have consequently shown the IMP can be used from 15°C to as high as 40°C without any impact to the

quality of the product. All temperature excursions beyond these temperatures must be reported to the BLIPA study team. OM Pharma will be informed by the trial manager.

Storage conditions, including the minimum and maximum temperatures will be monitored on a daily basis (Monday to Friday only) and according to temperature logging policy at the local site. Further details may be found in the study pharmacy manual.

Broncho-Vaxom granules (within capsules) dispensed to the parents or guardians for take-home dosing should remain in the blister packaging and stored as noted above until the point of use. Comprehensive instructions will be provided to the parent or guardian in order to ensure compliance with dosing and storage procedures.

10.9 Prescription and Dispensing of IMP(s), placebo(s)

A trial specific prescription form must be completed by the PI/delegated sub-investigator in order to prescribe the IMP. Study drug will be administered or dispensed only to parents or guardians of eligible children under the supervision of the PI/delegated sub-investigators.

10.10 Administration of IMP(s), placebo(s)

Both IMP and placebo will be supplied for the first twelve months during the baseline visit and provided once at the 12 months visit after this. Broncho-Vaxom granules are for oral use. They can be taken independently or mixed with a suitable age-related food (such as milk, yoghurt or puree). Supplementary study specific material including posters, presentations and a short video explaining IMP administration will be available to sites and participant parents/care givers. The IMP video will be available both at the baseline visit and as a link for remote access.

10.11 Destruction, return, and recall of IMP(s) and placebo(s)

Used and unused IMP prescribed to children must be returned at the patient's face to face or clinic visit and recorded on the drug accountability log for trial monitoring. Study drug (expired or remaining at end of study) should be destroyed on site for safe disposal as per the site's local policy following written sponsor permission only. Delegated pharmacy personnel will document removal and destruction on drug accountability logs. OM Pharma will be informed by the trial manager that all residual IMP has been destroyed once full drug reconciliation is complete.

Should a recall need to be completed it should be conducted in accordance with the manufacturer's procedure and Pharmacy Manual.

10.12 Dosage schedules

Broncho-Vaxom 3.5mg granules will be the dose prescribed to all children and this will not change with age or size.

Study medication will be administered for 10 days of each month for the 24 month period. The dose can be taken at any time of day. The first treatment course in month one must be started within five weeks, plus or minus one week, of hospital discharge for the initial bronchiolitis episode. Subsequent treatment courses for the remaining 23 months should be started within the first 10 days of the month.

If a dose is missed or not taken on the scheduled day, patients will be instructed not to take double

or extra doses. In the case of a missed dose, the patient must take the missed dose on the following day and extend the number of days in that treatment course according to the treatment schedule. The treatment course needs to be extended by the number of days of missed doses within the same month. Forgotten capsules can be taken up to 10 days before the next dose or 10 days after the previous dose. Therefore, there always needs to be 10 days between 10-day treatment periods.

Should a child become unwell and vomits within one hour of taking the IMP, parents and guardians should notify the research team via text or email and extend the treatment course by one day. If vomiting occurs one hour or more after administration, full absorption of the compound can be assumed, and no further action is required.

10.13 Dosage modifications and delays

There is no modification to the study drug dosage due to age or otherwise.
If the child is unable to tolerate the trial medication or there are significant laboratory AEs deemed to be related to the drug, then the drug will be discontinued as per protocol. The child will remain in trial follow-up.

10.14 Management of Broncho-Vaxom-specific adverse events

Hypersensitivity reactions should be reported to a medical professional (Primary care/A+E) and treated appropriately.

10.15 Known drug reactions and interventions with other therapies

Broncho-Vaxom drops contain methyl p-hydroxybenzoate (E 218) which may cause allergic reactions (possibly delayed).

No drug interactions are known up to now.

10.16 Recommended concurrent treatment

Not applicable.

10.17 Prohibited medication

As there are no known drug interactions, there are no impacts on other medications being prescribed or taken.

10.18 Study restrictions

There are no specific restrictions.

10.19 Management of overdose

Due to the nature of Broncho-Vaxom and the results of toxicity tests performed in animals, consequences of overdosage seems unlikely to happen. Overdose with Broncho-Vaxom did not reveal any safety concern. Lethal dose was determined to be higher than 2,000 mg/kg in mice and higher than 1,400 mg/kg in rats. Moreover, no signs of toxicity were observed after 2,000 mg/kg/day OM-85 PO administration for 6 months in rats or 100 mg/kg/day for 3 months in dogs.

10.20 ARRANGEMENTS FOR POST-STUDY ACCESS TO IMP AND CARE

As this is primarily an effectiveness study, study drug will not be available after the trial.

11 Equipment and Devices

No additional medical devices or equipment will be used in this study.

12 Pharmacovigilance

12.1 General definitions

Term	Definition
Adverse Event (AE)	Any untoward medical occurrence in a participant to whom a medicinal product has been administered, including occurrences which are not necessarily caused by or related to that product. (See section 12.6 for AEs which do not require reporting).
Adverse Reaction (AR)	An untoward and unintended response in a participant to an investigational medicinal product which is related to any dose administered to that participant. The phrase "response to an investigational medicinal product" means that a causal relationship between a study medication and an AE is at least a reasonable possibility, i.e. the relationship cannot be ruled out. All cases judged by either the reporting medically qualified professional or the sponsor as having a reasonable suspected causal relationship to the study medication qualify as adverse reactions.
Serious Adverse Event (SAE)	A serious adverse event is any untoward medical occurrence that: Results in death. Is life-threatening. Requires inpatient hospitalisation or prolongation of existing hospitalisation. Results in persistent or significant disability/incapacity. Consists of a congenital anomaly or birth defect. Other 'important medical events' may also be considered serious if they jeopardise the participant or require an intervention to prevent one of the above consequences. NOTE: The term "life-threatening" in the definition of "serious" refers to an event in which the participant was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe.
Serious Adverse Reaction (SAR)	An adverse event that is both serious and, in the opinion of the reporting Investigator or medical assessor, believed with reasonable probability to be due to one of the study treatments, based on the information provided.
Suspected Unexpected Serious Adverse Reaction (SUSAR)	A serious adverse reaction, the nature and severity of which is not consistent with the information about the medicinal product in question set out in the Reference Safety Information (RSI): In the case of a product with a marketing authorisation, in the summary of product characteristics (SmPC) for that product. In the case of any other investigational medicinal product, in the investigator's brochure (IB) relating to the study in question.

12.2 Site investigator assessment

The Principal Investigator is responsible for the care of the participant, or in their absence an authorised medic within the research team is responsible for assessment of any event for:

- **Seriousness**: Assessing whether the event is serious according to the definitions given in section 12.1.

- **Causality**: Assessing the causality of all serious adverse events/reactions in relation to the study treatment according to the definition given. If the SAE is assessed as having a reasonable causal relationship, then it is defined as a SAR.

- **Expectedness:** Assessing the expectedness of all SARs according to the definition given. If the SAR is unexpected (as per the RSI), then it is a SUSAR.

- **Severity:** Assessing the severity of the event according to the following terms and assessments. The intensity of an event should not be confused with the term "serious" which is a regulatory definition based on participant/event endpoint criteria.
    - **Mild**: Some discomfort noted but without disruption of daily life
    - **Moderate**: Discomfort enough to affect/reduce normal activity
    - **Severe**: Complete inability to perform daily activities and lead a normal life

12.3 Reference Safety Information (RSI)

Reference Safety Information (RSI) is the information used for assessing whether an adverse reaction is expected.

The IB for Broncho-Vaxom is being used in this study, the IB will be updated annually (and/or as required) by OM Pharma.

12.4 Notification and recording of Adverse Events (AEs) or Reactions (ARs)

All AE and AR's are to be documented in the child's medical notes or other source data documents and the CRF by local site staff. AES will be collected monthly via the monthly questionnaire.

Once assessed, if the AE is not defined as SERIOUS, the AE is recorded in the participant's study file and the child is followed up by the research team.

12.5 Notification of AEs of Special Interest (AESIs)

There are no AEs the manufacturer or sponsor deem to be of special interest.

12.6 Adverse events that do not require reporting

Respiratory

- Cough
- Wheeze
- Lower respiratory tract illness (LRTI)

Baby symptoms would not be required to be reported as AEs:

- teething etc
- loss of appetite
- unsettled

12.7 Adverse Events that are not unexpected

Adverse event that are not unexpected still require reporting.

Gastrointestinal

- Diarrhoea
- Abdominal pain
- Nausea and vomiting

Skin and Subcutaneous tissue disorders

- Rash (non-itchy rashes)
- Urticaria

12.8 Notification and reporting of Serious Adverse Events (SAEs) and Suspected Unexpected Serious Adverse Reactions (SUSARs)

All Serious Adverse Event (SAEs) and Suspected Unexpected Serious Adverse Reactions (SUSARs) will be recorded in the child's notes, the CRF, the sponsor SAE form and reported to the sponsor (administered by the Joint Research Management Office or agreed representative) and the IMP provider (OM Pharma) as per IMP supply agreement within 24 hours of the site becoming aware of the event (except those specified in this protocol as not requiring reporting). Parents or guardians will be instructed to notify the local research team if there is any illness or hospitalisations during the study by telephone, text or email.

Nominated co-investigators (as listed) will be authorised to sign the SAE forms in the absence of the PI at the participating sites.

Suspected Unexpected Serious Adverse Reactions (SUSARs) that occur during the study will be reported to the Sponsor (JRMO or agreed representative) and IMP provider (as per IMP supply agreement) within 24 hours of the PI or co-investigator becoming aware of the event.

12.8.1 Sponsor medical assessment

The sponsor has delegated the responsibility for oversight of IMP safety profile and medical assessment of AEs, ARs, SAEs and SUSARs to the CI as medical assessor.

The CI must review all SAEs within 72 hours of receipt. This review should encompass seriousness, relatedness, and expectedness. Day 0 for all SUSARs is when the SAE / SUSAR is received by the CI and / or coordinating team and / or sponsor (whichever is first).

It is noted that the CI cannot downgrade the PI assessment of an event's causality. If there is disagreement between CI and PI assessment, no pressure should be placed on the PI to alter their assessment, but the CI can liaise with the site PI before the CI's final decision. The CI and PI assessment can differ.

12.8.2 Procedures for reporting blinded SUSARs

As a regulatory requirement, all SUSAR reporting to the MHRA and REC should be performed in an un-blinded manner. Investigators will only receive blinded information unless un-blinded information is judged necessary for safety reasons. The site PI should only un-blind the treatment allocation during the trial if this is relevant to the safety of the subject. With regards to the Sponsor, when an event which is a SUSAR, the blind should be broken by the sponsor only for that specific child, via the online randomisation/unblinding system.

The blind should be maintained for persons responsible for the ongoing conduct of the study (such as the site PI and sub-investigators, research nurses, study co-ordinators, data managers, pharmacists) and those responsible for data analysis and interpretation of results at the conclusion of the study, such as the statistician. Un-blinded information should only be accessible to those who need to be involved in the safety reporting to national competent authorities, Ethics Committees and IDMC, or other persons performing ongoing safety evaluations during the trial.

The CI, as sponsor medical assessor, will assess the event blinded for all possible IMPs, placebos, and combinations.

12.9 Urgent safety measures

The CI may take urgent safety measures to ensure the safety and protection of the clinical study participants from any immediate hazard to their health and safety, in accordance with Regulation 30 of the Medicines for Human Use (Clinical Trials) regulations. The measures should be taken immediately. In this instance, the approval of the Competent Authority prior to implementing these safety measures is not required. However, it is the responsibility of the CI to attempt, where possible, to discuss the proposed change with the sponsor and Medical Advisor at the MHRA (via telephone) prior to implementing the change if possible.

The CI has an obligation to inform both the MHRA and Research Ethics Committee in writing **within 3 days** of implementing the Urgent Safety Measure. They must also submit a substantial amendment documenting the changes with 14 days of implementing the urgent safety measure. The JRMO must be sent a copy of the correspondence with regards to this matter as soon as it is sent.

13 Annual reporting

13.1 Development Safety Update Report (DSUR)

The DSUR will be written by the CI (following Sponsor procedures) and submitted to the sponsor for review prior to submission to the MHRA. The DSUR is due for submission within 60 days of the end of the reporting period. The reporting period is annually from the date on the "*Notice of acceptance letter*" from the MHRA. The sponsor's delegated Medical Assessor, usually the CI, will carry out a risk benefit analysis of the IMPs encompassing all events having arisen on the study. REC will be sent a copy of the DSUR.

13.2 Annual Progress Report (APR)

The APR will be written by the CI (using the HRA's template) and submitted to the sponsor for review prior to submission to the REC. The APR is due within 30 days of the anniversary date of the "favourable opinion" letter from the REC.

14 Statistical and data analysis

14.1 Sample size calculation

Since Razi et al, in a secondary prevention study, reported a 38% reduction in wheeze episodes with oral BV, our effect size is based on a reduction of 40% of the number of children developing parent-reported doctor-confirmed wheeze (22% to 13.2%) [13]. To detect this difference with 90% power at the 5% significance level, we need a total sample size of N=778 children (calculated in Stata using the ssi command). We will recruit 894 children in total from the UK and Australia, which allows for 116 (13%) to be lost to follow-up.

We chose a 40% reduction in the development of wheeze after 24 months of treatment since;

1. our parent panel regarded this to be an important and meaningful effect that justifies treating some children who are not going to develop asthma.
2. a 40% reduction is compatible with the effect reported in several therapeutic studies of BV. First, as mentioned above, Razi et al demonstrated a 38% reduction in wheezing attacks in the BV-treated group of infants [13]. Second, Esposito et al in a retrospective matched analysis of children receiving oral BV for 2 years reported a 50% reduction in risk of new respiratory tract episodes [24]. Third, Gutiérrez-Tarango et al reported a 37% reduction in acute respiratory tract infections in a trial of children 1 to 12 years of age who were randomized to receive either OM-85 BV or placebo at the beginning of the trial, and then at 6 months later using the same schedule [30].
3. a 58% reduction in development of wheeze reported for raw milk in the recent meta-analysis of Brick et al, and a 40% reduction is compatible with effect of asthma therapies in active preschool wheeze [31]. The Prevention of Asthma in Kids (PEAK) Trial 45 randomised children 2 to 3 years of age without wheeze to either daily inhaled fluticasone propionate or placebo for 2 years. After 2 years, study therapy was discontinued, and children were followed for an additional year to determine wheeze. Although the proportion of episode-free days during the 3rd year of the study did not differ between treatment groups in the PEAK trial (i.e. there was no disease modifying effect of inhaled steroids), during active treatment inhaled steroid-treated children had a 34% reduction in wheeze exacerbations [32].

14.2 Planned recruitment rate

Power calculations are shown in section 14.1 above and an overview of recruitment is also covered in section 3.

Recruitment will take place over an 18-month period with particular focus on the two winter seasons within this timeframe. Winter hospital admissions for bronchiolitis in children in the 3 to 12 months age range for the five UK study sites are; n= 406 for Barts Health, n=229 for King College, n=396 for Edinburgh, n=153 for Aberdeen and n=263 for Southampton; total n=1,447.

With recruitment continuing over a Summer/Spring season and two winter seasons gives a conservative estimate of n=2894. With a sample size of 894, the recruitment rate is therefore 31%.

In the winter seasons, for the months September through to February we assume a realistic recruitment rate of 17 patients at each of the five UK sites. For the months March through to August we assume a recruitment rate of one child per site.

14.3 End of trial (EOT) definition

EOT is defined as three months after the Last Participant Last Visit (LPLV).

The CI is delegated the responsibility of submitting the EOT notification to REC and MHRA once reviewed by the sponsor. The EOT notification must be received by the REC and MHRA within 90 days of the end of the study. If the study is ended prematurely, the Chief Investigator will notify the Sponsor, REC, and MHRA within 15 days, including the reasons for the premature termination.

14.4 Statistical Analysis Plan

To maintain blinding for any interim reports an independent statistician will prepare any information which requires knowledge of treatment allocations.

A full detailed statistical analysis plan will be developed prior to final analysis. The purpose of this document is to provide details of the statistical analyses and presentation of results to be reported within the principal paper(s) of the trial. Any exploratory, post hoc or unplanned analysis will be clearly identified as such in the respective study analysis report.

The trial statisticians conducting statistical analysis work will be blinded in relation to allocation status until the SAP is signed off, all follow up data is collected, and data cleaning has occurred.

Site has been used as a stratification in the randomisation, Site will be accounted for in all analyses by including site as a random effect. A fixed effect for the stratification factor of paternal asthma will be included in all analyses.

For the analysis of each outcome, the following information will be presented:

- The number of patients included in each analysis, by treatment group.
- A summary statistic of the outcome (e.g. mean (SD), number (%), by treatment group
- The estimated treatment effect
- A 95% confidence interval for the estimated treatment effect
- A two-sided p-value

Statistical significance will be assessed at the 5% level.

14.5 Summary of baseline data and flow of participants

Baseline characteristics and clinical data will be summarised by treatment group. Numbers (%) for categorical variables and means (SD) or medians (IQR) for continuous variables as appropriate will be provided separately for each group. Participant flow through the trial will be illustrated by a CONSORT flow diagram [33].

14.6 Analysis of participant populations

The intention-to-treat population consists of all randomised children with a recorded outcome analysed according to the treatment to which they were randomised.

14.7 PRIMARY OUTCOME ANALYSIS

The primary analysis will be conducted on the ITT population.

The primary outcome of parent-reported, healthcare professional-confirmed, wheeze between 19 and 24 months will be analysed using a mixed-effect logistic regression model. The magnitude of the treatment effect will be reported as an odds ratio with a 95% confidence interval. Significance will be set at $p<0.05$.

Only site and parental asthma will be accounted for in the analysis, no other important covariates have been identified. Should any variables of interest be identified in the scientific literature during the course of the trial their analysis will be considered exploratory and detailed in the SAP.

The primary analysis will be repeated on the compliant population, defined as those who take at least 80% of the assigned medication over the 24 months of treatment, evidenced by the parent-reported monthly questionnaire.

14.8 SECONDARY OUTCOME ANALYSIS

14.8.1 Secondary Outcomes

The analysis of secondary outcomes will be conducted on the ITT population. Binary outcomes will be analysed using a mixed effects logistic regression model and will be presented as an odds ratios (95% CI). Count outcomes will be analysed using a mixed effects negative binomial regression model to account for overdispersion and will be presented as a rate ratio (95% CI). Time to event outcomes will be analysed using a frailty model and will be presented as a hazard ratio (95% CI).

14.9 MECHANISTIC OUTCOME ANALYSIS

14.9.1 Blood Markers

Variations in atopy cell markers will be examined by appropriate statistical methods.

14.10 SAFETY ANALYSIS

Safety outcomes will be reported descriptively and will include the number and proportion of children experiencing an SAE or adverse event, summarised by treatment group and relationship to IMP.

14.11 SUBGROUP ANALYSES

No subgroup analyses have been planned. Should any subgroups of interest be identified in the scientific literature during the course of the trial their analysis will be considered exploratory and detailed in the SAP.

14.12 INTERIM ANALYSIS AND CRITERIA FOR THE PREMATURE TERMINATION OF THE STUDY

To maintain blinding all unblinded analyses for the DMC will be performed by an independent statistician who is not otherwise involved in the trial.

An interim analysis for safety will be done if requested by the DMC, the DMC will be made aware of any Suspected Unexpected Serious Adverse Reactions (SUSARs) and Severe Adverse Events (SAEs).

14.13 PROCEDURE(S) TO ACCOUNT FOR MISSING OR SPURIOUS DATA

The primary analysis will not account for missing data. Sensitivity analyses around missing data assumptions will be detailed in the SAP.

15 Data handling and record keeping

15.1 SOURCE DATA AND SOURCE DOCUMENTS

Study data will be captured in source documents such as the hospital medical notes, and study-specific source document questionnaires. Data will be entered into the electronic case report form (eCRF) by delegated members of the study team at each site (paper CRFs will be used as a backup if required).

All parent (or guardian) reported data such as study questionnaire CRFs, will constitute as source data for the study.

Source documents will likely contain personal identifiable data and therefore will be stored securely at investigative sites and will not leave Trust premises. All documents will be stored safely in confidential conditions.

Sites that use electronic source (e-source) data should ensure to provide access to e-source systems and database(s) to the BLIPA monitor (and all other authorised personnel) at onsite visits. It is the site's responsibility to maintain these e-source databases, to ensure that they are GCP and MHRA guidelines compliant and provide a suitable audit trial, and that systems are in place to demonstrate that the PI at site has clinical oversight of e-source data. Printouts from e-source data must be documented to be verified copies, dated and signed.

Direct access will be granted to authorised representatives from the sponsor, host institution, and the regulatory authorities to permit study-related monitoring, audits, and inspections.

15.2 CASE REPORT FORMS (CRFS)

The trial data will be captured electronically in a bespoke eCRF and database system. The system will be designed and developed by PCTU in accordance with its own SOPs and the Sponsor's SOP 38b "Trial Data Management Systems", with input from the CI and study team.

A database specification and data management plan will be written and agreed prior to the development of the database to ensure only data required in the protocol is captured in the CRF. The data management plan will also cover all aspects of managing the data such as, the CRF design, the data management systems, data entry, data checking, secure integration of pharmacy data, query management and cleaning, data transfer, quality control procedures, data extractions, database freeze and lock. Once built, the database will undergo validation including user acceptance testing. Once testing has been completed and the system has been approved, a test report will be documented. The database will be hosted on a secure server within Queen Mary and secure backups of the trial data will also be maintained.

Access to the database will be by password-protected user accounts to prevent unauthorised access, and the database will be encrypted at rest. The CI will have overall responsibility for the data stored

within the database. Data will be entered into the eCRF by delegated study team members. Further detail on data management may be found in the study data management manual.

CRFs will be pseudo-anonymised using a participant identification code allocated at time of recruitment. This code will consist of the trial site number followed by the consecutive recruitment number starting at 001, for example 01-001. A separate screening identification code will be used prior to a child's enrolment in the trial. This screening code will consist of the trial site letters followed by the consecutive screening number starting at 0001, for example ABC-0001.

15.3 Data capture

Data will be recorded from a variety of sources including parent (or guardian)-reported information, physical examination findings, results from the primary care records, and questionnaires. All efforts will be made to maximise completeness of data, e.g. telephoning parents or guardians to obtain missing data. All interactions with parents and guardians will be documented (including telephone conversations and emails) and filed within the child's study file. Questionnaires will be completed by parents or guardians and every questionnaire should be dated and signed by the person completing it (if they complete the paper version). The PI/delegate will keep records of all children (sufficient information to link records e.g., CRFs, hospital records and samples), all original signed informed consent forms and copies of the CRF pages. Further detail on data management may be found in the data management plan.

15.4 Transferring and transporting data

All paper records of person-identifiable information will be kept securely on site. Local research teams and sites will be reminded not to send or store any person-identifiable information on any portable device, for example laptops, memory sticks, recording devices, CD/DVDs, unless it is encrypted. Where study data need to be transferred or stored outside of sites no personal identifiable information will be used; only the participant identification code will be used.

15.5 Data Management

Data management for BLIPA-UK will be undertaken by PCTU at Queen Mary. Standard operating procedures will be in place for the collection and handling of data received at the Unit. All study data will be entered into a database by appropriately trained staff with restricted access. Data collected on the various CRFs will be stored in an electronic database in which the participant will be identified by a unique trial number. The database validity and quality can be ensured and monitored by validation and audit trails.

Electronic storage is on a restricted area of a file server. The server is in a secure location and access is restricted to a few named individuals. Access to the building in which the Queen Mary server is situated is via an electronic tag and individual rooms are kept locked when unoccupied. Data will be processed on a workstation by authorised staff. The workstations access the network via a login name and password. No data are stored on individual workstations. Database data is stored on secure servers and has validated procedures in place to ensure data security, back-up and disaster recovery.

The Chief Investigator will ensure that this information is kept confidential. All documents will be stored securely and kept in strict confidence in compliance with the GDPR and Data Protection Act 2018.

Direct access will be granted to authorised representatives from the Sponsor, host institution and the regulatory authorities to permit trial-related monitoring, audits and inspections.

16 Confidentiality

The Chief Investigator will be the data custodian for all data generated during the study.

The Chief Investigator and the study team will ensure that all children's identities are protected at every stage of the study. To ensure this, at time of consent each child will be allocated a unique screening number by the local team before undergoing any screening procedures.

The Principal Investigator is responsible for protecting the identity of children at their site. Children will be referred to only by their unique study identifier whenever data is transferred outside of the site, and in all correspondence between the site and the coordinating centre, co-investigators, sponsor, or anyone associated with the study.

No children will be individually identifiable from any publications resulting from the study.

Information regarding study children will be kept confidential and managed in accordance with the GDPR and Data Protection Act (2018), the Research Governance Framework for Health and Social Care and Research Ethics Committee approval. All study data will be stored in line with the Medicines for Human Use (Clinical Trials) Regulations 2004 and subsequent amendments and the Data Protection Act. Study data will be archived in line with the Medicines for Human Use (Clinical Trials) Regulations 2004 and all subsequent amendments, and as defined in the JRMO SOP 20 Archiving.

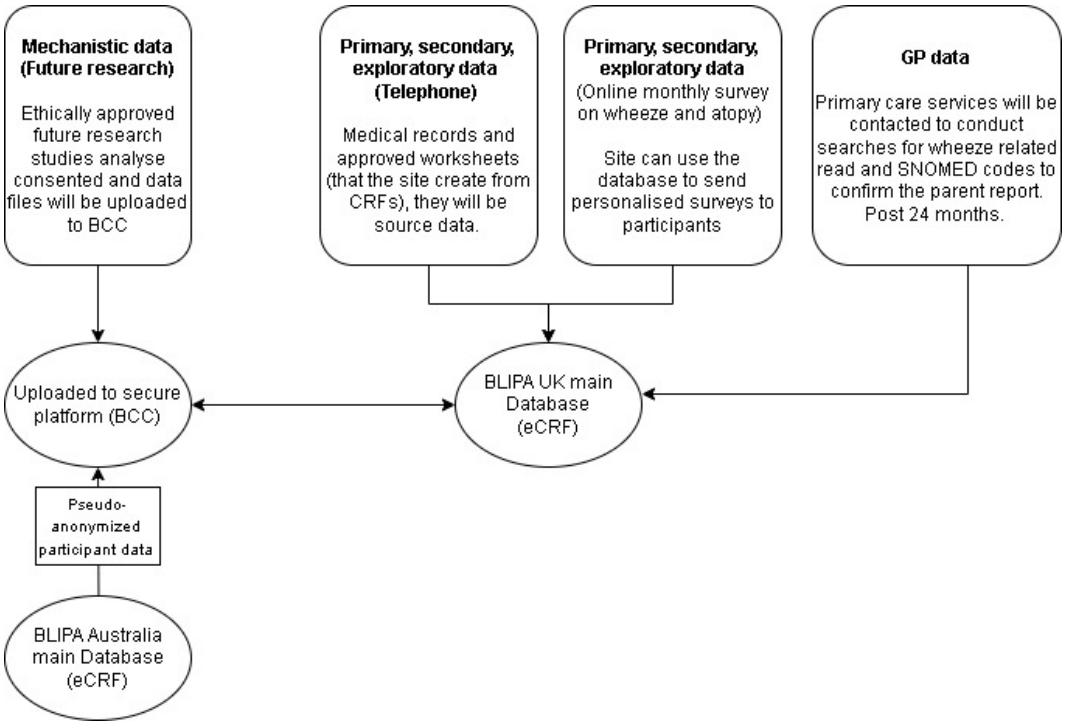


Figure 3: Study data flow between sites, PCTU and laboratories

16.1 De-identification of participants

Locally, children will be identified by their NHS and hospital number. For screening, a unique screening identifier will be used as a means of pseudo-anonymising parameters. This will consist of the local trial site letters followed by a consecutive screening number starting at 0001, for example ABC-0001. This information will be kept on a screening log, which should be updated accordingly throughout the study. Once the participant has completed screening procedures and is enrolled onto the study, the participant will be allocated a unique participant identification number from the enrolment log. The unique participant identification number can only be de-coded at the hospital.

All data will be pseudo-anonymised using a unique identifier before it is used or transferred to the site. Consent form for each child will be collected and recorded locally at the recruitment site. We will collect the child's age in years and months only, no full date of birth.

The participant identification code will be a unique identifier composed of two parts. The first part will be made up of two numbers referring to the recruitment site e.g. 01. The second part will be made up of a series of consecutive numbers, e.g. the first enrolled subject may be allocated the subject number 001, the second subject enrolled, subject 002, etc. Thus, all study documents (apart from those source documents which by default contain identifying data, such as hospital medical notes, laboratory results, etc.), including the case report forms and study database will be labelled with site number and participant number, e.g. 01-001.

This unique participant identification code will be assigned by the PI/delegate once the child is enrolled in the study. This information will be kept in an enrolment log, which will be kept updated throughout the study, and will be stored in the investigator site file (ISF) with access limited to members of the study team and authorised individuals. The unique participant identification code (site numbers and participant number) will be used to randomise the child into a treatment group using the study online randomisation system. All data will be anonymised before it is used or sent/transferred from the site. Only the participant identification code will be used. The unique participant code will be used to link BLIPA participants and their data with consented samples taken for future research.

17 Monitoring, Audit, and Inspection

17.1 Monitoring

A Trial Monitoring Plan will be developed and agreed by the sponsor and Chief Investigator based on the sponsor's risk assessment, which will include on site monitoring. Monitoring procedures are detailed in the Trial Monitoring Plan.

17.2 Auditing

The sponsor retains the right to audit any aspect of the study, study sites, or central facilities. In addition, any part of the study may be inspected by the regulatory bodies, and funders where applicable.

All sites and vendors are asked to inform the sponsor if notified of any Audit or inspection affecting this study.

18 Compliance

The CI will ensure that the protocol and study is conducted in compliance with the principles outlined in the Medicines for Human Use (Clinical Trials) Regulations 2004 and subsequent amendments, current UK Policy Framework for Social and health care research (2017), GCP guidelines, the World Medical Association Declaration of Helsinki, the Sponsor's and study specific SOPs, and other regulatory requirements.

The study will not commence until sponsor permission to activate sites is received.

Sites will be individually activated by the CI and team; this will not occur until site approval is granted.

18.1 Non-Compliance

Prospective, planned deviations or waivers to the protocol are not allowed under the UK regulations on Clinical Trials and must not be used (i.e. it is not acceptable to enrol a participant if they do not meet the eligibility criteria or restrictions specified in the study protocol).

Accidental protocol deviations can happen at any time. They must be adequately documented on the relevant forms and reported to the Chief Investigator and Sponsor immediately. Deviations from the protocol which are found to frequently recur are not acceptable, will require immediate action and could potentially be classified as a serious breach. Systematic failure of both the CI and the study staff adhering to SOPs/protocol/ICH-GCP and UK regulations, which leads to prolonged collection of deviations, breaches or suspected fraud.

Non-compliances may be captured from a variety of different sources including self-reporting, monitoring visits, CRFs, communications and updates. The CI and the study team will maintain a log of the non-compliances to ascertain if there are any trends developing which need to be escalated. CI and the study team should assess the non-compliances and action a timeframe in which they need to be dealt with. This assessment should include the need to escalate to the sponsor. Any event with the potential to affect participant safety or data integrity should be reported to the sponsor within 24 hours of the Site, CI or Coordinating team becoming aware.

Where applicable corrective and preventative actions (CAPA) should be assigned. Each action will be given a different timeframe dependent on the severity. If the actions are not dealt with accordingly, the Sponsor will agree an appropriate action, including an on-site audit.

18.2 Notification of Serious Breaches to GCP and/or the protocol

A 'serious breach' is a breach which is likely to affect to a significant degree:

- The safety or physical or mental integrity of the participants of the study; or
- The scientific value of the study.

The site Principal investigator is responsible for reporting any potential serious breaches to the sponsor (research.safety@qmul.ac.uk) within **24 hours** of becoming aware of the event.

The Chief Investigator is responsible for reporting any potential serious breaches to the JRMO **within 24 hours** of becoming aware of the event.

The sponsor is responsible for determining whether a potential serious breach constitutes a serious breach and will work with the CI to investigate and notify and report to the MHRA and REC (as applicable) within 7 working days of becoming aware of the serious breach.

19 Declaration of interests

The CI, PIs at each site, and committee members for the overall study management, will provide detail on:

1. *All competing interests.*
2. *Ownership interests that may be related to products, services, or interventions considered for use in the study or that may be significantly affected by the study.*
3. *Commercial ties (e.g. pharmaceutical, behaviour modification, and/or technology companies).*
4. *Non-commercial potential conflicts (e.g. professional collaborations that may impact on academic promotion).*
5. *These will be held within the Trial master file. Please address enquiries to*
6. *The sponsor requires all study committee members complete competing interest declarations.*

20 Peer review

The protocol will be reviewed and approved by the funder and sponsor.

The study has been funded by the NIHR through a competitive grant process and has undergone independent peer review.

21 Public and Patient Involvement (PPI)

Parents and guardians of children admitted to hospital with Bronchiolitis were involved in the trial's initial design and in the development of the full grant application.

For the trial itself, we will recruit a panel of up to 10 parents whose children have previously been admitted with bronchiolitis and invite at least one to be a member of the Trial Steering Committee (TSC) and Data Monitoring Committee. The parent panel will be invited to face to face meetings held in the different recruitment centres – with a Zoom/Skype option. We will offer training to parents on randomised clinical trials and PPI.

22 Indemnity/ Insurance

The insurance that Queen Mary University of London has in place provides cover for the design and management of the study as well as "No Fault Compensation" for participants, which provides an indemnity to participants for negligent and non-negligent harm.

23 Study committees

BLIPA-UK and BLIPA-Australia will have the following joint trial oversight committees: Trial Management group (TMG), Trial Steering Committee (TSC) and Data Monitoring Committee (DMC). During the study the UK and Australia Data Monitoring Committees to be able to review summary data from both trials to make informed recommendations to the respective sponsors about stopping recruitment and about the safety of the participants.

In addition, the BLIPA study (which incorporates the BLIPA-UK and BLIPA-Australia trials) will have an overarching Study Management Group that includes representatives of both TMGs.

23.1 Trial Management Group (TMG)

The Trial Management Group (TMG) will meet regularly (in person or virtually) throughout the study to discuss all aspects of trial progression including but not limited to; site set up, recruitment, data collection and study milestones and any issues that may impact these. These individuals will also be invited to attend the steering committee meetings.

The TMG will be primarily composed of the CI, trial manager, trial statistician and researchers.

23.2 Trial Steering Committee (TSC)

The Trial Steering Committee (TSC) will comprise of several independent members, one of which will be allocated as chair. The CI, trial manager and statistician will also attend these meetings.

The TSC will meet regularly (in person or virtually) to review safety data, the progress of the trial, adherence to the protocol and consider new information of relevance to the research question. The TSC will be expected to provide advice, through its chair, to the CI, Sponsor, Funder, Host Institution and the Contractor on all appropriate aspects of the trial.

Further detail on the membership and full remit of this committee may be found in the TSC Charter.

23.3 Data monitoring committee (DMC)

The Data Monitoring Committee (DMC) will comprise of three independent members, one of which will be allocated as chair. The CI, trial manager and statistician will also attend the open sessions of these meetings.

The DMC will meet regularly (in person or virtually) to assess safety data, data quality, recruitment figures, compliance and provide advice to the TSC.

Further detail on the membership and full remit of this committee may be found in the DMC Charter.

24 Publication and dissemination policy

24.1 Publication

Trial data may be presented and published nationally and internationally. Only fully anonymised results will be presented and published. Publication of the final study results will be in a peer-reviewed journal. Responsibility for ensuring accuracy of any publication from this study is delegated to the CI. The sponsor retains the right to review all publications prior to submission or publication. All publications will be sent to the JRMO prior to publication.

A copy of any proposed disclosure will be provided to OM Pharma for review, as per the IMP Supply Agreement.

The full study report will be accessible via EudraCT or other suitable public website within one year of the End of the Trial Notification.

24.2 Dissemination policy

The data arising from the study will be under the ownership of the Sponsor. However, any confidential or proprietary information in relation to the IMP will remain under the ownership of OM Pharma. The Sponsor, Funder and OM Pharma will be acknowledged in any presentation or publication of the study data.

On completion of the trial, the data will be analysed and tabulated and a final study report will be prepared. The full study report will be accessible via EudraCT. Contributing centres (and participating investigators) will be acknowledged in the final manuscript.

Participants will be advised that they should contact the study team if they wish to be informed of the study results.

The results will be published in high impact and specialist journals and will be presented at international meetings dealing with the environment and disease. In line with the 'NIHR policy on clinical trial registration and disclosure of results' the results of BLIPA will be published in a peer-reviewed Open Access journal.

We also intend on communicating with politicians and learned societies directly e.g. the Royal College of Paediatrics and Child Health. We will disseminate our findings more widely to the public community through public engagement events. We will also engage with health services via our links with the Royal Colleges, Asthma UK, the British Lung Foundation, and our direct link with the Asthma UK Centre for Applied Research jointly based at QMUL and Edinburgh.

24.3 Access to the final study dataset

The CI, TSC and DMC will have access to the final trial dataset. Additionally, and subject to compliance with applicable data protection laws and regulations, the sponsor shall provide OM Pharma, its affiliates or their designated business partners with access to the study data, in an anonymized or pseudonymized form, as required and agreed. For the avoidance of doubt, this shall in no way be interpreted as an obligation for the sponsor to provide, or a right for OM Pharma to be provided with any medical records or any other individually identifiable patient information (such as name, address, etc.).

25 Archiving

During the course of the research, all records are the responsibility of the Chief Investigator and will be kept in secure conditions. When the research study is complete, it is a requirement of the Barts Health Policy that the records are kept for a further 25 years.

Site files from other sites must be archived for 25 years at the external site and will not be stored at the Barts Health Modern Records Centre or within Queen Mary.

Destruction of essential documents will require authorisation from the Sponsor.

Once the study is completed, the data collected over the trial period will be archived by QMUL using Arkivum, who are contracted to provide archiving solutions.

During the course of research, all records are the responsibility of the Principal (site) Chief (coordination) Investigator and must be kept in secure conditions. Once all Sites have been closed and documentation archived, it is a requirement of the Sponsor Policy that the records are kept for a further 25 years.

Archiving of CI and Coordinating documentation will be authorised by the sponsor following the submission and publication of the end of study report.

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