Non-Interventional Study Protocol	
Study Code	R02616
Version	V1.3
Date	30 Jan 2016

AURA

Assessing the Use of fractional exhaled nitric oxide and blood eosinophils as biomarkers in predicting asthma exacerbations and evaluating the subsequent healthcare Resource utilisAtion

Contents

List of Abbreviations

Abbreviation	Explanation
A&E	Accident and Emergency
AQLQ	Asthma Quality of Life Questionnaire
ATS	American Thoracic Society
AZ	AstraZeneca
BDP	Beclomethasone Dipropionate
ENCePP	European Network of Centres for Pharmacoepidemiology and Pharmacovigilance
ERS	European Respiratory Society
FeNO	Fractional exhaled Nitric Oxide
FEV_1	Forced Expiratory Volume in one second
FP	Fluticasone propionate
FVC	Forced Vital Capacity
GCP	Good Clinical Practice
GERD	Gastro-oesophageal reflux disease
GINA	Global Initiative for Asthma
GP	General Practitioner
GPP	Good Pharmacoepidemiology Practice
HES	Hospital Episode Statistics
HRU	Healthcare Resource Utilization
ICD	International Classification of Diseases
ICS	Inhaled Corticosteroids
IgE	Immunoglobulin E
LABA	Long-Acting Beta Agonists
LAMA	Long-Acting Muscarinic Antagonists
LTRA	Leukotriene Receptor Antagonists
MI	Myocardial Infarct
NIS	Non-Investigational study
OCS	Oral corticosteroid
OPCRD	Optimum Patient Care Research Database
OPRI	Observational & Pragmatic Research Institute
ppb	Part per billion
PSSRU	Personal Social Services Research Unit
iNOS	Inducible Nitric Oxide Synthase
SABA	Short-Acting Beta Agonists
SAMA	Short-Acting Muscarinic Antagonists
SC	Steering Committee
SD	Standard Deviation
Th-2	T-helper 2
UK	United Kingdom

Responsible Parties

Name	Professional Title	Role in Study	Affiliation	Email Address
Sarang Rastogi	MPharm	AZ Researcher	AZ	sarang.rastogi@astrazeneca.com
Dr Gokul Gopalan	MD	AZ Researcher	AZ	gokul.gopalan@astrazeneca.com
Sadia Halim	MBBS	AZ Researcher	AZ	sadia.halim@astrazeneca.com
Danny Gibson		AZ Researcher	AZ	danny.gibson@astrazeneca.com
Prof. David Price	Professor	Chief Investigator	University of Aberdeen, OPRI	dprice@opri.sg
Dr Sen Yang	Vice President OPRI Affiliates	Scientific input	OPRI	sen@opri.sg
Andrew McLoughlin	Researcher	Researcher	OPRI	andrew@opri.sg
Dr Marjan Kerkhof	Research Scientist	Lead Epidemiologist	OPRI	marjan@opri.sg
Dr Simon Wan Yau Ming	Medical Science Lead	Scientific input	OPRI	simon@opri.sg
Catherine Hutton	Commercial Director	Contracting	OPRI	catherine@opri.sg
Liz Gardener	Head of Statistics	Lead Statistician	OPRI	lizg@crs-ltd.org
Dr Alessandra Cifra	Medical writer	Medical Writer	OPRI	alessandra@crs-ltd.org

Synopsis

Background/Rationale:

Of the 300 million people worldwide who have asthma, 5-10% of patients have severe asthma that is refractory to standard inhaled corticosteroid (ICS) treatment. The number of blood eosinophils has been shown to be positively correlated with the frequency of severe asthma exacerbations, and is a promising marker for responsiveness to monoclonal antibody therapy in the presence of corticosteroid resistance. FeNO is another biomarker for corticosteroid responsiveness. FeNO levels and blood eosinophilia together, may predict patients with uncontrolled corticosteroid-resistant asthma who may respond positively to monoclonal antibody therapy.

The aim of the study is to correlate the level of FeNO and blood eosinophils to the number of severe exacerbations. To further explore the implications of these biomarkers in severe asthma, we will characterise prospective burden of disease, healthcare resource utilisation costs, quality of life, and stability of biomarkers over time and changes in ICS dosage for patients with different categories of FeNO and blood eosinophils.

Objectives

Phase 1 primary objective:
To find the relative risk of having an increased number of severe exacerbations in patients categorised by different biomarker levels[1] in the year prior to the FeNO measurement.

Phase 1 secondary objective:
To describe demographic characteristics, lung function, comorbidities, respiratory medication and healthcare resource utilisation in patients groups categorised by biomarkers.

The results of phase one will be discussed by a panel of experts including the steering committee, and client who will decide whether it is feasible or of clinical interest, to continue to pursue the following phases. Based on this decision, some, all or none of the following objectives will be studied.

Phase 2 primary objective:
To find the relative risk of having an increased number of severe exacerbations in patients categorised by different biomarker levels[1] in the outcome period after the FeNO measurement.

Phase 2 secondary objectives:
To describe the patient characteristics by group[1] in the outcome period after FeNO measurement.

To find the relative risk of having a shorter time to exacerbation in patients categorised by different biomarker levels[1] in the outcome period after the FeNO measurement.

---

[1] 6 patient groups will be categorised by eosinophils ($\geq 0.3 \times 10^9$/L and $< 0.3 \times 10^9$/L) and FeNO levels (≥ 50ppb – high, ≥ 25ppb < 50ppb – medium, < 25ppb - low). Combinations of these groups can be considered eg low FeNO/high eosinophil + medium FeNO/low eosinophil + medium FeNO/high eosinophils.

Phase 3 primary objective:

To compare quality of life data gathered either from a postal survey (if objective 4 is not pursued) or from questionnaires issued during a clinic visit (if objective 4 is studied) for matched patients in the 2 different groups/combinations of groups of interest[1].

Phase 3 secondary objective:

To compare quality of life data gathered either from a postal survey (if objective 4 is not pursued) or from questionnaires issued during a clinic visit (if objective 4 is studied) for unmatched patients in all 6 different groups[1].

Phase 4 primary objective:

To study the consistency in FeNO levels (initial reading and corresponding blood eosinophil level) and FeNO and blood eosinophil level taken at a specialist clinic for patients with stable, stepped down and stepped up ICS treatment.

Phase 4 secondary objective:

To study the consistency in FeNO levels (initial reading and corresponding blood eosinophil level) and FeNO and blood eosinophil level taken at a specialist clinic for patients with stable, stepped down and stepped up ICS treatment in each patient group[1] if numbers allow.

Methods

Study design

This study uses a bespoke dataset from the OPCRD, which includes FeNO and blood eosinophil measurements from patients with asthma in the UK. The index date is defined as the date of the most recent FeNO measurement. The baseline period is defined as one year prior to index date (used for objective 1). If the study is extended to phase 2, data will be gathered for the prospective period, defined as one year after index date (used for phase 2). If the study is extended to phase 3 or phase 3 and 4, a postal survey or a biomarkers clinic will be conducted respectively. The postal survey will be delivered to patients after the prospective period is over (used for phase 3). Patients will be invited to the specialist clinic after the prospective period has ended (phase 4).

Data Sources

A combined dataset of patients with asthma using data extracted from the OPCRD, which is a bespoke database inclusive of: GP electronic health records and best-practice respiratory review data from questionnaires and nurse-lead respiratory clinics, will be used for analyses.

Sample Size Estimations

Based on a 20% increase in the rate of severe exacerbations, a simulated Poisson regression model with 3 independent variables (FeNO, eosinophils and one other predictor) and 6 different groups ($\geq 0.3 \times 10^9$ and $<0.3 \times 10^9$ eosinophils, stratified by FeNO readings of ≥ 50ppb, between 25ppb and 50ppb and <50ppb) will provide 90% power. This will require 850 patients between the 6 groups for phase 1 and

2. A matched analysis will take place between 2 groups/combinations of groups of interest identified after the unmatched analysis. This will require 425 patients across the 2 groups/combination of groups (212 patients in each group/combination of groups) for 90% power to identify a 20% increase in exacerbation rate in one group. A similar number will be required to compare the difference between time to exacerbation between treatment groups.

For phase 3, based on a 0.05 difference and a standard deviation of 0.13 in EuroQol 5 dimensions (EQ-5D) the study will need 170 patients in each group with a power of 90%, and an alpha of 0.025 to detect a difference in questionnaire scores between groups. For the Asthma Quality of Life Questionairre (AQLQ) with the same assumptions as above, to detect a difference of 0.4 between the groups with standard deviation of 0.92 the study will need approximately 140 patients in each group. Power for the St Georges Respiratory Questionnaire (SGRQ) will be conducted if this option is chosen instead of the AQLQ. Phase 4 will be an exploration of step up/step down patients and is not powered for any outcome.

Amendment History

Date	Brief description of change	Administrative Change / Amendment / New Protocol Version.
12/12/2016		Version 1.1
20/1/2017	Primary analysis cut off and split of FeNO for primary analysis changed from low/medium/high (<25ppb, 25-49ppb, and ≥50ppb) to low and high (<35ppb cut off for low)	Version 1.2
	Title change	Version 1.3

Milestones

Date	Milestone
12 December 2016	Revised protocol delivered to AstraZeneca

1. Background

An estimated 5-10% of the 300 million people worldwide who have asthma have a severe form of the disease, defined as patients treated with high dose ICS who continue to be uncontrolled. Approximately 40–60% of this population with severe asthma have eosinophilic airway inflammation (1).

Eosinophils, proliferation and survival of which is elicited by IL-5 are considered a key effector arm of the allergic response in asthma (1). ICS subdues the cellular inflammatory response and encourages the anti-inflammatory response in the airway, but in some patients, the allergic response is under-responsive to ICS (2). Fractional exhaled nitric oxide (FeNO) and airway eosinophilia have been identified as biomarkers for Th2-driven allergic airway inflammation in patients with asthma, and both are considered reliable predictors of responsiveness to ICS (3).

Although FeNO levels are correlated with blood eosinophils and sputum eosinophils in patients with mild-to-moderate asthma (3, 4), this association attenuates in patients with severe asthma. FeNO is reduced by ICS in a dose dependent manner in asthma patients with elevated FeNO levels, which is correlated to improvement in ACQ (5). Increases and decreases in FeNO levels are positively correlated with improvement and deterioration in asthma symptoms (6, 8). Despite treatment with ICS, FeNO is detectable with high doses of ICS (5). High FeNO levels are also associated with poorer adherence to ICS, which may partially explain continuing uncontrolled severe asthma despite high-dose ICS prescription in some patients (9).

The use of airway eosinophilia as a biomarker for exacerbation risk is a topic of ongoing study (10, 11). Interestingly, it identifies an inflammatory phenotype that responds to anti-Th2 biologic agents (12). Anti-IL5 therapy has been shown to reduce sputum eosinophils, but not FeNO levels (12). Studies have found that exhaled nitric oxide is related to the cellular targets of IL4 and IL13, which are also candidates for monoclonal antibody therapy (13). This suggests that the measurement of FeNO may provide predictive value for severe exacerbations in patients with a strong Th2 allergic response.

Assessment of airway eosinophilia is impractical in non-specialised settings. Peripheral blood eosinophil counts are easily obtained, and high blood eosinophil counts ($\geq 0.3 \times 10^9$/L) has been found to be a useful predictor of sputum eosinophilia ($\geq 2\%$) in patients with severe asthma (14). Significant reduction in severe exacerbations with have also been seen in patients with severe asthma with blood eosinophils $\geq 0.3 \times 10^9$/L treated with an IL-5 receptor antagonist (15).

Further support for blood eosinophils as a valid biomarker includes a previous OPRI study found that a count–response relation existed between blood eosinophil counts and asthma-related outcomes, suggesting a causal relationship between eosinophilic inflammation and lack of asthma control (10, 16, 18).

Results from two large mepolizumab trials showed that a blood eosinophil count greater than or equal to 150 cells/µL was not inferior to sputum counts $\geq 3\%$ in predicting treatment response to anti-IL5 therapy, and suggested that high blood eosinophil counts may even be a better predictor of therapy response than sputum eosinophils (19).

The presence of raised FeNO levels and/or high blood eosinophil counts, despite adherence to medium to high doses of ICS, may identify corticosteroid-resistant asthma patients who continue to suffer from

severe exacerbations. These patients may be candidates for drugs targeting IgE, IL-4, IL-5 or IL-13 which have yielded positive outcomes in severe asthma patients with evidence of type 2 airway inflammation (10, 17, 20).

This study aims to determine if blood eosinophil levels combined with FeNO levels can also be used to predict the frequency of severe asthma exacerbations. In addition, options for follow up studies are proposed to investigate healthcare resource utilisation and / or quality of life prospectively in different patient populations, defined by FeNO and eosinophil levels at baseline.

2. Rationale and objectives

Although both FeNO and high blood eosinophil counts are considered useful biomarkers of asthma control in most patients who respond to ICS, no studies have examined whether both can be used to determine severe exacerbation risk. Previous studies suggest that their value as a biomarker is independent from each other, and the biochemical pathways in which they are linked to allergic disease also has differences (10, 13, 16, 18). Assessment of both biomarkers together may provide a novel method to identify patients at higher risk of exacerbations who may benefit from monoclonal antibody treatment (phase 1 and 2). Phase 1 and 2 will also compare the extent of healthcare resource utilisation of patients categorised into different groups through their biomarkers. Phase 3 will compare the quality of life of patients with different biomarkers who may have more disease burden than their exacerbation rate suggests. Phase 4 will assess whether the biomarkers used in assessing which patient has a higher risk of exacerbations remain stable through time and medication change.

Phase 1 primary objective:

To find the relative risk of having an increased number of severe exacerbations in patients categorised by different biomarker levels[2] in the year prior to the FeNO measurement.

Phase 1 secondary objective:

To describe demographic characteristics, lung function, comorbidities, respiratory medication and healthcare resource utilisation in patients categorised by biomarkers[2].

Decision to Extend Study

The results of objective one will be discussed by a panel of experts including the steering committee, who will decide whether it is feasible or of clinical relevance, to continue to pursue the following objectives. Based on this decision, the study will be extended to phase 2, with an option to proceed to phases 3 and/or 4 or no further analyses will be made. Based on this decision, the protocol will be updated and the chosen phases will be described in greater detail.

Phase 2 primary objective:

To find the relative risk of having an increased number of severe exacerbations in patients categorised by different biomarker levels[2] in the outcome period after the FeNO measurement.

Phase 2 secondary objective:

To describe the patient characteristics by group[2] in the outcome period after FeNO measurement.

Phase 2 secondary objective:

To find the relative risk of having a shorter time to exacerbation in patients categorised by different biomarker levels in the outcome period after the FeNO measurement.

Phase 3 primary objective:

To compare quality of life data gathered either from a postal survey (if objective 4 is not pursued) or from questionnaires issued during a clinic visit (if objective 4 is studied) for matched patients in 2 different groups of interest[2].

Phase 3 secondary objective:

To compare quality of life data gathered either from a postal survey (if objective 4 is not pursued) or from questionnaires issued during a clinic visit (if objective 4 is studied) for unmatched patients in all 6 different groups[2].

Phase 4 primary objective:

To study the consistency in FeNO levels (initial reading and corresponding blood eosinophil level) and FeNO and blood eosinophil level taken at a specialist clinic for patients with stable, stepped down and stepped up ICS treatment.

Phase 4 secondary objective:

---

[2] 6 patient groups will be categorised by eosinophils ($\geq 0.3 \times 10^9$/L and $< 0.3 \times 10^9$/L) and FeNO levels (≥ 50ppb – high, ≥ 25ppb < 50ppb – medium, < 25ppb - low). Combinations of these groups can be considered eg low FeNO/high eosinophil + medium FeNO/low eosinophil + medium FeNO/high eosinophils)

To study the consistency in FeNO levels (initial reading and corresponding blood eosinophil level) and FeNO and blood eosinophil level taken at a specialist clinic for patients with stable, stepped down and stepped up ICS treatment in each patient group[2] if numbers allow..

3. Methodology

3.1 Study Design – General Aspects

3.1.1 Data Sources

A dataset of patients from the Optimum Patient Care Research Database (OPCRD) will be used for analyses. OPCRD is a respiratory-focused primary care research database, maintained by Optimum Patient Care (OPC), who provide chronic respiratory review services. Primarily it contains anonymous, longitudinal data extracted from electronic health records in over 600 UK general practices (>2,862,000 patients). It is approved by Trent Multi Centre Research Ethics Committee for clinical research use and offers a high-quality data source that is used regularly in clinical, epidemiological and pharmaceutical research. Additionally, OPCRD is able to provide anonymised data from previous clinical trials conducted by OPRI (22).

![AstraZeneca]

3.2 Phase 1

The index date will be the most recent date of a FeNO reading recorded for active asthma patients who have at least one year of data prior to the index date in which they have been prescribed ≥ 1 ICS inhaler (Figure 1).

The study population will be divided into six groups.

- Group 1: High blood eosinophils (≥ 0.3 x 10^9/ L) and high FeNO (≥ 50 ppb)
- Group 2: High blood eosinophils (≥ 0.3 x 10^9/ L) and low FeNO (< 25 ppb)
- Group 3: Low blood eosinophils (< 0.3 x 10^9/ L) and high FeNO (≥ 50 ppb)
- Group 4: Low blood eosinophils (< 0.3 x 10^9/ L) and low FeNO (< 25 ppb)
- Group 5: High blood eosinophils (≥ 0.3 x 10^9/ L) and medium FeNO (25-<50 ppb)
- Group 6: Low blood eosinophils (≥ 0.3 x 10^9/ L) and medium FeNO (25-<50 ppb)

The number of exacerbations in the year prior to the FeNO reading that serves as the index date will be compared in all 6 unmatched groups. Two groups (or combinations of groups eg: groups 1+2 vs groups 3+4+5) will be selected for further matched comparison, these will be referred to as the two *primary analysis groups*. Number of exacerbations will be the primary outcome.

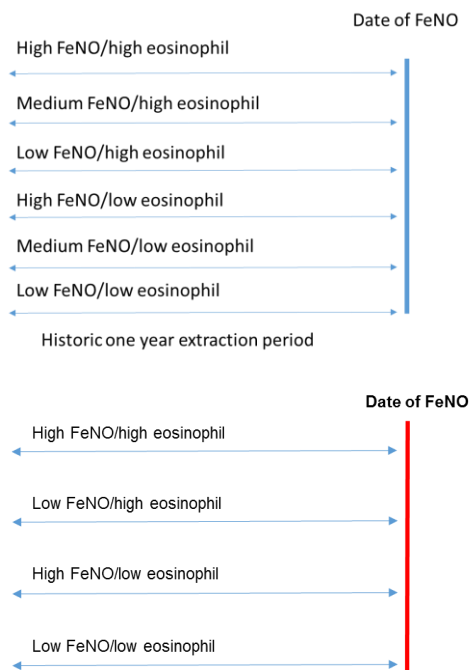On discussion with the steering committee, the cut off for FeNO groups was changed to low and high (<35ppb for low).

Figure 1. Study design for Phase 1 including the original and new primary cut offs

3.3 Phase 2 (optional)

Data will be sourced from the year following the index date. This will enable the opportunity to study the value of FeNO and blood eosinophil readings on prospective asthma control.

Summaries of the same characteristics across the same six groups as in phase 1 will be presented.

The number of exacerbations in the year after the FeNO reading that serves as the index date will be compared in all 6 unmatched groups. Two groups will be selected for further matched comparison. Two groups (or combinations of groups) will be selected for further matched comparison, these will be referred to as the two *primary analysis groups*. Number of exacerbations will be the primary outcome, time to exacerbation will be the secondary outcome.
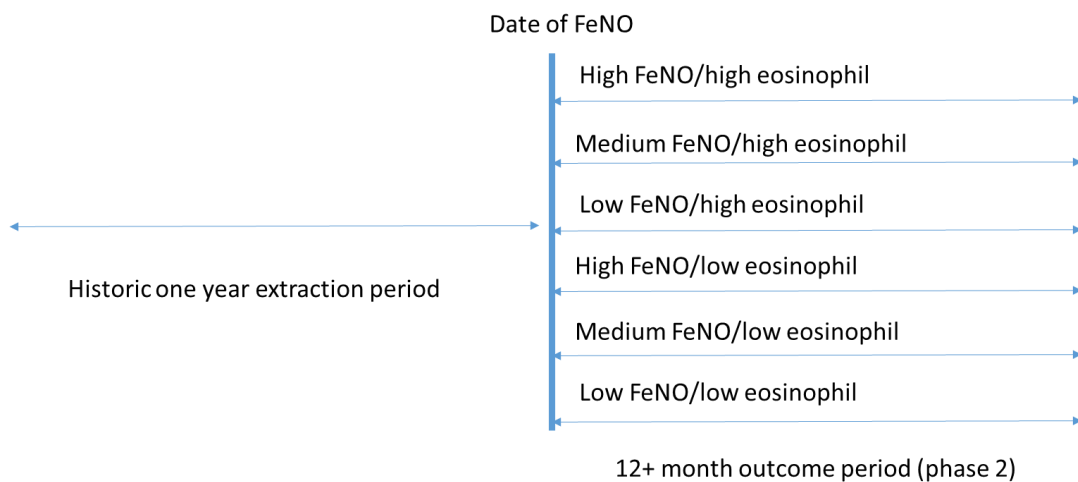

Figure 2. Study design for Phase 2

3.4 Phase 3 (optional)

The study design will be dependent on which options are chosen. If phase 3 is chosen and phase 4 is not chosen, the questionnaires will be send to patients in the form of a postal survey; however, if both phases 3 and 4 are to be pursued, then the questionnaires will be collected during an asthma review clinic visit.

Quality of life will be measured using the AQLQ (Asthma Quality of Life Questionnaire) or St George's Respiratory Questionairre (SGRQ). The AQLQ was developed to measure the functional problems (physical, emotional, social and occupational) that are most troublesome to adults (17-70 years) with asthma. The SGRQ is designed to measure health impairment in patients with asthma and COPD, hereas the EQ5D is a standardized instrument for measuring generic health status.

Both options involve the AQLQ/SGRQ and the EQ5D being targeted at the same primary analysis groups of patients of interest for the primary outcome (those identified in phase 1 and 2). An unmatched comparison will be performed for the secondary objective between all the groups.
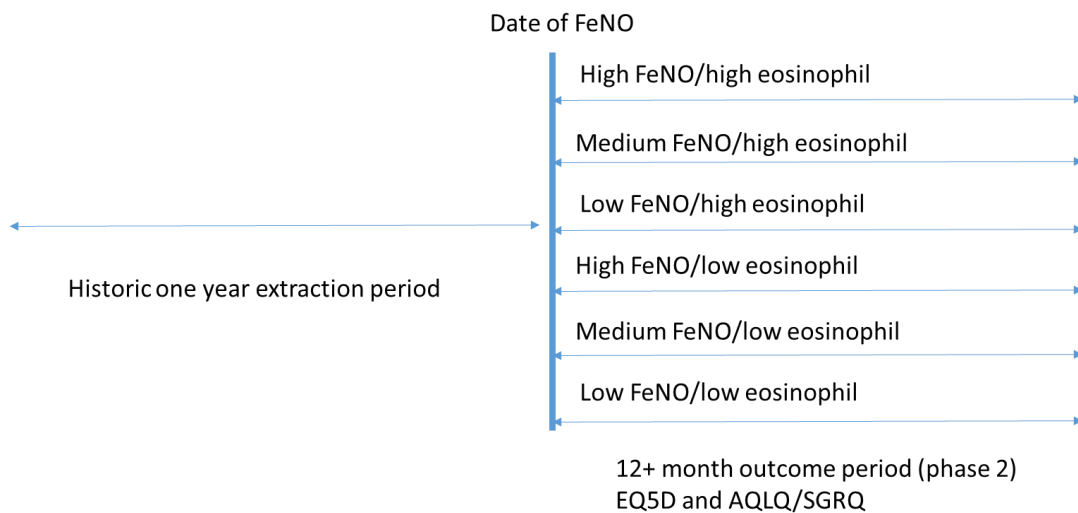

Figure 3. Study design for Phase 3

![AstraZeneca logo]

3.5 Phase 4 (optional)

Data will be sourced from data provided by an asthma review clinic that provides QoL questionnaires, FeNO and blood eosinophil readings as routine. Patients who have a prescription code for ICS in the 8 weeks following their initial FeNO reading will be categorised into:

- Stable/Baseline Group: patients with no change in dosage of prescribed ICS (exploratory only)
- Step-Up: Patients who have been prescribed an increased dosage of ICS (exploratory only)
- Step-Down: Patients who have been prescribed a reduced dosage of ICS (exploratory only)

The outcomes include biomarker readings taken at clinic. This will enable study of whether the biomarkers remain consistent over time during stable and stepped up/down treatment. If numbers allow, this study will be split into groups of patients categorised by biomarker readings at the first FeNO reading.
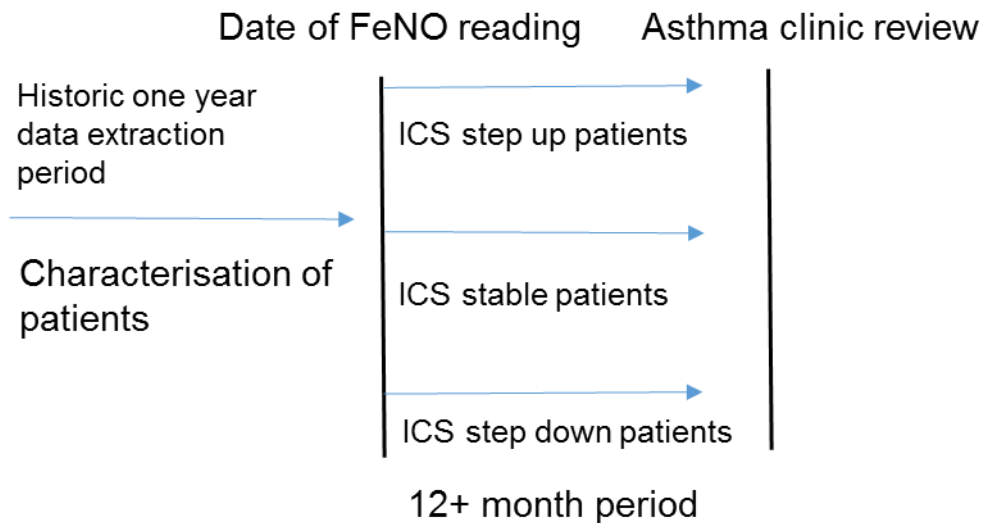

Figure 4: Study design for Phase 4; The asthma clinic review will include a second FeNO and eosinophil reading. The 12+ month period will be the minimum period where the ICS dose has been changed/remained stable post initial FeNO reading

4 Study Population

The study population consists of patients with asthma and who are registered at a general practice which provides data to OPC.

4.1 Inclusion Criteria (all phases unless specified)

- A diagnostic Read code for asthma (ever, without an asthma resolution Read code at the index date) qualifying for inclusion in the register of patients with asthma, maintained by GPs for the Quality Outcomes Framework (QOF) OR prescription of ≥2 asthma related medications, one of which must be an ICS, none of which are a LAMA, and without a $FEV_1/FVC<0.70$
- A FeNO reading in the last 2 years prior to the extraction date that serves as the index date
- ≥ 1 prescription for ICS in the year prior to the most recent FeNO reading
- Age 18-80 inclusive at the date of the most recent FeNO reading
- ≥ 1 valid blood eosinophil count measurement within 2 years prior to the index date
- Continuous data prior to index date for one year
- Completion of relevant QoL questionnaire (phase 3)
- Attendance and completion of asthma review clinic (phase 4)

4.2 Exclusion Criteria (all phases unless specified)

- Diagnostic Read code for other chronic lower respiratory conditions ever:
  - o Bronchiolitis Obliterans
  - o Lung disease due to external agents other than smoking, such as occupational agents
  - o Pulmonary fibrosis
  - o Pulmonary hypertension
  - o Cystic fibrosis
- Prescription of maintenance corticosteroids in the baseline year.

5 Epidemiological Measurements

5.1 Exposures and Covariates

The following exposures and covariates will be studied:

5.1.1 Blood eosinophil counts

The presence of blood eosinophilia will be assessed at the last recorded blood eosinophil count within 2 years and without a prescription of oral corticosteroids 2 weeks prior to the reading. Blood eosinophilia will be defined as $\geq0.3 \times 10^9/L$ and low blood eosinophils will be defined as $<0.3 \times 10^9/L$.

5.1.2 FeNO levels

High FeNO is considered ≥50 ppb. Medium FeNO is 25-<50ppb and low FeNO is <25 ppb. These categorization of clinically significant cut points for high medium and low FeNO are based on recommendations on standardizing the measurement of FeNO published by 2011 ATS FeNO guidelines. ATS Clinical Practice Guideline recommends that FeNO greater than 50 ppb be used to indicate that eosinophilic inflammation and, in symptomatic patients, responsiveness to corticosteroids are likely, whereas low FeNO less than 25 ppb be used to indicate that eosinophilic inflammation and responsiveness to corticosteroids are less likely (6).

5.1.3 Prescribed dose of inhaled corticosteroids

Prescribed cumulative doses of ICS will be averaged and expressed as dose per day based on number of prescriptions of inhalers, the strength of inhalers and the number of puffs per day over the baseline or outcome period.

5.1.4 Demographic and baseline characteristics

The following factors will be presented for phases 1 and 2:

- Age at index date
- Gender
- Smoking status, Read code closest to and within 5 years prior to index date
    - Never smoker
    - Ex-smoker
    - Current smoker
- BMI, calculated from height and weight data if available and taken from practice recorded BMI value if not, within 10 years prior to index date
    - Underweight: <18.5
    - Normal weight: ≥18.5 and <25
    - Overweight: ≥25 and <30
    - Obese: ≥30
- Asthma related factors
    - Type of ICS prescribed and cumulative dose in baseline year
    - Number of severe asthma exacerbations in baseline year (this will be the outcome variable of interest in phase 1 due to the limited study range)
    - Acute respiratory events in baseline year
    - Lower respiratory-related hospitalisations in baseline year
    - Lower respiratory-related GP consultations in baseline year
    - Respiratory-related medications (ICS, ICS/LABA, LAMA, LABA, SABA, SAMA, LTRA, Cromones, Theophylline and Omalizumab (Xolair®)) (Read codes) in baseline year
    - Lower respiratory-related outpatient visits in baseline year
    - Lower respiratory-related day cases in baseline year
    - Concomitant asthma treatment in baseline year (Read codes)
    - FEV_1/FVC ratio prior to index date
    - Percent predicted peak flow prior to index date

AstraZeneca

- o Respiratory-related antibiotics prescribed in the baseline year
- o Total dose of acute respiratory related oral corticosteroids prescriptions in baseline year
- o Number of respiratory related acute oral corticosteroids prescribed in baseline year
- o Total dose of short-acting bronchodilators in baseline year
- o Adherence to prescribed ICS, measured as medication possession ratio in baseline year
- Comorbidities
  - o Allergic rhinitis diagnosis prior to index date
    - ▪ Never
    - ▪ Active (Read code + medication)
    - ▪ Ever, not active (Read code)
  - o COPD (Read codes) prior to index date
  - o Sleep disorders diagnosis ever prior to index date (Read codes)
  - o Obstructive sleep apnoea diagnosis ever prior to index date (Read codes)
  - o Eczema diagnosis prior to index date (Read codes)
    - ▪ Never
    - ▪ Active (Read code + medication)
    - ▪ Ever, not active (Read code)
  - o Nasal polyps ever prior to index date (Read codes)
  - o Chronic sinusitis diagnosis ever prior to index date (Read codes)
  - o Gastro-oesophageal reflux disease (GERD) (Read codes & medication)
  - o Diabetes Mellitus type I and type II diagnosis ever prior to index date (Read codes)
  - o Osteopenia / Osteoporosis diagnosis ever prior to index date (Read codes)
  - o Cataract diagnosis ever prior to index date (Read codes)
  - o Glaucoma prior to index date(Read codes)
  - o Cardiovascular disease diagnosis (MI or heart failure) ever prior to index date (Read codes)
  - o Hypertension diagnosis ever prior to index date (Read codes)
  - o Anxiety / depression diagnoses ever prior to index date (Read codes)
  - o Chronic kidney disease diagnosis ever (Read codes & eGFR < 60 ml/min)
- Charlson co-morbidity index: based on diagnoses ever prior to index date (Read codes)

6 Outcome Variables

Phase 1 Outcome

Number of severe exacerbations in the baseline period as defined by the 2015 ATS/ERS position statement (7).

Phase 2 Outcome

Number of severe exacerbations in the outcome period as defined by the 2015 ATS/ERS position statement (7).

Time to severe exacerbation (in days) from the index date (secondary)

Phase 3 Outcome

AstraZeneca
EQ5D and AQLQ score

Phase 4 Outcome

FeNO and blood eosinophil levels as continuous variables at the asthma review clinic

6.1 Other outcome variables

The following variables will be presented to characterise the patients:
- Respiratory-related asthma medications (ICS, ICS/LABA, LAMA, LABA, SABA, SAMA, LTRA, Cromones, Theophylline and Omalizumab (Xolair®), Oral Corticosteroids) (Read codes)
- Acute respiratory events defined as the occurrence of:
    o Non-routine lower respiratory related Hospital admissions
    o Lower respiratory related A&E admissions
    o Oral corticosteroid courses with evidence of a lower respiratory consultation AND / OR
    o Antibiotics prescribed with evidence of a lower respiratory consultation
- Use of Short-acting ß2-agonists (SABA), defined as an average daily dose.
- Average daily SABA dosage during outcome year, calculated as average number of puffs per day over the year multiplied by strength (in mcg) and categorised as appropriate to the data:

$$\frac{Number\ of\ inhalers * doses\ per\ inhaler}{365} * strength$$

- Risk domain asthma control, defined as none of the following:
    o Non-routine lower respiratory related Hospital admissions
    o Lower respiratory related A&E admissions
    o Oral corticosteroid courses with evidence of a lower respiratory consultation
    o Antibiotics prescribed with evidence of a lower respiratory consultation
- Overall asthma control, defined as risk domain asthma control AND no excessive SABA use ($\geq$ 200 mg / day)

6.2 Healthcare Resource Utilisation

- Lower respiratory-related GP consultations
    o Lower Respiratory read codes (including Asthma, COPD and LRTI read codes);
    o Asthma/COPD review codes excluding any monitoring letter codes;
    o Lung function and/or asthma monitoring
    o Any additional respiratory examinations, referrals, chest x-rays or events.
- Lower respiratory-related Outpatient visits
- Lower respiratory-related A&E attendances

- Lower respiratory-related Hospital admissions (definite and probable) defined as hospitalisations occurring within a 7-day window (either side of the hospitalisation date) of a lower respiratory Read code.
- Lower respiratory-related asthma medications (ICS, ICS/LABA, LAMA, LABA, SABA, SAMA, LTRA, Cromones, Theophylline and Omalizumab (Xolair®), Oral Corticosteroids) (Read codes)
- Antibiotics associated with lower respiratory consultations
- Events costs will be based on the most recent PSSRU costs (2015) (27)
- The NHS Dictionary of Medicines and Devices browser will be used to estimate medication costs

7 Statistical Analysis Plan

7.1 Statistical Methods

General Methods

All statistical analyses will be conducted using STATA version 14 (StataCorp, College Station, TX: StataCorp LP) and SAS version 9.3 (SAS Institute, Cary, NC). Variables measured on interval or ratio scale will be summarised using the following summary statistics: number of non-missing records (n), minimum, maximum, mean and standard deviation (SD) and median, (25th and 75th percentile). Categorical data will be summarised as the number and percentage of patients in each category. Although the data are derived from routine clinical records, the presence of biomarker data in routine data is related to the health of the subject, and thereby the dataset is inherently biased; this will be discussed as a limitation.

Matching

This section describes the approach used to handle confounding. Potential confounders are identified based on a combination of baseline imbalance, bias potential and expert judgement, and the most relevant confounders will be used for direct matching.

Direct matching can only use a limited number of variables to match on without restricting the patient population too much, and it is therefore necessary to exclude variables that do not relevantly affect the association of interest.

After matching this approach will be repeated in the matched sample to identify any residual confounding, selecting confounders for direct adjustment in the outcome analyses.

The exact method for matching will depend on the unmatched results.

Confounder identification

Baseline balance

A characterisation of all baseline demographic, co-morbidity, indicators of disease severity and other patient characteristic variables will be carried out and presented for each arm. The difference between

the arms will be quantified using the Standardized Mean Difference (SMD) (Stuart 2010, Rosenbaum & Rubin 1985). This measure is not affected by the number of observations, and thus a better way to judge imbalance than a p-value of a hypothesis test of difference. The SMD will be calculated for both continuous and categorical variables as described below: An SMD ≤ 0.1 indicates sufficient balance between the treatment and the reference (control) groups.

Table 1. Formulae for Standardised Mean Difference

Covariate type	Formula
Continuous	$SMD = \dfrac{(\overline{x_t} - \overline{x_r})}{\sqrt{\dfrac{s_t^2 + s_r^2}{2}}}$, where $\overline{x_t}$, $\overline{x_r}$ denote the sample means and s_t, s_r the standard deviations
Binary	$SMD = \dfrac{(\widehat{p_t} - \widehat{p_r})}{\sqrt{\dfrac{\hat{p}_t(1-\hat{p}_t) + \hat{p}_r(1-\hat{p}_r)}{2}}}$, where $\widehat{p_t}$, $\widehat{p_r}$ denote the proportion of patients in each category
Categorical (>2 categories)	$SMD = \sqrt{(T - C)'S^{-1}(T - C)}$ where S is a $(k-1) \times (k-1)$ covariance matrix: $S = [S_{kl}] = \begin{cases} \dfrac{\hat{p}_{1k}(1 - \hat{p}_{1k}) + \hat{p}_{2k}(1-\hat{p}_{2k})}{2} &, k = l \\ \dfrac{\hat{p}_{1k}\,\hat{p}_{1l} + \hat{p}_{2k}\,\hat{p}_{2l}}{2} &, \quad k \neq l \end{cases}$ $, T = (\hat{p}_{12}, \dots, \hat{p}_{1k})'$, $C = (\hat{p}_{22}, \dots, \hat{p}_{2k})'$ and $\hat{p}_{jk} = P(category\ k|treatment\ arm\ j)$, $j = 1,2$, $k = 2,3,\dots,k$

Bias potential

Bias potential assesses the degree to which the observed association between the exposure of interest and the outcome is affected by conditioning on the variable. Bias potential will be measured using the relative change in co-efficient (RCC) of the exposure when the covariate is added into the model predicting outcome.

Table 2. Formulae for Relative Change in Co-efficient

Outcome type	Regression type	Formula
Continuous	Linear	$RCC = abs\left\{ \dfrac{\left(\beta_{crude} - \beta_{adjusted} \right)}{\beta_{crude}} \right\}$
Binary	Logistic	
Time-to-event	Cox-Proportional Hazard	$RCC = abs(1 - e^{(\beta_{crude} - \beta_{adjusted})})$
Count	Poisson	

> Where β_{crude} is the co-efficient of exposure in the crude model and $\beta_{adjusted}$ is the co-efficient of exposure after adding the covariate in the model.

It is called *bias potential* since the bias was estimated without other covariates in the model. To what extent a variable introduces bias into a model will depend on the total model.

Statistics References:

Rosenbaum PR, Rubin DB, 1985. Constructing a control group using multivariate matched sampling

methods that incorporate the propensity score. The American Statistician. 39:33–38

Rubin, D.B., 2007. The design versus the analysis of observational studies for causal effects:

parallels with the design of randomized trials. Stat. Med. 26, 20–36.

Stuart, E.A., 2010. Matching methods for causal inference: a review and a look forward.

Stat. Sci. Rev. J. Inst. Math. Stat. 25, 1–21.

7.1.1 Phases 1, 2 and 3:
Primary Analysis

The primary analysis will be carried out in accordance with the above section on matching, following discussion with the steering committee of the results of the secondary analysis. This will initially require the numbers of exacerbations to be collected and then compared through matched groups, with the exact matching method based on baseline characteristics collected through the secondary analysis. Results will be presented additionally as a relative risk.

Secondary Analysis

Descriptive statistics will be carried out in accordance with the general methods. Characteristics of cohorts will be compared using the chi-squared test and the Kruskal Wallis test, as appropriate for unmatched groups.

7.1.2 Phase 4

The difference between initial biomarker (FeNO and blood eosinophil readings) and those taken at the asthma review clinic will be studied. Limits of agreement (mean difference +/- 1.96 x standard deviation of the differences) will provide insight into how much random variation may be influencing the measurements. Limits of agreement will be calculated for

- difference in FeNO levels (treated as a continuous variable)
- difference in eosinophil levels (treated as a continuous variable)

grouped by:

- stable ICS treatment
- stepped up ICS treatment
- stepped down ICS treatment

If there are sufficient subjects to allow more granular analysis, these groups would further be divided by high/low FeNO and high/low eosinophil levels.

8 Feasibility and sample size estimation

Over 1000 patients are available from the unique OPCRD database with asthma diagnoses, FeNO readings and blood eosinophil counts.

Based on a 20% increase in the rate of severe exacerbations, a simulated Poisson regression model with 3 independent variables (FeNO, eosinophils and one other predictor) and 4 different groups (≥ 0.3 x 10^9 and <0.3 x 10^9 eosinophils, stratified by FeNO readings of $\geq$50ppb and $<$50ppb) will provide 90% power. This will require 850 patients between the 6 groups for phase 1 and 2. Approximately 1200 patients are available for analysis. A matched analysis will take place between 2 groups of interest identified after the unmatched analysis. This will require 425 patients across the 2 groups (212 patients in each group) for 90% power to identify a 20% increase in exacerbation rate in one group.

For phase 3, based on a 0.05 difference and a standard deviation of 0.13 in EuroQol 5 dimensions (EQ-5D) the study will need 170 patients in each group with a power of 90%, and an alpha of 0.025 to detect a difference in questionnaire scores between groups. For the Asthma Quality of Life Questionairre (AQLQ) with the same assumptions as above, to detect a difference of 0.4 between the groups with standard deviation of 0.92 the study will need approximately 140 patients in each group. Phase 4 will be an exploration of step up/step down patients and is not powered for any outcome. The expected response rates will be approximately 10% based on previous OPRI studies, and 80-90% response with a clinic.

9. Study Limitations

The study dataset comprises of information collected for clinical and routine use rather than specifically for research purposes. Although extensive quality control and validity checks are conducted on the practice level, the validity and completeness of individual patient records can be limited. Hospital admissions and A&E attendances are not systematically recorded in GP databases. The applied definition to identify asthma-related hospital admissions or A&E events may give false positive events. Exposure to ICS is estimated based on the number of prescribed doses over time periods, but correct use cannot be guaranteed.

The study included only patients who had a recorded blood eosinophil measurement. Such measurements are not collected routinely, so it possible that patients with asthma who have had blood eosinophils measured, are not representative of the overall asthma population.

A limitation of all observational studies is the possibility of confounding of the results, arising from systematic differences between the patients being compared.

Prescriptions are recorded in the general practice records and not pharmacy dispensation records and thus may overestimate adherence.

10 Study Conduct and Regulatory Details

10.1 Data Management

The OPCRD is owned and managed by OPC (a company affiliated with OPRI). Database construction will be performed by OPC and data analyses, by OPRI.

The Non-Interventional Study (NIS) will be performed in accordance with ethical principles that are consistent with the Declaration of Helsinki, ICH GCPs, GPP and the applicable legislation on Non-Interventional Studies. The Investigator will perform the NIS in accordance with the regulations and guidelines governing medical practice and ethics in the country of the NIS and in accordance with currently acceptable techniques and know-how. The final protocol of the Non-Interventional Study, including the final version of the Subject Informed Consent Form, must be approved or given a favourable opinion in writing by the Ethics Committee/Institutional Review Board (IRB)/Independent Ethics Committee (IEC). The Ethics Committee/IRB/IEC must also approve any amendment to the protocol and all advertising used to recruit subjects for the study, per local regulations.

10.1.1 Compliance with Study Registration and Results Posting Requirements.

The study will be registered at ENCePP (http://www.encepp.eu/) and ADEPT.

10.1.2 Compliance with Financial Disclosure Requirements

Any information that may be a conflict of interest in terms of compensation or financial interests will be disclosed for each investigator.

11 Steering Committee

- Sinthia Bosnic-Antievich
- Ian Pavord
- Nicolas Roche
- David Halpin
- Leif Bjermer
- Omar Usmani
- Rohit Katial
- Guy Brusselle

12 Publication Plan

Publication plans will be discussed with AZ after the final reports have been delivered. The aim is to publish the results in at least two manuscripts. Abstracts of the results will be submitted to international conferences, preferably the ERS and ATS conferences in 2017.

13 Estimated Study Timelines

Part 1:	Duration	Date of Completion
Protocol development, data extraction for phase 2 begins, clinic recruitment as extractions occur	3 weeks	29 November 2016
Steering committee review	1 week	2 December 2016
AZ sign-off	2 weeks	13 December 2016
Data extraction and ethical approval (phase 1)	1 week	20 December 2016
Total cohort analysis	1 week	27 December 2016
Report writing (slides and word document)	1 week	19 December 2016
Steering committee review	1 week	26 December 2016
Client review of phase 1, go ahead for clinics/extraction	1 week	1 January 2017
Part 2:		
Phase 2 extraction complete	5 months	21 May 2017
Data extraction and ethical approval	1 month	18 June 2017
Total cohort analysis	1 month	16 July 2017
Report writing (slides and word document)	1 month	13 August 2017
AZ review and SC review	2 weeks	27 August 2017
Phase 3 postal option	3 months	19 November 2017
Phase 3 clinic option	20 months	2 June 2019
Further analysis	1 month	30 June 2019
Manuscript production	3 months	22 September 2019
AZ review and SC review	2 weeks	6 October 2019

14 List of References

1. Spencer LA, Weller PF. Eosinophils and Th2 immunity: contemporary insights. Immunology and cell biology. 2010;88(3):250-6.
2. Airway remodeling and lack of bronchodilator response in steroid-resistant asthma
3. Bjermer L, et al. Current evidence and future research needs for FeNO measurement in respiratory diseases. Respir Med 2014; 108(6): 830-41.
4. Sweeney J, Brightling CE, Menzies-Gow A, Niven R, Patterson CC, Heaney LG. Clinical management and outcome of refractory asthma in the UK from the British Thoracic Society Difficult Asthma Registry. Thorax. 2012;67(8):754-6.
5. Anderson WJ, Short PM, Williamson PA, Lipworth BJ. Inhaled corticosteroid dose-response using domiciliary exhaled nitric oxide in persistent asthma: the FeNO type trial. Chest. 2012;142(6):1553-61.
6. Dweik RA, Boggs PB, Erzurum SC, Irvin CG, Leigh MW, Lundberg JO, et al. An official ATS clinical practice guideline: interpretation of exhaled nitric oxide levels (FeNO) for clinical applications. Am J Respir Crit Care Med. 2011;184(5):602-15.
7. Chung KF, Wenzel SE, Brozek JL, Bush A, Castro M, Sterk PJ, et al. International ERS/ATS guidelines on definition, evaluation and treatment of severe asthma. The European respiratory journal. 2014;43(2):343-73.
8. Price D, et al. Using fractional exhaled nitric oxide (FeNO) to diagnose steroid-responsive disease and guide asthma management in routine care. Clin Transl Allergy 2013; 3(1): 37.
9. Price DB, Rigazio A, Campbell JD, Bleecker ER, Corrigan CJ, Thomas M, et al. Blood eosinophil count and prospective annual asthma disease burden: a UK cohort study. Lancet Respir Med. 2015;3(11):849-58.
10. Price D, et al. Predicting frequent asthma exacerbations using blood eosinophil count and other patient data routinely available in clinical practice. J Asthma Allergy 2016; 9: 1-12.
11. Wilson AM, Lipworth BJ. Dose-response evaluation of the therapeutic index for inhaled budesonide in patients with mild-to-moderate asthma. The American journal of medicine. 2000;108(4):269-75.
12. Staton TL, Choy DF, Arron JR. Biomarkers in the clinical development of asthma therapies. Biomarkers in medicine. 2016;10(2):165-76.
13. Bjemer L, Alving K, et Diamant Z. Current evidence and future research needs for FeNo measurement in respiratory diseases. Respiratory medicine. 2014;108(6):830-841.
14. Fowler SJ, Tavernier G, Niven R. High blood eosinophil counts predict sputum eosinophilia in patients with severe asthma. The Journal of allergy and clinical immunology. 2015;135(3):822-4.e2.
15. Malinovschi A, Fonseca JA, Jacinto T, Alving K, Janson C. Exhaled Nitric Oxide levels and blood eosinophil counts independently associate with wheeze and asthma events in National Health and Nutrition Examination survey subjects. J Allergy Clin Immunol. 2013 Oct;132(4):821-7.e1-5. doi: 10.1016/j.jaci.2013.06.007. Epub 2013 Jul 26.

16. Price DB, Pavord ID, Thomas M, Corrigan CJ, Wilson AM, Hillyer EV, Kerkhof M Inhaled corticosteroid dose-response on blood eosinophils in asthma. Lancet Respir Med. 2016;4 e1-e2.

17. Bleecker, Eugene R., et al. "Efficacy and safety of benralizumab for patients with severe asthma uncontrolled with high-dosage inhaled corticosteroids and long-acting β 2-agonists (SIROCCO): a randomised, multicentre, placebo-controlled phase 3 trial." The Lancet 388.10056 (2016): 2115-2127.

18. Price D, et al. Predicting frequent asthma exacerbations using blood eosinophil count and other patient data routinely available in clinical practice. J Asthma Allergy 2016; 9: 1-12.

19. Ortega H, Katz L, Gunsoy N, Keene O, Yancey S. Blood eosinophil counts predict treatment response in patients with severe eosinophilic asthma. The Journal of allergy and clinical immunology. 2015;136(3):825-6.

20. British Thoracic Society. BTS/SIGN asthma guidelines 2014

21. Pavord ID, et al. Emerging biologics in severe asthma. Immunol Allergy Clin North Am 2016; 36(3): 609-23.

22. Price D, Ryan D, Burden A, Von Ziegenweidt J, Gould S, Freeman D, Gruffydd-Jones K, Copland A, Godley C, Chisholm A and Thomas M. Using fractional exhaled nitric oxide (FeNO) to diagnose steroid-responsive disease and guide asthma management in routine care. Clin. Trans. Aller. 2013, 3:37.

23. Wenzel SE. Asthma: defining of the persistent adult phenotypes. Lancet. 2006; 368:804–813.

24. Anderson GP. Endotyping asthma: new insights into key pathogenic mechanisms in a complex, heterogeneous disease. Lancet. 2008; 372: 1107–1119.

25. Walford HH, Doherty TA. Diagnosis and management of eosinophilic asthma: a US perspective. Journal of Asthma and Allergy 2014:7 53–65.

26. Cisternas MG, Blanc PD, Yen IH, et al. A comprehensive study of the direct and indirect costs of adult asthma. J Allergy Clin Immunol. 2003; 111:1212–1218.

27. Chung KF, Wenzel SE, Brozek JL, Bush A, Castro M, Sterk PJ, et al. International ERS/ATS guidelines on definition, evaluation and treatment of severe asthma. The European respiratory journal. 2014;43(2):343-73.

28. Optimum Patient Care Service and Database Specification: iHARP November 2014

29. Personal Social Services Research Unit: http://www.pssru.ac.uk/project-pages/unit-costs/2015/index.php