Study Protocol

PhEnotypic Characteristics, coMorBidities and response to thErapeutic inteRventions associated with non-type 2 asthma (EMBER)

An investigation of non-T2 asthma phenotypes, clinical characteristics of phenotypic groups, and treatment responsiveness

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TITLE	**PhEnotypic Characteristics, coMorBidities and response to thErapeutic inteRventions associated with non-type 2 asthma (EMBER)**
Subtitle	An investigation of non-T2 asthma phenotypes, clinical characteristics of phenotypic groups, and treatment responsiveness
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Study aims and objectives	**Study Aims:** To describe distributions of biomarkers for patients with severe asthma, identify patients displaying evidence of non-T2 phenotype, and assess how patients with T2 and non-T2 phenotypes respond to therapeutic interventions. We additionally aim to investigate disease burden through considering comorbid diseases **Study Objectives:** **Objective 1**: To illustrate distributions for asthma biomarkers (BEC, FeNO, and IgE), and identify phenotypes according to level of T2 inflammatory involvement **Objective 2**: To describe the demographic and clinical characteristics (including comorbid diseases) associated with phenotypic groups identified as being non-T2 asthma, relative to phenotypic groups with higher levels of T2 inflammatory involvement **Objective 3:** To investigate patient response to therapeutic interventions with biologics for non-T2 and T2 asthma patients, and whether there are differences according to phenotypic group **Objective 4:** To explore rates of hospitalisations for asthma patients according to their T2 phenotype
Countries of study	Argentina, Australia, Bulgaria, Canada, Colombia, Denmark, Greece, India, Ireland, Italy, Japan, Korea, Kuwait, Mexico, Portugal, Saudi Arabia, Spain, Taiwan, United Arab Emirates (UAE), United Kingdom (UK), United States of America (USA)
Author(s)	Sarah Burkill

Contents

LIST OF ABBREVIATIONS

Abbreviation or special term	Explanation
ANOVA	Analysis of variance
BEC	Blood eosinophil count
BMI	Body mass index
FeNO	Fractional exhaled nitric oxide
FEV_1	Forced expiratory volume in the first second
GINA	Global Initiative for Asthma
IgE	Immunoglobulin E
ICS	Inhaled corticosteroids
IL-4, -5, -13	Interleukin-4, -5, -13
ISAR	International Severe Asthma Registry
ISC	ISAR Steering Committee
LABA	Long-acting beta-agonist
LAMA	Long-acting muscarinic antagonist
LTRA	Leukotriene receptor antagonist
OCS	Oral corticosteroids
OPC	Optimum Patient Care
OPRI	Observational and Pragmatic Research Institute
R	R software from the R Project for Statistical Computing
T2	Type 2 inflammation

1.0 Background

Asthma is an inflammatory condition affecting the airways. Severe asthma is defined as asthma which remains uncontrolled despite treatment, or which requires extensive treatment according to steps 4 and 5 of the Global Initiative for Asthma(1). Most asthma can be attributed to the effects of type 2 (T2) inflammation(2), characterized by high IgE antibody titers and eosinophilia. The majority of therapies currently available for asthma are targeted toward patients displaying evidence of T2 asthma(3), however, asthma is a heterogenous condition, and can present on a spectrum of T2 inflammation, from high to low T2 involvement. Asthma which presents through mechanisms other than T2 inflammation, or with low T2 involvement has been categorized as non-T2 asthma, and is associated with higher levels of neutrophilic inflammation relative to patients presenting with asthma due to higher levels of T2 inflammation(4).

There is no consensus on the definition of non-T2 asthma, which can be difficult to diagnose due to a lack of clear biomarkers(5). The current literature indicates a sizeable proportion of patients with severe asthma present with underlying T2 inflammation, and the symptoms of many severe asthma patients persist despite T2 suppression (6–8). Currently, much less is known about immunological responses in non-T2 patients(9). Criteria for diagnosing non-T2 asthma are therefore somewhat arbitrary, and a diagnosis is usually dependent on the absence of T2 markers such as high peripheral blood eosinophil count (BEC), coupled with low fractional exhaled nitric oxide (FeNO) levels(10,11). Additionally, neutrophils are generally present in non-T2 patient sputum(10). However, much remains unknown about the prevalence of non-T2 asthma, and there is debate surrounding whether non-T2 patients constitute a separate group with a distinct diagnosis, or whether asthma exists on a spectrum of T2 involvement. Identifying possible phenotypes will assist with answering this question. Several pulmonary comorbid diseases, including obstructive sleep apnoea, nasal polyposis, and rhinitis have been associated with particularly T2 asthma, alongside extrapulmonary conditions such as anxiety and depression(12,13). Additionally, non-T2 patients have been noted to be more likely to be overweight or obese, be smokers, and have pre-existing lung disease(10,14). If there are differences in prevalence of comorbid conditions according to asthma phenotype, this could be clinically useful and provide insight into how different phenotypes manifest and present in patients.

An understanding of underlying clinical attributes and patient phenotypes can also be important when estimating how well patients are likely to respond to certain treatment

regimens. The extent to which patients are responsive to treatments can be important for ascertaining their risk of comorbid diagnoses and other end points. When treating T2 asthma patients, monoclonal antibodies which target IgE or interleukin (IL) IL-4, IL-5, and IL-13, all type 2 cytokines, are generally prescribed to reduce exacerbation rates(15). However, these treatments tend to be effective in patients displaying stronger evidence of T2 inflammation because they target T2 immune response, meaning they are generally not appropriate therapies for primarily non-T2 patients, whose asthma is activated through different biological pathways. Severe non-T2 asthma patients are often treated with anti-inflammatory corticosteroids, often with reduced responsiveness and slower reduction in exacerbations relative to patients displaying more clearly as T2(3). Patients displaying less evidence of T2 inflammatory involvement in their asthma are not necessarily a homogenous group, and can present with varying clinical characteristics. Therefore, it is possible and indeed likely that some patients will have better responses to currently available therapies. Ascertaining which patient groups respond best to certain treatments through for example assessing their exacerbation rate, or lung function can be an important insight when considering treatment options for non-T2 asthma patients(16).

Previous studies have worked towards categorising patients according to the extent of their T2 inflammation, however this tends to have been done by considering biomarkers separately, and defining cut-offs through discussion amongst clinicians, which means such approaches are at least partially open to subjective opinions(7,17). We propose a data driven investigation of non-T2 asthma patients through use of the International Severe Asthma Registry (ISAR). This resource is uniquely placed to provide insight into potential phenotypes on the T2 inflammatory spectrum, comorbidities and demographic characteristics, and responsiveness to currently available therapies. This database can also provide an understanding of the risk of serious outcomes including hospitalization for patients displaying lower levels of T2 inflammation, relative to patients with more evidence of T2 involvement in their asthma presentation.

2.0 Study Aims and Objectives

2.1 Study Aims

To describe distributions of biomarkers for patients with severe asthma, identify patients displaying evidence of non-T2 phenotype, and assess how patients with T2 and non-T2 phenotypes respond to therapeutic interventions. We additionally aim to investigate disease burden through considering comorbid diseases.

2.2 Study Objectives

Objective 1: To illustrate distributions for asthma biomarkers (BEC, FeNO, and IgE), and identify phenotypes according to level of T2 inflammatory involvement

Objective 2: To describe the demographic and clinical characteristics (including comorbid diseases) associated with phenotypic groups identified as being non-T2 asthma, relative to phenotypic groups with higher levels of T2 inflammatory involvement

Objective 3: To investigate patient response to therapeutic interventions with biologics for non-T2 and T2 asthma patients, and whether there are differences in response according to phenotypic group

Objective 4: To explore rates of inpatient hospitalisations for asthma patients according to their T2 phenotype

3.0 Study Design

3.1 Objective 1:

This will utilize **a cross-sectional** study design including all asthma patients enrolled into ISAR. The distributions of biomarkers (highest measurements for each patient) will be described. K-means cluster analysis, using three biomarkers (BEC, FeNO, and IgE) to identify clustered groups, will assist with identification of asthma phenotypes. Using this approach removes the need for pre-determined, subjective biomarker cut-offs for distinguishing between T2 and non-T2. Non-T2 phenotypes will be identified by considering the biomarker characteristics of each cluster. If the results indicate clearly distinctive clusters, this would provide evidence for a separate, non-T2 presentation of asthma. If the results do not show clear differences between clusters, this would be an indication there is less evidence for a separate, non-T2 phenotype.

3.2 Objective 2:

Cross sectional data at baseline for asthma patients according to the phenotypic groups identified in objective 1 will be used to establish whether associations exist between certain characteristics and each asthma type at baseline. This includes examination of comorbid conditions, and asthma control, alongside demographic variables.

3.3 Objective 3:

This will be a **longitudinal cohort** design including identified phenotypes as cohorts. These phenotypic groups would be used to examine whether any groups respond better than others throughout the course of a year. The focus will be on biologics. Response will be measured through four primary outcomes: exacerbation rate in the year following treatment initiation, lung function, use of rescue systemic steroids in the year following treatment initiation, and the extent to which the patient's asthma is considered to be controlled. Start of follow up will be date of treatment initiation.

In this longitudinal cohort, it would be interesting to investigate the stability of the non-T2 phenotype over time by considering how biomarker values are affected for each phenotypic group after treatment initiation.

3.4 Objective 4:

This will be a primarily **descriptive exploratory** study, illustrating hospitalization rates amongst different phenotypic groups in order to gain an understanding of health burden amongst different asthma phenotypes.

For this study, we hypothesize that the different phenotypic clusters are stable over time; however, if this hypothesis appears to be incorrect, we need to differentiate for example "permanent non-T2-phenotype" patients and "permanent T2-phenotype patients" from switchers, i.e. "non-T2 → T2-phenotype" patients and "T2 → non-T2-phenotype" patients. This, however, would be difficult using the currently available ISAR data, and not feasible for many patients.

4.0 Study Population

4.1 Data Sources

ISAR is an international collaborative initiative aiming to gather longitudinal data on severe asthma patients. Those eligible for enrolment are patients aged 18 or over visiting a participating centre. They must have been diagnosed with severe asthma and provided informed consent to their data being collected. Severe asthma is defined as asthma which is uncontrolled despite treatment, or which requires extensive treatment as outlined by steps 4 and 5 of GINA(1).

Data collection began in 2018, and as of July 2021, there were **11,439** participants from 24 countries enrolled into ISAR. Of these enrolled participants, **6,169** were prospective level patients(18). There are currently **3938** (preliminary number) patients with available data on all three biomarkers. The data is comprised of relevant information collected from patients at each visit and extracted medical records.

4.2 Inclusion and Exclusion Criteria

Inclusion Criteria

Objectives 1 and 2:

- All eligible severe asthma patients with available data on biomarkers. The patient will need data on BEC, FeNO, and IgE in order to be included.

Objective 3:

- All eligible severe asthma patients with available data on biomarkers (BEC, FeNO, and IgE), information on bx treatment, and at least 1 year of follow-up time to assess treatment responsiveness

Objective 4:

- All eligible severe asthma patients with available dates of asthma diagnosis or age at asthma onset

Exclusion Criteria

Objectives 1, 2, 3, and 4:

- None

The final study population will depend on whether data is missing for any of the key variables needed to complete the objectives. The population used for objective 3 will follow the same criteria as used for the study population for objectives 1 and 2 minus those without adequate follow-up time for studying treatment responsiveness.

5.0 Study Variables

The following variables will be used to derive an analysis dataset suitable for the objectives of the study. Whether the variables are part of the core ISAR database, or whether they are part of the bolt-on datasets is also indicated in the variable list. Bolt-on data is available for approximately half of ISAR participants as of July 2021 and applies to co-morbidity variables for this study.

5.1 Patient Identifier and Demographic Variables

Label	Type	Values	Core	Optional
Identifying patients				
Patient identifiers	String	String	✓	
Identifying visits				
Visit identifiers for each patient	String	String	✓	
Demographic and background variables				
Gender	Binary	Male, Female, Missing	✓	
Ethnicity	Categorical	Caucasian, Southeast Asian, Northeast Asian, African, Mixed, Other, Unknown	✓	
Country from which patient was recruited	String	Argentina, Australia, Bulgaria, Canada, Colombia, Denmark, Greece, India, Ireland, Italy, Japan, Korea, Kuwait, Mexico, Portugal, Saudi Arabia, Spain, Taiwan, UAE, UK, USA	✓	
Age at asthma onset	Numeric	Numeric value	✓	
Age at study entry	Numeric	Numeric value	✓	
Body mass index (BMI)	Numeric	Numeric value	✓	
Smoking status	Categorical	Current smoker, ex-smoker, never smoked	✓	

5.2 Biomarker Variables**

Label	Type	Values	Core	Optional
Biomarkers for defining phenotype				
When IgE was measured	String	Date variable	✓	
Highest recorded IgE levels	String	Numeric value	✓	
Were FeNO levels taken in the past year	Binary	Yes, No, Missing	✓	
When FeNO levels were taken	String	Date variable	✓	
Highest recorded FeNO levels	Numeric	Numeric value	✓	
When BEC was measured	String	Date variable	✓	
Highest recorded BEC	Numeric	Numeric value	✓	
Whether any IgE levels were taken prior to treatment initiation	Binary	Yes, No, Missing	✓	
Whether any FeNO levels were taken prior to treatment initiation	Binary	Yes, No, Missing	✓	
Whether any BEC levels were measured prior to treatment initiation	Binary	Yes, No, Missing	✓	

*The highest value for each biomarker for BEC, FeNO, and IgE for each patient will be taken for objectives 1 and 2, as this provides insight into how T2 a patient can be. This means the values for each biomarker may not be taken at the same time for each patient. Pre-biologics initiation values will be used for objective 3 when treatment responsiveness is the focus.

5.3 Defining Treatment

Label	Type	Values	Core	Optional
Defining treatments				
When treatment was prescribed	String	Date variable	✓	
Was treatment a biologic	Binary	Yes, No	✓	
Was treatment an oral corticosteroid (OCS)	Binary	Yes, No	✓	
Specific treatment name	Categorical	OCS, ICS, ICS/LABA, LABA, LAMA, theophylline, LTRA, anti-IgE, anti-IL5, anti-IL4, macrolide, steroid sparing, LABA/LAMA	✓	
When treatment ended	String	Date variable	✓	
Whether patient ever had bronchial thermoplasty	Binary	Yes, No, No data		

5.4 Defining Treatment Responsiveness

Label	Type	Values	Core	Optional
Defining treatment responsiveness				
Number of exacerbations in last year	Numeric	Numeric value	✓	
When exacerbation occurred	String	Date variable	✓	

Highest lung function measure	Numeric	Numeric value	✓
Change in lung function 1 year after treatment initiation			
Date lung function measured	Numeric	Date variable	✓
Were FeNO levels taken in the past year	Binary	Yes, No	✓
When FeNO levels were taken	String	Date variable	✓
FeNO levels	Numeric	Numeric value	✓
How well is asthma currently controlled	Categorical	Well controlled, Partially controlled, Not controlled	✓
Highest degree of asthma control over year under review	Categorical	Well controlled, Partially controlled, Not controlled	✓
Type of rescue systemic steroid used during exacerbation	Categorical	Betamethasone, Deflazacort, Dexamethasone, Hydrocortisone, Prednisone, Prednisolone, Methylprednisolone, Triamcinolone acetonide, Other	✓
Date rescue steroid initiated	String	Date variable	✓
Date rescue steroid stopped	String	Date variable	✓
Dose of rescue steroid prescribed	String	Dosing value	✓
Frequency per day of prescribed rescue steroid use	String	Daily frequency value	✓

5.5 Comorbidities

Label	Type	Values	Core	Optional
Comorbidity				
Obstructive sleep apnea	Binary	Yes, No		✓
Rhinitis	Binary	Yes, No	✓	
Nasal polyposis	Binary	Yes, No		✓
Allergic aspergillus	Binary	Yes, No		✓
Anxiety	Binary	Yes, No		✓
Depression	Binary	Yes, No		✓
Cardiovascular disease	Binary	Yes, No		✓
Metabolic disease	Binary	Yes, No		✓
Positive allergy test	Binary	Yes, No	✓	
Other allergies	Binary	Yes, No	✓	
Type of allergy	Categorical	Dust Mite, Grass mix, Cat hair, Mould mix, Dog hair, Aspergillus, Weed Mix, Trees, Food mix, Animal Mix, Other	✓	
Osteoperosis	Binary	Yes, No		✓
Heart failure	Binary	Yes, No		✓
Myocardial infarction	Binary	Yes, No		✓
Stroke	Binary	Yes, No		✓
Glaucoma	Binary	Yes, No		✓
Cataract	Binary	Yes, No		✓
Renal failure	Binary	Yes, No		✓
Peptic ulcer	Binary	Yes, No		✓
Gastroesophogeal reflux disease	Binary	Yes, No		✓
COPD	Binary	Yes, No		✓
ABPA	Binary	Yes, No		✓
Dysfunctional breathing	Binary	Yes, No		✓
Pneumonia	Binary	Yes, No		✓
Pulmonary embolism	Binary	Yes, No		✓
Osteoperosis	Binary	Yes, No		✓
Type 2 diabetes	Binary	Yes, No		✓
EGPA	Binary	Yes, No		✓
Hypertension	Binary	Yes, No		✓

5.6 Hospital Admissions

Label	Type	Values	Core	Optional
Identifying hospital admissions				
Number of hospital visits in the past year	Numeric	Numeric value	✓	
Number of hospital admissions in the past year	Numeric	Numeric value	✓	

6.0 Study Outcomes

6.1 Objective 1:

This objective will be focused towards identifying phenotypes according to degree of T2 inflammatory involvement using measurements of BEC, FeNO, and IgE taken for each patient. The outcome will be phenotype, which will be identified using cluster analysis and therefore unknown until the analysis is complete. Clusters will be dependent on the biomarkers included in the cluster analysis, and those allocated to clusters displaying most evidence of non-T2 asthma will be identified. Once phenotypes have been identified, they will be used as the exposure variables for the subsequent objectives. The cluster analysis approach is particularly suited to this objective, because whether or not the clusters are clearly defined will be insightful in itself. If clusters are indistinct and there is a large amount of overlap for biomarker distributions, it suggests non-T2 is less likely to be a separate clinical phenotype.

6.2 Objective 2:

Outcomes for this objective will include associations between phenotype as identified in objective 1, and demographic and clinical variables. This will allow consideration of potential differences between non-T2 and T2 patients, with particular focus on differences in disease burden through consideration of comorbid diseases. The demographic variables include sex, age at asthma onset, and country of residence. Clinical variables include asthma therapy, lung function, and the extent to which asthma is considered to be controlled.

6.3 Objective 3:

Treatment responsiveness to biologics according to phenotypic group will be the primary focus of this objective. Outcomes measuring responsiveness will include exacerbation rates (defined using the ISAR variable which asks 'the number of asthma exacerbations that the patient had encountered during the past 12 months that required the use of rescue steroids'), lung function measures, asthma control status, and use of rescue steroids in the year following treatment initiation. Analysis will be stratified according to treatment type including class of biologic.

6.4 Objective 4:

For the final objective, severe outcomes for patients according to phenotypic group are the main focus. Data is not available on death, so this cannot be analysed using ISAR data. The outcomes will be hospitalisations, and emergency hospital visits, for which rates will be compared across phenotypes. This will provide insight into disease burden according to phenotypic group.

7.0 Statistical Analysis

7.1 Sample Size

The sample size will be dependent on the number of patients for which data is available for key variables. For objectives 1 and 2 which will be completed first, **3,938** (preliminary number) patients are available with information on all three biomarkers.

7.2 Software

The study will use STATA version 14 and R for analysis. Datasets will be received from the data analytics team in CSV, which can be easily imported into both STATA and R.

7.3 Analysis

Statistical analysis will be undertaken according to the best approach for each objective. The planned analysis for each objective is summarised as follows:

7.4 Objective 1:

Descriptions of key variables including distributions for clinical biomarkers will be provided. Cluster analysis will be undertaken to consider the possible existence of groups of patients displaying similar phenotypes. Highest biomarker value for each patient will be used as a measure of the extent to which the patient can experience T2 inflammation. Cluster analysis relies on knowledge of which variables are likely to influence phenotype, and in this instance data on T2 inflammatory biomarkers (here defined as IgE, BEC, and FeNO measures) as continuous variables will be included in the analysis. The number of clusters will be decided based on the Calinski-Harabaz pseudo F-value. Essentially, this value enables us to identify which number will provide the most distinctive clusters. Once the clusters are clarified, the characteristics of each would be explored, and those displaying most evidence of non-T2 asthma identified. If clusters are indistinct and biomarker distributions appear similar across clusters, it suggests no clear evidence of non-T2 asthma as a separate phenotype, which is still clinically useful information. Given that some patients do not have any biomarker readings taken prior to treatment initiation, a sensitivity analysis will be undertaken.

The influence of treatment on biomarker values differs depending on both the treatment, and the biomarker. Therefore, we aim to conduct a sensitivity analysis for objective 1, which identifies phenotypes, to consider whether conclusions drawn would differ when the possible effects of treatment are taken into consideration. These alternative phenotypes will also be used to conduct objectives 2 to 4. Therefore, there will be an overall analysis using phenotypes

which take the highest biomarker values into account, and a sensitivity analysis which considers the potential effect of treatment on biomarker values, for each objective.

Table 1 shows how each biomarker is believed to respond to particular treatments, and therefore how the final sensitivity analysis criteria was arrived at.

Table 1:

Table 1:

	BEC	**IgE**	**FeNO**
Anti-IL4/13	No change	No change	Reduced ↓↓↓
Anti-IL5	Reduced ↓↓↓	No change	No change
Anti-IgE	Reduced ↓	Slight increase	Reduced ↓↓

We aim to develop phenotypes based on an individuals' 'natural' level of inflammation. Therefore, for all treatments which reduce biomarker measurements, the sensitivity analysis will exclude patients on these treatments if they do not have any measurements taken prior to treatment initiation, as their highest measurement is likely to be taken at a time when their T2 inflammation is suppressed. For patients initiating IgE, their highest biomarker value for IgE levels will be taken as their highest value prior to treatment initiation, and if no biomarker values are present prior to this, these patients will be excluded.

7.5 Objective 2:

Descriptive statistics for demographic and clinical characteristics among patients of different phenotypic groups will be reported. Statistical tests comprising chi-square for categorical and t-tests or analysis of variance (ANOVA) for continuous variables will be conducted where appropriate. This will include assessment of whether there are differences in distributions of comorbidities and demographic characteristics according to phenotypic group. Finally, a backward stepwise model will be conducted to identify which variables appear to explain the most variance in terms of differences between higher T2 and non-T2 phenotypic groups.

7.6 Objective 3:

For objective 3, data and distributions for variables indicating treatment responsiveness will be described. Patient phenotypic groups, as identified by the cluster analysis, will be used as exposure variables in a longitudinal cohort design. Responsiveness to treatment, with a focus on biologics, will be assessed through four main outcomes: number of exacerbations in the year following treatment initiation, lung function measurements, use of rescue steroids over

the year following treatment initiation, and degree of asthma control. Firstly, the data will be described, including proportions and frequencies in each phenotypic group, and for each responsiveness category. Statistical tests including chi-square, t-tests and ANOVA will be performed depending on the variable format. Secondly, the data will use longitudinal modelling to assess whether the best responders belong to certain phenotypic groups. For count data, such as number of exacerbations, Poisson or negative binomial regression will be used for analysis depending on the distribution of the data. For some patients, data on date of exacerbation is available. Therefore, as a sensitivity analysis, Cox proportional hazard models using this subset of patients can be conducted as a time to event analysis. If results are similar, with direction and magnitude of effect remaining roughly the same, this will give reassurance in future studies that valid conclusions can be drawn using only patients with an exact exacerbation date available for time to event analyses. For biomarker measurements, changes will be examined through generalised estimating equations (GEE's). Analysis will be stratified according to treatment type including biologic class. For switchers, patients will exit the study at the point they switch.

7.7 Objective 4:

Hospitalisation rates across the period covered by ISAR for asthma patients according to their T2 phenotype will be described. Statistical tests will consider potential differences according to rates between phenotypic group. This will provide some information on disease burden according to asthma presentation.

8.0 Feasibility Assessment

As of August 2021, the ISAR database contains **11,439** patients with available data for the cross-sectional design intended for objectives 1 and 2. All these patients will be described as part of these objectives, however full data will not be available for all patients. In terms of conducting cluster analysis, the largest limitation is likely to be the lack of information on certain biomarkers for some patients. For example, only patients who have had a FeNO measurement taken in the past year have this measurement included in their data. Missing data for any of the biomarker variables included in the cluster analysis will result in a missing value for cluster allocation. Therefore, for a sizeable proportion of ISAR patients, it won't be possible to estimate their clustered group. Given the number of patients with complete biomarker data, which we currently believe to be **3,938** (preliminary number), we should still have adequate statistical power to conduct cluster analysis and therefore gain an understanding of asthma phenotypes according to biomarkers. The focus will be on the patients with complete biomarker information, however it is possible that these patients are particular, and selection bias could be occurring. Therefore, patient characteristics for those who have 0, 1, 2, and 3 biomarkers available will be described, which will give some insight into potential differences between patients with more biomarker measurements available, and those with fewer values available. For objective 2, the same feasibility issues apply in that if the patient has a missing cluster value, we cannot know which group they most likely belong to, and therefore it is not possible to fully describe each phenotype. Additionally, it is possible that treatment regimen could act as a confounding factor between biologic treatment, and clinical outcomes. Therefore, it may be worth considering only diagnoses given prior to treatment initiation as a sensitivity analysis, which will remove the potential pathway from treatment regimen to clinical characteristic. In these instances, the direction of effect would be from clinical characteristic to treatment regimen, which would remove the possibility of treatment regimen acting as a confounder. However, numbers may be small since not all patients will have this data available prior to their ISAR enrolment, and power therefore limited.

For objective 3, the same issues surrounding phenotype classification apply, however the main obstacle to undertaking time to event analysis will be gaining an accurate and unbiased measurement of study time. Patients are not necessarily enrolled into ISAR at the initiation of particularly non-biologics, therefore we cannot know exposure time for many patients on these treatments. Some patients are enrolled at the point of biologics initiation, therefore the index date for these treatments is known. Full exposure time is therefore available for some patients, whereas only partial exposure time is available for other treatment groups. Additionally, only

pharmacotherapy intended for the treatment of asthma can be studied using the ISAR data. Treatments for comorbid conductions such as obstructive sleep apnea (OSA) for example, could affect the outcome, however we cannot account for this in analysis since the data is not collected. Additionally, physical or psychological therapies for asthma cannot be assessed since data is not collected on these treatment regimens.

The final objective may suffer from a lack of statistical power, due to relatively few patients having a recorded hospitalization in the ISAR database.

For missing data, proportion missing will be reported for each variable. Any variables with unexpected missing values for particular countries may be checked through contacting the relevant country site in order to solve any potential issues.

9.0 Regulatory and Ethical Compliance

This study was designed and shall be implemented and reported in accordance with the criteria of the "European Network Centres for Pharmacoepidemiology and Pharmacovigilance (ENCePP)" and follows the ENCePP Code of Conduct (EMA 2014). Once a final version of the protocol has been agreed and reviewed by the advisory group, this study will be registered with ENCePP (www.encepp.eu).

ISAR is approved by the Health Research Authority for clinical research use and governed by the Anonymised Data Ethics & Protocol Transparency (ADEPT) Committee. We will submit the finalised version of this protocol to the ADEPT committee (https://www.regresearchnetwork.org/adept-committee/) for approval.

All sites will enter into a regulatory agreement in compliance with the specific data transfer laws and legislation pertaining to each country and its relevant ethical boards and organisations. Further, all data extracted to be transferred from sites will be hashed and will enter the research database in the form of anonymised patient IDs. The data will be retrieved by Optimum Patient Care (OPC) data analysts and utilised as an anonymised dataset to perform the analysis according to protocol. This study will be performed in compliance with all applicable local and international laws and regulations, including without limitation ICH E6 guidelines for Good Clinical Practices.

10.0 Data Dissemination

This study will be the first we are aware of to attempt identification of phenotypic groups according to biomarker values, and through this to determine which patients should be considered to present with non-T2 asthma. This will provide important insight into patient prognosis, comorbidities, and expectations for treatment effectiveness.

The results will be disseminated to the public through publications in peer reviewed journals, alongside abstract presentations at relevant conferences. Authorship will be determined through the ISAR authorship policy.

11.0 Advisory Group

Professor David Price, Chief Investigator for the study, is the chair of the ISAR Steering Committee (ISC). Other members of the committee, as listed in the following table, will form the Advisory Group.

Project Steering Committee Member	Country/Funder
Mark Hew	Australia
Matthew Peters	
Peter G. Gibson	
Sinthia Bosnic-Anticevich	
Jorge Maspero	Argentina
Guy Brusselle	Belgium
George C. Christoff	Bulgaria
Todor A. Popov	
J. Mark FitzGerald	Canada
Mohsen Sadatsafavi	
Ken Chapman	
Shawn Aaron	
Carlos A. Torres-Duque	Colombia
Celeste M. Porsbjerg	Denmark
Charlotte S Ulrik	
Susanne Hansen	
Alan Altraja	Estonia
Lauri A. Lehtimäki	Finland
Arnaud Bourdin	France
Camille Taillé	
Christian Taube	Germany
Nikolaos G. Papadopoulos	Greece
Andriana I. Papaioannou	
Konstantinos Kostikas	
Mina Gaga	
Unnur Steina Björnsdóttir	Iceland
Sundeep Salvi	India
Richard W. Costello	Ireland
Enrico Heffler	Italy
Giorgio Walter Canonica	
Concetta Sirena	
Puggioni Francesca	
Takashi Iwanaga	Japan
Takahiko Horiguchi	
Tatsuya Nagano	
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13.0 Timelines

Action	Timeline
Protocol finalisation	September 2021
Ethics approval	October 2021
Dataset preparation	October 2021
Analysis & preliminary results	November 2021
Study report	January 2022
Conference abstract	January 2022
Manuscript	February 2022

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