"TAILOR" Study

Biomarkers in preschool children with wheeze to **TA**rget therapy w**I**th inha**L**ed c**OR**ticosteroids (**TAILOR**): a feasibility study.

Version 9.0 – 22nd September 2023

MAIN SPONSOR:	Imperial College London
FUNDERS:	Asthma UK Centre for Applied Research
STUDY COORDINATION CENTRE:	Oxford University Hospitals NHS Foundation Trust
IRAS Project ID:	300524
REC reference:	21/PR/1195

Signature Page

The undersigned confirm that the following protocol has been agreed and accepted and that the Chief Investigator agrees to conduct the study in compliance with the approved protocol and will adhere to the principles outlined in the Declaration of Helsinki, the Sponsor's SOPs, and other regulatory requirement.

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 investigation without the prior written consent of the Sponsor.

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

This protocol has been written in accordance with the Sponsor's guidance for writing non-CTIMP protocol.

PROTOCOL MODIFICATIONS		
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PROTOCOL AUTHORISED BY:

NAME AND ROLE	**DATE**	**SIGNATURE**

STUDY MANAGEMENT GROUP

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STUDY COORDINATION CENTRE: Imperial College London

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SPONSOR

Imperial College London is the main research Sponsor for this study. For further information regarding the sponsorship conditions, please contact the Head of Regulatory Compliance at:

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FUNDER

Asthma UK Centre for Applied Research

This protocol describes the "**TAILOR**" study and provides information about procedures for entering child participants. Every care was taken in its drafting, but corrections or amendments may be necessary. These will be circulated to investigators in the study. Problems relating to this study should be referred, in the first instance, to the Chief Investigator.

This study will adhere to the principles outlined in the UK Policy Framework for Health and Social Care Research. It will be conducted in compliance with the protocol, the Data Protection Act and other regulatory requirements as appropriate.

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1. GLOSSARY OF ABBREVIATIONS

AE	Adverse Event
APR	Annual Progress Report
AR	Adverse Reaction
CI	Chief Investigator
CRF	Case Report Form
ED	Emergency Department
EVW	Episodic Viral Wheeze
FeNO	Fractional Exhaled Nitric Oxide
GCP	Good Clinical Practice
GDPR	General Data Protection Regulation
GP	General Practitioner
HRA	Health Research Authority
ICF	Informed Consent Form
ICS	Inhaled Corticosteroids
ID	Identification Number
IL	Interleukin
LPLV	Last Patient Last Visit
MHRA	Medicines and Healthcare Products Regulatory Agency
MTW	Multiple-Trigger Wheeze
Non-CTIMP	Non-Clinical Trial of an Investigational Medicinal Product
OCS	Oral Corticosteroids
PI	Principal Investigator
PIS	Patient Information Sheet
REC	Research Ethics Committee
R&D	Research and Development
RGF	Research Governance Framework
SAE	Serious Adverse Event
SMF	Study Master File
SOP	Standard Operating Procedure
SPTs	Skin Prick Tests

KEYWORDS:

Wheezing, preschool, children, atopic sensitisation, atopy, aeroallergens, blood eosinophils, eosinophilia, FeNO.

2. STUDY SUMMARY

TITLE: Biomarkers in preschool children with wheeze to TArget therapy wIth inhaLed cORticosteroids (TAILOR): a feasibility study".

AIM: To obtain pilot data to test the hypothesis that aeroallergen sensitisation (confirmed by a wheal formation with a diameter ≥ 3 mm after a skin prick test is performed), FeNO (offline method) ≥ 10 ppb and a peripheral blood eosinophil count ≥ 300/µl, alone or in combination, can predict future wheezing exacerbations in preschool children as well as response to treatment with inhaled corticosteroids, as a preliminary to a large, randomised controlled trial. Also, that these tests are acceptable to families.

OBJECTIVES:

- To recruit 100 preschool children with a history of wheeze, aged one to five years old, and determine atopic status, peripheral blood eosinophil count (point of care test) and FeNO (offline method).
- To evaluate the feasibility and acceptability of these tests
- To categorize the children according to the biomarkers tested and assess whether any or all, alone or in combination, predict future wheezing exacerbations as well as determine the clinical outcome over one year
- To obtain parents' and doctors' insights into whether the biomarkers mentioned above could be used in a future intervention study.

OUTCOME MEASURES:

Primary outcome:

- Acute attacks of wheeze defined as requiring an unscheduled health care visit

Secondary outcomes:

- Carer days off work
- Children unable to attend childcare facility
- Use of oral corticosteroids
- Use of inhaled β-2 agonist (rescue therapy)
- Assessment if children's respiratory or wheezing condition is under control by using the TRACK™ score questionnaire (Test for Respiratory and Asthma Control in Kids) (Appendix 3).

POPULATION: Children aged one to five years old presenting either to primary care with wheezing episodes or to emergency departments with an acute attack of wheeze or to secondary care (e.g., outpatients) or children identified from primary care records and have been diagnosed with wheezing by their GP or paediatrician.

ELIGIBILITY: (children)

Inclusion criteria:

1. Patients aged one to five years old presenting either to primary care or to the emergency department or to secondary care (e.g., outpatients) or identified from primary care records and have been diagnosed with wheezing by their GP or paediatrician who has decided to prescribe any bronchodilator, ICS or montelukast on clinical grounds
2. Parents/Carers able to understand and familiarize themselves with the study and are willing to provide informed consent

Exclusion criteria:

1. Inability to understand and cooperate with study procedures
2. Significant co-morbidity (respiratory or otherwise), for example cystic fibrosis (excluding atopic disorders such as eczema, allergic rhinitis and food allergy)
3. Withholding or withdrawal of informed consent
4. Severe procedural anxiety (needle phobia)
5. Child is already enrolled in another study involving investigational medicinal product (CTIMP)
6. History of anaphylaxis or near-fatal asthma that resulted in intubation / assisted ventilation.

ELIGIBILITY: (Healthcare professionals)

Inclusion Criteria:

1. Doctors and nurses working in GP practices.

Exclusion Criteria:

1. Inability to understand and cooperate with study procedures.

DESIGN:

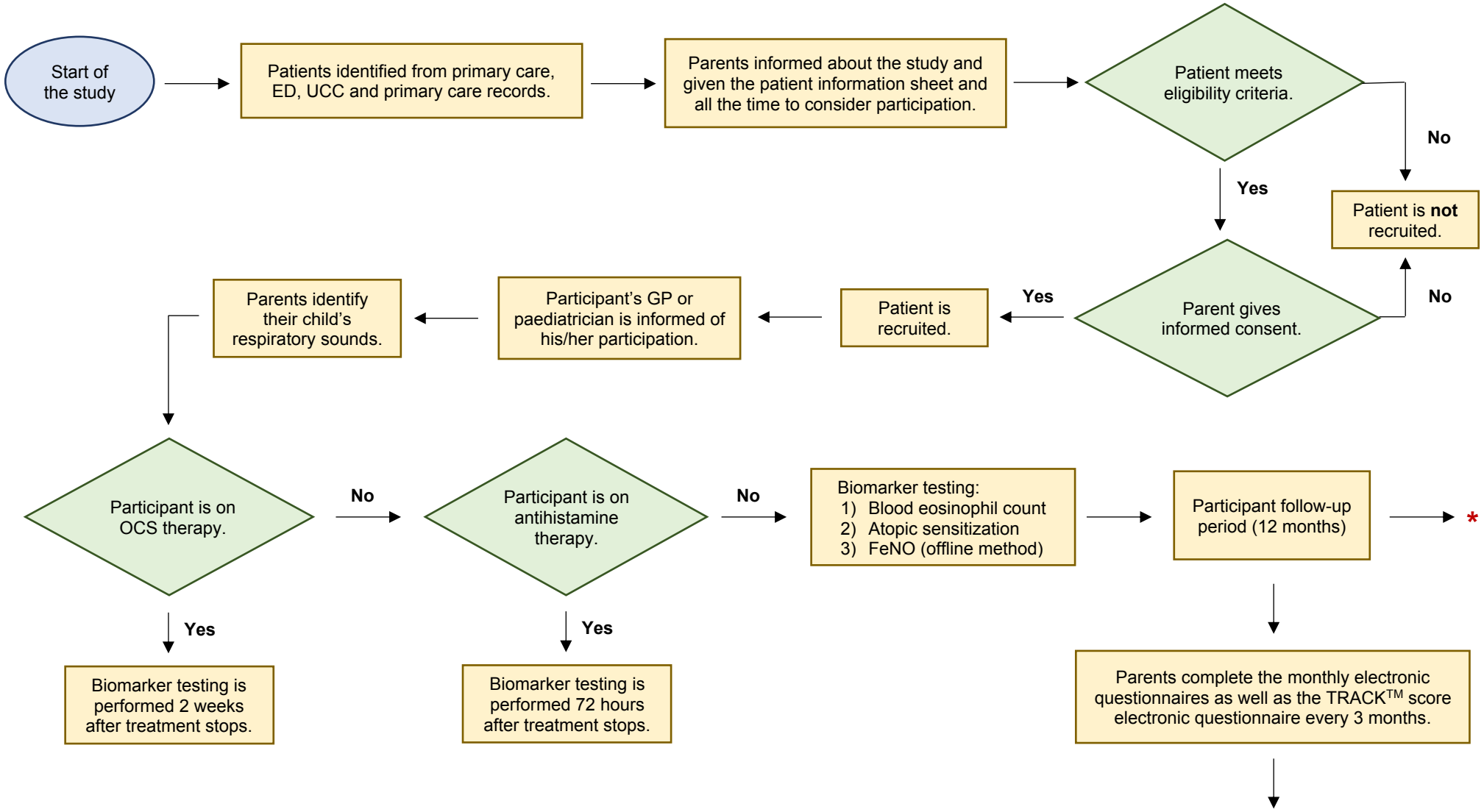
This is a pragmatic, observational study in children with pre-school wheeze. After recruitment and at the first study visit, biomarkers (allergic sensitisation, exhaled nitric oxide and peripheral blood eosinophil count) will be measured. The children will be followed for a year, with monthly electronic questionnaires and study visits every 3 months. All treatment and routine monitoring decisions will be at the discretion of the children's general practitioner (GP) or paediatrician (as per usual clinical practice), blinded to the study measurements. There will be a second optional biomarker measurement 3 months after the first one, in their second study visit. Parents/Carers of preschool children presenting either to primary care or to the Emergency Department (ED) or to secondary care (e.g., outpatients) as well as of those identified from primary care records and have been diagnosed by their GP or paediatrician with wheezing, will be identified by collaborating doctors and nurses who will ask the parents if they are willing to have their child participate in the study (the list of recruitment sites is given in Appendix 1 – Recruitment Plan). A patient information sheet will be given, containing all the information regarding the

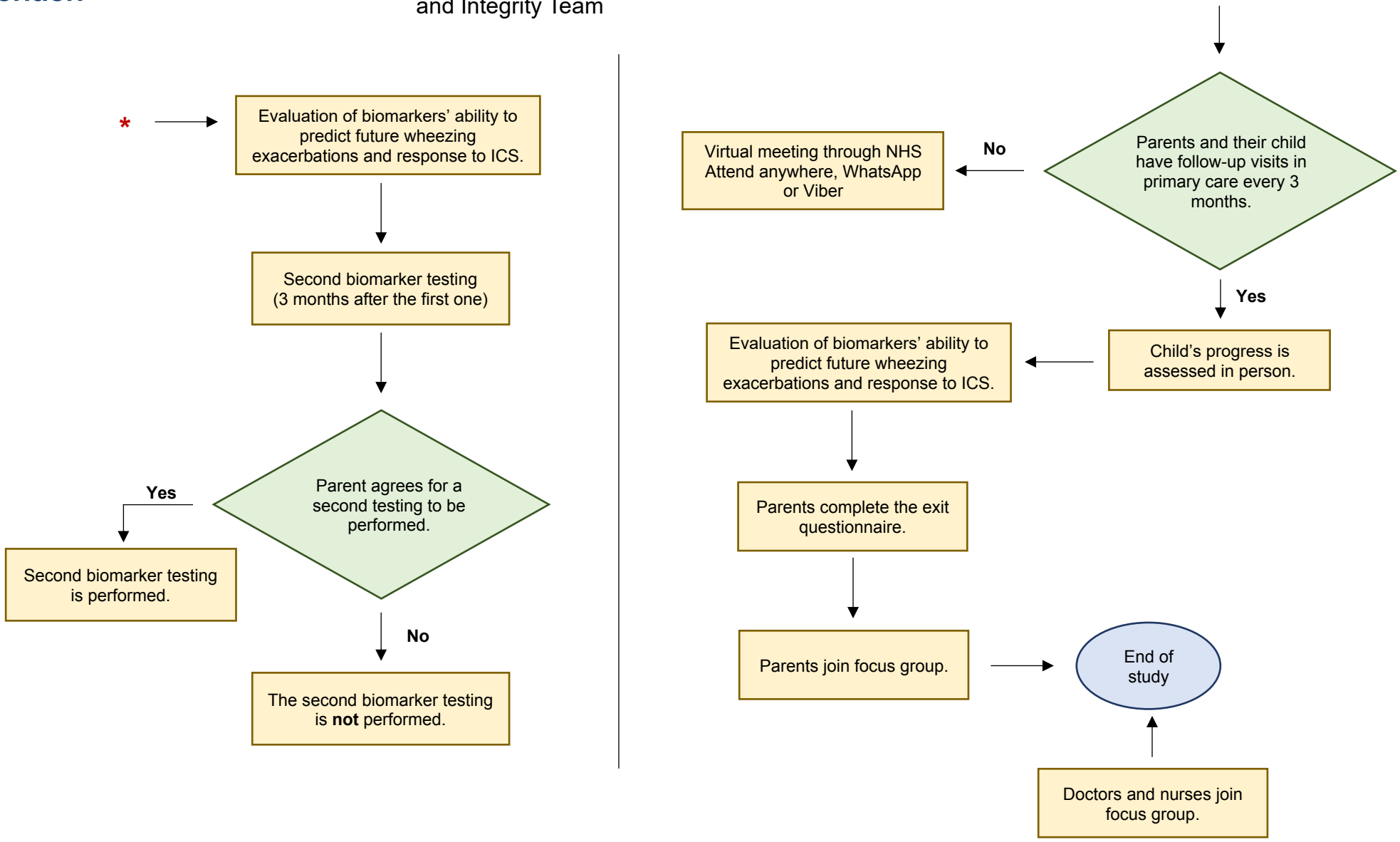
project and the contact details of the research team, for further questions. They will be asked for their willingness to give the study team permission to contact them and discuss recruitment. After clinical consultation and following submission of the electronic informed consent, the parent/carer will be asked to give the contact details of the child's GP or paediatrician, who will be informed about child's participation in this study through a letter sent to his/her email along with the patient information sheet. In addition, we will ask them to provide some of the child's personal data. The parent carer will be then shown a standard video questionnaire and will be asked to identify what respiratory sounds their children have been making. The child will be booked for the above biomarker tests at a single visit and at a time of clinical stability. If the child has been prescribed OCS, biomarker testing will be delayed by 2 weeks. Firstly, a sample of the child's exhaled tidal breath will be collected in a suitable bag for offline measurement of FeNO. Skin prick tests will be performed to (a) house dust mite, (b) grass pollen, (c) tree pollen, (d) cat hair € dog hair, as well as negative and positive controls and these will be applied on either the child's back, leg or arm. If the child has been prescribed antihistamines, we will ask for these to be withheld for 72 hours prior to skin prick testing. Finally, the peripheral blood eosinophil count will be measured from a finger prick blood sample, using the Haemocue machine, giving a result in approximately 2 minutes. All tests will normally take no more than one hour.

Follow-up: We will arrange for a short monthly electronic questionnaire to be sent by email to the parents/carers, to record on a monthly basis, the days their child was out of childcare, days the carer missed from work, unscheduled health care visits and use of rescue therapy or oral corticosteroids (including dosage) (Appendix 4: Monthly Questionnaire). We will record the children's TRACK^{TM} score (Test for Respiratory and Asthma Control in Kids) (Appendix 3: TRACK^{TM} score questionnaire) every 3 months to assess wheeze control. In addition, review visits will occur every 3 months, but if not feasible, video calls will be arranged through Microsoft Teams. Correct inhaler use will be ensured by parental education. If a child has had their ICS treatment discontinued, we will record whether this was because the treatment was ineffective (treatment failure) or if the child was so well that the family or GP did not wish to continue (treatment success or spontaneous improvement). If an additional medication is prescribed, or the child is referred to secondary care, this will be considered as treatment failure. When the study ends, parents/carers will be given the opportunity to join a focus group and give their insights into the investigations and the conduct of the study and inform the design of the future intervention study. In addition, we will ask them to fill in a very short questionnaire after the study is finished, to check their perspective

	and acceptability on these biomarkers being used in clinical settings to guide children's therapy in the future (Appendix 5: Exit Questionnaire). Finally, a focus group for doctors and nurses will also be conducted to check the feasibility of the 3 biomarker tests being used in clinical practice.
DURATION:	36 months total study duration, with 1-year follow-up for each child participant included. Study's start date will be 01.09.2021 and end date will be 31.12.2023.

3. TAILOR STUDY – PROTOCOL FLOWCHART





4. INTRODUCTION

4.1. BACKGROUND

Wheezing in preschool children is a common and troublesome problem, leading to impaired quality of life and repeated admissions to hospital (1). Management hitherto has been largely on the basis of history, rather than biomarker driven, and bedevilled by meaningless questions such as 'At what age can you diagnose asthma?'(2,3). There is some merit in dividing these children into episodic viral wheeze (EVW, children who only wheeze with a usually clinically diagnosed viral respiratory tract infection) and multiple trigger wheeze (MTW, children who wheeze both with respiratory viral infections and with triggers such as exercise and allergen exposure between colds) (4), but such a distinction relies on accurate parental perception of symptoms (5). This is notoriously inaccurate, and for, example, when compared with a video questionnaire, it is clear that many sounds which are not the result of intrathoracic airway narrowing are described as wheeze by parents/carers.

The Lancet Asthma Commission has highlighted that 'asthma' is an umbrella term for a clinical syndrome comprising wheeze, chest tightness, breathlessness and sometimes increased cough (2). The focus has moved to defining so-called 'treatable traits', such as whether there is eosinophil airway inflammation, usually driven by the signature Type 2 cytokines Interleukin (IL) -4, -5, and -13, which are responsive to inhaled corticosteroids (ICS) (6,7). Nowhere is this concept of treatable traits more important than in preschool wheeze, wherein only a few children who wheeze have any evidence of allergic sensitisation or Type 2 inflammation. At age one year, even children with such severe symptoms as to merit bronchoscopy in a tertiary referral children's hospital had no evidence of any inflammation or airway remodelling (8). By 30 months of age, in another cross-sectional study, some but by no means all had evidence of eosinophil airway inflammation, reticular basement membrane thickening and airway smooth muscle hypertrophy and hyperplasia (9). Clinical experience reflects this diversity, and although atopic preschool wheezers are more likely to respond to ICS, as we have shown, many atopic wheezers do not have an eosinophil airway phenotype (10), and are thus unlikely to be ICS responsive. We have recently confirmed that there is a good correlation between bronchoalveolar lavage and peripheral blood eosinophil concentrations (11).

Another biomarker for eosinophil airway inflammation is exhaled nitric oxide (FeNO). In older children and adults, titrating ICS dose using FeNO has been shown to reduce asthma attacks and improve outcomes (12). The use of FeNO to guide management not been studied in pre-school children. Young children usually cannot perform the standard slow exhalation, but we have experience in measuring FeNO off-line by collecting tidally exhaled air, which is the technique we propose to use.

Allergic sensitisation as determined by skin prick tests, is another association of airway Type 2 inflammation in children, which has been used to predict response to ICS (13). Any biomarker which is of practical use in the management of preschool wheeze has to be able to provide an answer quickly and be available in primary care where most of these children are treated. A portable point of care measurement of peripheral blood eosinophil count from a finger prick blood sample will be carried out in a network of primary health care centres in and around Oxford (a finger prick being the sort of test done repeatedly at home for diabetic children to monitor insulin requirements) and exhaled nitric oxide measurements, meaning that both tests are practical by the above criteria.

Finally, it should be noted that atopy is not an 'all-or-nothing' phenomenon (14). We propose to perform exploratory, hypothesis generating analyses quantifying atopy by the sum of skin prick test wheals, to see if the predictive power of this test can be further improved.

4.2. RATIONALE FOR CURRENT STUDY

Inhaled corticosteroids are effective treatment at all ages for patients with eosinophil airway disease, but have side-effects, including growth suppression, adrenal failure and increased susceptibility to respiratory infection. Only some children with preschool wheeze have airway eosinophilia, and thus benefit from inhaled corticosteroids, but these cannot be distinguished from the non-eosinophil phenotypes clinically. By using three simple point of care tests (blood eosinophil count, skin prick test to aeroallergens and FeNO) we aim to select out the subgroup of responders who will benefit, while avoiding exposing non-responders to potential side-effects.

4.3. PRE-CLINICAL/CLINICAL DATA

This highly variable pathophysiology of pre-school wheeze is reflected in the response to treatment, making the determination of phenotype much more than a matter of academic importance. In the first serious attempt to apply personalised medicine to this group of patients, the INFANT study investigators used a three way crossover design to compare the differential response to therapy with intermittent ICS, regular ICS and montelukast in 300 children (15). Sixty improved spontaneously over the duration of the study. Atopic sensitisation predicted a good response to ICS, shown by reduced numbers of attacks of wheeze and better control of chronic symptoms. In a retrospective analysis, an elevated blood eosinophil count and even better, a combination of positive SPTs and peripheral blood eosinophils >300/µl were the best predictors of a good response to regular ICS. Leukotriene receptor antagonists were not beneficial in any group. Those with no positive SPTs or elevated eosinophil count rarely had acute attacks of wheeze. These findings, especially the predictive power of blood eosinophil count, need to be tested prospectively in primary care, the setting in which most of these children are treated, in a large prospective randomised controlled trial. Prior to this, pilot data to inform power calculations, and the feasibility and

acceptability of this approach needs to be determined. Additionally, although FeNO in preschool children is not well standardized there is one study which proposed FeNO values ≥ 10 ppb to be predictive of eosinophil inflammation in preschool children (16).

5. STUDY'S AIM AND OBJECTIVES

AIM: To obtain pilot data to test the hypothesis that aeroallergen sensitisation (confirmed by a wheal formation with a diameter ≥ 3 mm after a skin prick test is performed), FeNO (offline method) ≥ 10 ppb and a peripheral blood eosinophil count ≥ 300/µl, alone or in combination, can predict future wheezing exacerbations in preschool children as well as response to treatment with inhaled corticosteroids, as a preliminary to a large, randomised controlled trial. Also, that these tests are acceptable to families.

OBJECTIVES:

- To recruit 100 preschool children with a history of wheeze, aged one to five years old, and determine atopic status, peripheral blood eosinophil count (point of care test) and FeNO (offline method).
- To evaluate the feasibility and acceptability of these tests
- To categorize the children according to the biomarkers tested and assess whether any or all, alone or in combination, predict future wheezing exacerbations as well as determine the clinical outcome over one year
- To obtain parents' and doctors' insights into whether the biomarkers mentioned above could be used in a future intervention study.

5.1. STUDY RISK / BENEFIT ANALYSIS

Benefits: The only tests of clinical significance to the child are the skin prick tests, which might for example inform a decision about the purchase of a pet. The family will be informed about these results. The other tests won't be of clinical significance. The ultimate benefit will be in the future if we can show with a large intervention trial that measuring one or more of these biomarkers helps predict future wheezing exacerbations as well as guide treatment more efficiently, thus children stop receiving unnecessary medications. The benefit generated by this trial is to show that these markers can be measured in practice, and they are acceptable to the families.

Risk analysis: Child participants' routine clinical management is not changed in this observational study. Clinicians have no access to study data. Risks relate to study procedures: all procedures [blood sampling through finger prick test, skin prick tests and FeNO collection (offline method)] will be performed by trained research personnel.

Skin prick tests are safe and used clinically for many years, but adrenaline and antihistamines will be available while the study procedures are being performed, in the highly unlikely event of an allergic reaction (Figure 1). All research staff will be trained in management of acute reactions / anaphylaxis. The finger prick test is the same as used by diabetic patients to monitor blood glucose. Collecting breath into a bag is devoid of risk.

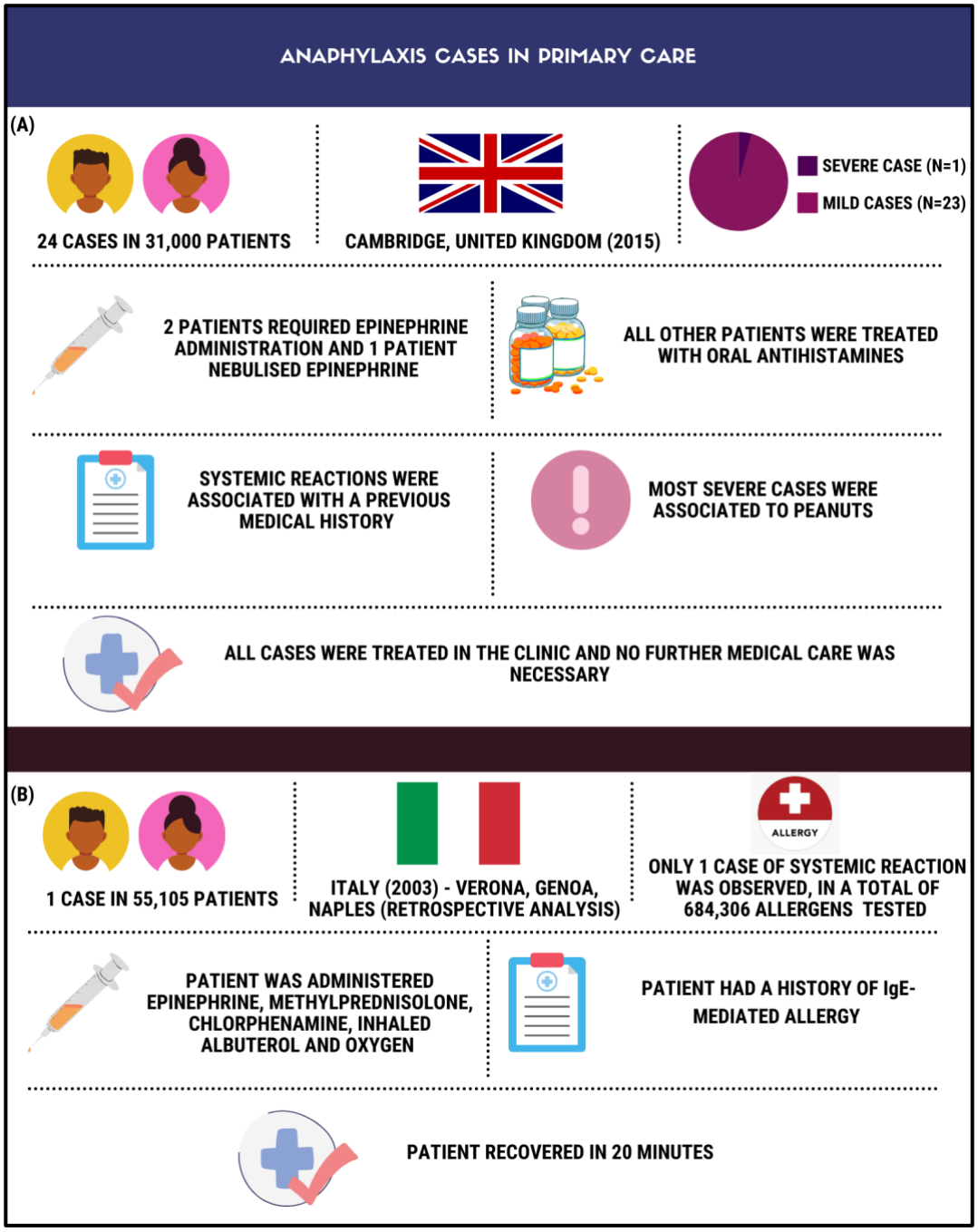


Figure 1. Anaphylaxis case reports after skin prick test. (A) Clinical study in Cambridge, UK involving 31,000 participants (17), (B) Retrospective analysis from 4 allergy centres in Italy (Verona, Genoa and Naples) involving 55,105 participants (18).

6. STUDY DESIGN

This is a pragmatic, observational study involving preschool children with wheeze aged one to five years old. All treatment and routine monitoring decisions will be at the discretion of their treating general practitioner (GP) or paediatrician (as per usual clinical practice), blinded to the study measurements. After clinical consultation and following submission of the electronic informed consent through the Qualtrics® platform (offered by Imperial College London), the parent/carer will be asked to give the contact details of the child's GP or paediatrician, who will be informed about child's participation in this study through a letter sent to his/her email along with the patient information sheet. The parent carer will be then shown a standard video questionnaire and will be asked to identify what respiratory sounds their children have been making. The child will be booked for the three biomarker tests [atopic sensitisation, blood eosinophil count and FeNO (off-line method)]. The tests will be carried out once and at a time of clinical stability. In this visit, a sample of the child's exhaled tidal breath will be collected in a suitable bag in duplicate for offline measurement of FeNO later on with chemiluminescence. Skin prick tests will be performed to: (a) house dust mite, (b) grass pollen, (c) tree pollen, (d) cat hai€(e) dog hair, as well as normal saline and histamine and these will be applied on either the child's back, leg or arm. If the child has been prescribed antihistamines, we will ask for these to be withheld for 72 hours prior to skin prick testing. FeNO values (offline method) ≥ 10 ppb will indicate Type 2 inflammation and wheal diameter, measured with a specialised skin prick test ruler, ≥ 3mm will indicate sensitisation to the applied aeroallergen. In addition, skin prick tests will allow the assessment of which aeroallergen is the most useful predictor of outcomes in preschool children. Finally, the peripheral blood eosinophil count will be measured from a finger prick blood sample, using the Haemocue machine, allowing a result in approximately 2 minutes. Values ≥ 300 cells/µl indicate Type 2 inflammation. All tests will normally take no more than one hour.

The recruitment period will approximately last 9 months, and all preschool wheezers can join the study at any point (Appendix 1: Recruitment Plan). Parents/Carers of preschool children presenting to primary care or to the Emergency Department (ED) or to secondary care (e.g., outpatients) as well as those identified from primary care records and who have been diagnosed by a GP or paediatrician with wheezing, will be approached by collaborating doctors and nurses who will ask their parents if they are willing to have their child participate in the study (Appendix 1: Recruitment Plan). A patient information sheet will be given, containing all the information regarding the project and the contact details of the research team, for further questions. They will be asked for their willingness to give the study team permission to contact them and discuss recruitment, and if so, they will submit their contact details in an electronic form made with Qualtrics® questionnaire platform (offered by Imperial College London), which allows gathering data according to the GCP guidelines and as used for years in the Breathing Together study (19). This form will be available online in the Asthma UK Centre for Applied Research's (AUKCAR) website. The initial introduction

will be made either by the clinical team (above) with the study team contacting them at a later date to give more information or by Mr. Andreas Perikleous if he is there in person.

Additionally, the Emergency Department of Oxford Universities Hospital (OUH) Trust will identify patients who have previously presented and diagnosed with wheezing in the Emergency Department. A regular search of the OUH databases will be performed by the clinical care team to identify patients who meet the eligibility criteria and have attended the Emergency Department. Parents/carers of eligible participants will be informed about the study by phone by a member of their clinical care team and asked whether they agree to be contacted by the study team to give them more information and send them the participant information sheet. Their contact details will be then submitted in the TAILOR study contact details form.

If the child has been prescribed OCS, testing will be delayed by 2 weeks for biomarker testing as soon as the electronic informed consent is submitted. Finally, parents will be informed through the patient information sheet as well as, in person, that a second optional testing will take place 3 months after the first one, in their second study visit. This will allow assessing the reproducibility of the obtained biomarker results, thus additionally assess their stability.

Patient recruitment at a site will only commence once the study team has ensured the following:

- HRA approval
- Completed Local Site Delegation of Duties and Signature Log
- Confirmation of Capacity and Capability

All recruitment procedures will be in accord with the GCP, and parents/carers will be given detailed information regarding the study as well as all the time they need to understand it. Parents/guardians have at least 24 hours to consider participation. Recruitment will only commence after parents/carers have fully understood the project and given informed consent online through the Qualtrics® platform (offered by Imperial College London).

Child participants entering the study upon their parents' wish, will be fully screened by the Principal Investigator (PI) or someone else from the research team who is suitably qualified and delegated to do so, with the latter taking informed consent as well. After the electronic informed consent sheet is submitted, parents will be asked to provide the contact details of their child's GP or paediatrician, to inform him/her about the child's participation in this study through a letter sent by email along with this patient information sheet. In addition, we will ask them to provide some of the child's personal data (Appendix 1: Recruitment Plan, Table 3: The personal data that will be obtained

by each individual participant). Children's full name, date of birth, gender, ethnicity as well as, their parent's full name, telephone number and email address will be added in the child's patient card. All other data obtained from the study will be added in the Case Report Form (CRF).

Follow-up: We will arrange for a short monthly electronic questionnaire to be sent by email to the parents/carers, in order to record on a monthly basis, the days their child was out of childcare, days the carer missed from work, unscheduled health care visits and use of rescue therapy or oral corticosteroids (including dosage) (Appendix 4: Monthly Questionnaire). In addition, we will record the children's TRACK™ score (Test for Respiratory and Asthma Control in Kids) (Appendix 3: TRACK™ score questionnaire) every 3 months, to assess wheeze control. The questionnaires will be made and sent through the Qualtrics® questionnaire platform (offered by Imperial College London). There will be review visits every 3 months, to evaluate their progression in person, but if not feasible, video calls will be arranged through Microsoft Teams. Correct use of spacers will be ensured after guiding parents on how to use them and will be done by their GP or paediatrician as well as from Mr. Andreas Perikleous who is a registered pharmacist in the UK.

In the event that a child has had their ICS treatment discontinued, we will record whether this was because the treatment was ineffective (treatment failure) or if the child was so well that the family or GP did not wish to continue (treatment success or spontaneous improvement). If an additional medication is prescribed, or the child is referred to secondary care, this will be considered as treatment failure. When the study ends, participants' parents/carers will be invited to a face-to-face focus group interview, or a one-to-one interviews, or remotely through Microsoft Teams, depending on their availability at the end of the study period. The focus group and one to one interviews will utilise a semi structured questionnaire, to provide qualitative feedback on the acceptability of the 3 biomarker tests. In addition, a separate focus group for doctors and nurses will be conducted to assess the feasibility of the 3 biomarker tests being used in clinical practice. They will be given all the information regarding the study as well as the study's patient information sheet. Those agreeing to take part, will sign a consent form. They will be attending either a face-to-face focus group interview, or a one-to-one interview, or remotely through Microsoft Teams, depending on their availability. A semi structured questionnaire will be utilised. Where possible a second independent researcher will sit in the focus groups discussion and interviews to ensure there is no bias. The conversations from the focus group and one to one interviews will be transcribed and coded into themes. We will utilise an external company for transcribing the conversations called 1st Class. https://www.1stclass.uk.com/

The focus group and one to one interviews will be audio recorded, transcribed and put into themes to conduct an inductive thematic analysis. 25% of the transcripts will be

double coded by an independent researcher, experienced in qualitative research to ensure that the correct themes have been used and to ensure that there is no bias. The focus group and one to one interviews will be used to assess the acceptability and feasibility of measuring biomarkers in primary care and using them to help determine management of preschool wheeze.

In addition, we will ask them to fill in a very short electronic questionnaire after the study is finished, to check their perspective and acceptability on these biomarkers being used to guide children's therapy in the future (Appendix 5: Exit Questionnaire).

The whole duration of this study is expected to be 20-21 months, with 1-year follow-up for each patient included.

6.1. STUDY OUTCOME MEASURES

Primary outcome:
Acute attacks of wheeze defined as requiring an unscheduled health care visit

Secondary outcomes:

- Carer days off work
- Days the child unable to attend childcare facility
- Use of oral corticosteroids
- Use of inhaled β-2 agonist (rescue therapy)
- Assessment if children's respiratory or wheezing condition is under control by using the TRACK™ score questionnaire (Test for Respiratory and Asthma Control in Kids) (Appendix 3).

7. CHILD PARTICIPANT ENTRY

7.1. PRE-REGISTRATION EVALUATIONS AND RECRUITMENT

This is a pragmatic, observational study involving preschool children with wheeze aged one to five years old. All treatment and routine monitoring decisions will be at the discretion of their treating general practitioner (GP) or paediatrician (as per usual clinical practice), blinded to the study measurements. After clinical consultation and following submission of the electronic informed consent through the Qualtrics® questionnaire platform (offered by Imperial College London), the parent/carer will be asked to give the contact details of the child's GP or paediatrician, who will be informed about child's participation in this study through a letter sent to his/her email along with the patient information sheet. The parent carer will be then shown a standard video questionnaire and will be asked to identify what respiratory sounds their children have been making. The child will be booked for the three biomarker tests [atopic sensitisation, blood eosinophil count and FeNO (off-line method)]. The tests will be carried out once and at a time of clinical stability. In this visit, a sample of the child's exhaled tidal breath will be collected in a suitable bag in duplicate for offline

measurement of FeNO later on with chemiluminescence. Skin prick tests will be performed to: (a) house dust mite, (b) grass pollen, (c) tree pollen, (d) cat hair, (e) dog hair, as well as normal saline and histamine and these will be applied on either the child's back, leg or arm. If the child has been prescribed antihistamines, we will ask for these to be withheld for 72 hours prior to skin prick testing. FeNO values (offline method) ≥ 10 ppb will indicate Type 2 inflammation and wheal diameter, measured with a specialised skin prick test ruler, ≥ 3mm will indicate sensitisation to the applied aeroallergen. In addition, skin prick tests will allow the assessment of which aeroallergen is the most useful predictor of outcomes in preschool children. Finally, the peripheral blood eosinophil count will be measured from a finger prick blood sample, using the Haemocue machine, allowing a result in approximately 2 minutes. Values ≥ 300 cells/μl indicate Type 2 inflammation. All tests will normally take no more than one hour.

The recruitment period will approximately last 9 months, and all preschool wheezers can join the study at any point (Appendix 1: Recruitment Plan). Parents/Carers of preschool children presenting to primary care or to the Emergency Department (ED) or secondary care (e.g., outpatients) as well as those identified from primary care records and who have been diagnosed by a GP or paediatrician with wheezing, will be approached by collaborating doctors and nurses who will ask their parents if they are willing to have their child participate in the study (Appendix 1 – Recruitment Plan). A patient information sheet will be given, containing all the information regarding the project and the contact details of the research team, for further questions. They will be asked for their willingness to give the study team permission to contact them and discuss recruitment, and if so, they will submit their contact details in an electronic form made with the Qualtrics® questionnaire platform (offered by Imperial College London), which allows gathering data according to the GCP guidelines and as used for years in the Breathing Together study (19). This form will be available online in the Asthma UK Centre for Applied Research's (AUKCAR) website. The initial introduction will be made either by the clinical team (above) with the study team contacting them at a later date to give more information or by Mr. Andreas Perikleous if he is there in person. If the child has been prescribed OCS, testing will be delayed by 2 weeks for biomarker testing as soon as the electronic informed consent is submitted. Finally, parents will be informed through the patient information sheet as well as, in person, that a second optional testing will take place 3 months after the first one, in their second study visit. This will allow assessing the reproducibility of the obtained biomarker results, thus additionally assess their stability.

Patient recruitment at a site will only commence once the study team has ensured the following:

- HRA approval
- Completed Local Site Delegation of Duties and Signature Log
- Confirmation of Capacity and Capability

All recruitment procedures will be in accord with the GCP, and parents/carers will be given detailed information regarding the study as well as all the time they need to understand it. Parents/guardians have at least 24 hours to consider participation. Recruitment will only commence after parents/carers have fully understood the project and submitted the electronic informed consent form through the Qualtrics® platform (offered by Imperial College London).

Child participants entering the study upon their parents' wish, will be fully screened by the Principal Investigator (PI) or someone else from the research team who is suitably qualified and delegated to do so, with the latter taking informed consent as well. After the electronic informed consent sheet is submitted, parents will be asked to provide the contact details of their child's GP or paediatrician, to inform him/her about the child's participation in this study through a letter sent by email along with this patient information sheet. In addition, we will ask them to provide some of the child's personal data (Appendix 1: Recruitment Plan, Table 3: The personal data that will be obtained by each individual participant). Children's full name, date of birth, gender, ethnicity as well as, their parent's full name, telephone number and email address will be added in the child's patient card. All other data obtained from the study will be added in the Case Report Form (CRF).

Follow-up: We will arrange for a short monthly electronic questionnaire to be sent by email to the parents/carers, in order to record on a monthly basis, the days their child was out of childcare, days the carer missed from work, unscheduled health care visits and use of rescue therapy or oral corticosteroids (including dosage) (Appendix 4: Monthly Questionnaire). In addition, we will record the children's TRACK™ score (Test for Respiratory and Asthma Control in Kids) (Appendix 3: TRACK™ score questionnaire) every 3 months, to assess wheeze control. The questionnaires will be made and sent through the Qualtrics® questionnaire platform (offered by Imperial College London). There will be review visits every 3 months, to evaluate their progression in person, but if not feasible, video calls will be arranged through Microsoft Teams. Correct use of spacers will be ensured after guiding parents on how to use them and will be done by their GP or paediatrician as well as from Mr. Andreas Perikleous who is a registered pharmacist in the UK.

In the event that a child has had their ICS treatment discontinued, we will record whether this was because the treatment was ineffective (treatment failure) or if the child was so well that the family or GP did not wish to continue (treatment success or spontaneous improvement). If an additional medication is prescribed, or the child is referred to secondary care, this will be considered as treatment failure. When the study ends, participants' parents/carers will be invited to a face-to-face focus group interview, or a one-to-one interviews, or remotely through Microsoft Teams, depending on their availability at the end of the study period. The focus group and one to one interviews will utilise a semi structured questionnaire, to provide qualitative

feedback on the acceptability of the 3 biomarker tests. In addition, a separate focus group for doctors and nurses will be conducted to assess the feasibility of the 3 biomarker tests being used in clinical practice. They will be given all the information regarding the study as well as the study's patient information sheet. Those agreeing to take part, will sign a consent form. They will be attending either a face-to-face focus group interview, or a one-to-one interview, or remotely through Microsoft Teams, depending on their availability. A semi structured questionnaire will be utilised. Where possible a second independent researcher will sit in the focus groups discussion and interviews to ensure there is no bias. The conversations from the focus group and one to one interviews will be transcribed and coded into themes. We will utilise an external company for transcribing the conversations called 1st Class. https://www.1stclass.uk.com/

The focus group and one-to-one interviews will be audio recorded, transcribed and put into themes to conduct an inductive thematic analysis. 25% of the transcripts will be double coded by an independent researcher, experienced in qualitative research to ensure that the correct themes have been used and to ensure that there is no bias. The focus group and one to one interviews will be used to assess the acceptability and feasibility of measuring biomarkers in primary care and using them to help determine management of preschool wheeze.

In addition, we will ask them to fill in a very short electronic questionnaire after the study is finished, to check their perspective and acceptability on these biomarkers being used to guide children's therapy in the future (Appendix 5: Exit Questionnaire).

The whole duration of this study is expected to be 20-21 months, with 1-year follow-up for each patient included.

7.2. INCLUSION AND EXCLUSION CRITERIA

7.2.1. CHILDREN

INCLUSION CRITERIA	EXCLUSION CRITERIA
1. Patients aged one to five years old presenting to primary care or to the emergency department or to secondary care (e.g., outpatients) or identified from primary care records and have been diagnosed with wheezing by their GP or paediatrician who has decided to prescribe any bronchodilator, ICS or montelukast on clinical grounds	**1.** Inability to understand and cooperate with study procedures
	2. Significant co-morbidity (respiratory or otherwise), for example cystic fibrosis (excluding atopic disorders such as eczema, allergic rhinitis and food allergy)
	3. Withholding or withdrawal of informed consent
2. Parents/Carers able to understand and familiarize themselves with the study and are willing to provide informed consent	**4.** Severe procedural anxiety (needle phobia)
	5. Child is already enrolled in another study involving investigational medicinal product (CTIMP)
	6. History of near-fatal asthma that resulted in intubation / assisted ventilation.

7.2.2. HEALTHCARE PROFESSIONALS

INCLUSION CRITERIA	EXCLUSION CRITERIA
1. Doctors and nurses working in GP practices.	**1.** Inability to understand and cooperate with study procedures.

7.3. WITHDRAWAL CRITERIA

Parents can withdraw their child at any time without having to justify their decision. Upon withdrawal, patients will continue in their usual clinical care. In addition, the investigator may withdraw a child participant from the study if it is considered necessary to protect their wellbeing or that of others. Child participants' data will be retained and included in the final study analysis, unless the parent/carer specifies otherwise. Reasons for withdrawal, where available, and timing of withdrawal will be collected and recorded. Attempts to replace child participants whose parents decided to withdraw, will be made in line with recruitment procedures outlined in section 4. If the child participant is withdrawn due to an adverse event, the research team will

arrange for follow-up visits or telephone calls to monitor the adverse event until it has resolved or stabilised.

8. ADVERSE EVENTS

8.1. DEFINITIONS

Adverse Event (AE): any untoward medical occurrence in a patient or clinical study subject.

Serious Adverse Event (SAE): any untoward and unexpected medical occurrence or effect that:

- Results in death
- Is life-threatening – *refers to an event in which the subject was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe*
- Requires hospitalisation, or prolongation of existing inpatients' hospitalisation
- Results in persistent or significant disability or incapacity
- Is a congenital anomaly or birth defect

Medical judgement should be exercised in deciding whether an AE is serious in other situations. Important AEs that are not immediately life-threatening or do not result in death or hospitalisation but may jeopardise the subject or may require intervention to prevent one of the other outcomes listed in the definition above, should also be considered serious.

8.2. REPORTING PROCEDURES

All adverse events will be recorded in the Case Report Form (CRF). Depending on the nature of the event the reporting procedures below should be followed. Any questions concerning adverse event reporting should be directed to the Chief Investigator in the first instance.

8.2.1. Non serious AEs

All such events, whether expected or not, should be recorded- it should be specified if only some non-serious AEs will be recorded, any reporting should be consistent with the purpose of the trial end points.

8.2.2. Serious AEs

An SAE form should be completed and emailed to the Chief Investigator within 24 hours. All SAEs should be reported to the <name of REC> where in the opinion of the Chief Investigator, the event was:

- '**related'**, i.e., resulted from the administration of any of the research procedures; and
- '**unexpected'**, i.e., an event that is not listed in the protocol as an expected occurrence

Reports of related and unexpected SAEs should be submitted within 15 days of the Chief Investigator becoming aware of the event, using the NRES SAE form for non-

IMP studies. The Chief Investigator must also notify the Sponsor of all related and unexpected SAEs. Local investigators should report any SAEs as required by their Local Research Ethics Committee, Sponsor and/or Research & Development Office.

CONTACT DETAILS FOR REPORTING SAEs
RGIT@imperial.ac.uk
CI email (and contact details below)
Fax: 02073518113, attention Professor Andrew Bush
Please send SAE forms to: Professor Andrew Bush
Telephone: 02073528121 (Mon to Fri 09:00 – 17:00)

CI: a.bush@imperial.ac.uk
Fax: 0044-2073518113

8.3. THE TYPE AND DURATION OF THE FOLLOW-UP OF SUBJECTS AFTER AEs

No related SAEs are anticipated as a result of this study. Unexpected SAEs will be managed according to clinical need. Child participants will continue routine clinical care throughout and after the study; routine follow up appointments may be expedited if required due to clinical need as a result of SAEs.

8.4. PREGNANCY

N/A as study involves preschool children

8.5. ANNUAL PROGRESS REPORTS (APRs)

The Chief Investigator (CI) will prepare the APRs for the study. It will be reviewed by the study site Research Office and sent to the REC by the CI within 30 days of the anniversary date on which the favourable opinion was given by the REC, and annually until the study is declared ended.

8.6. REPORTING URGENT SAFETY MEASURES

The Sponsor and/or the Investigator may take appropriate urgent safety measures in order to protect the subjects of a clinical study against any immediate hazard to their health or safety. If safety measures are taken, REC approval is not required before the measure is taken. The Investigator will immediately and in any event no later than 3 days from the date the measures are taken, give written notice to the REC and the study Sponsor of the measures taken and the circumstances giving rise to those measures. In order to prevent any delays in the reporting timelines the Sponsor has delegated this responsibility to the CI. Therefore, the CI must report any urgent safety measures to the REC directly, and in parallel to the Sponsor. The REC coordinator will acknowledge receipt of urgent safety measures within 30 days.

8.7. MANAGEMENT OF POTENTIAL STUDY RISKS

Child participants routine clinical management is not changed in this observational study. Clinicians have no access to study data. Risks relate to study procedures. All procedures [blood sampling through finger prick test, skin prick tests on either the child's back, leg or arm and FeNO collection (offline method)] will be performed by trained research personnel. Skin prick tests are safe and used clinically for many years, but adrenaline and antihistamines will be available while the study procedures are being performed, in the highly unlikely event of an allergic reaction. All research staff will be trained in management of acute reactions / anaphylaxis. The finger prick test is the same as used by diabetic patients to monitor blood glucose. Collecting breath into a bag is devoid of risk.

9. ASSESSMENT AND FOLLOW-UP

We will arrange for a short monthly electronic questionnaire to be sent by email to the parents/carers, in order to record on a monthly basis, the days their child was out of childcare, days the carer missed from work, unscheduled health care visits and use of rescue therapy or oral corticosteroids (including dosage) (Appendix 4: Monthly Questionnaire). In addition, we will record the children's TRACK™ score (Test for Respiratory and Asthma Control in Kids) (Appendix 3: TRACK™ score questionnaire) every 3 months, to assess wheeze control. There will be review visits every 3 months, to evaluate their progression in person, but if not feasible, video calls will be arranged through Microsoft Teams. In the case of any incidental findings, there will be thorough evaluation of potential benefits or harms before disclosing the information to the child's parent. As this is an observational study, no interventions can be done in the case of incidental findings and parents will be advised to discuss this with their child's GP or paediatrician.

10. END OF STUDY

The end of the study will be when the last of the 100 participants completes his follow-up period of 12 months with his/her last study visit. The anticipated finishing date is December 2023.

11. STATISTICS AND DATA ANALYSIS

The statistical analysis of the project will be done by Mr. Andreas Perikleous in collaboration with Dr Steff Lewis (University of Edinburgh) and the Asthma UK Centre for Applied Research (AUKCAR), the funder of the study. Statistical analyses will use the GraphPad PRISM software package. A normality test will be performed to assess normal (Gaussian) distribution (e.g., the Shapiro-Wilk test. If there is normal distribution, then parametric tests will be used, otherwise non-parametric tests. Level of significance is set as a p value <0.05. The tests that are currently planned to be used are:

- **Chi-Squared test (x2-test)**

This test allows assessing if a variable is similar between two or more groups or to test if two or more variables are related/dependent

- **Fisher's Exact test**

This test is used when chi-squared values are <5, usually with a small sample size.

- **Student t-test or Mann-Whitney test**

These tests are used for comparison between two study groups. The first is for normally distributed quantitative variables and the second for non-normally distributed quantitative variables.

Data and all appropriate documentation will be stored for a minimum of 10 years after the completion of the study, including the follow-up period.

12. REGULATORY ISSUES

12.1. ETHICS APPROVAL

The Study Coordination Centre has obtained approval from the Queen Square Research Ethics Committee (REC) and Health Regulator Authority (HRA). The study must also receive confirmation of capacity and capability from each participating NHS Trust before accepting participants into the study or any research activity is carried out. The study will be conducted in accordance with the recommendations for physicians involved in research on human subjects adopted by the 18th World Medical Assembly, Helsinki 1964 and later revisions.

12.2. CONSENT

The Informed Consent form will be electronic and submitted through the Qualtrics® platform (offered by Imperial College London). After submission by the parent/carer, an automatic email will be sent to his/her email containing a link to access and save their true copy of the informed consent form. All will be performed in line with guidance from the HRA and MHRA, taken by a member of the research team who has been delegated the duty by the PI as recorded on the Sponsor's Delegation of Responsibilities Log and recorded in writing which will be sent to the researcher via email. No hard copies will be retained. Those confirming informed consent will have had formal training in informed consent and completed GCP training. Consent to enter this study will be obtained after a full account has been provided of its nature, purpose, risks, burdens and potential benefits, and the patient has had the opportunity to deliberate. The patient will be allowed to specify the time they wish to spend deliberating. Patients are encouraged to consider taking part, and discuss the study with their physician, family, friends and whoever else they wish before deciding. The PI or designee will explain to the parents that their child is under no obligation to enter the study and they can withdraw at any time during the study, without having to give a reason. Participant's parent/carer will be given a copy of the most recent approved PIS

as well. The electronic consent form will be retained at the study site [one filed in the medical notes, and one filed in the Study Master File (SMF)].

12.3. CONFIDENTIALITY

The Chief Investigator will preserve the confidentiality of participants taking part in the study and is registered under the Data Protection Act. Data will be anonymised.

12.4. INDEMNITY

Imperial College London holds negligent harm and non-negligent harm insurance policies which apply to this study.

12.5. SPONSOR

Imperial College London will act as the main Sponsor for this study. Delegated responsibilities will be assigned to the NHS trusts taking part in this study.

12.6. FUNDING

Asthma UK Centre for Applied Research (AUKCAR) is funding this study.

12.7. AUDITS

The study may be subject to audit by Imperial College London under their remit as sponsor and other regulatory bodies to ensure adherence to GCP and the UK Policy Framework for Health and Social Care Research.

13. DATA MANAGEMENT AND QUALITY ASSURANCE

13.1. CONFIDENTIALITY

All data will be handled in accordance with the General Data Protection Regulation (GDPR) (2018), NHS Caldecott Principles, The UK Policy Framework for Health and Social Care Research, and the condition of the REC approval. The Case Report Forms (CRFs) will not bear the child participants' name or other personal identifiable data. Identification will be performed through the participants' Identification Number (ID). No data will be shared with any external organisation without appropriate consent and data sharing agreement in place, as applicable.

13.2. DATA COLLECTION TOOL

Case Report Forms (CRF) will be designed by the trial coordinator and PI, and the final version will be reviewed and discussed with the study Sponsor. Researchers will complete electronic versions of the CRF to reduce errors compared to transcribing from paper to an electronic database. It is the Investigator's responsibility to ensure the accuracy of all data entered and recorded in the CRFs. The Delegation of Responsibilities Log will identify all study personnel responsible for data collection, entry, handling and managing the database.

13.3. DATA HANDLING AND ANALYSIS

The Informed Consent form will be electronic and submitted through the Qualtrics® platform (offered by Imperial College London). After submission by the parent/carer, an automatic email will be sent to his/her email containing a link to access their true copy of the informed consent form. All data will be stored in the Imperial College London Microsoft Office 365 cloud account of Mr. Andreas Perikleous, and all will be done in accordance with the Imperial College London's data policy. Data will be backed up in a second Imperial College London Microsoft Office 365 cloud account of Mr. Andreas Perikleous and in accordance with the aforementioned policy. The CI will act as the Data Custodian for the study. Mr. Andreas Perikleous (study coordinator) will be responsible for data entry and quality. He will also be responsible for data analysis, with support from the study statistician. Data analysis will be performed on separate occasions to data entry.

13.4. ARCHIVING ARRANGEMENTS

Data will be kept in a secure storage unit at the Sponsor site for 10 years. The study documents (including the Study Master File (SMF), Case Report Forms (CRFs), Informed Consent Forms along with the study database) will be kept for 10 years. The study database will be kept electronically on Imperial College London's computer network, in a password-protected encrypted folder. The PI is responsible for the secure archiving of study documents. The approved repository for longer retention of local materials for studies that involve Imperial College London patients is Box-It Storage UK. The study documentation will be prepared for archiving by the research team in line with the Research Office Archiving SOP and the transfer will be arranged by the Research Office. After 10 years all data, including audio and video recordings, will be destroyed in accordance with Imperial College London's data policy.

13.5. DIRECT ACCESS TO SOURCE DATA

The Investigator(s)/institution will permit study-related monitoring, audits, REC review, and regulatory inspection(s), providing direct access to source data/documents. Parents will be informed of this during the informed consent discussion. Parents will be asked to consent to provide access to their medical notes.

14. INSURANCE AND INDEMNITY

NHS bodies are liable for clinical negligence and other negligent harm to individuals covered by their duty of care. NHS Institutions employing researchers are liable for negligent harm caused by the design of studies they initiate. The provision of such indemnity for negligent harm will be stated to the parent/carer of the child participant.

15. STUDY MANAGEMENT

The day-to-day management of the study will be co-ordinated by Mr. Andreas Perikleous.

16. PUBLICATION POLICY

Data ownership rights will lie with the institution.

17. STATEMENT OF COMPLIANCE

The study will be conducted in compliance with the protocol, Sponsor's Standard Operating Procedures (SOPs), GCP and the applicable regulatory requirement(s).

The study conduct shall comply with all relevant laws of the EU if directly applicable or of direct effect and all relevant laws and statutes of the UK country in which the study site is located including but not limited to, the Human Rights Act 1998, the General Data Protection Regulation (GDPR) (2018), the Medicines Act 1968, and with all relevant guidance relating to medicines and clinical studies from time to time in force including, but not limited to, the ICH GCP, the World Medical Association Declaration of Helsinki entitled 'Ethical Principles for Medical Research Involving Human Subjects' (2008 Version), the NHS Research Governance Framework for Health and Social Care (Version 2, April 2005).

This study will be conducted in compliance with the protocol approved by HRA and according to RGF standards. No deviation from the protocol will be implemented without the prior review and approval of the Sponsor and HRA except where it may be necessary to eliminate an immediate hazard to a research subject. In such case, the deviation will be reported to the Sponsor and the REC as soon as possible.

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19. APPENDICES

19.1. APPENDIX 1: RECRUITMENT PLAN

The recruitment period will approximately last 9 months, and all preschool wheezers can join the study at any point. Parents/Carers of preschool children presenting to primary care or to the Emergency Department (ED) or to secondary care (e.g., outpatients) as well as those identified from primary care records and who have been diagnosed by a GP or paediatrician with wheezing, will be approached by collaborating doctors and nurses who will ask their parents if they are willing to have their child participate in the study. The list of recruitment sites is given below (Table 1). A patient information sheet will be given, containing all the information regarding the project and the contact details of the research team, for further questions. They will be asked for their willingness to give the study team permission to contact them and discuss recruitment, and if so, they will submit their contact details in an electronic form made with the Qualtrics® questionnaire platform (offered by Imperial College London), which allows gathering data according to the GCP guidelines and as used for years in the Breathing Together study (19). Additionally, the questionnaire form submission will not collect parents' IP address or geolocation. This form will be available online in the Asthma UK Centre for Applied Research's (AUKCAR) website. The initial introduction will be made either by the clinical team (above) with the study team contacting them later to give more information or by Mr. Andreas Perikleous if he is there in person.

Name of Participating NHS Organisation	**University of Oxford NHS Hospitals**

Location	**Activity**
Oxford University Hospitals NHS Foundation Trust	Participant Identification, Review Visits, Biomarker testing (finger prick test, skin prick test, collection of FeNO in a bag)
John Radcliffe Hospital	Participant Identification
Oxfordshire Clinical Commissioning Group	
Bicester Health Centre	
White Horse Medical Practice	
Oxfordshire BCSC (Horton Hospital)	
Whitchurch Surgery	
Norden House Surgery	
The Swan Practice	
Windrush Surgery	
CRN East Midlands	

Table 1. Patient identification and recruitment sites

Study procedures	Eligibility assessment	Before starting the study
Child participant identification	X	
Contact information submission	X	
Telephone/email contact with parents	X	
Inclusion/Exclusion criteria		X
Patient Information Sheet		X
Face-to-face project discussion		X
Electronic Informed Consent		X

Table 2. Patient recruitment plan

PERSONAL DATA OBTAINED	
a) Child's Full Name	e) Medical History
b) Child's Date of Birth	f) Child's GP contact details
c) Gender	g) Parents' smoking history
d) Ethnicity	h) Parent's telephone and email address

Table 3. The personal data that will be obtained by each individual participant.

COLLABORATING HEALTHCARE PROFESSIONALS – PARTICIPANT IDENTIFICATION	
EMERGENCY DEPARTMENT (ED)	
NAME	**DETAILS**
1) Dr. Alex Novak	• Consultant in Emergency Medicine and Ambulatory Care, Oxford University Hospitals NHS FT • Work Address: Emergency Department, John Radcliffe Hospital, Headley way, Oxford OX3 9DU
2) Dr. Moya Dawson	• Consultant in Paediatric Emergency Medicine • Emergency Department, John Radcliffe Hospital, Headley way, Oxford OX3 9DU • Oxford University Hospitals NHS FT

Table 4. The list of collaborating healthcare professionals in the ED for participant identification.

19.2. APPENDIX 2: FOLLOW-UP PLAN

We will arrange for a short monthly electronic questionnaire to be sent by email to the parents/carers, in order to record on a monthly basis, the days their child was out of childcare, days the carer missed from work, unscheduled health care visits and use of rescue therapy or oral corticosteroids (including dosage) (Appendix 4: Monthly Questionnaire). In addition, we will record the children's TRACK™ score (Test for Respiratory and Asthma Control in Kids) (Appendix 3: TRACK™ score questionnaire) every 3 months, to assess wheeze control. The questionnaires will be made and sent through the Qualtrics® questionnaire platform (offered by Imperial College London), which allows data gathering data according to the GCP guidelines and as used for years in the Breathing Together study (19). Additionally, the questionnaire form submission will not collect parents' IP address or geolocation. There will also be review visits every 3 months, to evaluate their progression in person, but if not feasible, video calls will be arranged through Microsoft Teams.

	Months											
	1	2	3	4	5	6	7	8	9	10	11	12
Primary Care Visit			X			X			X			X
Video Call (Microsoft Teams)[1]			X			X			X			X
Biomarker Testing[2]			X[3]									
TRACK™ Score Questionnaire[4]			X			X			X			X
Monthly Questionnaire	X	X	X	X	X	X	X	X	X	X	X	X
Focus Group												X

Table 2. The follow-up plan after preschool wheezers' recruitment.

[1] In case they are not available for primary care visit

[2] Testing the 3 biomarkers, blood eosinophils, atopic sensitisation and FeNO (offline method).

[3] A second testing will be performed 3 months after the first one and will be **optional**. Parents will be informed through the patient information sheet, as well as in person that they have the option to deny participation of their child in this second testing. It is written in the informed consent form as well.

[4] The TRACK™ score will be sent to the parents/carers by email or SMS every 3 months and will be incorporated to the monthly questionnaire. They will be sent through the Qualtrics® questionnaire platform.

19.3. APPENDIX 3: TRACK™ SCORE QUESTIONNAIRE (TEST FOR RESPIRATORY AND ASTHMA CONTROL IN KIDS)

1. During the past 4 weeks, how often was your child bothered by breathing problems, such as wheezing, coughing or shortness of breath?					
Not at all ☐ 20	Once or twice ☐ 15	Once every week ☐ 10	2 or 3 times a week ☐ 5	4 or more times a week ☐ 0	☐
2. During the past 4 weeks, how often did your child's breathing problems (wheezing, coughing, shortness of breath) wake him or her up at night?					
Not at all ☐ 20	Once or twice ☐ 15	Once every week ☐ 10	2 or 3 times a week ☐ 5	4 or more times a week ☐ 0	☐
3. During the past 4 weeks, to what extend did your child's breathing problems, such as wheezing, coughing or shortness of breath, interfere with his or her ability to play, go to school or engage in usual activities that a child should be doing at his or her age?					
Not at all ☐ 20	Slightly ☐ 15	Moderately ☐ 10	Quite a lot ☐ 5	Extremely ☐ 0	☐
4. During the past 3 months, how often did your need to treat your child's breathing problems (wheezing, coughing or shortness of breath) with quick relief medications (e.g., albuterol, Ventolin®)					
Not at all ☐ 20	Once or twice ☐ 15	Once every week ☐ 10	2 or 3 times a week ☐ 5	4 or more times a week ☐ 0	☐
5. During the past 12 months, how often did you child need to take oral corticosteroids (e.g., prednisone, prednisolone, Decadron®)					
Not at all ☐ 20	Slightly ☐ 15	Moderately ☐ 10	Quite a lot ☐ 5	Extremely ☐ 0	☐

Total Score ☐

Figure 1. The TRACK™ Score Questionnaire. TRACK (Test for Respiratory and Asthma Control in Kids) is a trademark of AstraZeneca group of companies © 2009. TRACK score less than 80 means that the child's breathing problems are out of control. TRACK score above 80 means that the child's breathing problems seem to be in control.

19.4. APPENDIX 4: MONTHLY QUESTIONNAIRE

Introduction: Please take a moment to complete the following questionnaire relating to your child's wheezing condition. The questions will help us identify wheezing exacerbations and assess your child's quality of life.

Question 1: Did your child experience any wheezing symptoms during the last month?				
Not at all ☐	Once or twice ☐	Once every week ☐	2 or 3 times a week ☐	4 or more times a week ☐

Question 2: Was the child unable to go to childcare because of breathing problems?		
Yes ☐	No ☐	N/A ☐
If **yes**, please state how many days: _____(Number)		

Question 3: Did you have to take your child for an emergency appointment with your GP, urgent care or hospital because of wheezing?				
Not at all ☐	Once or twice ☐	Once every week ☐	2 or 3 times a week ☐	4 or more times a week ☐

Question 4: Was your child treated with inhaled or oral corticosteroids?		
Yes ☐	No ☐	
If **yes**, what was the dose, frequency and duration?		
Dose: _____	Frequency: _____	Duration: _____

Question 5: Was your child given β2 agonists (blue inhaler, rescue therapy)?		
Yes ☐	No ☐	
If **yes**, what was the dose, frequency and duration?		
Dose: _____	Frequency: _____	Duration: _____

Question 6: Did you have to take days off work because your child had wheezing or breathing difficulties?	
Yes ☐	No ☐
If **yes**, how many days did you take? Specify number: _____ (days)	

Survey's ending message: "We thank you for your time spent taking this survey. Your response has been recorded."

19.5. APPENDIX 5: EXIT QUESTIONNAIRE

Introduction: Thank you for participating in this study. We are really grateful for your willingness to help and volunteer for a study that aims to provide better wheezing treatment for our children in the future. Please take a moment to give us your feedback relating to the 3 biomarkers that were tested in this study as well as some comments on what you think the future definitive study should entail?

Question 1: How satisfied are you with this whole 1-year follow-up period study?				
Very Dissatisfied ☐	Dissatisfied ☐	Neutral ☐	Satisfied ☐	Very Satisfied ☐

Question 2: As a parent would you be interested in having the biomarker-based approach for your child in the future?		
Yes ☐	No ☐	Maybe ☐

Question 3: Did the study's 1-year follow up period help you change your mind on the 3 biomarkers (blood eosinophil count, FeNO and atopic sensitisation) being used in clinical settings for personalising treatment?			
Not at all ☐	Slightly ☐	Moderately ☐	Quite a lot ☐

Question 4: Would you recommend this approach to other parents with children experiencing the same wheezing conditions?			
Not at all ☐	Maybe ☐	Recommend ☐	Highly recommend ☐

Question 5: What else would you like to see in the future clinical trial? Would you change anything on the current process? What was good, what was not good?
Comment:

Survey's ending message: "Thank you for participating in our survey!"