BALLOON

Bacterial mucosal immunotherapy for prevention of lower respiratory tract infections in preterm born infants

A RANDOMISED, DOUBLE-BLIND, PARALLEL GROUP, PLACEBO CONTROLLED, TRIAL OF BACTEK FOR THE PREVENTION OF LOWER RESPIRATORY TRACT INFECTIONS IN PRETERM INFANTS

PROTOCOL VERSION 1.3 DATED 10.12.2024

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SIGNATURE PAGE

The undersigned confirm that the following protocol has been agreed and accepted and that the Chief Investigator agrees to conduct the trial in compliance with the approved protocol and will adhere to the principles outlined in the relevant trial regulations, GCP guidelines, and CTR's SOPs.

I agree to ensure that the confidential information contained in this document will not be used for any other purpose other than the evaluation or conduct of the clinical investigation without the prior written consent of the Sponsor.

I also confirm that I will make the findings of the trial publicly available through publication or other dissemination tools without any unnecessary delay and that an honest accurate and transparent account of the trial will be given; and that any discrepancies from the trial as planned in this protocol will be explained.

Trial Sponsor:			
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Natalie Richards	**Research Integrity & Governance Officer**	E-signature	**16/12/2024**
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Chief Investigator:			
Professor Sailesh Kotecha			
Name	**Signature**	**Date**	
Sailesh Kotecha	**E-signature**	**16/12/2024**	

General Information This protocol describes the BALLOON clinical trial and provides information about the procedures for entering participants into the trial. The protocol should not be used as a guide, or as an aide-memoire for the treatment of other patients. Every care has been taken in drafting this protocol; however, corrections or amendments may be necessary. These will be circulated to the known Investigators in the trial. Problems relating to the trial should be referred, in the first instance, to CTR

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Trial Co-ordination:

The BALLOON trial is being coordinated by the Centre for Trials Research (CTR) Cardiff University, a United Kingdom Clinical Research Collaboration (UKCRC) registered trials unit.

This protocol has been developed by the BALLOON Trial Management Group (TMG).

For **all queries** please contact the BALLOON team through the main trial email address. Any clinical queries will be directed through the Trial Manager to either the Chief Investigator or a Co-Investigators

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Randomisations:

Randomisation
Online randomisation is through the Trial Randomisation Database: https://w3.abdn.ac.uk/hsru/BALLOON **(See section 9.4 for more details).**

Clinical queries
BALLOON@Cardiff.ac.uk **All clinical queries will be directed to the most appropriate clinical person.**

SAE reporting
Where the adverse event meets one of the serious categories, an SAE form should be completed by the responsible clinician and submitted to CTR within 24 hours of becoming aware of the event (See section 16 for more details). **Contact: ctr-safety @cardiff.ac.uk**

Table of Contents

Glossary of abbreviations

AE	Adverse Event
AR	Adverse Reaction
CI	Chief Investigator
CRF	Case Report Form
CTA	Clinical Trials Authorisation
CTIMP	Clinical Trial of Investigational Medicinal Product
CTR	Centre for Trials Research
CTU	Clinical Trials Unit
DSUR	Development Safety Update Report
EHR	Electronic Health Records
GCP	Good Clinical Practice
GDPR	General Data Protection Regulations
GMP	Good Manufacturing Practice
GP	General Practitioner
IB	Investigator Brochure
ICH	International Conference on Harmonization
IDMC	Independent Data Monitoring Committee
IMP	Investigational Medicinal Product
IMPD	Investigational Medicinal Product Dossier
ISF	Investigator Site File
ISRCTN	International Standard Randomised Controlled Trial Number
LRTI	Lower Respiratory Tract Infection
MedDRA	Medical Dictionary for Regulatory Activities
MHRA	Medicine and Healthcare products Regulatory Agency
MRC	Medical Research Council
NHS	National Health Service
NIMP	Non-Investigational Medicinal Product
PI	Principal Investigator
PIAG	Participant Information Advisory Group
PIS	Participant Information Sheet
QA	Quality Assurance
QL (QoL)	Quality of Life
QP	Qualified Person

R&D	Research and Development
RCT	Randomised Controlled Trial
REC	Research Ethics Committee
SAE	Serious Adverse Event
SAR	Serious Adverse Reaction
SmPC	Summary Product Characteristics
SOP	Standard Operating Procedure
SUSAR	Suspected Unexpected Serious Adverse Reactions
TMF	Trial Master File
TMG	Trial Management Group
TSC	Trial Steering Committee

1 Amendment History

The following amendments and/or administrative changes have been made to this protocol since the implementation of the first approved version.

Amendment No.	Protocol version no.	Date issued	Summary of changes made since previous version
Response to MHRA and ethics initial submission	1.1	18.07.2024	1. Version number and date updated 2. Exclusion criteria updated to include primary immune deficiencies 3. Inclusion criteria updated to include specific common exemplars 4. Dose rationale. 5. Safety run in period 6. Composition of IDMC
1	1.2	14.08.2024	1. Version number and date updated 2. REC and ISRCTN ref added 3. Update to note parents will return swabs directly to the virology laboratory at PHW 4. Bactek and placebo provided free of charge 5. Timing of randomisation 6. IS systems 7. Update to statistical section 8. Update to primary and secondary outcomes and objectives . 9. Update to study aim. 10. Update to timing of pilot and interim analysis. 11. Removal of sentinel dosing 12. Update to withdrawal section
2	1.3	10.12.2024	1. Addition of randomisation information 2. IS system update

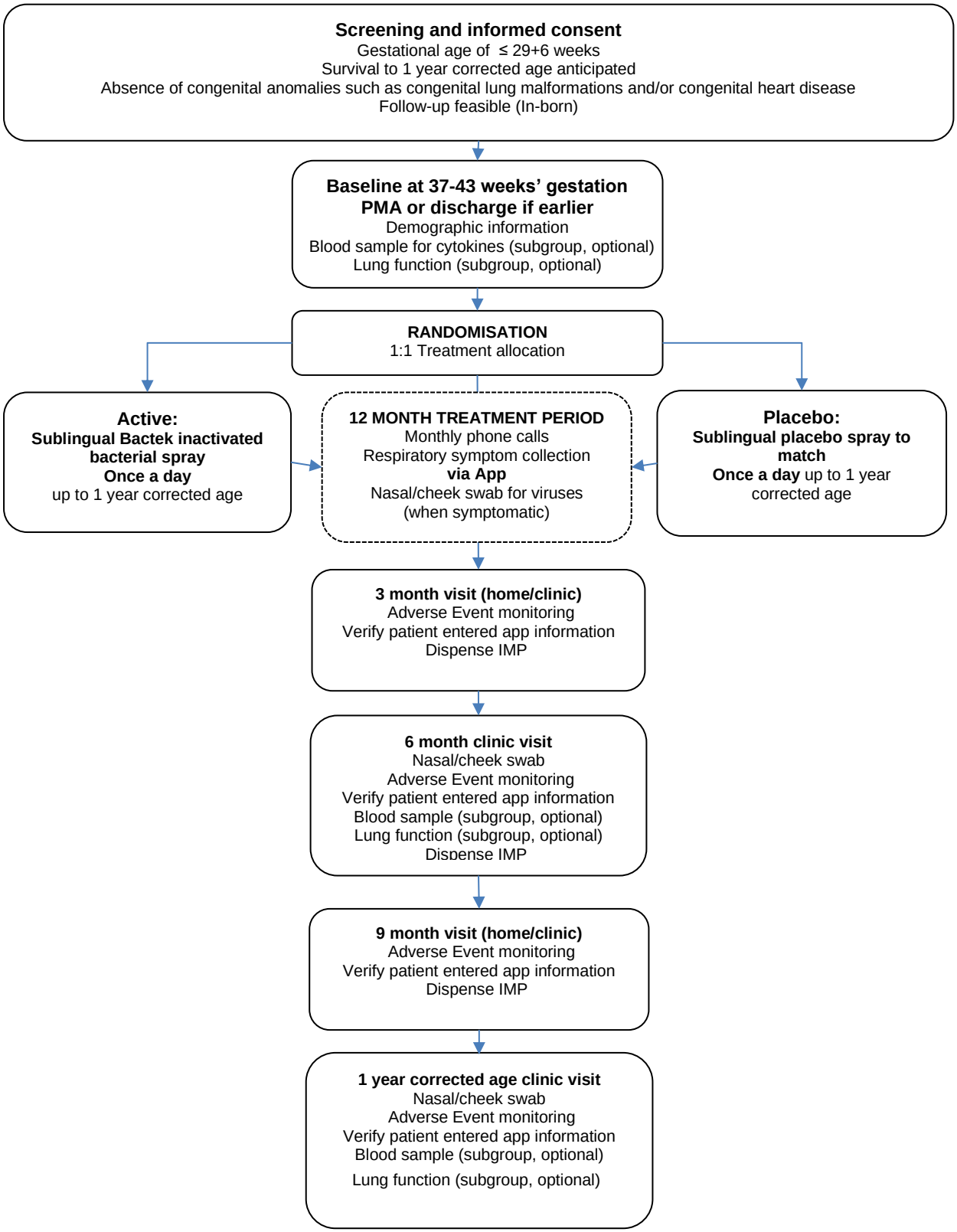
2 Synopsis

Short title	Bacterial mucosal immunotherapy for prevention of lower respiratory tract infections in preterm born infants
Acronym	BALLOON
Internal ref. no.	SPON 1939-23
Clinical phase	III
Funder and ref.	NIHR156651
Trial design	Double blind, multicentre, randomised, placebo-controlled
Trial participants	Infants born ≤29+6 weeks' gestational age
Planned sample size	542
Planned number of sites	12
Inclusion criteria	• Birth at gestational age ≤29+6 weeks' • Follow-up is likely to be feasible • Survival to one year corrected age is anticipated • In addition to infants without an eventful course, the following groups are eligible for inclusion: • Infants diagnosed with bronchopulmonary dysplasia • Infants discharged home on oxygen • Infants who have had necrotising enterocolitis • Infants on oral or nasogastric tube feeding • Infants with treated patent ductus arteriosus • Infants with neurological disorders • Infants with resolved sepsis This list is not exhaustive and others can be discussed with the PI in the first instance and trial team if necessary.
Exclusion criteria	• Presence of major surgical, cardiac or congenital abnormalities (not including patent ductus arteriosus or patent foramen ovale) • Contraindication of Bactek™ as specified in the Investigator's Brochure • Participation in other interventional trial that precludes participation in BALLOON • Primary immune deficiencies
Treatment duration	From randomisation, up to one year corrected age
Follow-up duration	30 days following completion of IMP
Planned trial period	36 months (two years recruitment, one year to complete all follow up)
Primary objective	To investigate if sublingual Bactek spray, when compared to placebo, decreases the risk of health professional diagnosed LRTIs (after unscheduled visits to general practitioners (GPs), accident and emergency departments (A&Es) and hospital admissions) between term-equivalent 37-43 weeks' gestation or discharge if earlier and one year of corrected age.
Secondary objectives	• To establish if sublingual Bactek spray is superior to placebo in decreasing the number of health professional diagnosed LRTIs between term-equivalent 37 weeks' gestation or discharge if earlier and one year of corrected age. • To establish if sublingual Bactek spray is superior to placebo in increasing the

	time to first health professional diagnosed LRTI between term-equivalent 37 weeks' gestation or discharge if earlier and one year of corrected age. • To establish if sublingual Bactek spray is superior to placebo in preventing parent-reported, health professional confirmed, wheezing from 37-43 weeks' corrected gestation or discharge if earlier to one year of corrected age in infants born at ≤29+6 weeks' gestational age. • To compare the use of respiratory medications including inhalers (bronchodilators, inhaled corticosteroids), antibiotics and systemic corticosteroids between the two groups. • To investigate the effect of Bactek on growth (weight, length and head circumference) between the two groups. • To establish effects of LRTI on the family, including time missed from work and/or nursery time missed for the infants. • To explore differential intervention effects by gestational age and anti-RSV prophylaxis. • To assess safety (volume of serious adverse events).
Primary outcome	The presence/absence of parent-reported, health professional-confirmed unscheduled visits for LRTIs to GPs, A&Es and hospital admissions between 37-43 weeks' corrected age (or discharge if earlier) and one-year corrected age.
Secondary outcomes	• Number of parent-reported, health professional-confirmed unscheduled visits for LRTIs to GPs, A&Es and hospital admissions between 37 weeks' corrected age (or discharge if earlier) and one-year corrected age. • Time to first episode of parent-reported, health professional-confirmed unscheduled visits for LRTIs to GPs, A&Es and hospital admissions between 37-43 weeks' corrected age (or discharge if earlier) and one-year corrected age. • Parent-reported wheeze episode between 37-43 weeks' corrected age or discharge if earlier and one-year corrected age. Wheeze episode is defined as an episode of wheezing: o 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. • To establish effect of Bactek on growth between the two groups • Parent-reported use of respiratory medications including bronchodilators, antibiotics and systemic corticosteroids • Parent(s)/guardian(s) reported time missed from work and/or nursery time missed for the infant • Volume of adverse reactions • Identification of virus(es) associated with LRTIs
Investigational medicinal products	Bactek (MV130) or placebo
Form	Metered-dose actuation spray pump
Dose	300 FTU/mL (2x 150 FTU/mL sprays), once daily, from 37-43 weeks corrected age or discharge if earlier up to 1 year of corrected age.
Route	Sublingual

3 Trial summary & schema

3.1 Participant flow diagram



3.2 Trial lay summary

BACKGROUND

What is the problem?
Babies born early or prematurely have under-developed lungs. Many will need help with their breathing after birth, sometimes for several weeks. Some babies will go on to have lifelong lung problems.

Like all babies, premature babies will get infections which they generally deal with well. The main problem is that over half will get chest infections ("pneumonia", commonly due to viruses) which will further damage the lungs.

Why do we need this study?
There are very few treatments available to prevent viral chest infections except against the virus called RSV (Respiratory Syncytial Virus).

We want to use a new approach to prepare the babies' immune system to fight chest infections. Bactek (MV130) is an under the tongue spray containing harmless dead bacteria, so it works like a vaccine. The dead bacteria should help the baby build a strong immune system, preventing further damage to the lungs by helping to fight chest infections. Studies show that Bactek decreases chest infections and wheezing safely in children and babies. Because only some premature babies have been studied so far, a larger study is needed to show if Bactek works in premature babies born at ≤29+6 weeks of gestation.

WHAT ARE THE STUDY AIMS?

What is the main question we want to address?
We want to show if Bactek spray decreases the risk of a premature baby having to visit the GP, A&E or is admitted to hospital for chest infections when compared to babies who get a placebo (dummy) spray in the first year of life.

We will do a study called "a blinded, randomised, placebo-controlled trial" or RCT. This means with consent from parents, we will use a computer to allocate their baby to one of two treatments.

One group will get Bactek and the other will get a placebo ("dummy" treatment spray that looks the same as Bactek but does not contain any dead bacteria). The parents and the researchers will not know which group the baby is in.

The allocated treatment will be given daily from when the baby reaches 37-43 weeks' corrected gestation (close to their "due date") or discharge if earlier until they are one year from their expected due date.

How many babies will we study?
We wish to include 542 babies to show if chest infections decrease in an important way – which we feel is to decrease the current rate of chest infection from 55% (55 babies in very 100) to 40% (40 babies in every 100). We have allowed for one in seven babies not being able to complete the study.

Does this trial represent good value for money?
If this treatment works, there will be large savings. The cost to the health service for each hospital admission for a chest infection is over £6,000 (in 2012). More importantly, it will benefit the parents and their babies.

PATIENT AND PUBLIC INVOLVEMENT

Involvement to date
Babies and their parents are central to the whole study. We have consulted many parents during the planning of the study. Two parents, who had prematurely born babies are co-applicants on the study. We have also talked to parents on the neonatal units at Cardiff, Newcastle and Leicester. They have advised us on: if the treatment is important, is possible to deliver, and what outcome is most important to them. We have taken all their comments into account when we designed the study.

Involvement in the conduct of the research
Two parents have agreed to attend the monthly Trial Management Group meetings which means they will contribute to the daily running of the trial including help us to produce leaflets for parents and for the staff.

DISSEMINATION

We will present our results at international and national medical meetings and will publish the results in reputable medical journals. We will show the results via our trial website, social media and regular newsletters to inform the parents and the wider public.

4 Background

A significant proportion of infants born at ≤29+6 weeks' gestation develop lung disease during the neonatal period thus putting them at risk of significant lung disease in child- and adult-hood including premature development of chronic obstructive pulmonary disease (COPD). At discharge from the neonatal unit, the pre-existing lung disease is exacerbated by frequent respiratory viral infections requiring far greater health utilisation, including hospital admissions, than their term-born equivalents. Even in term-born infants, evidence, after such viral infections points towards development of preschool wheezing but in preterm-born infants, the consequences are greater due to added insults to the significant pre-existing lung disease. Opportunities to prevent viral infections in infancy are largely limited to anti-RSV antibody prophylaxis, but in term-born infants trained immunity-based vaccines (TIBVs) such as Bactek (MV130) are increasingly used (Montalbán-

Hernández et al., 2024, Nieto et al., 2021). One approach that is increasingly used in term-born infants at risk of developing wheezing is the use of inactivated bacterial vaccines such as Bactek to provide protection via activation of innate and adaptive immunity. The efficacy of Bactek, and other bacterial-based therapies, in studies of infants and young children (which have included significant numbers of preterm-born infants/children) in decreasing wheezing and respiratory exacerbations is convincing. Therefore, Bactek provides a promising therapeutic avenue for preterm-born infants to target post-discharge respiratory viral infection in this vulnerable group of infants.

4.1 Rationale for current trial/Justification of Treatment Options

Worldwide, 15 million preterm births occur each year (De Costa et al., 2021). From 654,741 UK births in 2018, 7.9% were preterm (<37 weeks' gestation) with 17,678 (2.7%) born at 29-34 weeks' gestation and 3,273 (0.5%) at ≤28 weeks' gestation (ONS 2018). Encouragingly, survival after preterm birth, especially at extremes of prematurity, has improved markedly over the two decades (Morgan et al., 2022) thus it has become necessary to focus on improving longer term outcomes including respiratory health after discharge from neonatal units to optimise quality of life (Kotecha et al., 2013). In particular, intervening early in life is most likely to improve outlook in adulthood. Respiratory disease, including the neonatal lung disease bronchopulmonary dysplasia (BPD), is common in preterm born survivors and is associated with long term respiratory compromise including increased respiratory symptoms (Been et al., 2014), decreased lung function (Kotecha et al., 2022), increased hospitalisation (Paranjothy et al., 2013) and increased inhaler use. Parent reported wheezing occurs in 65% of preterm-born infants born at ≤29+6 weeks' gestation during their first year of life (Edwards et al., 2016). Symptoms occur even in those born late preterm at 33-34 weeks' gestation (Kotecha et al., 2012) - prematurity-associated lung disease (PLD) is now considered a risk factor for early development of chronic obstructive pulmonary disease (COPD) (Stocks et al., 2013).

There is little doubt that preterm birth leads to compromised respiratory health later in life. Our recent systematic review showed that BPD is associated with ~17% decrease in percent predicted forced expired volume in 1 s (FEV1) in later life (Kotecha et al., 2022). The burden on health services is substantial. It was estimated in 2012, that a preterm infant with rhinovirus or respiratory syncytial virus (RSV) had excess costs of £5,769 when compared to equivalent preterm infants without LRTIs (Drysdale et al., 2013). Thus, minimising viral LRTIs will result in significant NHS savings but also improve longer term respiratory outcomes (Coathup et al., 2020).

Lung injury in preterm infants in the neonatal period is driven by neutrophil-mediated inflammation largely due to ante- and post-natal exposures such as chorioamnionitis and respiratory support with mechanical ventilation and oxygen therapy after delivery at an early stage of lung development, often complicated by infection (Davies et al., 2010, Chakraborty et al., 2010). Subsequent repair/remodelling results in significantly abnormal lung growth (Joshi and Kotecha, 2007). Lung function, assessed using non-invasive oscillometry, is already sub-optimal before discharge home with increased airway resistance, and decreased compliance and reactance (Zannin et al., 2022). Drysdale reported that 73 of 159 (46%) discharged infants with median gestation of 34 (range: 23-

36) weeks developed LRTIs (Drysdale et al., 2011) during the first year of life. Importantly, those who had viral infections had worse lung function at one year of corrected age when compared to those who did not (Drysdale et al., 2014). More recent UK data using record linkages reported that 1,666 preterm infants born at <32 weeks' gestation followed up to 5 years of age had adjusted incidence rate ratios for LRTIs of 2.79 (2.59, 3.01) when compared to 253,277 term-born controls (Tan et al., 2020). The AZTEC follow up data showed admission rates of 55% (of 385 survivors with available data) by one year of corrected age (unpublished data).

During the early neonatal period, these infants have immature innate and adaptive immunity including impaired complement production (McGreal et al., 2012), decreased cellular phagocytic activity and limited Th1 and Th2 cell responsiveness to pathogens (Melville and Moss, 2013), which limits their ability to present antigens, express cytokines and counter infections. Infants born preterm also have impaired adaptive immunity with reduced T cell responses including low memory T cell frequencies (Walker et al., 2011). However, preterm babies have lower frequencies of T cells (Walker et al., 2011). Encouragingly, the immune system rapidly matures being comparable by discharge to term-born infants but whether the immune system is as robust during viral infections is less well studied (Kamdar et al., 2020, Koblin et al., 1988, Slack et al., 2005).

The development of appropriate T cell responses may determine outcomes such as wheezing after viral infections in infants and young children. Asthma in children has typically been considered to be dominated by Th2 allergic immune responses, although Th17 responses are also implicated in the pathogenesis of asthma (Cosmi et al., 2011). Importantly, during foetal life, T cell responses are biased towards Th2 with Th1 responses linked to increased risk of spontaneous abortion (Basha et al., 2014, Szekeres-Bartho, 2002). Furthermore, cord blood monocytes from preterm born infants produce fewer Th1-skewing cytokines than cord blood monocytes from full term infants (Tatad et al., 2008), suggesting that the innate immune compartment in neonates is less likely to promote antiviral immunity or counter Th2 development.

Bactek (MV130, Inmunotek, Spain) is a polybacterial preparation of heat-inactivated Staphylococcus aureus, S. epidermidis, Streptococcus pneumoniae, Haemophilus influenzae, Klebsiella pneumoniae and Moraxella catarrhalis which can boost innate immunity and enhance T-cell responses (Cirauqui et al., 2018, Del Fresno et al., 2021, Brandi et al., 2022). Bactek is not a conventional vaccine but is generally regarded as a trained immunity-based vaccine (TIbV), working by generating broad heterologous protection against related and unrelated to pathogens in its formulation (Sánchez-Ramón et al., 2021, Baydemir et al., 2024). It exerts its actions via activation of both the innate and adaptive immune systems, this way generating both short and long-term memory (Figure 1) (Martín-Cruz et al., 2023).

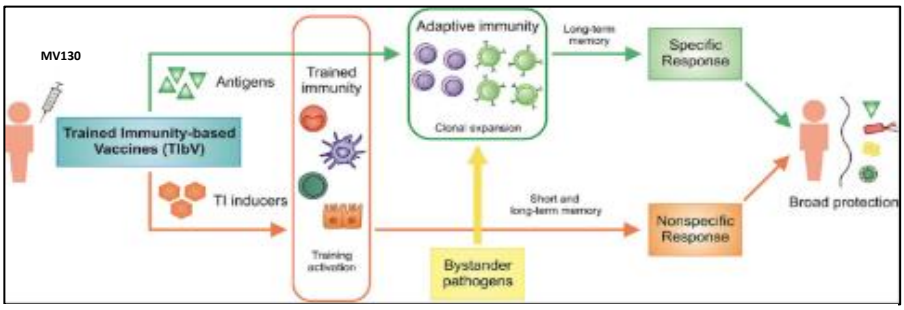


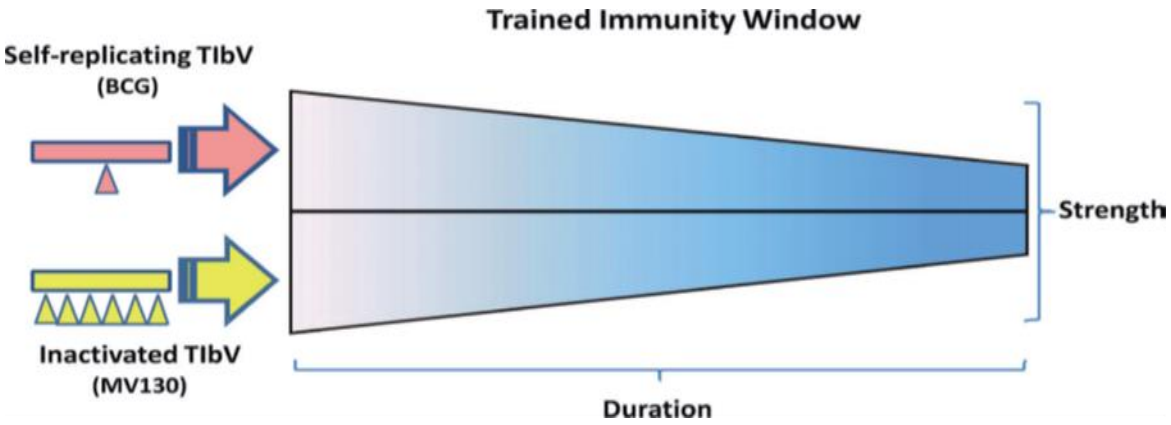
Figure 1: Mechanism of action of trained immunity-based vaccines (TIbVs) for infections (Martin-Cruz 2023)

There are many pre-clinical and clinical studies which support the effectiveness, efficacy and safety of Bactek at the recommended dose of 300 FTU/mL. Pre-clinical *in vivo* studies in mice have shown that repeated dose administration of Bactek via intranasal and/or sublingual route (50 µL or 10-20 µL, respectively) induces both local cellular and humoral response in the airways (Del Fresno et al., 2021, Brandi et al., 2022) as well as systemic immune responses in the spleen (Cirauqui et al., 2018). Such mucosal administration of Bactek in mice has shown heterologous protection against not only to influenza, Vaccinia and SARS-CoV-2 intranasal viral infections but also against systemic *Candida albicans* challenge (Del Fresno et al., 2021, Brandi et al., 2022). In addition, a recent study also supports an antiviral role of MV130 through cross-reactive immunity, a mechanism independent of trained immunity(Bodas-Pinedo et al., 2023).

Bactek has been used in many human clinical studies including in infants and young children. In all studies, including adults with recurrent respiratory tract infections (RRTI) (Alecsandru et al., 2011, Ochoa-Grullón et al., 2020), vulnerable populations such as patients with COVID, B cell haematological malignancy, or subjects with autoimmune diseases, the dose 200 µL of Bactek (300 FTU/mL) daily through the sublingual route has been used (Ochoa-Grullón et al., 2020, Sánchez-Ramón et al., 2021, Guevara-Hoyer et al., 2020, Pérez-Sancristóbal et al., 2023). In addition, a similar dose was used in a phase III clinical trial conducted in infants and young children (including 17% born preterm), aged 6-36 months with recurrent wheezing, establishing both safety and efficacy of Bactek with significantly lower number of wheezing attacks (Bactek: 3.0, IQR 2.0–4.0; and placebo: 5.0, IQR, 3.0–7.0, $p<0.001$) when used at a similar dose of 300 FTU/mL (Nieto et al., 2021). Therefore, all results thus far including in adults and children support using the dose of 300 FTU/mL.

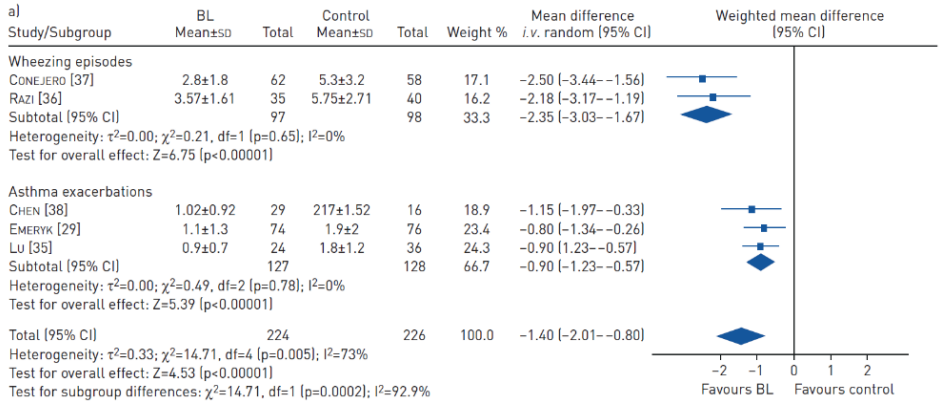
Bactek is used to generate immune training thus repeated doses are needed to induce this effect. As shown in Figure 2 repeated doses of Bactek, which contains inactivated respiratory tract bacteria, are needed to maintain the same strength over time as that observed with self-replicating TIbV such as BCG (Conejero et al., 2021). The two-phase III trials in children with wheezing attacks and adults with COPD have shown Bactek administration to be safe and, most importantly, effective following 6 months and 12 months immunisation, respectively. Although clinical data has shown protection is maintained after treatment finalisation at least for another 6 months, considering preterm-born infants are a vulnerable population and given their exposures during the winter period, it would be reasonable to ensure that each discharged infant is covered during their first winter period thus we have chosen a treatment period of 12 months to ensure that each infant is covered during the winter period regardless of the season when they are discharged.

Figure 2: Trained immunity window of TIbVs (Conjero 2021)



Several systematic reviews of studies using bacterial lysates including the one by de Boer have confirmed decreased wheezing in preschool children (most studies included preterm-born children) (mean difference of -2.35, 95%CI -3.03, -1.67), reduced duration of each wheezing episode and absence of adverse reactions for pooled studies of Bactek and the bacterial lysate OM-85 (de Boer et al., 2020). Furthermore, rates of LRTIs are also decreased (Figure 3))

Figure 3: Forest plot showing improvement in wheezing episodes and asthma exacerbations after lysate therapy



The current extensively published data are wholly applicable to the preterm population, especially as preterm-born children were included in many of these studies. For instance, 16.7% were preterm born in Nieto's MV130 study (39) showing fewer wheezing attacks (3.0 vs 5.0). However, a trial of extremely preterm-born infants when they reach their due date is required. Mechanistically, when preterm-born infants reach their 'due date' around 37 weeks' corrected gestation, they are considered equivalent to term-born infants as they have similar physiology and immune responses. Once discharged, both groups have similar respiratory viral exposures with identical immune responses. However, these viral infections will further compromise the already injured lungs resulting in significant life-long respiratory morbidity. Robust evidence of immunocompetence similar to term-born infants was shown in the 1980s when it was shown that preterm-borns did not need 4+ routine vaccinations (instead of usual 3) as their immune responses were similar to term-borns (28, 29). Recent data also confirm rapid immune maturation after birth (Kamdar et al., 2020).

In summary, preterm infants born ≤29+6 weeks' gestation are at increased risk from additional damage to their already impaired lungs through LRTIs over their first year of life. Data supports reductions in wheeze/asthma in other populations which include small numbers of preterm infants, not currently separately analysed. This study aims to apply this potentially effective treatment specifically to a very preterm population.

5 Trial objectives/endpoints and outcome measures

Does treatment with the trained immunity-based vaccine Bactek™ (MV130) decrease the risk of an unscheduled visits to health care professionals for Lower Respiratory Tract Infections (LRTIs), when compared to placebo, in infants born at ≤29+6 weeks' gestation when treated from term-equivalent (37-43 weeks' correct gestation) or discharge if earlier to one-year corrected age?

5.1 Primary objective

To investigate if sublingual Bactek spray, when compared to placebo, decreases the risk of health professional diagnosed LRTIs (after unscheduled visits to general practitioners (GPs), accident and emergency departments (A&Es) and hospital admissions) between term-equivalent 37-43 weeks' gestation or discharge if earlier and one year of corrected age.

5.2 Secondary objectives

- To establish if sublingual Bactek spray is superior to placebo in decreasing the number of health professional diagnosed LRTIs between term-equivalent 37-43 weeks' gestation or discharge if earlier and one year of corrected age.
- To establish if sublingual Bactek spray is superior to placebo in increasing the time to first health professional diagnosed LRTI between term-equivalent 37 weeks' gestation or discharge if earlier and one year of corrected age.
- To establish effect of Bactek on growth between the two groups
- To establish if sublingual Bactek spray is superior to placebo in preventing parent-reported, health professional confirmed, wheezing from 37-43 weeks' corrected gestation or discharge if earlier to one year of corrected age in infants born at ≤29+6 weeks' gestational age.
- To compare the use of respiratory medications including inhalers (bronchodilators, inhaled corticosteroids), antibiotics and systemic corticosteroids between the two groups.
- To establish effect of Bactek on growth (weight, length and head circumference) between the two groups.
- To establish effects of LRTI on the family, including time missed from work and/or nursery time missed for the infants.
- To explore differential intervention effects by gestational age, anti-RSV prophylaxis, and centre.
- To assess safety (volume of serious adverse events).

5.3 Primary outcomes measure(s)

The presence/absence of parent-reported, health professional-confirmed unscheduled visits for LRTIs to GPs, A&Es, paediatric assessment units and hospital admissions between 37-43 weeks' corrected age (or discharge if earlier) and one-year corrected age.

"Corrected age" is calculated using the expected date of delivery, therefore taking the degree (number of weeks of prematurity) into account.

An LRTI will be defined as:

- Fever (≥38°C) or a runny nose (rhinitis) for at least 12 hours, plus one additional symptom from the following:
    - moist cough
    - wheezing
    - shortness of breath
    - tachypnoea
    - intercostal recession
    - poor feeding

5.4 Secondary outcomes measure(s)

- The number of parent-reported, health professional-confirmed unscheduled visits for LRTIs to GPs, A&Es, paediatric assessment units and hospital admissions between 37 weeks' corrected age (or discharge if earlier) and one-year corrected age. The LRTI will be considered to be a new episode if there is an intervening period of at least 72 hours between episodes when the infant is well.
    - The time to first parent-reported, health professional-confirmed unscheduled visit for LRTI to GPs, A&Es, paediatric assessment units and hospital admissions between 37-43 weeks' corrected age (or discharge if earlier) and one-year corrected age.
- Parent-reported wheeze episode between 37-43 weeks' corrected age or discharge if earlier and one-year corrected age.

    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. A WheezeScan device will be provided to parent(s)/guardian(s) and may be used to help parents detect these symptoms.

- Parent-reported use of respiratory medications including bronchodilators, antibiotics and systemic corticosteroids
- To establish effect of Bactek on growth (weight, length and head circumference) between the two groups.
- Parent(s)/guardian(s) reported time missed from work and/or nursery time missed for the infant
- Volume of adverse reactions
- Identification of virus(es) associated with LRTIs

6 Trial design and setting

Research design: BALLOON will be a multi-centre, randomised, double-blinded, two stage, placebo-controlled trial of Bactek to assess its efficacy in reducing unscheduled visits for LRTIs when compared to placebo in expreterm infants being discharged from neonatal units in the UK. We plan to enrol 542 infants from approximately 12 centres over a 2-year recruitment period. Enrolment will take place on neonatal units during preparations for discharge planning of eligible infants who were born prematurely (≤29+6 weeks' gestation). Following informed consent from parents, they will be randomised to receive Bactek™ subligual spray or matching placebo (50:50 treatment allocation) up to one year of corrected age, to be administered by parents once daily (2x actuations of spray). Formal follow up will be conducted by the research nurse at month 3, 6, 9 and 12 (one year corrected) ages.

6.1 Risk assessment

A Trial Risk Assessment has been completed to identify the potential hazards associated with the trial and to assess the likelihood of those hazards occurring and resulting in harm. This risk assessment has been completed in accordance with the MRC/DH/MHRA Joint project guidance document 'Risk-adapted approaches to the management of Clinical Trials of Investigational Medicinal Products' and includes:

- The known and potential risks and benefits to human subjects
- How high the risk is compared to normal standard practice
- How the risk will be minimised/managed

This trial has been categorised as TYPE B where the level of risk is somewhat higher than the risk of standard medical care. A copy of the trial risk assessment may be requested from the Trial Manager. The trial risk assessment is used to determine the intensity and focus of monitoring activity (see section 25.1).

7 Site and Investigator selection

This trial will be carried out at participating sites within the UK. All sites who are interested in participating in the trial will be required to complete a registration form to confirm that they have adequate resources as well as expertise and experience to conduct the trial. Where electronic health records are being used, Site teams must be willing and able to take steps to avoid unintentional unblinding through EHR system.

Before any Site can begin recruitment a Principal Investigator (PI) at each site must be identified. The following documents must be in place and copies sent to the BALLOON Trial email account.

- Confirmation of Capacity and Capability from the NHS organisation's R&D Department, following submission of the local information pack

- Attendance of key study personal at site initiation visit (at minimum: PI, nominated study nurse, pharmacy representative)
- A signed Clinical Trial Agreement
- Current Curriculum Vitae (CV) and GCP training certificate of the PI
- Completed Site Delegation Log and Roles and Responsibilities document
- Completed Source Data Verification Log
- Full contact details for all host care organisation personnel involved, indicating preferred contact

Upon receipt of all the above documents, the Trial Manager (TM) will send written confirmation to the PI/lead Research Nurse detailing that the centre is now activated. This letter/email must be filed in each site's Site File. Along with the written confirmation, the site should receive their trial drug supplies and a trial pack holding all the documents required to recruit into the Trial.

Occasionally during the trial, amendments may be made to the trial documentation listed above. CTR will issue the site with the latest version of the documents as soon as they become available. It is the responsibility of the CTR to ensure that they obtain local R&D approval for the new documents.

Site initiation will be by site visit, regional launch meeting, or by video conference.

8 Participant selection

Participants are eligible for the trial if they meet all of the following inclusion criteria and none of the exclusion criteria apply. All queries about participant eligibility should be directed to the Trial Manager before randomisation.

8.1 Inclusion criteria

- Birth at gestational age ≤29+6 weeks (including infants born as one of a multiple birth)
- In the opinion of the PI, follow-up is likely to be feasible (i.e routine outpatient appointments will be at the recruiting site, locality of baby's residence so follow up to one year corrected age is possible.)
- Survival to one-year corrected age is anticipated
- In addition to infants without an eventful course, the following groups are eligible for inclusion:
- Infants diagnosed with bronchopulmonary dysplasia
- Infants discharged home on oxygen
- Infants who have had necrotising enterocolitis
- Infants on oral or nasogastric tube feeding
- Infants with treated patent ductus arteriosus

- Infants with neurological disorders
- Infants with resolved sepsis

This list is not exhaustive and others can be discussed with the PI in the first instance and trial team if necessary.

8.2 Exclusion criteria

- Presence of major surgical, cardiac or congenital abnormalities (not including patent ductus arteriosus or patent foramen ovale)
- Contraindication of Bactek™ as specified in the Investigator's Brochure
- Participation in other interventional trial that precludes participation in BALLOON
- Primary immune deficiencies

8.3 Co-enrolment

To avoid potentially confounding issues, ideally participants should not be recruited into other clinical trials of investigational medicinal products during their participation in BALLOON.

Where recruitment into another trial is considered to be appropriate and without having any detrimental effect on the BALLOON trial this must first be discussed with the TM, who will contact the Chief Investigator (CI).

9 Recruitment, Screening and registration

9.1 Participant identification

Screening will be performed by local clinical care team who will regularly review the clinical progress potentially eligible infants regarding they suitability for the study. Parent(s)/legal guardian(s) of a potentially eligible infant will then be approached by a member of the local research team to consider participation. The decision to confirm eligibility and enter the infant into the trial will be made by an appropriate clinician.

9.2 Screening logs

A screening log of all ineligible and eligible but not consented/not approached will be kept at each site so that any biases from differential recruitment will be detected. De-identified screening information will be entered to the eCRF on a monthly basis (see section 19 for further detail on data monitoring/quality assurance).

9.3 Informed consent

The parents(s)/guardian(s) will be approached by a member of the local trial team. Consent may be taken by a delegated member of the trial team who, in the opinion of the local PI, be experienced at imparting important information and with experience in obtaining informed consent in the neonatal environment.

Upon reviewing the informed consent documents, the trial team member will explain the research trial to the parent(s)/guardian(s). This information will emphasise that participation in the trial is voluntary and that the parent(s)/guardian(s) may withdraw the participant from the trial at any time and for any reason. All parent(s)/guardian(s) will be given opportunity to ask any questions that may arise, and time to consider the information prior to agreeing to participate. A full explanation of the treatment options, including the conventional and generally accepted methods of treatment, will be given. A contact point where further information about the trial may be obtained will be provided. The rights and welfare of the infant participants will be protected by emphasising that the quality of medical care will not be adversely affected if the parent(s)/guardian(s) declines to consent to the infant's participation in this trial, or subsequently withdraws them from either further protocol treatment or trial follow-up.

The consent process will include a discussion of planned linkage to routine data sources: NHS Digital, Digital Health and Care Wales, ISD Scotland, GP data and the National Neonatal Research Database up to 2 years corrected age for the purposes of longer-term follow-up, and consequently participant consent will be requested to collect NHS/CHI Numbers for the mother and infant.

Consent for an infant's participation in the BALLOON trial must be provided by those who have parental responsibility for the infant. Although consent is only needed from one person with parental responsibility, it is recommended that all persons with parental responsibility be involved in the discussions and agree to the infant's participation. The participant's written informed consent must be obtained using the trial Consent Form, which follows the Parent Information Sheet. The parent(s)/guardian(s) should be given sufficient time after the initial invitation to participate before being asked to sign the Consent Form. Informed consent must be obtained prior to the parent(s)/guardian(s) undergoing procedures that are specifically for the purposes of the trial.

Please note, only when written informed consent has been obtained from the parent(s)/guardian(s) and the infant has been randomised/enrolled into the will they be considered as a trial participant.

Parent(s)/guardian(s) should always be asked to sign a consent form. One copy should be given to the Parent(s)/guardian(s), but the original copy should be kept in the investigator site file and a further copy should be kept with participant's hospital notes. A copy will also be sent to CTR.

The right of the parent(s)/guardian(s) to refuse permission for their infant to participate in the trial without giving reasons must be respected. After the infant has entered the trial, the investigator must remain free to give alternative treatment to that specified in the protocol, at any stage, if he/she feels it to be in the best interest of the infant. However, the reason for doing so should be recorded and the infant will remain within the trial for the purpose of follow up and data analysis according to the treatment option to which he/she has been allocated. Similarly, the parent(s)/guardian(s) must remain free to withdraw at any time from the protocol treatment without giving reasons and without prejudicing further treatment. If the parent(s)/guardian(s) are willing to provide a reason this should be recorded to inform ongoing trial decisions.

9.3.1 Consent for respiratory function testing and blood sampling

At sites participating in ancillary blood sample collection, the consent process will include an additional description of the procedures involved and plans to retain the collected samples for use in future research.

At sites participating in the ancillary respiratory function testing (oscillometry) the consent process will include an additional description of the procedures involved.

9.4 Randomisation

Participants can be randomised at 37-43 weeks' gestation or prior if discharged earlier.

Participants will be randomised 1:1 using minimisation with a random element (p = 0.8). Allocations will be minimised by gestational age and anti-RSV prophylaxis – both assumed to have a strong prognostic relationship with outcome. The randomisation program will be developed by the CTR and will be embedded within the study database with controlled access maintaining blinding. To avoid any issues with inadvertent unblinding or dosing errors, multiple births will be randomised to the same arm.

All assessments for enrolment must be performed before randomisation and before administration of trial treatment. The infant may only be randomised once full eligibility has been confirmed by a medical doctor and written informed consent has been obtained.

At randomisation, designated Visit 0, an authorised member of the site research team will access the web randomisation system. After confirming that the participant qualifies for randomisation, the randomisation system will assign both a study ID and a pack ID number. The Pack ID links the participant to a unique treatment pack of the trial drug to be allocated. An automated email confirming the randomisation will be sent to the site team and a copy of the email will also be sent to the local site pharmacy team and to the CTR BALLOON trial team.

In the event the online randomisation system is unavailable at site, then the local investigator may contact the CTR (during office hours). Randomisation may be performed by CTR staff via the randomisation system provided by The Centre for Healthcare Randomised Trials (CHaRT) on request of the local investigator.

If necessary, the code may be unblinded for an individual infant at the request of the site PI or clinician in charge of the baby. See Section 15.2 for the procedure for unblinding treatment allocation.

9.4.1 Treatment initiation

The first 4 infants recruited and randomised will participate in the safety run-in period as described in section 16.1.1.

10 Withdrawal & lost to follow-up

10.1 Withdrawal

Parent(s)/guardian(s) have the right to withdraw consent for their infant's participation in any aspect of the trial at any time. The infants medical care will not be affected at any time by declining to participate or withdrawing from the trial.

If a parent(s)/guardian(s) initially consents but subsequently withdraws from the trial, clear distinction must be made as to what aspect of the trial the participant is withdrawing from. These aspects could be:

1. Withdrawal of Trial Treatment/ Intervention
2. Withdrawal from questionnaires (collected via Trial App)
3. Withdrawal from further routine trial samples (virology samples)
4. Withdrawal from routine trial samples already collected that are unprocessed (these will be destroyed)
5. Withdrawal from follow-up visits
6. Withdrawal from blood collection sub-study and further blood sample donations (if applicable)
7. Withdrawal of blood samples already collected (these will be destroyed) if applicable
8. Withdrawal from oscillometry sub-study (if applicable)
9. Withdrawal of Consent to all of the above

The withdrawal of consent shall not affect the trial activities already carried out and the use of data collected prior to participant withdrawal. The use of the data collected prior to withdrawal of consent is based on informed consent before its withdrawal.

In the event a withdrawal form is not completed, it must be assumed that the parent(s)/guardian(s) withdraw their infant from all aspects of the trial. However, the information and consent form will state that and any unprocessed viral swab samples will still be used. In respect of sub-study samples, these must be disposed unless the parent(s)/guardian(s) have signed the optional statement in the consent form that allows the samples to be retained in the event the parent(s)/guardian(s) do not complete the withdrawal form.

NB: If a participant is withdrawn for medical reasons by a clinician, the withdrawal form does not need to be completed. However, confirmation would be needed that the parent(s)/guardian(s) agrees to their infants samples being retained and used as per original consent.

Furthermore, it is important to collect safety data ongoing at the time of withdrawal, especially if the participant withdraws because of a safety event. There is specific guidance on this contained in the Participant Information Sheet but briefly:

If a participant wishes to fully withdraw from the trial they will need to be assessed for a final safety assessment. If the participant is suffering a serious adverse reaction to the trial treatment when are withdrawn, their research team will be required to continue to collect information about that reaction until the adverse reaction has resolved.

An infant may be withdrawn or be withdrawn from trial treatment for the following reasons:

- Intolerance to trial medication

- Any alteration in the infant's condition which justifies the discontinuation of the treatment in the Investigator's opinion
- Non-adherence

In all instances where a parent(s)/guardian(s) who consent and subsequently withdraw a withdrawal form should be completed on the infant's behalf by the researcher/clinician based on information provided by the participant, using the electronic Case Report Form (eCRF). Any queries relating to potential withdrawal of a participant should be forwarded to the CTR team.

10.2 Lost to follow up

An infant will be considered lost to follow-up if they repeatedly fail to return for scheduled visits and the parent(s)/guardian(s) are unable to be contacted by the trial site.

The following actions must be taken if an infant fails to return to the clinic for a required trial visit:

- The site must attempt to contact the parent(s) and reschedule the missed visit as soon as possible. The site must counsel them on the importance of maintaining the assigned visit schedule and data collection and ascertain whether or not the infant wishes to and/or should continue in the trial.
- Before an infant is deemed lost to follow up, the PI or designee must make every effort to regain contact with the the parent(s)/guardian(s). Where possible, this will require at least 2 telephone calls or local equivalent methods of contact. These contact attempts should be documented in the infant's medical record.
- If a recruited infant moves to a different country or moves without advising the recruiting site. In this instance the parent(s)/guardian(s) has not explicitly removed their consent, and their data can still be used.

The site PI will make every effort to reduce loss to follow-up by:

- Emphasising the importance of obtaining follow-up data to all parent(s)/guardian(s) at baseline and during their follow-up assessments, irrespective of treatment adherence
- Arranging mutually acceptable dates for the baseline visit and follow-up appointments; where possible, the next trial visit will be scheduled, and agreed with the parent(s)/guardian(s) at the previous visit.

In respect to sample collection, If the participant is lost to follow up, then they are not subject to the withdrawal processes, and the original consent stands.

11 Trial Intervention

BALLOON is a placebo-controlled trial. The active IMP and the placebo are sublingual sprays. The placebo is identical to the active IMP except for the absence of the active ingredients. The trial is being carried out under a Clinical Trial Authorisation. The intervention is therefore only to be used by the named investigators, for the participant s specified in this protocol, and within the trial.

11.1 Treatment(s)

Active IMP: A sublingual spray comprising of a polybacterial preparation of heat-inactivated *Staphylococcus aureus, S. epidermidis, Streptococcus pneumoniae, Haemophilus influenzae, Klebsiella pneumoniae and Moraxella catarrhalis* (Bactek™, Inmunotek, Spain).

Placebo: A sublingual spray comprising of all excipients of the active IMP, aside from the inactivated bacteria.

Dosage: 300 FTU/mL (2x 150 FTU/mL sprays), once daily, from 37 weeks corrected age or discharge if earlier up to 1 year of corrected age

IMP must be administered separately from the intake of food and drinks (at least, 30 minutes prior to, or 30 minutes after the consumption of food and/or drink).

11.2 Treatment supply and storage

Bactek™ or placebo to match will be packaged, labelled and QP released by <<Inmunotek's>> manufacturer. St Mary's Pharmaceutical Unit (SMPU, Cardiff and Vale University Health Board) will import the IMP to the UK, and provide final regulatory release. SMPU will warehouse the IMP supply and distribute to study sites. The IMP management will be managed as per established CTR SOPs. Inmunotek are supplying the Bactek and placebo free of charge.

- **Packaging**

Bactek™ or placebo will be packaged as pairs of sprays, which in combination provide sufficient supply for at least 3 months of treatment. 5 boxed pairs of sprays will be presented in a "treatment pack", sufficient to cover the treatment period.

- **Labelling**

Labelling will comply with Annex 13 of GMP (including provision for labelling of small packaging units).

- **Distribution & Supply**

The IMP and placebo will be distributed to the sites after technical and regulatory release has been confirmed by the Sponsor, and the site has completed the CTR site initiation procedure. The IMP and placebo will be distributed via specialised courier to the address of the pharmacy provided by the PI of each site.

- **Storage & Stability**

The supplied products will be stored under the responsibility of the local pharmacist according to the particulars of the IB.

On receipt of the IMP shipment at each of the individual trial sites, the responsible pharmacist will ensure treatment packs are stored in an appropriate secure location accessible only to authorised personnel. The temperature for storage of the IMP is between 2 and ≤8°C.

Short temperature deviations during transport or storage are not critical and no further actions are required: In the case of temperature deviations >8°C, stability data has shown that the IMP is stable and safe for use for up to 24 months, even if stored up to 25°C for 3 months.

- **Expiry date and resupply management**

Initial supply and resupply of treatment packs to sites will be arranged via the Trial Manager at the CTR. The randomisation system will not permit expired IMP to be assigned to participants. The Trial Manager will closely monitor IMP batch expiry.

11.3 Treatment prescribing and dispensing

The IMP will be prescribed on a trial-specific prescription which will be submitted to the pharmacy, specifying the pack ID to be dispensed. IMP for each recruited infant shall be prescribed and dispensed on a 3-monthly basis. Further guidance on IMP management and supply will be provided to study sites.

11.4 Dose modification

There are no dose modifications permitted.

11.5 Management of dosing errors

In the event a dosing error is identified by parents or any member of the site team, this should be reported to the CTR as per SOPs. Sites will receive training on management of dosing errors.

11.6 Prohibited medications and interaction with other drugs

No drug interactions are expected due to the nature of Bactek (MV130) and its mechanism of absorption. There are no prohibited medications.

11.7 Accountability procedures

Accountability procedures will be as per the trial IMP Management plan, which will be signed-off prior to commencement of the trial.

11.8 Adherence

Adherence will be closely monitored during the treatment period, utilising a parent-reported question collected via the Trial App. Local site research nurses will utilise the App dashboard to regularly assess adherence and contact parents when gaps in questionnaire completion are identified.

Parents will be instructed to return used sprays at each visit for review. The research nurse will be responsible for weighing of each spray to determine the volume of IMP given.

12 Sample management

12.1 Virology samples

Nasal swabs for virology analysis will be taken from infants by parents on an ad-hoc basis, based on symptoms reported via the Trial App. The App will alert both parents and the site research nurse when a sample is required.

Sites will be provided with the required materials for sample collection, labelling and transport to the virology laboratory in Cardiff, which will be given to parents at each study visit. The parents will post the swabs directly to the Virology laboratory at Public Health Wales in Cardiff, for batch analysis.

Detailed information will be provided to parents on the collection, and transport of these samples. After taking a swab the parents will post the swab straight away to the Virology laboratory at Public Health Wales using the provided materials.

12.2 Blood samples

A sub-set of sites, as determined by the site assessment form, will be selected to collect blood samples at Baseline, 6 month and one-year corrected age. Collection of these blood samples for use in future research will be an optional aspect of consent at these sites.

Where possible, blood sampling will be aligned to a clinically required blood-tests, however, parents will be informed that separate blood letting may be necessary for the trial. Blood collection volume at baseline will be between 0.5 to 1mL, and between to 1-2mL at 6 and 12 months, via the most minimally invasive procedure possible (e.g. venepuncture).

Detailed information will be provided to sites on the collection, storage, and batch shipment of blood samples back to Cardiff University laboratories, for use in future research. The sites will freeze the samples at -80 degrees centigrade in a secure location and transport them in batches to Cardiff University on dry ice via an approved courier service.

13 Trial procedures

13.1 Baseline & Randomisation

- Screening to assess eligibility of baby for entry into the trial according to inclusion/exclusion criteria
- Confirmation of eligibility (by medically qualified doctor)
- Informed consent
- Collect baseline neonatal and maternal demographic details (data collection as per eCRF)
- Blood samples (selected sites only)
- Respiratory function measurement (selected sites only)
- Randomisation
- IMP dispensing
- Weight, length and head circumference.

13.2 Months 1&2 (telephone call)

- Adverse event monitoring
- Treatment adherence checks, via Trial App
- Symptom diary review, via Trial App
- Ad-hoc viral swab if symptomatic

13.3 Month 3 (home/clinic visit)

- Adverse event monitoring
- Treatment adherence checks, via Trial App
- Symptom diary review, via Trial App
- IMP accountability check
- IMP dispensing
- Ad-hoc viral swab if symptomatic
- Verify patient entered app information

13.4 Months 4&5 (telephone call)

- Adverse event monitoring
- Treatment adherence checks, via Trial App
- Symptom diary review, via Trial App
- Ad-hoc viral swab if symptomatic

13.5 Month 6 (clinic visit)

- Blood samples (selected sites only)
- Respiratory function measurement (selected sites only)

- Adverse event monitoring
- Treatment adherence checks, via Trial App
- Symptom diary review, via Trial App
- IMP accountability check
- IMP dispensing
- Ad-hoc viral swab if symptomatic
- Weight, length and head circumference.
- Verify patient entered app information

13.6 Months 7&8 (telephone call)

- Adverse event monitoring
- Treatment adherence checks, via Trial App
- Symptom diary review, via Trial App
- Ad-hoc viral swab if symptomatic

13.7 Month 9 (home/clinic visit)

- Adverse event monitoring
- Treatment adherence checks, via Trial App
- Symptom diary review, via Trial App
- IMP accountability check
- IMP dispensing
- Ad-hoc viral swab if symptomatic
- Verify patient entered app information
-

13.8 Month 12 (clinic visit)

- Blood samples (selected sites only)
- Respiratory function measurement (selected sites only)
- Adverse event monitoring- final review
- Symptom diary review, via Trial App
- Treatment adherence checks, via Trial App
- IMP accountability check
- Ad-hoc viral swab if symptomatic
- Weight, length and head circumference.
- Verify patient entered app information

Visit and telephone call time points are approximate and can be +/- 1 month around parent's convenience.

Table 1: Schedule of procedures

Assessment	Baseline	Randomisation	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6	Month 7	Month 8	Month 9	Month 10	Month 11	Month 12
Invitation and PIS provision	X													
Informed consent	X													
Assessment of eligibility	X													
Randomisation		X												
Dispense IMP		X			X			X			X			
Nasal/throat swab			X*											
Blood sample		(X)						(X)						(X)
Lung function: Impulse oscillometry (Tremoflo)		(Y)						(Y)						(Y)
Telephone call			X	X		X	X		X	X		X	X	X
Home/clinic visit					X						X			
Clinic visit								X						X
Treatment compliance monitoring (via App)		X	X	X	X	X	X	X	X	X	X	X	X	X
Verify patient entered app information					X			X			X			X
Weight, length and head circumference	X							X						X
Adverse event monitoring		X	X	X	X	X	X	X	X	X	X	X	X	X

*Nasal swab to be taken at the time of suspected LRTI
(Y) Oscillometry undertaken at 5 mechanistic sites only, if optional consent from parents is in place
(X) Blood sample taken at 5-6 mechanistic sites only, if optional consent from parents is in place

13.9 Follow-up

End of follow-up is defined as completion of the 30 days after completion of IMP.

14 Pharmacovigilance

The Principal Investigator is responsible for ensuring that all site staff involved in this trial are familiar with the content of this section.

All SAEs must be reported immediately (and within 24 hours of knowledge of the event) by the PI at the participating site to the CTR PV and Safety Specialist. This includes SAEs related to IMPs.

14.1 Definitions

Table 2

Term	Definition
Adverse Event (AE)	Any untoward medical occurrence in a participant or clinical trial participant administered a medicinal product and which are not necessarily caused by or related to that product
Adverse Reaction (AR)	Any untoward and unintended response in a clinical trial participant to an investigational medicinal product which is related to any dose administered to that participant
Serious Adverse Event (SAE)	Any adverse event that - • Results in death • Is life-threatening* • Required hospitalisation or prolongation of existing hospitalisation** • Results in persistent or significant disability or incapacity • Consists of a congenital anomaly or birth defect • Other medically important condition***
Serious Adverse Reactions (SARs)	Any SAE occurring in a clinical trial participant for which there is a reasonable possibility that it is **related** to the IMP at any dose administered.
Suspected Unexpected Serious Adverse Reactions (SUSARs)	A SAR, the nature and severity of which is not consistent with the Reference Safety Information (RSI) for the IMP.

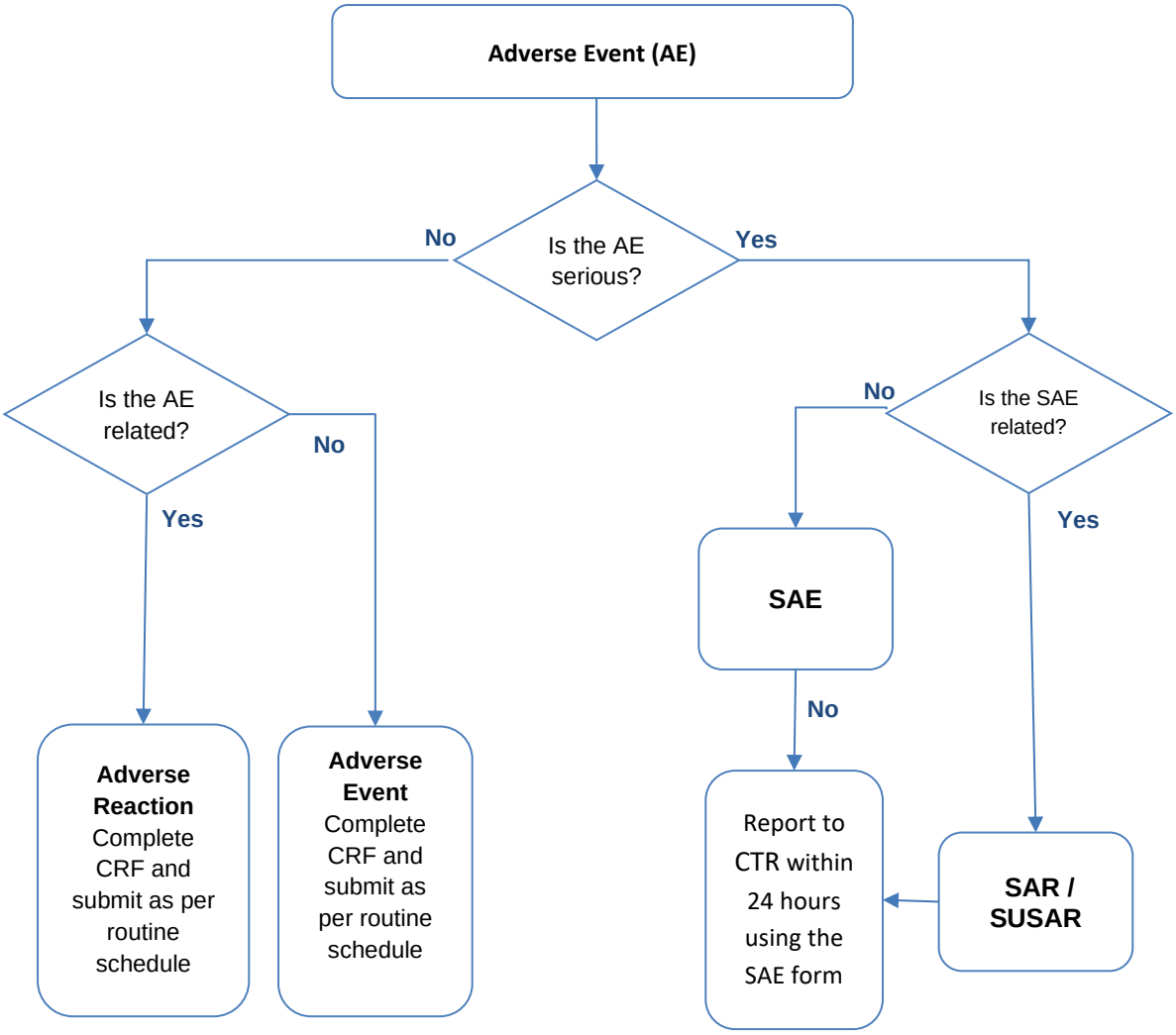
***Note:** The term 'life-threatening' in the definition of serious refers to an event in which the trial participant was at risk of death at the time of the event or it is suspected that used or continued used of the product would result in the subjects death; it does not refer to an event which hypothetically might have caused death if it were more severe.

**** Note:** Hospitalisation is defined as an inpatient admission, regardless of the length of stay, even if the hospitalisation is a precautionary measure for continued observation. Pre-planned hospitalisation e.g. for pre-existing conditions which have not worsened, or elective procedures, does not constitute an SAE.

***** Note:** other events that may not result in death, are not life-threatening, or do not require hospitalisation, may be considered as an SAE when, based upon appropriate medical judgement, the event may jeopardise the participant and may require medical or surgical intervention to prevent one of the outcomes listed above.

14.2 Trial-specific SAE reporting requirements

The figure below demonstrates the pharmacovigilance reporting process for the BALLOON trial. The PI is responsible for ensuring that all site staff involved in this trial are familiar with the content of this section.



14.2.1 Forseeable Adverse Events

For the purposes of this trial the following events are considered Foreseeable will not require reporting as AEs at any severity:

- Fever (≥38°C)
- Rhinitis
- moist cough
- wheezing
- shortness of breath/increased work of breathing
- tachypnoea
- poor feeding
- Lower respiratory tract infection

- Upper respiratory tract infection

NB: Foreseeable AEs should be formally reported as AEs if considered more severe than expected in the trial population or if considered related to the study drug.

14.3 Severity

The assignment of the severity grading for each reportable SAE should be made by the investigator responsible for the care of the participant using the definitions below.

Mild: does not interfere with routine activities

Moderate: interferes with routine activities

Severe: impossible to perform routine activities

A distinction is drawn between serious and severe AEs. Severity is a measure of intensity (see above) whereas seriousness is defined using the criteria in section 14.1, hence, a severe AE need not necessarily be a Serious Adverse Event.

14.4 Causality

Causal relationship will be assessed for IMPs, other trial treatments (nIMPs) and procedures:

Table 3

IMPs: Bactek (MV130)

The Principal Investigator (or another delegated medically qualified doctor from the trial team) and Chief Investigator (or another medically qualified doctor from the Trial Management Group) will assess each SAE to determine the causal relationship:

Table 4

Relationship	Description	Reasonable possibility that the SAE may have been caused by the IMP?
Unrelated	There is no evidence of any causal relationship with the trial/intervention	No
Unlikely	There is little evidence to suggest there is a causal relationship with the trial/intervention (e.g. the event did not occur within a reasonable time after administration of the trial medication). There is another reasonable explanation for the event (e.g. the participant's clinical condition, other concomitant treatment).	No

Possible	There is some evidence to suggest a causal relationship with the trial/intervention (e.g. because the event occurs within a reasonable time after administration of the trial medication). However, the influence of other factors may have contributed to the event (e.g. the participant's clinical condition, other concomitant treatments).	Yes
Probable	There is evidence to suggest a causal relationship and the influence of other factors is unlikely.	Yes
Definite	There is clear evidence to suggest a causal relationship and other possible contributing factors can be ruled out.	Yes

The causality assessment given by the Principal Investigator (or delegate) cannot be downgraded by the Chief Investigator (or delegate), and in the case of disagreement both opinions will be provided.

14.5 Expectedness

The Chief Investigator (or another delegated appropriately qualified individual) will assess each SAR to perform the assessment of expectedness.

The expectedness assessment should be made with reference to the current Reference Safety Information (RSI), contained in the Investigator's Brochure, for the IMP. Expectedness decisions must be based purely on whether the event is listed in the RSI; other factors such as the participant population and participant history should not be taken into account. Expectedness is not related to what is an anticipated event within a particular disease. SARs which add significant information on specificity or severity of a known, already documented adverse event constitute unexpected events. Fatal and life-threatening (LT) SARs should not be considered expected (unless explicitly stated in the RSI and approved by the NCA). For example, an event more specific or more severe than that described in the RSI is considered unexpected.

The table below lists the RSI's that should be referenced:

Table 5

IMP	RSI to be used for expectedness assessment	Relevant section to be used for expectedness assessment
Bactek (MV130)	Investigator's Brochure	6.b.2.1

Reference Safety Information (RSI) on any CTR trial will be reviewed regularly according to CTR procedures.

14.6 Reporting procedures

14.6.1 Participating Site Responsibilities

The PI (or delegated medically qualified doctor from the trial team) should sign and date the SAE CRF to acknowledge that he/she has performed the seriousness and causality assessments. Investigators should also report SAEs to their own health boards or trust in accordance with local practice.

A completed SAE form for all events requiring immediate reporting should be submitted via email to the CTR within 24 hours of knowledge of the event. A separate form must be used to report each event, irrespective of whether or not the events had the same date of onset.

The participant will be identified only by trial number, date of birth or year of birth and initials. The participant's name (or any other personal identifiers) should not be used in any correspondence.

It is also required that sites respond to and clarify any queries raised on any reported SAEs and report any additional information as and when it becomes available through to the resolution of the event. Additionally, CTR/pharmaceutical companies may request additional information relating to any SAEs/SARs and the site should provide as much information as is available to them in order to resolve these queries.

Serious Adverse Event (SAE) email address:

CTR-Safety@Cardiff.ac.uk

Serious adverse events should be reported from time of signature of informed consent, throughout the treatment period up to, and including attendance at the 12-month visit. Serious adverse reactions (such as long term side effects of trial treatment under investigation) should continue to be reported until the end of follow up as defined in the protocol.

An SAE form should contain at least the minimum information:

- Full participant trial number
- An Adverse Event / Adverse Reaction
- IMP or trial intervention
- A completed assessment of the seriousness, and causality as performed by the PI (or another appropriately medically qualified doctor registered on the delegation log)

If any of these details are missing, the site will be contacted and the information must be provided by the site to the CTR within 24 hours.

All other AEs should be reported.

14.6.2 The CTR responsibilities

Following the initial report, all SAEs should be followed up to resolution wherever possible, and further information may be requested by the CTR. Follow up information must be provided on a new SAE form.

The CTR should continue reporting SAEs until 30 days after the participant receives their last dose of the investigational medicinal product. Serious adverse reactions should continue to be reported until the end of follow up.

Once an SAE is received at the CTR, it will be evaluated by staff at the CTR and sent to the Chief Investigator (or their delegate) for an assessment of expectedness.

Investigator reports of suspected SARs will be reviewed immediately and those that are identified as SUSARs are reported to the MHRA, Research Ethics Committee and Inmunotek.

14.7 SUSAR reporting

Cardiff University is undertaking the duties of trial Sponsor and has delegated to the CTR the responsibility for reporting SUSARs and other SARs to the MHRA, REC and to Inmunotek as follows:

SUSARs which are fatal or life-threatening must be reported to the MHRA and REC within 7 calendar days of receipt at the CTR.

SUSARs that are not fatal or life-threatening must be reported to the MHRA and REC within 15 days of receipt at the CTR.

If report is incomplete then additional follow-up information should be reported within a further 8 calendar days of submitting the initial report, for all fatal and non fatal, life-threatening and non life-threatening

Any additional, relevant information must be reported within a further 15 days.

14.8 Unblinding for the purposes of SUSAR reporting

To enable processing of a SAR in this blinded trial, expectedness should be assessed initially using the assumption that the IMP has been given to the trial participant.

If it is assessed as unexpected as per the RSI of the IMP the SUSAR will be unblinded by the independent CTR safety group prior to reporting to any onward reporting.

If after unblinding it is evident that the trial participant received the placebo, this event will not require expedited reporting to the MHRA and REC, unless in the opinion of the Principal Investigator or Chief Investigator the event was related to the placebo (for example an allergic reaction to an excipient).

14.9 Safety Reports

A list of all SARs (expected and unexpected) will be reported annually to the MHRA, REC, trial sponsor and Inmunotek in the form of a Development Safety Update Report (DSUR). This report must be submitted within 60 days of the anniversary of the MHRA CTA approval date.

The CTR will report a list of all SARs (expected and unexpected) and any other safety recommendations to all PIs annually throughout the course of the trial. This frequency may be reviewed and amended as necessary. This reporting will be done via the Investigator safety report (ISR).

14.10 Urgent Safety Measures (USMs)

An urgent safety measure is an action that the Sponsor, Chief Investigator or Principal Investigator may carry out in order to protect the subjects of a trial against any immediate hazard to their health or safety. Any urgent safety measure relating to this trial must be notified to the MHRA and Research Ethics Committee immediately by telephone, and in any event within 3 days in writing, that such a measure has been taken. Inmunotek will also be informed within the same timeframe. USMs reported to the CTR will be handled according to CTR processes.

15 Statistical considerations

15.1 Randomisation

Participants will be randomised 1:1 using minimisation with a random element (p = 0.8). Allocations will be minimised by gestational and anti-RSV prophylaxis – both assumed to have a strong prognostic relationship with outcome. The randomisation program will be developed by the CTR and will be embedded within the study database with controlled access maintaining blinding. To avoid any issues with inadvertent unblinding or dosing errors, multiple births will be randomised to the same arm.

15.2 Blinding

Treatment allocation of Bactek or placebo will be blinded such that the allocation will not be known by clinicians, the baby's family or the trial outcome assessors.

15.2.1 Unblinding of individual participants during the trial

Unnecessary or unintended decoding of the blinding should not occur during the trial. Unblinding is only performed in emergency situations where knowledge of the identity of the trial drug is considered absolutely necessary for the clinical management of the infant, or if there is another compelling medical or safety reason to do so.

The Site PI is responsible for requesting emergency unblinding. Where authority for requesting unblinding is delegated to another member of the research team this should be clearly identified in the site delegation. Where possible, permission to unblind an individual case should be requested via the TM, who will then seek the agreement of the CI (or delegate). If the treating doctor/ healthcare professional is not the PI, the PI must be informed of the unblinding as soon as possible and the reasons for the actions taken, however, the allocation does not need to be revealed to the PI, unless required for the participant's medical treatment.

If unblinding is deemed necessary by authorised members of the local investigational team, the following procedure should be followed.

1. A web-based unblinding system will be made available to the local investigational team. Access will be controlled using a unique username and password.
2. Where possible (during office hours), consent for individual unblinding should be made via the TM at the CTR who will seek the opinion of the CI
3. An authorised member of the investigational team will log on to the unblinding system. On entering the required information, including the Pack ID of the infant, the treatment allocation will be revealed. The allocation will be transmitted to the person primarily responsible for their care.
4. A delegated member of the research team will complete the BALLOON Unblinding CRF, to be returned to the CTR within 24 hours of the event.
5. The trial treatment allocation should not be included on the CRF and the allocation should not routinely be revealed to CTR staff.

In the event the randomisation system is not available, during office hours the site may contact the CTR safety team, who will arrange for unblinding to be performed by a member of staff who is independent from the project team.

A copy of the completed unblinding CRF should be filed in the site file and the PI (or delegate) is also required to document the circumstances of the unblinding, including the reasons for doing so, in the participant's medical records.

The responsible PI should ensure that all necessary CRFs to the time of unblinding are completed and submitted to CTR (if possible, completed before unblinding is performed, although this is not always feasible).

15.2.2 Unblinding for safety reporting

The blinding will only be broken centrally for suspected SUSARs once assessed by the clinical reviewer. Only SUSARs from participants on active treatment (unless thought to be due to the excipient in the placebo) will be expediently reported to the MHRA and REC.

15.3 Sample size

We propose a two-arm, two-stage design where the trial may be stopped for lack of benefit. Our primary analysis will involve comparing health professional diagnosed LRTIs between 37-43 corrected weeks' or discharge if earlier and one-year corrected age between the two trial arms. Based on rates of LRTIs from previous cohorts of preterm-born infants of 46% (Drysdale et al., 2011, Drysdale et al., 2014), and the AZTEC follow up data showed admission rates of 55% (of 385 survivors with available data) by one year of corrected age (unpublished data), we have estimated that 55% of participants in the placebo arm will experience a health professional diagnosed LRTI between 37-43 weeks' corrected gestation or discharge if earlier and one year corrected age.

Our target effect size is a 15% absolute reduction (i.e. reduction to 40% in the intervention arm, translating to a relative difference of 27%). This is a realistic target and of clinical importance to both clinicians, health providers and parents. We assume a loss to follow-up rate of 15%, as data will be

obtained from healthcare professionals and followed up by Research Nurses and hence completion will be high.

In order to maximise trial efficiency and allow for stopping for lack-of-benefit, we have included an interim analysis as part of this trial with an intermediate outcome of health professional diagnosed LRTIs within 6-months post-randomisation (Bratton et al., 2013).

Assuming per-stage accrual rates of 19 per month in Stage 1 and 24 per month in Stage 2, a positive predictive value between intermediate and definitive outcomes of 1 (as $P(D=1|I=1) = 1$ by design given that I is D at an earlier time point), an estimate of the intermediate outcome proportion in the placebo arm of 0.37 (assuming that most infections occur during the first three-months of life), and identical absolute target effect sizes at both stage, we used the *nstagebinopt* command in Stata to identify the most admissible designs (and optimal in terms of minimising a weighted loss function using a Bayesian optimality criterion).

Stata code for replication:

```
nstagebinopt, nstage(2) arms(2) alpha(0.025) power(0.9) theta0(0 0) theta1(0.15 0.15) ctrlp(0.37 0.55) fu(0.5) ltfu(0.15 0.15) aratio(1) ppv(1) accrate(228 288)
```

This produces four admissible designs, of which we have chosen the one which strikes a reasonable balance between expected sample size under the null and maximum sample size (q-range = [0.22,0.67]). In our code below, we build in one month for interim analysis and reporting.

Stata code for replication:

```
nstagebin, nstage(2) accrate(19 24) alpha(0.43 0.025) power(0.97 0.92) extrat(1) probs ess arms(2 2) theta0(0 0) theta1(0.15 0.15) ctrlp(0.37 0.55) fu(6 12) ltfu(0.15 0.15) aratio(1) ppvc(1) ppve(1) tunit(4)
```

To maintain overall power of 90% and a pairwise one-sided alpha of 0.025, **we would require 346 recruited participants, of whom 182 will have analysable intermediate outcome data, to conduct an interim analysis with stopping for lack-of-benefit**. Our Stage 1 one-sided alpha is 0.43 and power is 97%. Based on our anticipated accrual rates, participant data will be accumulated for the interim analysis at recruitment month 21, and we plan one month for analysis and reporting.

Should we progress to the Stage 2, **we would require 542 total recruited participants of whom 460 have analysable definitive outcome data**. The Stage 2 one-sided alpha is 0.025 and power is 92%. Based on Stage 2 anticipated accrual rates, participant data would be accumulated at recruitment month 39 (i.e. study month 45).

In BALLOON, we plan to randomise infants who are born as part of a multiple to the same arm. This may induce clustering, as outcomes from infants born as part of a set of twins, triplets, etc. may be more similar to infants born to other mothers giving birth to singletons.

In the AZTEC trial (Lowe et al. 2024), our primary analysis included 796 infants born to 707 mothers, giving an average number of infants per mother of 1.13. We have no reliable assessment of the intra-cluster correlation coefficient for LRTI on which to calculate a design effect.

We have modelled various scenarios for ICCs and conclude that, when fixing the average cluster size at 1.13, we will retain at least 85% power regardless of clustering, 87% power for all but high degrees of clustering (e.g. ICCs > 0.6), and 88% power for moderate degrees of clustering (e.g. ICCs > 0.4). We therefore conclude that we will not inflate our current sample size for clustering. However, we will monitor key parameter estimates during our IDMC meetings and discuss the likely impact on power accordingly.

15.4 Missing, unused & spurious data

See 16.1.

15.5 Procedures for reporting deviation(s) from the original SAP

These will be submitted as substantial amendments where applicable and recorded in subsequent versions of the protocol and SAP.

15.6 Termination of the trial

See 16.1.1.

15.7 Inclusion in analysis

Our primary clinical question is: *In infants born at ≤29+6 weeks' gestation without a severely life-limiting condition/prognosis, does treatment with the trained immunity-based vaccine Bactek (MV130), from term-equivalent 37-43 weeks' correct gestation or discharge if earlier to one-year corrected age, decrease the risk of at least one unscheduled visit to a health care professionals for a lower respiratory tract infection (LRTI)?*

Therefore, our primary estimand is described by the following attributes:

Population: infants born at ≤29+6 weeks' gestation and aged term-equivalent 37-43 weeks' correct gestation or discharge if earlier **without a severely life-limiting condition/prognosis**.

Outcome/variable of interest: occurrence of at least one parent-reported, health professional-confirmed unscheduled visit for LRTI to GP, A&E and/or hospital admission between discharge for neonatal care and one-year corrected age.

Treatment condition: the Bactek (MV130) treatment or placebo, with advice to administer once daily (2 x actuations) at least 30 minutes prior or after the consumption of food and drink, from term-equivalent 37 weeks' correct gestation or discharge if earlier to up to one-year corrected age, regardless of adherence (including initiation, implementation, or persistence) of treatment (treatment policy strategy) (Vrijens et al., 2012).

Remaining intercurrent events: none expected, but death is an extremely rare possibility (infants expected to die within the first year of life will be excluded from the trial). These events will be handled using a "while on treatment" strategy, allowing for a healthcare provider perspective.

Population-level summary: Risk ratio of Bactek (MV130) compared to placebo adjusted for gestational age, and anti-RSV prophylaxis.

Rationale for estimand: To compare the strategy of providing Bactek (MV130) on LRTI incidence as might be expected in routine clinical practice.

As a sensitivity estimand, we will focus on the hypothetical effect of Bactek (MV130) on LRTI incidence in those infants who persist with treatment for the full 12-months. Further detail regarding this is given in Section 16.1 and will be provided in our Statistical Analysis Plan (SAP).

16 Analysis

16.1 Main analysis

Descriptive statistics and analysis plan

Descriptive statistics will be summarised using frequencies and percentages, means and standard deviations, or medians and interquartile ranges as appropriate.

A comprehensive SAP will be written and approved by the TMG, TSC, and IDMC prior to database lock and commencement of recruitment. The findings will be reported according to the Consolidated Standards of Reporting Trials (CONSORT) statement. We will incorporate the estimand framework within our protocol and statistical analysis plan to specify primary and sensitivity estimands and align analysis strategies accordingly.

Analysis of primary outcome

Analysis will be performed based on intention to treat. Our primary analysis will compare the proportion of participants receiving a health professional diagnosed LRTI post-randomisation between 37-43 weeks' corrected gestation or discharge if earlier and one-year corrected age between arms by fitting a two-level Poisson (or negative binomial) regression model, with multiple infants nested within mothers and robust standard errors. Models will also adjust for gestational age and anti-RSV prophylaxis (as per randomisation factors). Results will be reported as risk ratios, 95% confidence intervals, and p-values. Univariable, as well as adjusted models will be presented. Missing data are expected to be minimal but will be handled within a multiple imputation framework.

Sensitivity analyses around primary outcome measure

- As a sensitivity analyses, we will consider the use of inverse probability of treatment weights to estimate the effect of the intervention under the hypothetic scenario of different levels of adherence

Analysis of secondary outcome measures

Secondary outcomes will be analysed using appropriate regression models (e.g., linear, Poisson, negative binomial, flexible parametric survival models), adjusting for stratification and minimisation variables as per the primary analysis.

16.1.1 Safety run in period

Safety run-in period

- Recruit and randomise four infants. NB, we would expect only half of participants to be allocated to the active arm of the trial. The IDMC will review the allocation and advise if more participants need to be recruited.
- Administer study product as per protocol and monitor randomised infants for one-week post-randomisation, ideally while still in hospital.
- Record any adverse events. If no adverse events are reported, write an e-mail to the IDMC chair declaring this.
- If there are adverse events, generate a safety report for IDMC and hold open and closed meetings to discuss the progression of the study.

- Decisions which the IDMC could make following the end of the safety run in period:
    - **Do not proceed with any further recruitment according to the current protocol and indefinitely pause the trial.** This would be due to adverse events being reported which were deemed to be serious (defined as any untoward medical occurrence(s) that results in death, hospitalisation or prolongation of existing hospitalisation, persistent or significant disability/incapacity) and probably / definitely related to the study product. We would liaise with the site teams and manufacturer to determine the best way to resolve any issues that are identified.
    - **IDMC to meet at 3-months to review safety on a greater number of participants.** This would be due to either non-serious adverse events occurring which were probably or definitely related to study product or serious adverse events occurring which were possibly related to study product. Recruitment would continue during this period unless major safety concerns were identified by the TMG.
    - **Continue recruitment for the rest of the internal pilot.** This would be due to no adverse events occurring (confirmed via e-mail to IDMC), non-serious adverse events which were at most possibly related, or serious adverse events which were at most unlikely related to the study product.
- IDMC write a letter of recommendation to the TSC who in turn will write to the funder at the end of the safety run in period.

Table 6: Adverse event scenarios and decision-making in safety run-in for BALLOON

Seriousness of adverse event	Relatedness	Trigger pausing trial indefinitely	IDMC at 3-months to review safety on a greater number of participants	Trigger continuing to recruit into the internal pilot
N/A – no adverse event				X
Not serious	None			X
	Unlikely			X
	Possibly			X
	Probably		X	
	Definitely		X	
Serious	None			X
	Unlikely			X
	Possibly		X	
	Probably	X		
	Definitely	X		

16. 2 Internal pilot and progression criteria

An internal pilot will assess recruitment rates and data completeness for a 9-month period between recruitment months 4 – 12 staring immediately after completion of the 3-month safety run-in period as outlined in Table 6. The internal pilot report will be reviewed by the TMG and provided to the Independent Data Monitoring committee (IDMC)/Trial Steering Committee (TSC) for their consideration for a recommendation to be made to the funder during recruitment month 13 with guidance as follows:

Table 7: Pilot Study Criteria

	Red	Amber	Green
% Threshold*	<70%	70%-99%	100%
Recruitment rate per site	<0.65 infants per month	0.65 to <1.1 infants per month	≥1.1 infants per month
Number of sites open	<6	6-10	>10

Total number recruited	<95	95-135	≥ 136**

** the green category is based on recruiting 25% of total recruitment target of 542 i.e. 136.
* Threshold is the percentage of anticipated recruitment at nine months of the |Green category i.e. 70% of 136 = 95

If any of the criteria are red then a red outcome will be allocated. If none are red and any are amber then an amber category will be allocated. Otherwise, all criteria will have been met hence a green outcome will result. Since there will be many factors that can explain the outcome for the internal pilot study including those within the control of the TMG and recruiting teams or external ones beyond the Trial team's control, it will be important to carefully scrutinise the data including identifying specific factors which are within or outside the study team's control. These will be discussed with the TSC and IDMC as follows:

Red: Since we have used very stringent criteria, the BALLOON TMG will identify the underlying reasons for recruitment being low. These will identify if external factors beyond the remit of the TMG and BALLOON recruitment teams (including delays at regulatory bodies such as MHRA, HRA, etc., IMP supply issues, infections at the neonatal units, or due to national events as shown by the pandemic) or local factors (such as lack of infants, low rates of parents being approached or low rates of consent, etc.) were responsible for lower than target recruitment. These factors will be discussed robustly with the IDMC and the TSC to identify if the trial should be stopped or continue, addressing if the factors resulting in low recruitment are those beyond or within the TMG's control. Stopping the study or any mitigating factors to improve recruitment should result in robust confidence by the TSC, IDMC and EME that recruitment will improve or not in the future.

Amber: the TMG will identify any reasons why recruitment was not optimal. Again, external and internal factors, as mentioned above, will be identified and any potential remedial factors identified and discussed with the IDMC and TSC to implement to improve recruitment for the remaining recruitment period. The findings and recommendations will be provided and discussed with the EME team.

Green: whilst the study will continue, in consultation with the IDMC and TSC, we shall still aim to identify any areas of good practice and share these widely, but also aim to identify areas to improve recruitment, which will also be shared with the IDMC and TSC as well as the EME Team.

Sub-group analyses

We will extend our primary analysis by fitting trial arm by subgroup interaction terms to explore any differential intervention effects by gestational age and anti-RSV prophylaxis.

Interim analysis

At the interim analysis stage, we will compare the proportion of infants experiencing at least one health professional diagnosed LRTI within six months between trial arms by fitting a two-level Poisson (or negative binomial / logistic) regression model, with multiple infants nested within mothers and

robust standard errors. Models will also adjust for gestational age and anti-RSV prophylaxis (as per randomisation factors). Results will be reported to our IDMC as adjusted risk differences (using the margins command in Stata), 95% confidence intervals, and p-values. As per our design parameters, should our one-sided p-value be > 0.43, this would provide statistical indication of a lack of benefit of our intervention. Additional data (e.g. other outcomes, including safety data) may be considered by our IDMC when deciding whether to progress or stop at this stage.

17 Data Management

17.1 Source data

Table 8: Source data for the trial

Procedures (as per Schedule of Events, Figure 2)	CTR and CHaRT database	Participant medical notes	Participant Diary (Trial App)	Electronic System	Pharmacy File	SAE form	PIS & consent form	Laboratory report (PHW)	NHS Digital Data
Eligibility assessment		x							
Informed consent							x		
Randomisation	x								
Demographics and baseline characteristics		x							
Medical history		x							
Concurrent Medications & vaccinations		x							
Dispensing of drugs					x				
Withdrawal criteria	x								
Adverse events	x					x			
PROMS			x						
Hospital attendances									X
Virology								x	

Source Data is defined as *"All information in original records and certified copies of original records of clinical findings, observations or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents."* There is only one set of source data at any time for any data element, as defined in site source data agreement.

17.2 Data collection

It is intended to develop data recording for this trial as a web-based system. This is a secure encrypted system accessed by an institutional password, and complies with the General Data Protection Regulation 2016 standards.

Parents/Guardians will complete an App to record respiratory symptoms and adherence. Participants will be randomised on a Database provided by CHaRT and the IMP management system will also be on the CHaRTDatabase. Finally REDCap will be used by the research staff in the sites to record participant data.

Full details of data management are available in the Data Management Plan.

18 Sub-studies

18.1 Respiratory function training phase

To ensure adequate set-up, training and development of methodology for ancillary respiratory function testing, a training phase study will be undertaken recruiting approximately 5-10 babies in each participating centre. Participation in the sub-study training phase will be subject to a separate informed consent process, with independent written information, under the principles noted in section 9.3

The training phase studies will involve an oscillometry procedure as described in section 13 which may be performed 2 or 3 times on different days.

Participation in the sub-study training phase may include, but is not limited to, infants enrolled to the main trial.

Inclusion criteria

- At least 34 weeks' corrected age
- In the opinion of the PI, infants clinically well and able to tolerate the procedure(s)

Exclusion criteria

- Presence of major surgical, cardiac or congenital abnormalities (not including patent ductus arteriosus or patent foramen ovale)

19 Protocol/GCP non-compliance

The Principal Investigator should report any non-compliance to the trial protocol or the conditions and principles of Good Clinical Practice to the CTR in writing as soon as they become aware of it.

20 End of Trial definition

The end of the trial is defined as database hard lock.

Sponsor must notify the MHRA and main REC of the end of a clinical trial within 90 days of its completion or within 15 days if the trial is terminated early.

21 Archiving

The TMF and TSF containing essential documents will be archived at an approved external storage facility for a minimum of 25 years. The CTR will archive the TMF and TSFs on behalf of the Sponsor. The Principal Investigator is responsible for archival of the ISF at site on approval from Sponsor. Essential documents pertaining to the trial shall not be destroyed without permission from the Sponsor.

22 Regulatory Considerations

22.1 *Clinical Trial Authorisation*

This trial will be submitted for Clinical Trials Authorisation (CTA) from the UK Competent Authority: MHRA.

22.2 *Ethical and governance approval*

This protocol will be submitted for opinion from a Research Ethics Committee (REC) that is legally "recognised" by the United Kingdom Ethics Committee Authority for review and approval.

This trial protocol will be submitted through the relevant permission system for global governance review dependant on the location of the lead site.

Approval will be obtained from the host care organisation who will consider local governance requirements and site feasibility. The Research Governance approval of the host care organisation must be obtained before recruitment of participants within that host care organisation.

22.3 Data Protection

The CTR will act to preserve participant confidentiality and will not disclose or reproduce any information by which participants could be identified, except where specific consent is obtained. Data will be stored in a secure manner and will be registered in accordance with the General Data Protection Regulation 2016. The data custodian and the sample custodian for this trial is Cardiff University.

This includes collection of NHS number (or equivalent – e.g. CHI number in Scotland), name and postcode to register and trace participants with NHS Digital. Collection of NHS number or equivalent to utilise NHS data for future research is also required.

22.4 *Indemnity*

- Non-negligent harm: This trial is an academic, investigator-led and designed trial, coordinated by the CTR and Sponsored by Cardiff University. The Chief Investigator, local Investigators and coordinating centre do not hold insurance against claims for compensation for injury caused by participation in a clinical trial and they cannot offer any indemnity. The Association of the British Pharmaceutical Industry (ABPI) guidelines will not apply.

- Negligent harm: Where studies are carried out in a hospital, the hospital continues to have a duty of care to a participant being treated within the hospital, whether or not the participant is participating in this trial. Cardiff University does not accept liability for any breach in the other hospital's duty of care, or any negligence on the part of employees of hospitals. This applies whether the hospital is an NHS Trust or not. The Sponsor shall indemnify the site against claims arising from the negligent acts and/or omissions of the Sponsor or its employees in connection with the Clinical Trial (including the design of the Protocol to the extent that the Protocol was designed solely by the Sponsor and the Site has adhered to the approved version of the Protocol) save to the extent that any such claim is the result of negligence on the part of the Site or its employees.

All participants will be recruited at NHS sites and therefore the NHS indemnity scheme/NHS professional indemnity will apply with respect to claims arising from harm to participants at site management organisations.

22.5 *Trial sponsorship*

Cardiff University will act as Sponsor for trial. Delegated responsibilities will be assigned to the sites taking part in this trial.

The trial is being sponsored by Cardiff University with responsibilities delegated to the CTR. The CTR shall be responsible for ensuring that the trial is performed in accordance with the following:

- The Medicines for Human Use (Clinical Trials) Regulations 2004 (SI2004/1031) and subsequent amendments.
- Conditions and principles of Good Clinical Practice.
- Declaration of Helsinki (1996)
- UK Policy Framework for Health and Social Care Research.
- The General Data Protection Regulation 2018.
- The Human Tissue Act 2004.
- Other regulatory requirements as appropriate.

22.6 Funding

This Project is funded by the NIHR's Efficacy and Mechanisms Evaluation Programme.

24 Trial management

24.1 Trial Management Group

Trial Management Group (TMG) members will be required to sign-up to the remit and conditions as set out in the TMG Charter. The TMG will meet monthly to review trial progress.

24.2 Independent Trial Steering Committee

The independent Trial Steering Committee (TSC) members will be required to sign-up to the remit and conditions as set out in the TSC Charter. The IDMC will meet with frequency specified in the TSC charter.

24.3 Independent Data and Monitoring Committee

The Independent Data Monitoring Committee (IDMC) members will be required to sign-up to the remit and conditions as set out in the IDMC Charter. The IDMC will meet with frequency specified in the IDMC charter. The Committee consists of two experienced Clinicians and one experienced statistician:

1. Professor Eleanor Molloy – Professor of Paediatrics and Child Health, Dublin University
2. Professor Paul McNamara – Professor in Child Health, Liverpool University
3. Dr Zoe Hoare – Director and Principal Statistician at NWORTH, Bangor University

25 Quality Control and Assurance

25.1 Monitoring

Monitoring frequency will be determined by a clinical trial risk assessment performed prior to the start of the trial to determine the intensity and focus of central and on-site monitoring activity. A detailed monitoring plan will be generated detailing the frequency and scope of the monitoring for the trial. Throughout the course of the trial, the risk assessment will be reviewed, and monitoring frequency adjusted as necessary.

Investigators should agree to allow trial related monitoring, including audits and regulatory inspections, by providing direct access to source data/documents as required. Participant consent for this will be obtained. Where electronic health records (EHR) are being used, trial teams and monitors should check EHR process early in trial and periodically thereafter.

Findings generated from on-site and central monitoring will be shared with the Sponsor, CI, PI & local R&D.

25.2 Audits & inspections

The trial is participant to inspection by MHRA as the regulatory body. The trial may also be participant to inspection and audit by Cardiff University under their remit as Sponsor.

The CI or PI organisations/institution(s) will permit trial-related monitoring, audits, REC review, and regulatory inspection(s), providing direct access to source data / documents.

The site must inform the CTR of any MHRA inspections.

26 Public Involvement and Engagement

The PI&E plan will be documented in a separate document.

27 Publication policy

All publications and presentations relating to the trial will be authorised by the Trial Management Group.

28 References

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