Study Report

EffectIveness of biologics (by classes) in patieNts with dIfferent combinations of T2 biomarkErs (IGNITE)

An investigation into biomarker information needed to make informed predictions of patient responsiveness to biologic treatment by biologic class

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TITLE	**EffectIveness of bioloGics (by classes) in patieNts with dIfferent combination of T2 biomarkErs (IGNITE)**
Subtitle	An investigation into biomarker information needed to make informed predictions of patient responsiveness to biologic treatment according to biologic class
Study report version number	V3.0
Medicinal product	Not applicable
Product code	Not applicable
Marketing authorisation holder	Not applicable
Marketing authorisation number	Not applicable
ENCePP registration number	EUPAS 43806
ADEPT approval reference number	PROTOCOL 2305
Study aims and objectives	**Study aims:** To investigate whether T2 inflammatory biomarker measurements tend to be correlated within patients, and whether biomarker traits are associated with responsiveness to treatment with biologics **Study objectives:** **Objective 1**: To describe distributions of T2 inflammatory biomarkers in severe asthma patients, and examine whether different T2 biomarker measurements are correlated within patients

	Objective 2: To examine whether T2 biomarker measurements are associated with responsiveness to treatment with biologics **Objective 3:** To identify whether multiple biomarker measurements lead to better prediction of patient responsiveness to biologics
Countries of study	23 countries: Argentina, Australia, Bulgaria, Canada, Colombia, Denmark, Greece, India, Ireland, Italy, Japan, Korea, Kuwait, Mexico, Poland, Portugal, Saudi Arabia, Singapore, Spain, Taiwan, United Arab Emirates (UAE), United Kingdom (UK), United States of America (USA)
Data source	International Severe Asthma Registry (ISAR)
Author(s)	[redacted]

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LIST OF ABBREVIATIONS

Abbreviation or special term	Explanation
ADEPT	Anonymised Data Ethics & Protocol Transparency
BEC	Blood eosinophil count
BMI	Body mass index
CI	Confidence interval
COPD	Chronic obstructive pulmonary disease
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
IQR	Inter-quartile range
ISAR	International Severe Asthma Registry
ISC	ISAR Steering Committee
LABA	Long-acting beta-agonist
LAMA	Long-acting muscarinic antagonist
LTRA	Leukotriene receptor antagonist
LTOCS	Long-term oral corticosteroids
OCS	Oral corticosteroids
OPC	Optimum Patient Care
OPRI	Observational and Pragmatic Research Institute
ppb	Parts per billion
R	R software from the R Project for Statistical Computing
SD	Standard deviation
STATA	Stata software suite
T2	Type 2 inflammation

1.0 Executive Summary

Biomarker measurements are often collected and used in the selection of treatments for patients with severe asthma, based on evidence from clinical trials. However, relatively little is known about how well these predcit outcomes in real-world situations. Also the potential benefit of using combined information from multiple biomarkers to direct medical decisions is not known.

This study aimed to investigate whether T2 inflammatory biomarkers (blood eosinophil count (BEC), fractional exhaled nitric oxide (FeNO), and serum immunogloblin-E (IgE)) are correlated within patients. We also investigated whether biomarker traits are associated with responsiveness to treatment with biologics, and whether combinations of biomarker measurements gave better predictions of the outcomes.

Data from all patients in the ISAR database meeting the eligibility criteria (≥18 years and without bronchial thermoplasty) were used to investigate the associations between different biomarkers and between biomarkers and outcomes. For assessing the association between biomarkers and outcomes, the highest pre-biologic measurements were used as the baseline biomarker measurements. The outcomes were observed in the year before initiation of biologic treatments (baseline) and in the year following (follow-up) to assess the effect of the treatments. Biologic treatments were grouped into three classes; anti-IgE, anti-IL4, and anti-IL5/5R. Regression models were used to assess the associations between the baseline biomarker levels and the follow-up outcomes, adjusted for baseline levels of the outcomes.

Correlations between pre-biologic levels of the biomarkers were weak, whether we considered the highest pre-biologic measurements available or only pre-biologic measurements taken within 7 days of each other.

Change in exacerbations showed weak association with the biomarkers. This may be related to the lack of an assocation between baseline exacerbation rates and baseline biomarkers observed in the patients who went on to receive biologics, probably due to the selection criteria applied. Asthma control at follow-up also showed fairly weak assocations with the baseline biomarkers although a statistically significant association was seen with baseline BEC in the patients prescribed anti-IL5/5R. The strongest associations with the outcomes were seen for FEV_1 with baseline BEC and FeNO. High levels of these biomarkers were associated with the greatest improvements in FEV_1 with all three of the biologic classes.

The low correlations between biomarkers might suggest that different biomarkers would provide different information about outcomes and therefore a combination of biomarkers might be more useful. Whilst there were small statistical improvements in model predictions when multiple biomarkers were included, particularly for FEV_1, no improvements were found that appeared to be of clinical significance for predicting any of the three outcomes studied. We cannot rule out the possibility that multiple biomarkers would be useful for predicting compound outcomes such as improved asthma control + reduced steroid use.

The ISAR database is the largest database of its kind, collecting data on severe asthma patients in 23 countries. This offers unique opportunities to study associations between biomarkers and response to biologics in real-world settings. There are, however, limitations to using such data. The timing of biomarker measurements was not controlled to provide data on the patients at the start of their biologic treatments so did not necessarily capture the highest levels that the patients had experienced. Assessments of the outcomes of exacerbations and asthma control involve some degree of subjectivity by the patients and/or by medical staff. Some data, such as certain dates, also relied on patients' recollection and may have included some error. Also patients receiving different biologic treatments were not as well matched as would be expected in a clinical trial and there was no suitable control group available within the data. The baseline characteristics showed that this was, in general, a very ill cohort of patients (mean exacerbation rate = 2.2 per year, uncontrolled asthma = 70%, FEV_1 = 2.1 L, mean duration of asthma = 20 years). This may have limited the extent to which outcomes changed following treatment with biologics in patients in this study.

Despite the limitations, this study has shown a clear association between improvement in lung function (measured by FEV_1) and baseline levels of BEC and FeNO, measured in real-world settings, which could be used to help select which patients were likely to gain most from biologic treatments. The study also showed that patients with or without high levels of exacerbations at baseline were likely to benefit. IgE was not found to be strongly predictive of any of the outcomes.

2.0 Background

Severe asthma can be defined as asthma which remains uncontrolled, or which requires extensive treatment according to steps 4 and 5 of the Global Initiative for Asthma (GINA)[1], with recent estimates indicating 6.1% of asthma patients could fall into this category[2]. Biomarkers, defined as objectively measured characteristics which indicate biological processes[3], are increasingly used as indicators of disease presentation in many areas of medicine. Treatment of severe asthma is often determined based on biomarker measurements including blood eosinophil count (BEC), IgE measurements, and fractional exhaled nitric oxide (FeNO)[4]. This can be limiting if measurements are only considered as individual observations, rather than being taken in the context of other information including other biomarker values[4]. Additionally, biomarker measurements are often placed in a binary classification according to whether high levels of T2 inflammation are present or not, although where cut-offs should be drawn is still debated[5,6]. Using binary cut-offs in this way means information is lost, making it more difficult to tailor treatments to patients. Biomarker values are becoming increasingly important in understanding asthma endotype and treatment responsiveness[7], so it is crucial the right and most useful information is collected. Understanding whether using precise measurements rather than considering information only in terms of binary cut-offs could improve predictions of how well patients tend to respond to treatment appears to be an understudied area. Studying exactly what information is needed, and at what level of granularity, would therefore provide useful information for asthma clinicians when deciding what data needs to be collected from patients at each visit.

More recent asthma research has made a distinction between asthma control and asthma severity[8], with good asthma control being the intention of treatment. Asthma control refers to the extent to which presentations of asthma can be reduced or removed by therapy. In order to understand how well treatment works for individual patients, change in exacerbation rates before and after treatment initiation is often more insightful than simply considering exacerbation levels over the course of treatment exposure[9]. Exacerbations can have many causes, with susceptibility dictated by factors such as allergic sensitisation, genetic variation, and defective anti-viral immunity[10,11]. Comorbid diseases can also act as exacerbation triggers[12–14]. Knowledge on whether these exacerbation triggers can be identified prior to an exacerbation occurring through biomarker values would mean clinicians may be able to predict if an exacerbation is likely to occur and respond accordingly, improving overall asthma control for patients[11].

Asthma patients are a heterogenous group, and asthma patients with similar severity could present differently in terms of their biomarker measurements and responsiveness to treatments[15,16]. Severe asthma patients are thought to constitute 6.1% of all asthma patients when the GINA definition is used[2], however they are estimated to account for half of asthma healthcare-related costs[17]. Therefore, understanding how these patients respond best to treatment and how exacerbations can be reduced is of optimum importance. Differences in biomarker measurements between patients may assist with prediction of how well treatments are likely to perform, however, full information is not always collected or available for each patient. Understanding what information is needed in order to make more precise predictions as to patient responsiveness to treatment would provide useful insight into how much data should be collected on each patient during visits. Knowledge of specific patient presentation and phenotype through use of biomarkers can assist with making decisions on the most appropriate treatment regimens. We propose an investigation into which measurements should be taken when patients visit their clinicians, to generate useful predictions of how outcomes are likely to change when patients are treated with biologics.

3.0 Study Aims and Objectives

3.1 Study Aims

To investigate whether T2 inflammatory biomarker measurements tend to be correlated within patients, and whether biomarker traits are associated with responsiveness to treatment with biologics

3.2 Study Objectives

Objective 1: To describe distributions of T2 inflammatory biomarkers in severe asthma patients, and examine whether different T2 biomarker measurements are correlated within patients

Objective 2: To examine whether T2 biomarker measurements are associated with responsiveness to treatment with biologics

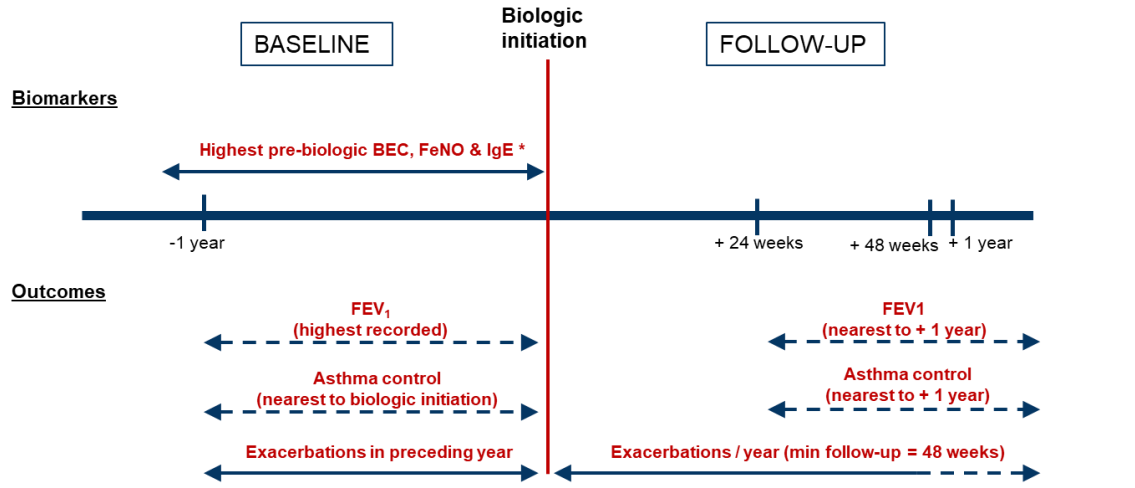
Objective 3: To identify whether multiple biomarker measurements lead to better prediction of patient responsiveness to biologics

4.0 Materials and Methods

4.1 Overall Study Design

This study aimed to consider firstly whether biomarker measurements tend to be correlated within patients, and secondly whether individual or multiple biomarkers may correlate with how certain outcomes change after biologics treatment. An overview of the study design is shown in Figure 1. The biomarkers studied were blood eosinophil count (BEC), fractional exhaled nitric oxide (FeNO) and serum immunoglobulin-E (IgE). The outcomes of interest in this study were annual exacerbations rates, forced expiratory volume in 1 second post-bronchodilator (FEV_1) and asthma control. For further details of the biomarker and outcome variables used see section 5.0. Biologic treatments were grouped by class: anti-IgE, anti-IL4 or anti-IL5/5R. Unless otherwise stated, any reference to anti-IL5 in this report also includes anti-IL5R drugs.

Figure 1. Overview of the study design



* For objective 1 associations between biomarkers collected pre-biologics and within 7 days of each other, as well as associations between highest pre-biologic biomarker values were studied; FeNO – fractional exhaled nitric oxide; IgE – serum immunoglobulin-E; FEV_1 – post-bronchodilator forced expiratory volume in 1 second

Objective 1: This objective considered associations between different biomarkers collected pre-biologic initiation and within 7 days of each other. Associations between the highest pre-biologic (baseline) biomarker measurements recorded in the ISAR database for each patient for each pair of the biomarkers (BEC, FeNO and IgE) were also assessed. For patients who were not prescribed biologics, highest biomarker measurements at any time were used, as all of these were pre- any biologic treatment.

Objective 2: This objective, which included only patients prescribed biologics, studied whether baseline biomarker levels (as define above) could be used to predict follow-up levels of the outcomes of interest (exacerbation rates, FEV_1 and asthma control) conditioned on the level prior to biologic initiation. These analyses were stratified by biologic class (anti-IgE, anti-IL5/5R, and anti-IL4) so that associations between the outcomes and biomarker levels could be compared between biologic classes. If differences exist, this information together with the pre-biologic biomarker levels, could be useful in selecting the most appropriate treatment for individual patients.

To assess the effect of the biologics on outcomes, exacerbation rates in the year preceding biologic initiation were compared with the annualised rate following biologic initiation. FEV_1 and asthma control scores were taken from assessments made in the year preceding biologic initiation and compared with assessments made as close as available to 1 year following biologic initiation (and at least 24 weeks after initiation). Further details of these outcome measures are given in section 5.3. Tests of association between the biomarkers and the outcomes were carried out for each biomarker / outcome combination individually. In order to maximise the use of data, patients were included in each analysis if they had all of the relevant biomarker / outcome data available, irrespective of whether they had data on the other outcomes or biomarkers.

Objective 3: This objective focused on whether information from multiple biomarkers could provide a useful improvement over using the best individual biomarker to predict patients' responsiveness to biologic treatments. Biomarker levels and outcomes were defined as for objective 2. However, only patients with baseline values of all three biomarkers available could be included in this analysis. If multiple biomarkers can provide improved predictions of outcomes, this would suggest there would be advantages to collecting multiple biomarker information prior to biologic initiation to inform the choice of treatment for patients.

4.2 Study Population and Data Sources

The International Severe Asthma Registry (ISAR) is an international collaborative initiative aiming to gather longitudinal data on patients with severe asthma. 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 for their data to be 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]. The data is comprised of relevant

information collected from patients at each visit, and extracted medical records. Patients' index dates for this study were the date of enrolment in ISAR for patients who were not prescribed biologics, and the date of biologic initiation for those who were.

Data collection began in [redacted], and as of [redacted], there were 15174 participants from 23 countries enrolled into ISAR. Patients meeting the eligibility criteria for this study numbered 11,373 (4901 biologic and 6472 non-biologic).

4.3 Inclusion and Exclusion Criteria

Inclusion Criteria:

Objective 1:

- All patients with sufficient biomarker information available to be included in any of the analyses.

Objective 2:

- All patients prescribed biologics and with relevant data available for biomarkers, biologics treatment, exacerbations, lung function and asthma control.

Objective 3:

- All patients prescribed biologics and with pre-biologic biomarker data for all three biomarkers, biologics treatment, and relevant outcomes information.

Exclusion Criteria:

Objectives 1, 2, and 3 :

- <18 years at the index date
- Patients treated with bronchial thermoplasty

5.0 Study Variables

5.1 Demographic variables

Demographic variables are collected in the ISAR database at the time the patient is registered. These were used to descibe the study population.

5.2 Clinical variables

Details of treatments and assessments are recoded in the ISAR database at registration and at follow-up visits. These were used to derive the following variables in IGNITE:

- Biologic patient (y / n) – Whether the patient had any biologic treatments.
- Biologic class (anti-IgE, anti-IL4, anti-IL5/5R) – For patients prescribed biologics, the first type of biologic treatment received. Note that patients with more than one type of biologic treatment were excluded from objectives 2 & 3 so that any effects could be attributed to a particular biologic class. Hence, no data from patients who switched to another biologic during follow-up were included in objectives 2 & 3. The term anti-IL5 used in some figures and tables includes anti-IL5R treatments.
- Biomarker results for the following biomarkers are collected in ISAR at registration and subsequent follow-up visits if the assessments have been carried out:
    - Blood eosinophil count (BEC) (cells/µL)
    - Fractional exhaled nitric oxide (FeNO) (ppb)
    - Serum IgE (IU/mL)

  These were used to derive the highest pre-biologic results for each patient for each biomarker, also referred to as the "baseline" biomarker results in this report. For objectives 2 & 3, positive outlying values of baseline biomarkers were excluded from the analysis to avoid issues created by points with high leverage and, effectively, extrapolating beyond the range where we had sufficient data. Outliers of baseline biomarker values were identified as > upper quartile + 1.5 x inter-quartile range[19].
- Long-term oral corticosteroid (LTOCS) use at biologic initiation – Whether the patient was on LTOCS at biologic initiation was derived from the biologic initiation date and the individual LTOCS treatment records in the ISAR database.
- Allergies (y / n) – Allergy testing varies considerably within and between countries. A single variable was derived to show whether the patient had any allergies recorded using any method (serum allergy test, skin prick test, or another method).
- Nasal polyps ever (y / n)

5.3 Outcome variables

The outcomes of interest in IGNITE were pre- and post- treatment exacerbation rates, asthma control scores, and lung function as measured by FEV_1.

- Exacerbations
    - Pre-biologic (baseline) exacerbations / year - The number of exacerbations in the year preceding biologic initiation is recorded in the ISAR database.
    - Post-biologic (follow-up) exacerbations / year – The number of exacerbations since the last visit are recorded for each follow-up visit. These were used to calculate the annual exacerbation rate during follow-up for the patient by dividing the number recorded by the length of follow-up in days and multiplying this by 365. This outcome was considered missing for patients with less than 48 weeks follow-up to avoid problems of seasonality if the patient had only been followed for part of a year.
- Asthma control scores – These are recorded in ISAR as 1 (well controlled), 2 (partially controlled), 3 (not controlled), based on the patient's GINA asthma control assessment (or ACT/ACQ score if GINA assessment is not available) collected at their most recent visit within 1 year prior to biologic initiation. Asthma control is also collected at each follow-up visit, reflecting symptoms in the 4 weeks prior to the visit. The assessment closest to 1 year post-biologic initiation (at least 24 weeks after biologic initiation) was used as the follow-up score. For the analyses in IGNITE asthma control scores were treated as a binary variable: uncontrolled (category 3) versus not uncontrolled (category 1 or 2).
- FEV_1 (measured post-bronchodilator) - Any previous spirometry results available are collected at the time of the patient's registration in ISAR. The baseline value for the analysis was taken as highest measurement up to 1 year pre-biologic initiation. The follow-up measurement for the analysis was taken as the nearest measurement available to 1 year post-biologic initiation (at least 24 weeks after biologic initiation).

6.0 Statistical Analysis

6.1 Sample Size

The final sample size was determined by number of individuals with available biomarker, biologic treatment, exacerbation, lung function, and asthma control data. As many patients as possible were included in each analysis if they had the relevant data available.

6.2 Descriptive Analysis

Patient characteristics are described using means and standard deviations, medians and inter-quartile ranges or counts and percentages, as appropriate.

Baseline characteristics of the patients were summarised for the following cohorts:

- Objective 1: Patients not prescribed biologics; patients who were prescribed biologics; overall
- Objective 2: Patients prescribed Anti-IgE, patients prescribed anti-IL5/5R; patients prescribed anti-IL4

6.3 Objective 1 analyses

This objective was to describe biomarker distributions for patients, and test associations between biomarker values within patients.

Distributions for each biomarker across the patients included in Objective 1 were plotted using histograms.

The associations between the different biomarker values (BEC vs FeNO, BEC vs IgE, and FeNO vs IgE) were tested using the continuous values for each biomarker, and binary recoded versions of the variables using cut-off values of 350 cells/µL for BEC, 25 ppb for FeNO and 75 IU/mL for IgE. These tests were carried out using only biomarker results collected within 7 days of each other. Since we used the highest pre-biologic biomarker values to test for associations with asthma outcomes, tests for association between these highest pre-biologic (baseline) biomarker values were also carried out in a similar way.

Due to the highly skewed distributions of all three biomarkers, associations for the continuous biomarkers were tested using Pearson's correlation coefficient calculated from the $\log_{10}$(biomarker values) and also using Spearman's rank correlation coefficient, which tests for associations between variables based on the rank order of results (i.e. whether higher values of one variable are associated with higher values of the other) irrespective of the shape of the distributions. This statistic is more robust to the effect of outliers. For the binary versions of the biomarkers, chi-square tests were used to test for associations.

6.4 Objective 2 analyses

Biomarkers (BEC, FeNO and IgE) were studied individually in objective 2. Exacerbations were modelled using negative binomial regression with follow-up exacerbation rate as the outcome. Predictors in the model were baseline exacerbation rate, biologic class (anti-IgE, anti-IL5/5R, anti-IL4), baseline biomarker level (BEC or FeNO or IgE), and interactions between baseline exacerbation rate and baseline biomarker level and between biologic type and baseline biomarker level. Hence the models can be used to study the association between follow-up exacerbation rate and baseline biomarkers for each biologic class, adjusting for the effect of baseline exacerbation rate in each biologic class. In order to be able to compare the degree of association for multiple biomarkers and multiple outcomes with three different biologics, similar models were used for each of the biomarkers and outcomes studied.

$$exacerbations_{follow-up} = \beta_0 + \beta_1 exacerbations_{baseline} + \beta_2 biologic\_class + \beta_3 biomarker_{baseline} + \beta_4 exacerbations_{baseline} \# biologic\_class + \beta_5 biomarker_{baseline} \# biologic\_class$$

[Equation 1]

(Note: # represents an interaction term in the model)

By rearranging the terms in the model it can be shown that this is equivalent to fitting a separate prediction equation for each biologic type (*x*) in the form below. In effect we fitted a model to predict follow-up exacerbations from the baseline biomarker value, adjusted for baseline exacerbations for each biologic type individually.

$$exacerbations_{follow-up} = \beta_{xa} + \beta_{xb} exacerbations_{baseline} + \beta_{xc} biomarker_{baseline}$$

[Equation 2]

The primary interest was whether the coefficients β_{xc} were different from zero (indicating there was an association between follow-up exacerbations and the baseline level of biomarker *x,* adjusted for baseline exacerbation rate).

Fitting the lines for all three biologics in the same model also allowed us to test whether the coefficient β_{xc} (i.e. slope of the exacerbations / biomarker association) differed between biologic types.

Results from the models were used to calculate the adjusted predictions of follow-up exacerbation rates for each biologic class over the range of the biomarker, for a patient with the mean rate of exacerbations at baseline (mean across all three biologic classes). These were presented graphically as the change from this baseline level. Separate models were fitted for each of the three biomarkers (BEC, FeNO and IgE).

Similar statistical models were used for the outcomes FEV_1 and uncontrolled asthma, using ordinary least squares and logistic regression respectively, adjusting for the baseline level of the relevant outcome in each case. For FEV_1, the change from baseline was presented graphically for a patient with the mean baseline FEV_1. For uncontrolled asthma, the probability of uncontrolled asthma at follow-up was presented for each biologic class as if all patients had been treated with that biologic, hence comparing the classes graphically for cohorts with the same baseline distribution of asthma control.

6.5 Objective 3 analyses

This objective aimed to examine the value of including multiple biomarkers to predict the effectiveness of biologics and hence to provide information about which class(es) of biologics would be most effective. For this objective, only patients with all three biomarkers available at baseline were included. The statistical models were as for objective 2 but included additional terms ($+biomarker_x + biomarker_x\#biologic\_class$) for each additional biomarker. In the notation of Equation 2 (section 6.4), this was equivalent to estimating a separate equation for each biologic, to estimate the follow-up outcome from the three biomarkers, adjusted for baseline exacerbation rate:

$$exacerbations_{follow-up} = \beta_{xa} + \beta_{xb} exacerbations_{baseline} + \beta_{xc} BEC_{baseline} + \beta_{xd} FeNO_{baseline} + \beta_{xe} IgE_{baseline}$$

[Equation 3]

For each outcome, the best individual biomarker model (i.e. the model including just BEC, just FeNO or just IgE) was selected as the one leading to the highest adjusted R^2 (for FEV_1) or the highest pseudo R^2 (for asthma control and exacerbations). The best individual biomarker model was then compared with a similar model including all three biomarkers as predictors, using a likelihood ratio test to test whether multiple biomarkers led to a statistically significant improvement in the model's predictions. A significant improvement in the overall fit of the model could be interpreted as showing that using multiple biomarkers would give us better ability to predict which patients would benefit most from biologic treatment. However, a statistically significant improvement in model fit does not necessarily show that predictions would be improved to a clinically relevant extent.

The practical improvement in predictions of the outcomes by adding additional biomarkers was assessed using:

i) Percentage of variance in follow-up FEV_1 explained by the model (as measured by the adjusted-R^2),

ii) Percentage of uncontrolled asthma outcomes predicted correctly (i.e. if predicted odds > 1 and actual outcome = uncontrolled, or predicted odds < 1 and actual outcome = not uncontrolled);

iii) Mean absolute error in the predicted exacerbation rate compared with the actual follow-up exacerbation rate.

6.6 Additional analyses

Additional analyses were suggested by members of the steering committee. These included testing for correlations between biomarkers collected at similar dates (see above) requested by [redacted] and analyses stratified by patients with low or high exacerbations at baseline, also suggested by [redacted]. Other analyses by subgroups were suggested by [redacted]. The analyses by patients on LTOCS or not was suggested by [redacted]. The analysis of changes in biomarkers following biologic initiation was chosen as the subject matter to be presented at ERS 2022. [redacted] suggested we include analyses of the

associations between outcomes and biomarkers at baseline. The methods used are described below.

6.6.1 Analysis by subgroups

Analysis of the associations between baseline eosinophil and the outcomes were also carried out by subgroups using similar methods to those described for comparing biologic classes. Due to the low numbers of patients with complete information about outcomes and other covariates of interest across the complete range of biomarkers, subgroup analyses have been carried out for patients prescribed all classes of biologics combined and not broken down by biologic class. The statistical models were:

$$outcome_{follow-up} = \beta_0 + \beta_1 outcome_{baseline} + \beta_2 subgroup + \beta_3 biomarker_{baseline} + \beta_4 outcome_{baseline} \# subgroup + \beta_5 biomarker_{baseline} \# subgroup$$

[Equation 4]

(Note: # represents an interaction term in the model)

This was equivalent to fitting a model to predict the follow-up level of the outcome from baseline biomarker level, adjusting for the effect of baseline outcome level in each subgroup. Fitting lines for both subgroups in the same model made it possible to test for differences in the slopes of the outcome / biomarker associations between subgroups. Analyses were carried out for the following pairs of subgroups:

- Patients on LTOCS at baseline (yes / no)
- Patients who had 0 or 1 exacerbations per year at baseline vs. patients who had ≥2 exacerbations per year (i.e. benefits to asthma control and FEV_1 for patients who did or did not require biologics to control exacerbations)
- Patients with allergies (yes / no)
- Patients with asthma onset at <18 years or ≥18 years

6.6.2 Associations between biomarkers and outcomes at baseline

The protocol focusses on the associations between baseline biomarkers and outcomes (exacerbations, FEV_1 and asthma control) at follow-up (**after** initiating biologics). In order to interpret these findings, we additionally analysed the associations between baseline biomarkers and the outcomes of interest at baseline, **before** the patients had received any

biologic treatments. This was subdivided by patients who were not prescribed biologics and patients who subsequently went on to receive biologics. The results were presented as forest plots.

6.6.3 Changes in biomarkers following biologic initiation

The study focussed on using baseline (pre-biologic) biomarker measurements to predict post-biologic outcomes, with a view to informing clinical decisions about which, if any, biologic treatment should be prescribed. Changes in biomarkers following biologic initiation were also studied and presented at ERS 2022. The highest biomarker measurement available in each period after starting biologics (first 3 months, 3-12 months, 12-24 months, 24-36 months and >36 months) was calculated as long as the patient was still on treatment with the biologic. Change from the baseline value, whether there had been >25% decrease in the biomarker compared with baseline, and whether the level was within the normal range for BEC (<150 cells/µL) and FeNO (<25 ppb) were also calculated. The median change in each period compared with patients' baseline values were presented graphically.

6.7 Software

Datasets were created by the OPRI data analytics team in CSV format and then converted to STATA. All analyses were undertaken in STATA v15.1.

6.8 Significance Testing

P-values ≤0.05 are considered statistically significant. No adjustments for multiple comparisons have been made, however results are interpreted accordingly if they are part of a group of significance tests.

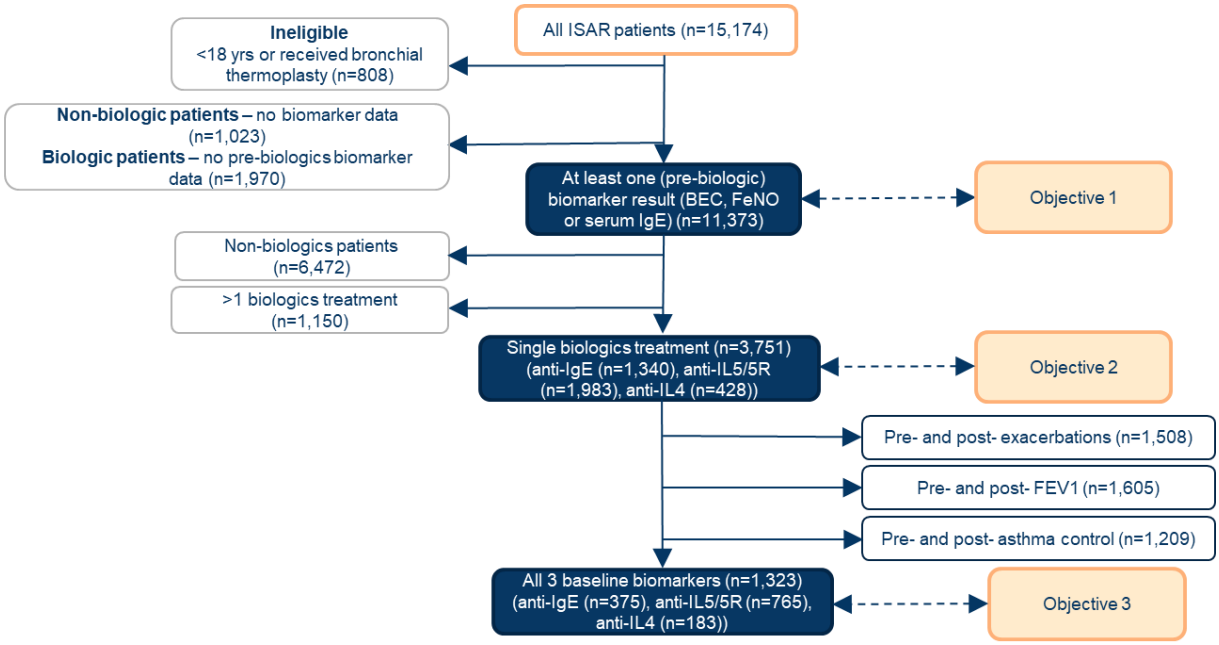
7.0 Results

7.1 Study cohort

All eligible patients with data for the relevant biomarkers and outcomes were included in the analyses if data were available. A total of 11373 patients (4901 biologic and 6472 non-biologic) met the eligibility criteria and had at least one baseline (pre-biologic) biomarker result available so were included in the objective 1 analyses.

Objectives 2 and 3 only included patients prescribed biologics. To simplify interpretation of the results, these analyses were restricted to patients who were prescribed only one type of biologic treatment (3751 patients) (1340 prescribed anti-IgE, 428 patients prescribed anti-IL4, and 1983 patients prescribed anti-IL5/5R). Hence any effects on the outcomes could be attributed to a particular class of biologics. Numbers included in the individual analyses for objectives 2 and 3 varied as patients needed to have the relevant outcome and biomarker variables available to be included in an analysis. In all cases, the maximum number with available data were included (e.g. for an analysis of the association between baseline BEC and change in FEV_1 in objective 2, patients were included if they had data available for pre- and post-biologic FEV_1 and baseline BEC, irrespective of whether they had data on the other outcomes or other biomarkers). For objective 3, patients needed to have baseline data for all three biomarkers to be included (1323 patients) in addition to pre- and post-biologic data for the relevant outcome.

Figure 2. Patient numbers included in the analyses for IGNITE



7.2 Data Availability for Important Study Variables

Of the patients included in objective 1 (N = 11373): 8880 (78%) had baseline eosinophil, 4886 (43%) had baseline FeNO, and 7515 (66%) had baseline IgE available.

Of the patients included in objective 2 (N = 3751): 3195 (85%) had baseline eosinophil, 1886 (50%) had baseline FeNO, and 2754 (73%) had baseline IgE available. 1605 (43%) of these had both baseline and follow-up FEV_1, 1209 (32%) had both baseline and follow-up asthma control, and 1508 (40%) had both baseline and follow-up exacerbation rates. 1323 (35%) of the objective 2 patients had all three baseline biomarkers available and were included in objective 3.

7.3 Demographic and Clinical Characteristics

Baseline characteristics for patients included in objective 1 are shown in Table 1. Patients were predominantly female with asthma onset at ≥18 years for both patients prescribed biologics and patients not prescribed biologics. Patients prescribed biologics were more likely to be on LTOCS at their index date and to have nasal polyps. Baseline eosinophil, FeNO and IgE were generally higher in the patients prescribed biologics. Baseline asthma control and exacerbations rates were worse in the patients who went on to receive biologics though there was little difference in FEV_1 between the two groups.

Of note, the patients who were prescribed biologics had a mean of 2.2 exacerbations per year at baseline and 70% had uncontrolled asthma, highlighting the severity of disease in this cohort of patients.

Table 1. Characteristics of patients included in objective 1

	Non-biologic (N=6472)	**Biologic** (N=4901)	**Total** (N=11373)
Sex (n (%))			
Female	4012 (62.2%)	3049 (62.3%)	7061 (62.2%)
Male	2436 (37.8%)	1847 (37.7%)	4283 (37.8%)
Missing (n)	24	5	29
Age at index date (mean (sd))			
	53.0 (15.0)	52.5 (14.5)	52.8 (14.8)
Age at asthma onset (mean (sd))			
	31.2 (19.3)	29.6 (18.4)	30.4 (18.9)
Missing (n)	3561	2153	5714
Age group at asthma onset (n (%))			
<18	852 (29.3%)	824 (30.0%)	1676 (29.6%)
18-40	1092 (37.5%)	1119 (40.7%)	2211 (39.1%)
41-64	843 (29.0%)	730 (26.6%)	1573 (27.8%)
65+	124 (4.3%)	75 (2.7%)	199 (3.5%)
Missing (n)	3561	2153	5714
Duration of asthma (years) (median (IQR))			
	19.1 (8.6-33.0)	19.8 (9.7-34.0)	19.3 (9.0-33.3)
Missing (n)	3561	2153	5714
Baseline eosinophil (cells/µL) (median (IQR))			
	300 (190-500)	400 (200-730)	300 (200-600)
Missing (n)	646	446	1092
Baseline FeNO (ppb) (median (IQR))			
	25.0 (14.0-49.0)	36.0 (18.0-72.0)	29.0 (15.0-60.0)
Missing (n)	3250	2187	5437
Baseline IgE (IU/mL) (median (IQR))			
	121 (37-370)	208 (78-532)	158 (52-456)
Missing (n)	1974	864	2838
Patient was on LTOCS at index date (n (%))			
Yes	488 (7.6%)	1411 (29.1%)	1899 (16.8%)
Missing (n)	16	55	71
Ever had nasal polyps (n (%))			
Yes	849 (13.4%)	1404 (28.8%)	2253 (20.1%)
Missing (n)	142	30	172
One or more allergies detected by any test (n (%))			
Yes	2045 (64.9%)	1939 (65.8%)	3984 (65.3%)
Missing (n)	3319	1953	5272
Baseline asthma control (n (%))			
Well controlled	541 (18.9%)	271 (12.0%)	812 (15.9%)
Partially controlled	731 (25.6%)	405 (18.0%)	1136 (22.2%)
Not controlled	1588 (55.5%)	1574 (70.0%)	3162 (61.9%)
Missing (n)	3612	2651	6263
Baseline FEV1 (mean (sd))			
	2.0 (0.8)	2.1 (0.8)	2.1 (0.8)
Missing (n)	1957	1074	3031
Baseline exacerbations (mean (sd))			

	0.8 (1.6)	2.2 (3.0)	1.3 (2.4)
Missing (n)	519	771	1290
Eosinophilic grade (n (%))			
Grade 0: Unlikely/Non-eosinophilic	723 (12.4%)	217 (4.7%)	940 (9.0%)
Grade 1: Least likely	1197 (20.5%)	310 (6.7%)	1507 (14.5%)
Grade 2: Likely	560 (9.6%)	266 (5.8%)	826 (7.9%)
Grade 3: Most likely	3346 (57.4%)	3800 (82.7%)	7146 (68.6%)
Missing (n)	646	308	954

Baseline characteristics of the patients included in objective 2 are shown in Table 2 according to which class of biologic they subsequently went on to receive. Patients prescribed anti-IL5/5R tended to be older at their index date (start of biologic therapy) and at their age of asthma onset. Median baseline eosinophil and FeNO were highest in the patients prescribed anti-IL5/5R but baseline IgE was highest in the patients prescribed anti-IgE. Patients prescribed anti-IgE were less likely to have nasal polyps but more likely to have one or more allergies. Baseline asthma control and FEV_1 were similar in the patients prescribed anti-IgE and anti-IL5/5R but less severe in those prescribed anti-IL4. Baseline exacerbation rates increased in the order anti-IL4 < anti-IgE < anti-IL5/5R.

Table 2. Characteristics of patients included in objective 2

	Biologic class		
	Anti-IgE (N=1340)	**Anti-IL4** (N=428)	**Anti-IL5** (N=1983)
Sex (n (%))			
Female	888 (66.4%)	265 (61.9%)	1192 (60.1%)
Male	450 (33.6%)	163 (38.1%)	790 (39.9%)
Missing (n)	2	0	1
Age at index date (mean (sd))	50.1 (14.5)	50.7 (15.2)	55.2 (13.9)
Age at asthma onset (mean (sd))	25.6 (17.4)	27.7 (19.4)	32.5 (18.2)
Missing (n)	538	303	665
Age group at asthma onset (n (%))			
<18	303 (37.8%)	45 (36.0%)	313 (23.7%)
18-40	334 (41.6%)	41 (32.8%)	541 (41.0%)
41-64	154 (19.2%)	36 (28.8%)	419 (31.8%)
65+	11 (1.4%)	3 (2.4%)	45 (3.4%)
Missing (n)	538	303	665
Duration of asthma (years) (median (IQR))	20.4 (11.0-35.8)	20.5 (9.0-35.0)	19.0 (9.0-33.7)
Missing (n)	538	303	665
Baseline eosinophil (cells/µL) (median (IQR))	245 (100-500)	400 (200-600)	475 (260-730)
Missing (n)	260	40	256
Baseline FeNO (ppb) (median (IQR))	23.0 (13.0-44.0)	33.0 (17.0-64.0)	38.0 (20.0-68.0)
Missing (n)	803	161	901

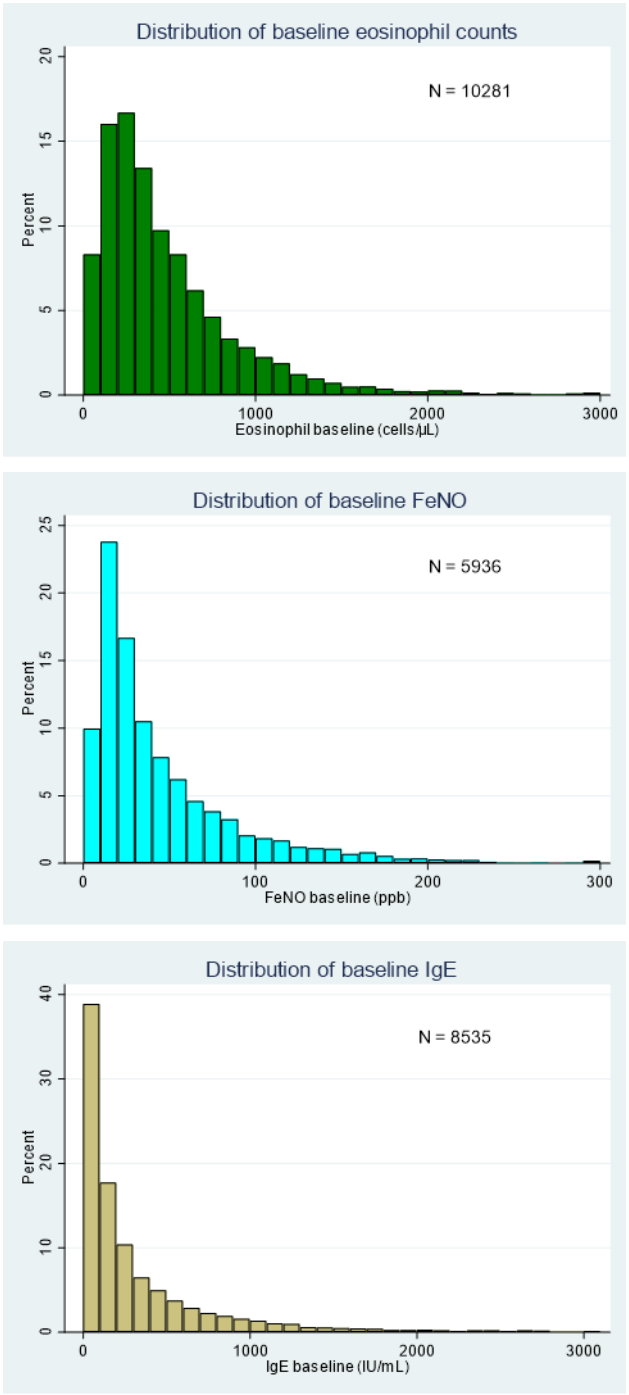
Baseline IgE (IU/mL) (median (IQR))			
	262 (118-528)	123 (37-320)	127 (50-319)
Missing (n)	268	124	605
Patient was on LTOCS at index date (n (%))			
Yes	264 (19.9%)	67 (15.7%)	675 (34.6%)
Missing (n)	16	1	30
Ever had nasal polyps (n (%))			
Yes	243 (18.2%)	140 (33.1%)	697 (35.3%)
Missing (n)	8	5	11
One or more allergies detected by any test (n (%))			
Yes	753 (81.7%)	136 (62.1%)	571 (55.3%)
Missing (n)	418	209	950
Baseline asthma control (n (%))			
Well controlled	83 (14.2%)	16 (16.3%)	128 (11.7%)
Partially controlled	93 (15.9%)	26 (26.5%)	223 (20.3%)
Not controlled	410 (70.0%)	56 (57.1%)	745 (68.0%)
Missing (n)	754	330	887
Baseline FEV1 (mean (sd))			
	2.1 (0.8)	2.3 (0.9)	2.1 (0.8)
Missing (n)	350	100	461
Baseline exacerbations (mean (sd))			
	1.8 (2.6)	0.8 (1.6)	2.7 (3.2)
Missing (n)	265	56	350
Eosinophilic grade (n (%))			
Grade 0: Unlikely/Noneosinophilic	145 (13.4%)	34 (8.8%)	0 (0.0%)
Grade 1: Least likely	198 (18.3%)	52 (13.4%)	0 (0.0%)
Grade 2: Likely	160 (14.8%)	41 (10.6%)	0 (0.0%)
Grade 3: Most likely	577 (53.4%)	261 (67.3%)	1983 (100.0%)
Missing (n)	260	40	0

7.4 Objective 1: Distributions and correlations of biomarkers

7.4.1 Distributions of biomarkers

Pre-biologic levels of all three biomarkers had a highly positive skew (Figure 3).

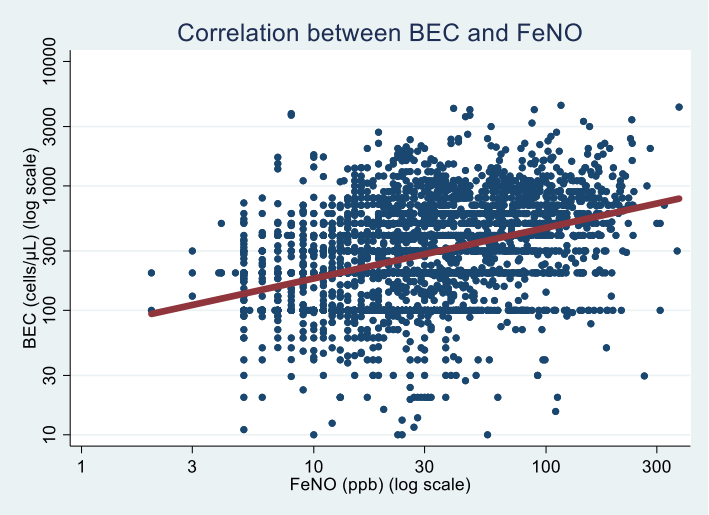
Figure 3. Distributions of highest pre-biologic values of biomarkers

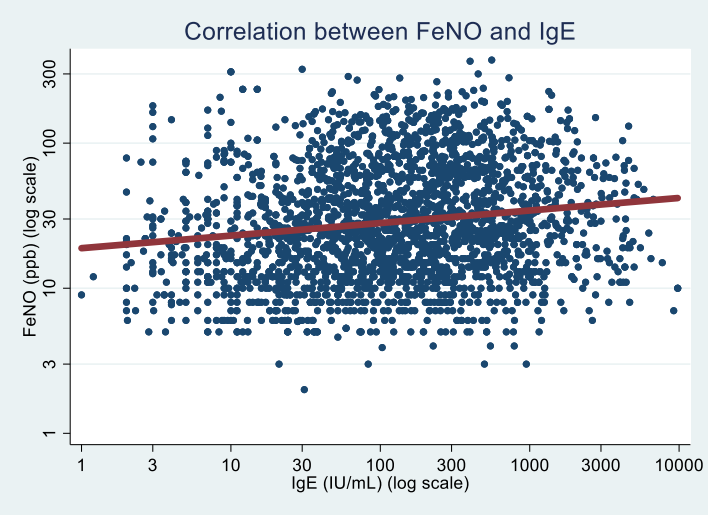


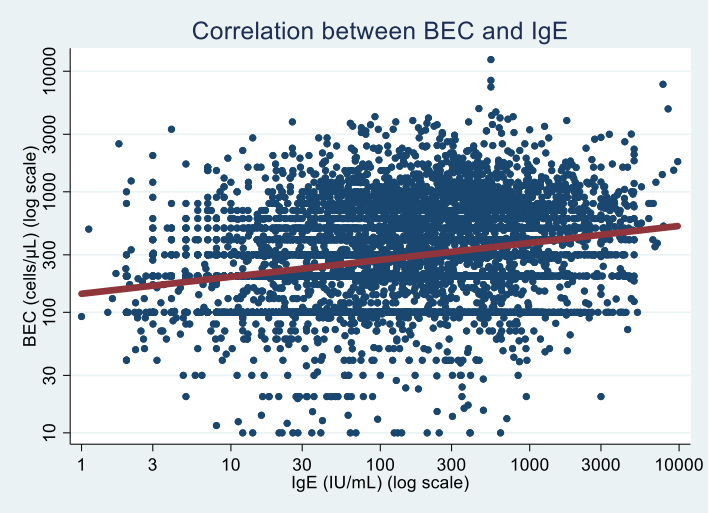
7.4.2 Correlations between biomarkers

Scatter plots of pre-biologic biomarker values collected within 7 days of each other, using $\log_{10}$ scales, are shown in Figure 4. Note that for the patients not prescribed biologics, "pre-biologic" includes measurements recorded at any time for this analysis.

Figure 4. Correlations between pre-biologic biomarker measurements made within 7 days of each other







Statistically significant positive correlations between all pairs of biomarkers were seen, although the strength of the correlations was low (≤0.4) in all cases (Table 3). When this analysis was repeated using the highest pre-biologic measurements for each patient, correlations between the biomarkers were slightly lower, although still significant (Table 3). The results were very similar using either Pearson's correlation coefficients calculated using the $\log_{10}$ biomarker values or Spearman's rank correlation coefficients, which use only the rank ordering of the values which are the same on a log or linear scale.

Table 3. Correlations between biomarker values taken before any biologic treatments

Correlation between	**Pre-biologic measurements taken within 7 days of one another**	**Highest pre-biologic measurement**
BEC and FeNO	r = 0.40 (p<0.001) r_s = 0.42 (p<0.001) N = 3099	r = 0.33 (p<0.001) r_s = 0.37 (p<0.001) N = 5126
FeNO and IgE	r = 0.16 (p<0.001) r_s = 0.16 (p<0.001) N = 2591	r = 0.15 (p<0.001) r_s = 0.16 (p<0.001) N = 4758
BEC and IgE	r = 0.25 (p<0.001) r_s = 0.26 (p<0.001) N = 7147	r = 0.20 (p<0.001) r_s = 0.22 (p<0.001) N = 7572

Note: r – Pearson's correlation coefficient calculated for the $\log_{10}$ values. r_s – Spearman's rank correlation coefficient.

Corresponding associations between the biomarkers were seen when binary cut-offs were used (Table 4).

Table 4. Associations between pre-biologic biomarker measurements made within 7 days of each other using binary cut-offs

	Baseline FeNO (ppb)		
	< 25	>= 25	Total
Baseline eosinophil (cells/µL)			
<300	930 (30.0%)	563 (18.2%)	1493 (48.2%)
>= 300	507 (16.4%)	1099 (35.5%)	1606 (51.8%)
Total	1437 (46.4%)	1662 (53.6%)	3099 (100%)

Chi-squared = 294
p < 0.001

	Baseline IgE (IU/mL)			
	< 75	>= 75	Total	
Baseline FeNO (ppb)				
< 25	509 (19.6%)	698 (26.9%)	1,207 (46.6%)	
>= 25	414 (16.0%)	970 (37.4%)	1,384 (53.4%)	Chi-squared = 42.2 p < 0.001
Total	923 (35.6%)	1,668 (64.4%)	2,591 (100%)	

	Baseline IgE (IU/mL)			
	< 75	>= 75	Total	
Baseline eosinophil (cells/µL)				
<300	1,561 (21.8%)	1,831 (25.6%)	3,392 (47.5%)	
>= 300	1,006 (14.1%)	2,749 (38.5%)	3,755 (52.5%)	Ch-squared = 286 p < 0.001
Total	2,567 (35.9%)	4,580 (64.1%)	7,147 (100%)	

Note: cell contents - count and % of total

7.5 Objective 2: Effectiveness of biologics by classes

As noted in section 7.3, there were some imbalances between the biologic classes in baseline levels of exacerbations, FEV_1 and asthma control. Therefore, all analyses in this section were adjusted for the baseline level of the outcome in question. Outcomes in relation to the biomarkers were predicted by fitting statistical models, as described in section 6.4.

Adjusted predictions of the means or probabilities of the outcomes at follow-up were calculated for selected values of the baseline biomarkers for each biologic type. For exacerbations the estimated follow-up rates were calculated for patients with a baseline exacerbation rate of 2.2 per year (mean value in the biologic population) and for FEV_1 the estimated follow-up values were calculated for patients with a baseline FEV_1 of 2.1 L (mean value in the biologic population). These estimates were plotted in the figures as the change from baseline, by subtracting the baseline value at which they were evaluated (2.2 exacerbations per year, or FEV_1 = 2.1 L). For asthma control the adjusted predictions for the probablity of uncontrolled

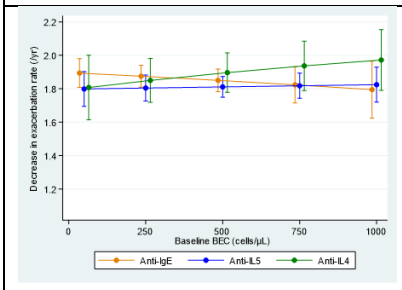
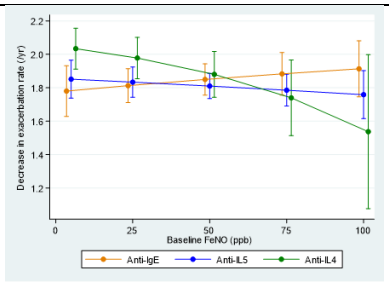
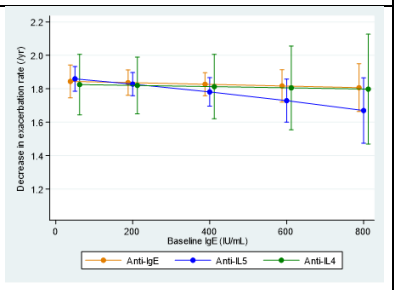
asthma at follow-up were estimated for each biologic type assuming the proportion of patients with uncontrolled asthma at baseline was equal to that amongst all of the included patients (i.e. as if all of the included patients had received each of the biologics). Appendix 1 shows the estimated values on which these plots are based.

This methodology allows the different biologic classes to be compared for patients with the same specified baseline level of the outcome or, for asthma control with the same distribution of baseline asthma control scores. 95% confidence intervals are displayed in the graphs. The strength of associations between the outcomes and baseline biomarkers can be seen from the slope of the lines, with steeper slopes indicating a stronger association.

Note, for exacerbations and FEV_1 the estimated follow-up values are plotted as change from the specified baseline value by subtracting the specified baseline value (at which they were evaluated) from the predicted follow-up value. As this only involves subtracting a constant from all of the predicted values for each outcome, the slopes of the lines would have been the same if we had plotted predicted follow-up exacerbation rate or FEV_1 (rather than change in these values) against the baseline biomarkers for the same baseline values of these outcomes.

7.5.1 Exacerbations

Figure 5. Association between decrease in exacerbations and baseline biomarkers

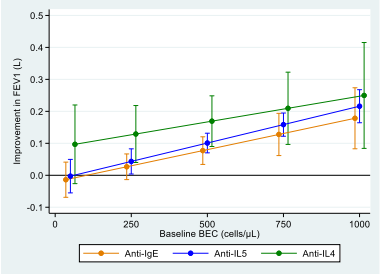
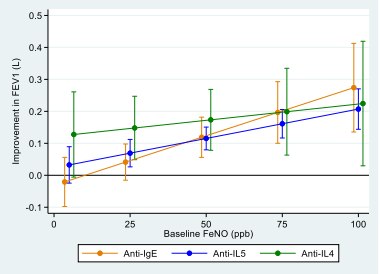
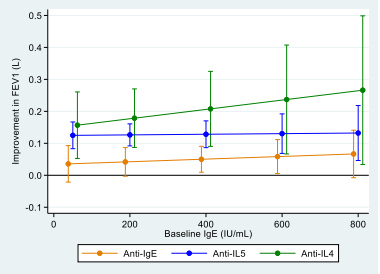
BEC		FeNO		IgE	
					
Anti-IgE	IRR = 1.343 N = 412 p = 0.354	Anti-IgE	IRR = 0.669 N = 211 p = 0.348	Anti-IgE	IRR = 1.143 N = 389 p = 0.708
Anti-IL5	IRR = 0.933 N = 772 p = 0.765	Anti-IL5	IRR = 1.282 N = 559 p = 0.381	Anti-IL5	IRR = 1.803 N = 654 p = 0.047
Anti-IL4	IRR = 0.565 N = 172 p = 0.312	Anti-IL4	IRR = 4.295 N = 116 p = 0.018	Anti-IL4	IRR = 1.098 N = 126 p = 0.875
Interactions		Interactions		**Interactions**	
Anti-IgE vs anti-IL5	p = 0.355	Anti-IgE vs anti-IL5	p = 0.205	Anti-IgE vs anti-IL5	p = 0.326
Anti-IgE vs anti-IL4	p = 0.182	Anti-IgE vs anti-IL4	p = 0.013	Anti-IgE vs anti-IL4	p = 0.954
Anti-IL5 vs anti-IL4	p = 0.411	Anti-IL5 vs anti-IL4	p = 0.075	Anti-IL5 vs anti-IL4	p = 0.454

Estimates (95% CI) of change in exacerbations for a patient with baseline exacerbation rate = 2.2 (mean of the biologic population). IRR – incidence rate ratio. Incidence rate ratios are for exacerbation rates at follow-up adjusted for baseline exacerbation rates. Note that decrease from baseline is presented on the graphs by subtracting the the predcited follow-up rate from the baseline exacerbation rate.
Incidence rate ratios for BEC are the ratio per 1000 cells/µL
Incidence ratios for FeNO are the ratio per 100 ppb
Incidence rate ratios for IgE are the ratio per 1000 IU/mL
Interactions tests are comparisons of the odds ratios for different biologic classes within that biomarker

From the slopes of the lines it can be seen that there was relatively little association between decrease in exacerbation rate (pre-biologic rate – post-biologic rate) and the baseline biomarkers. Although statistically significant associations were observed for FeNO in patients prescribed anti-IL4 (p=0.018) and for IgE in patients prescribed anti-IL5/5R (p=0.047), it should be noted that there were few anti-IL4 patients with high baseline FeNO measurements (leading to large 95% confidence intervals for high values of FeNO) and the strength of the association with IgE in patients prescribed anti-IL5/5R was not strong and only marginally significant. The general observation was therefore that the biomarkers were not strongly predictive of decrease in exacerbations following biologic treatment in this population.

7.5.2 FEV_1

Figure 6. Associations between improvement in FEV_1 and baseline biomarkers

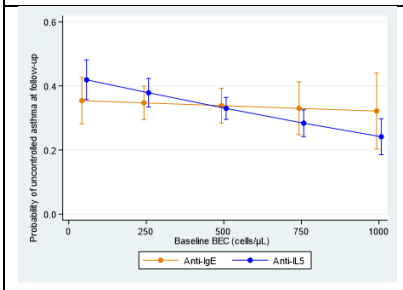
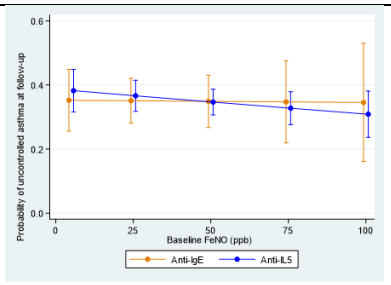
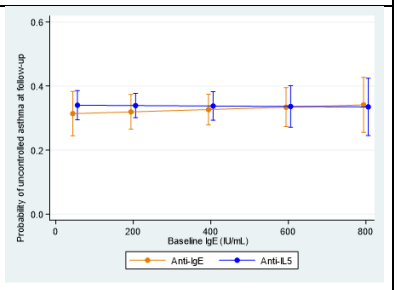
BEC		FeNO		IgE	
					
Anti-IgE	Coeff = 0.202 N = 512 p = 0.003	Anti-IgE	Coeff = 0.310 N = 254 p = 0.002	Anti-IgE	Coeff = 0.041 N = 494 p = 0.557
Anti-IL5	Coeff = 0.230 N = 789 p < 0.001	Anti-IL5	Coeff = 0.184 N = 588 p < 0.001	Anti-IL5	Coeff = 0.010 N = 668 p = 0.890
Anti-IL4	Coeff = 0.161 N = 125 p = 0.217	Anti-IL4	Coeff = 0.102 N = 92 p = 0.483	Anti-IL4	Coeff = 0.146 N = 94 p = 0.415
Interactions		**Interactions**		**Interactions**	
Anti-IgE vs anti-IL5	p = 0.733	Anti-IgE vs anti-IL5	p = 0.253	Anti-IgE vs anti-IL5	p = 0.749
Anti-IgE vs anti-IL4	p = 0.779	Anti-IgE vs anti-IL4	p = 0.234	Anti-IgE vs anti-IL4	p = 0.587
Anti-IL5 vs anti-IL4	p = 0.616	Anti-IL5 vs anti-IL4	p = 0.596	Anti-IL5 vs anti-IL4	p = 0.479

Estimates (95% CI) of improvement in FEV_1 for a patient with baseline FEV_1 = 2.1 (mean of the biologic population).
Coefficients for BEC are the increase per 1000 cells/µL
Coefficients for FeNO are the increase per 100 ppb
Coefficients for IgE are the increase per 1000 IU/mL
Interaction tests are comparisons of the coefficients for different biologic classes within that biomarker.

In the patients prescribed anti-IgE highly significant associations were seen between improvement in FEV_1 (post-biologic value – pre-biologic value) and baseline BEC (p=0.003) or FeNO (p=0.002) and simlarly in the patients prescribed anti-IL5/5R (p<0.001 and p<0.001 respectively). Larger improvements in FEV_1 were seen in patients with higher baseline values of these biomarkers. Similar trends were seen in the patients prescribed anti-IL4 though these were not significant, probably due to lower numbers of patients in the dataset. There was no significant association between improvement in FEV_1 and baseline IgE for any of the biologic classes. Overall it appeared that baseline BEC and FeNO were strongly predictive of which patients would benefit most in terms of improving FEV_1.

7.5.3 Asthma control

Figure 7. Associations between uncontrolled asthma and baseline biomarkers

BEC		FeNO		IgE	
					
Anti-IgE	OR = 0.852 N = 347 p = 0.697	Anti-IgE	OR = 0.968 N = 186 p = 0.956	Anti-IgE	OR = 1.189 N = 358 p = 0.658
Anti-IL5	OR = 0.392 N = 681 p = 0.001	Anti-IL5	OR = 0.692 N = 517 p = 0.209	Anti-IL5	OR = 0.967 N = 573 p = 0.923
Interaction Anti-IgE vs anti-IL5	p = 0.115	**Interactions** Anti-IgE vs anti-IL5	p = 0.605	**Interaction** Anti-IgE vs anti-IL5	p = 0.694

Estimates (95% CI) of probability of uncontrolled asthma as if each class of biologic was given to the whole cohort of anti-IgE and anti-IL5 patients. Anti-IL4 patients were excluded due to small numbers with this outcome available. OR – odds ratio.
Odds ratios for BEC are the ratio per 1000 cells/µL
Odds ratios for FeNO are the ratio per 100 ppb
Odds ratios for IgE are the ratio per 1000 IU/mL
Interaction tests are comparisons of the odds ratios for different biologic classes within that biomarker.

Only patients prescribed anti-IgE or anti-IL5/5R were included in this analysis due to the low numbers of anti-IL4 patients with sufficient data for this outcome. A significant association between baseline BEC and probability of uncontrolled asthma was seen in the patients prescribed anti-IL5 (p=0.001). Patients with higher baseline BEC had lower probability of uncontrolled asthma at follow-up. The other biomarkers showed little association with probability of uncontrolled asthma in either biologic class.

7.5.4 Subgroup analyses for objective 2

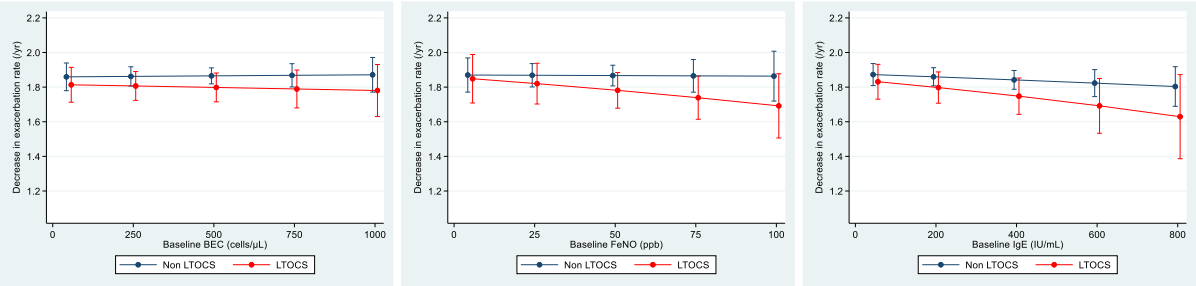
Due to low numbers of patients with certain subgroup/biomarker combinations available in the dataset it was impractical to repeat the analyses by biologic classes divided by subgroups. Instead, subgroup analyses were undertaken for all classes of biologics combined. The methods of estimation for comparing the subgroups were the same as described for comparing the biologic classes. This was intended to study some possible factors that may

be useful in interpreting the preceding results. In particular, this analysis was intended to reveal whether the associations between the outcomes and biomarkers described in sections 7.5.1-7.5.3 tended to be stronger or weaker in particular subgroups. The figures below compare the outcomes or changes in the outcomes adjusted for baseline levels of the relevant outcome. In effect, they compare the subgroups as if they were matched for that outcome at baseline. Numbers of patients and p-values for these associations are given in Appendix 2, with comparisons of particular interest described in the comments below.

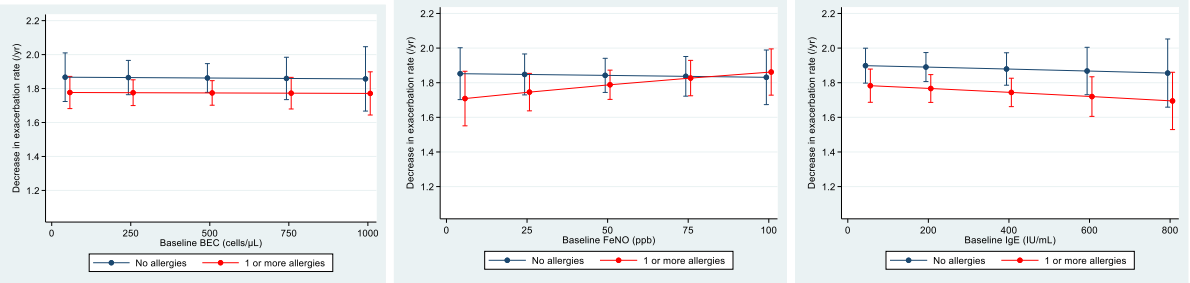
7.5.4.1 Exacerbations by subgroups

Figure 8. Associations between decrease in exacerbations and baseline biomarker levels by subgroups

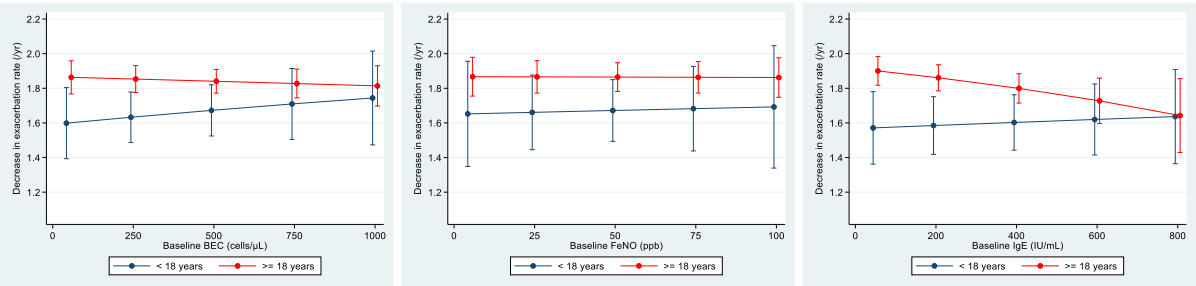
- By LTOCS use at biologic initiation



- By presence or absence of allergies



- By age at asthma onset



For exacerbations, associations between biomarkers and changes in exacerbation rates following treatment with biologics were relatively flat for all sub-groups. Only IgE for the group

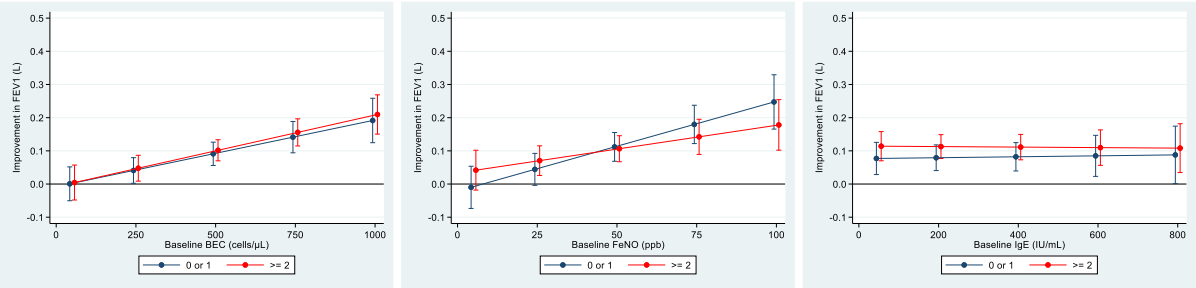
with asthma onset ≥18 years had a significant association with improvement in exacerbations (p=0.016), however this should be treated with caution as this was not significantly different from the <18 years onset group which showed little association between improvement in exacerbations and baseline IgE (p=0.074 for the interaction between asthma onset age and baseline IgE).

Generally decreases in exacerbation rates were better in patients who were not on LTOCS, had no allergies, or who were ≥18 years at asthma onset.

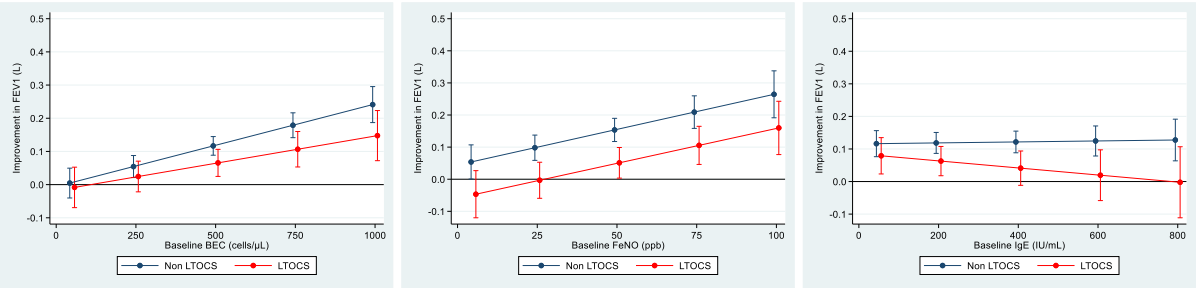
7.5.4.2 FEV_1 by subgroups

Figure 9. Associations between improvement in FEV1 and baseline biomarker levels by subgroups

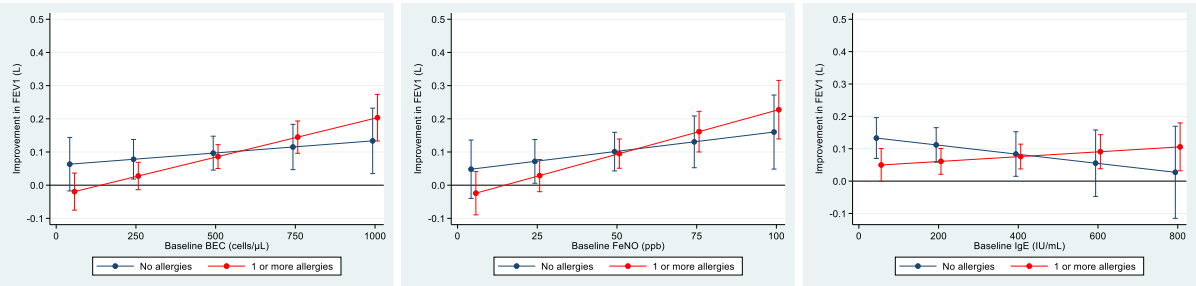
- By baseline exacerbation rates



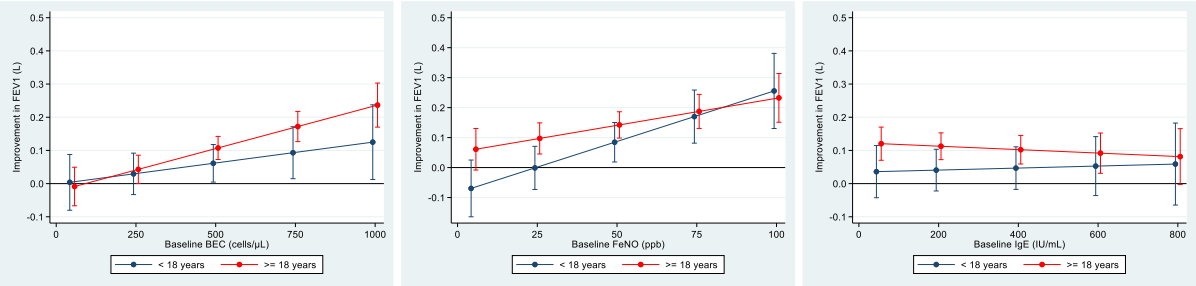
- By LTOCS use at biologic initiation



- By presence or absence of allergies



- By age at asthma onset



Improvement in FEV_1 showed a significant increasing trend with BEC and FeNO ($p<0.05$) for all subgroups except the no allergies group for BEC and FeNO and <18 years asthma onset for BEC. However, the general trend in these groups was also in the same direction and statistical significance may have been affected by the lower numbers of patients (<300) in these subgroups. No interactions (i.e. comparisons of the slopes of the lines) were significant (Appendix 2). However, the graphs suggest that the association between improvement in FEV_1 and biomarkers was strongest in patients who were not on LTOCS at baseline (for BEC) and patients with 1 or more allergies (for BEC and FeNO). Interestingly a clearer trend was seen in patients with asthma onset ≥18 years for BEC and <18 years for FeNO.

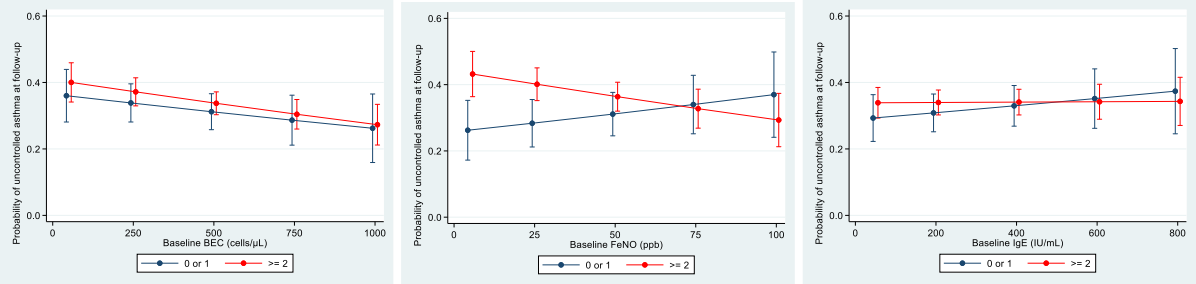
Patients with either high or low exacerbation rates at baseline showed similar associations between improvement in FEV_1 and BEC or FeNO.

Overall, improvement in FEV_1 was generally higher in patients who were not on LTOCS at baseline and patients with asthma onset at ≥18 years.

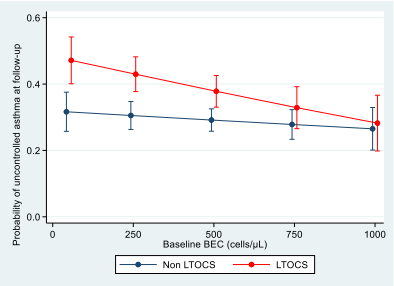
7.5.4.3 Asthma control by subgroups

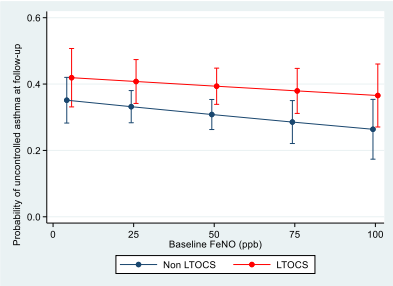
Figure 10. Associations between probability of uncontrolled asthma at follow-up and baseline biomarker levels by subgroups

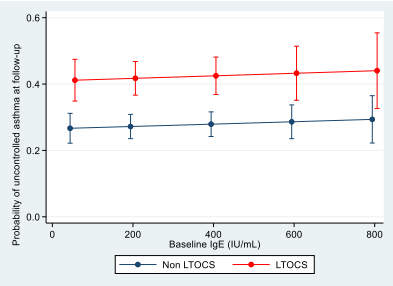
- By baseline exacerbation rates



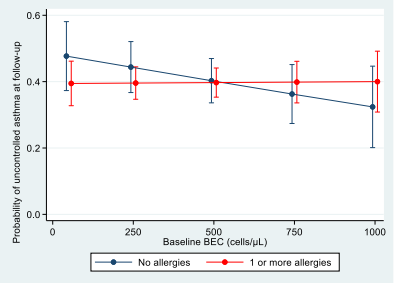
- By LTOCS use at biologic initiation

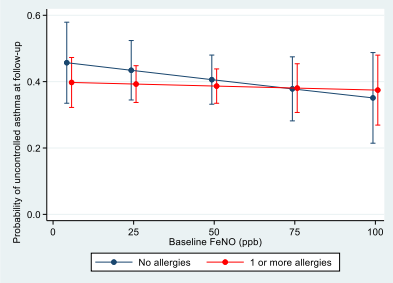


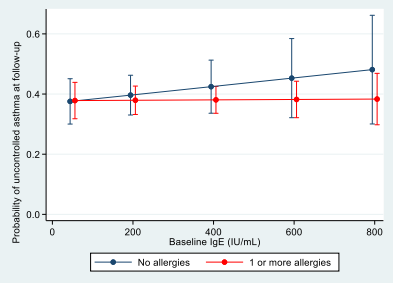




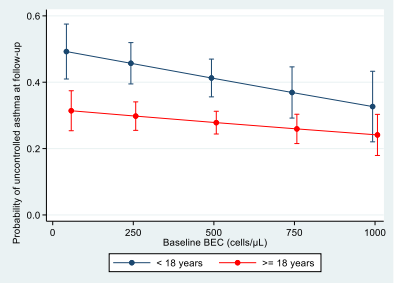
- By presence or absence of allergies

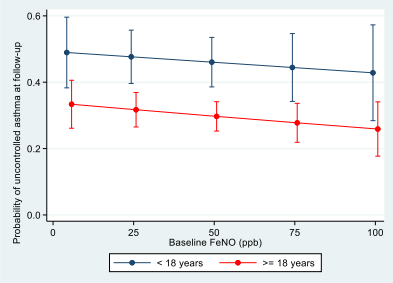


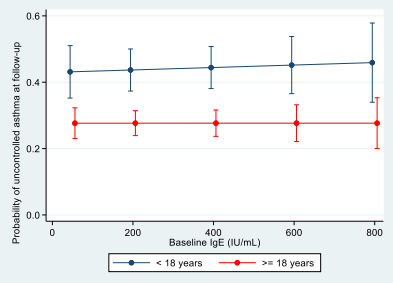




- By age at asthma onset







Associations between uncontrolled asthma at follow-up and the biomarkers were relatively flat, the only subgroups with a statistically significant association being LTOCS at baseline for BEC (p=0.005); patients with ≥2 exacerbations per year at baseline for BEC and FeNO (p=0.014 & p=0.031 respectively), and patients with asthma onset <18 years for BEC (p=0.043).

Interestingly, the association between uncontrolled asthma at follow-up and FeNO showed different trends for patients with high or low exacerbation rates at baseline with the significant interaction test suggesting there may be a real difference in these associations (p=0.028).

In general the probability of uncontrolled asthma at folllow-up (adjusted for baseline asthma control) was lower in patients with asthma onset at ≥18 years or patients who were not on LTOCS at baseline.

7.6 Objective 3: Value of using multiple biomarkers

The methods of assessing whether using mutiple biomarkers led to better predictions of the outcomes than any single biomarker are described in section 6.5. A summary of the predictive abilities of the models using single or multiple biomarkers is given in Appendix 3. Results from models where the baseline outcome level but no biomarkers were included are also shown. For all of the outcomes the baseline level of the outcome was the strongest predictor of the follow-up outcome.

7.6.1 Exacerbation rates

IgE was marginally the best single biomarker for predicting follow-up exacerbation rates (after adjusting for baseline exacerbations), with a pseudo R^2 of 0.049 (compared with 0.045 and 0.048 for the BEC and FeNO models respectively). Including all three biomarkers in the model led to a pseudo R^2 of 0.054, indicating a better fitting model. However, there was no statistically significant improvement in the overall fit (accuracy of predictions) of the model by including all three biomarkers compared with IgE alone ($p = 0.323$). Pseudo R^2 values can be used to compare logistic or negative binomial models but, unlike R^2 values in least squares regression models, have no direct interpretation as to the proportion of variance explained by the models. Instead the accuracy of these models was assessed by calculating the mean of the absolute error in the models' predictions of follow-up exacerbation rates. The model using IgE alone (after adjusting for baseline exacerbation rate) had a mean absoute error of 0.62 in the predicted exacerbation rates. The model using all three biomarkers had only a marginally lower mean absolute error of 0.60 in the predicted rates.

7.6.2 FEV_1

BEC was the best single biomarker model for predicting follow-up FEV_1 (after adjusting for baseline FEV_1), with an adjusted R^2 of 0.747 (compared with 0.743 and 0.736 for the FeNO and IgE models respectively). Including all three biomarkers in the model led to an adjusted R^2 of 0.750. There was a statistically significant improvement in the overall fit (i.e. accuracy of predictions) of the model by including all three biomarkers compared with BEC alone ($p = 0.029$). For ordinary least squares regression, adjusted R^2 measures the proportion of total variation in the outcome predicted by the statistical model. Although statistically significant,

including all three biomarkers only explained an additional 0.3% of the total variance in follow-up FEV_1 in this cohort, compared with using BEC alone.

7.6.3 Asthma control

BEC was the best single biomarker for predicting follow-up asthma control (after adjusting for baseline asthma control), with a pseudo R^2 of 0.062 (compared with 0.060 and 0.056 for the FeNO and IgE models respectively). Including all three biomarkers in the model led to a pseudo R^2 of 0.067. However, there was no statistically significant improvement in the overall fit (i.e. accuracy of predictions) of the model by including all three biomarkers compared with BEC alone ($p = 0.464$). The accuracy of these models was characterised by calculating the percentage of patients whose outcomes (well / patially controlled or uncontrolled asthma) were predicted correctly. The model using BEC alone (after adjusting for baseline asthma control) predicted 62% of outcomes correctly. The model using all three biomarkers predicted 64% of the asthma control outcomes correctly; a gain of only 2% in correct predictions.

7.7 Additional analyses

7.7.1 Pre-biologic associations between biomarkers and outcomes

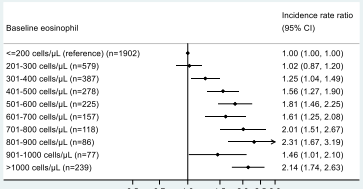
Objectives 2 and 3 studied the associations between baseline biomarkers and outcomes after biologic treatment, adjusting for the baseline level of the outcomes. By estimating these for a specific baseline level of the outcome it was possible to estimate the pre-treatment to post-treatment change in exacerbations and FEV_1. As improvement was characterised by the absolute change in these outcomes (follow-up value – baseline value), the patients with the worst outcome levels at baseline had the biggest opportunity for improvement. If the baseline levels of the outcomes are strongly associated with the biomarkers then the degree of improvement we observe might also be related to the biomarkers because of this. In order to interpret the results of objectives 2 and 3, an additional analysis was carried out to study the associations between the baseline biomarkers and exacerbations, FEV_1 and asthma control **before** the patients received any biologic treatments. These are shown in Figure 11. For comparison, the baseline associations between these outcomes and biomarkers in patients who did not receive biologics are also shown.

Figure 11. Associations between baseline biomarkers and baseline (pre-biologic) levels of the outcomes (exacerbations, FEV_1 and asthma control)

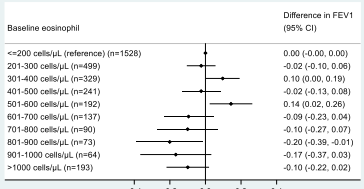
Associations with BEC

Non-biologic patients

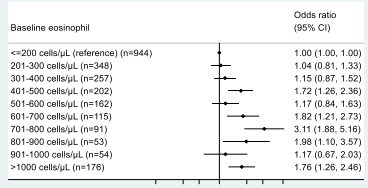
Exacerbations



FEV_1

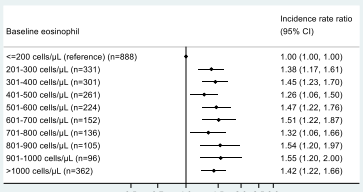


Asthma control

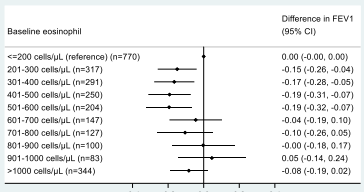


Pre-biologic patients

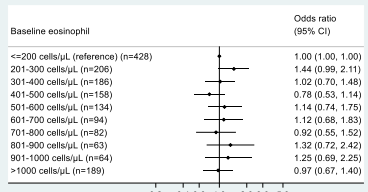
Exacerbations



FEV_1



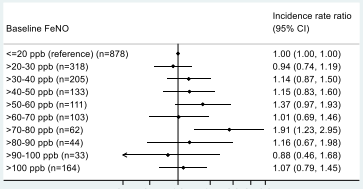
Asthma control



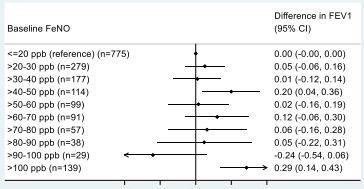
Associations with FeNO

Non-biologic patients

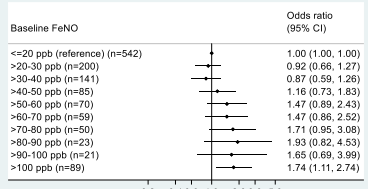
Exacerbations



FEV_1

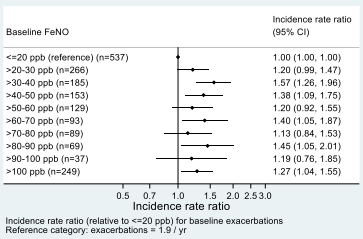


Asthma control

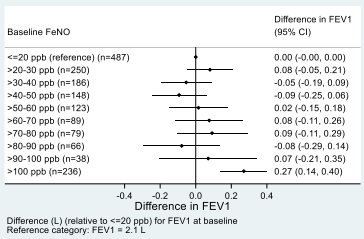


Pre-biologic patients

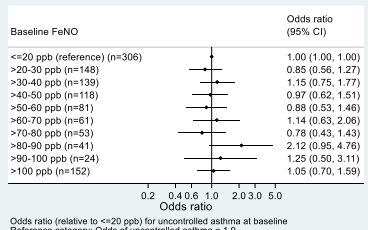
Exacerbations



FEV_1



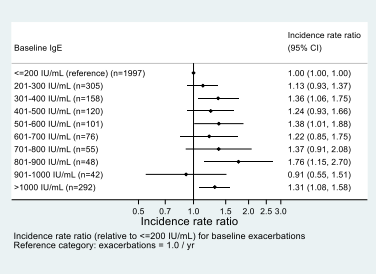
Asthma control



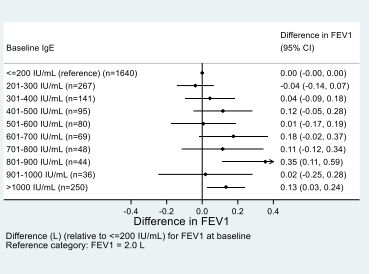
Associations with IgE

Non-biologic patients

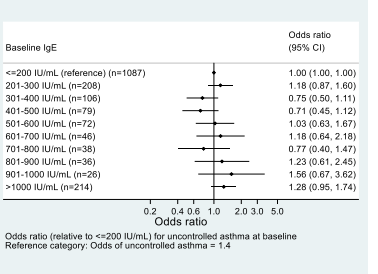
Exacerbations



FEV_1

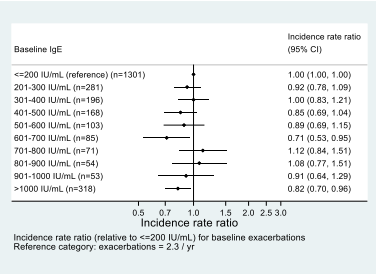


Asthma control

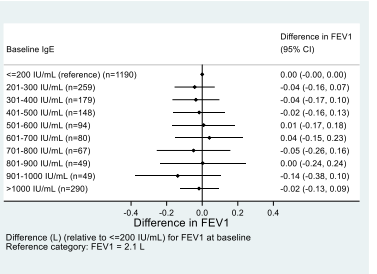


Pre-biologic patients

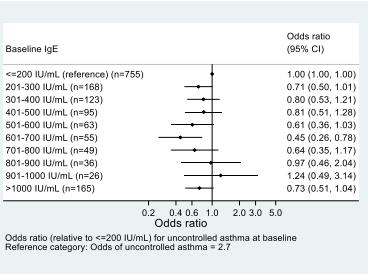
Exacerbations



FEV_1



Asthma control



Note: Pre-biologic patients refers to baseline levels of the outcomes in patients who subsequently went on to receive biologics.

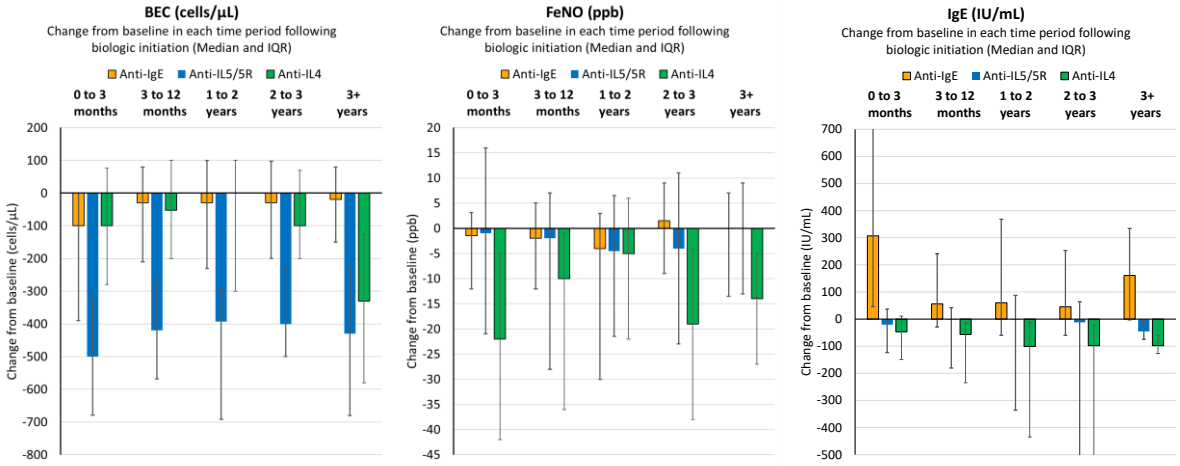
In the patients who were not prescribed biologics there were clear associations between increasing BEC and increasing exacerbation rates and odds of uncontrolled asthma. A similar trend was evident between increasing FeNO and increasing odds of uncontrolled asthma in the patients not prescribed biologics. Also, baseline FEV_1 appeared to be worse (lower) with high levels of baseline BEC in patients not prescribed biologics. These trends were much less evident or non-existent in patients who subsequently went on to receive biologics, which were the patients studied in objectives 2 and 3. The general lack of associations between baseline levels of the outcomes and biomarkers in the patients who later received biologics may be important for interpreting the relatively weak associations seen between post-treatment improvements and biomarkers in this study.

7.7.2 Change in biomarkers following initiation of biologics

Median changes in the biomarkers compared with baseline (pre-biologic) levels were determined for different intervals following biologic initiation whilst patients remained on treatment. An earlier version of this analysis was presented at ERS 2022. There was a

marked decrease in BEC following initiation of anti-IL5/5R treatments which was sustained over >3 years. FeNO levels decreased in the patients prescribed anti-IL4 but changed relatively little following initiation of anti-IgE or anti-IL5/5R treatments. IgE levels did not change in the anti-IL5/5R treatment but increased initially in patients treated with anti-IgE. This is believed to be due to the biologic causing complexes of IgE to form, which have a longer half-life and hence increased the total IgE[20]. Small decreases in IgE were seen in the anti-IL4 patients. The changes seen in the patients prescribed anti-IL4 should be treated with caution as there were relatively few patients in this group, particularly with >2 years of follow-up (Table 5).

Figure 12. Median biomarker changes compared with baseline, at different times after initiation of biologic therapy



Associated with the median changes seen in the levels of BEC in patients prescribed anti-IL5/5R, >80% of patients experienced >25% decrease in BEC and values within the normal range (<150 cells/µL) within the first 3 months, which was then sustained over >3 years. (Table 5).

Table 5. Changes in biomarkers compared with baseline at different times after initiation of biologics

Biologic treatment duration	BEC (cells/µL) (Anti-IgE baseline: N = 1113, median (IQR) = 280 (100, 500), <150/µL = 28%) (Anti-IL4 baseline: N = 403, median (IQR) = 400 (200, 700), <150/µL = 18%) (Anti-IL5 baseline: N = 1861, median (IQR) = 500 (300, 820), <150/µL = 15%)				FeNO (ppb) (Anti-IgE baseline: N = 553, median (IQR) = 23 (13, 47), <25ppb = 53%) (Anti-IL4 baseline: N = 285, median (IQR) = 38 (18, 72), <25ppb = 36%) (Anti-IL5 baseline: N = 1162, median (IQR) = 42 (22, 77), <25ppb = 30%)				IgE (IU/mL) (Anti-IgE baseline: N = 1182, median (IQR) = 306 (128, 659)) (Anti-IL4 baseline: N = 361, median (IQR) = 168 (46, 626)) (Anti-IL5 baseline: N = 1502, median (IQR) = 151 (56, 413))		
	N	Change in BEC (median (IQR))	>25% drop in BEC (N(%))	Within normal range (<150 cells/µL) (N(%))	N	Change in FeNO (median (IQR))	>25% drop in FeNO (N(%))	Within normal range (<25 ppb) (N(%))	N	Change in IgE (median (IQR))	>25% drop in IgE (N(%))
Anti-IgE											
0 to 3 months	51	-100 (-390, 0)	28 (55)	22 (43)	10	-2 (-12, 3)	5 (50)	8 (80)	19	307 (46, 814)	3 (16)
>3 to 12 months	252	-30 (-210, 80)	99 (44)	69 (31)	61	-2 (-12, 5)	23 (38)	32 (52)	95	56 (-29, 241)	18 (19)
>1 to 2 years	189	-30 (-230, 100)	80 (42)	47 (25)	53	-4 (-30, 3)	26 (49)	33 (62)	90	60 (-60, 368)	21 (23)
>2 to 3 years	115	-30 (-200, 97)	50 (43)	41 (36)	34	2 (-9, 9)	10 (29)	23 (68)	54	46 (-60, 253)	14 (26)
3+ years	201	-20 (-150, 80)	65 (32)	32 (16)	40	0 (-14, 7)	13 (33)	25 (63)	148	161 (-3, 335)	27 (18)
Anti-IL4											
0 to 3 months	42	-100 (-279, 77)	21 (50)	15 (36)	15	-22 (-42, -5)	11 (73)	10 (67)	11	-47 (-149, 11)	7 (64)
>3 to 12 months	104	-52 (-200, 100)	47 (45)	20 (19)	49	-10 (-36, -1)	29 (59)	33 (67)	46	-57 (-235, -13)	36 (78)
>1 to 2 years	54	0 (-300, 100)	22 (41)	12 (22)	35	-5 (-22, 6)	15 (43)	24 (69)	19	-101 (-435, -12)	14 (74)
>2 to 3 years	25	-100 (-200, 70)	13 (52)	6 (24)	11	-19 (-38, -4)	8 (73)	9 (82)	7	-98 (-1216, -22)	6 (86)
3+ years	12	-330 (-581, -125)	10 (83)	5 (42)	7	-14 (-27, -5)	5 (71)	6 (86)	3	-98 (-127, -60)	3 (100)
Anti-IL5											
0 to 3 months	136	-500 (-955, -205)	121 (89)	110 (81)	81	-1 (-15, 9)	30 (37)	26 (32)	23	-21 (-134, 0)	11 (48)
>3 to 12 months	595	-420 (-800, -150)	494 (83)	468 (79)	351	-2 (-24, 13)	133 (38)	97 (28)	152	-2 (-79, 14)	47 (31)
>1 to 2 years	510	-393 (-640, -110)	425 (83)	407 (80)	330	-5 (-28, 9)	136 (41)	119 (36)	118	-2 (-68, 22)	39 (33)
>2 to 3 years	269	-400 (-770, -150)	228 (85)	225 (84)	176	-4 (-24, 7)	80 (45)	64 (36)	49	-12 (-117, 6)	24 (49)
3+ years	188	-430 (-700, -200)	162 (86)	164 (87)	116	0 (-16, 19)	37 (32)	29 (25)	31	-45 (-207, 3)	17 (55)

8.0 Summary and Discussion

8.1 Objective 1

The distributions of baseline biomarkers in the ISAR population were highly skewed with low values being more common. Pre-biologic measurements of BEC, FeNO and IgE taken within 7 days of each other were positively but only weakly correlated within patients. BEC and FeNO were more correlated with each other than either was with IgE. Using highest pre-biologic measurements of the biomarkers instead made only small differences to the strength of the correlations which were then slightly weaker in all cases. This may be because there is little or no mechanistic link between these biomarkers in the body. Alternatively it may be because we looked at correlations between biomarker levels across the patients available in the ISAR population (i.e. whether the patients with a high level of one biomarker also tended to have a high level of another). This does not rule out the possibility that different biomarkers change synchronously over time within individual patients. If most patients had had multiple pairs of pre-biologic biomarker results available it would also have been possible to assess whether different biomarkers within an individual patient change together over time, but there was insufficient data to study this in IGNITE. However, our analysis of biomarker levels following biologic initiation (section 7.7.2) showed that changes in one biomarker can occur without corresponding changes in the others, suggesting that within patient levels of these biomarkers are also not strongly linked, particularly for patients who are on some form of asthma treatments.

8.2 Objective 2

Exacerbations

Decreases in exacerbations were only weakly related to pre-biologic biomarker levels. This was perhaps surprising considering that some clinical trials had found clear associations between baseline biomarkers and efficacy of biologics in reducing exacerbations[21,22]. One probable reason for this is that the clinical trials measured efficacy of the biologics against well matched control groups, whereas we measured the effects of the biologics against the pre-biologic levels of the outcomes in the same patients. Looking only at the patients treated with biologics in the clinical trials, there was a much smaller association between reduction in exacerbations and the biomarkers, similar to our findings. Additionally, when we studied the baseline (i.e. pre-treatment) levels of these outcomes in the ISAR patients who went on to receive biologics, there was little association between baseline exacerbation rates and the

biomarkers (section 7.7.1). This contrasted with the patients included in ISAR who did not receive biologics and probably reflected the selection criteria used for patients to be prescribed biologics.

FEV_1

Improvement in FEV_1 was strongly associated with baseline BEC and FeNO, with patients with the highest biomarkers tending to have the greatest improvements (when compared at the mean baseline FEV_1 of 2.1L). The trends and magnitude of these effects were very similar in patients prescribed anti-IgE and patients prescribed anti-IL5/5R (section 7.5.2). There were relatively few patients who had been prescribed anti-IL4 available for the analysis so trends, although similar, were not significant. No associations between baseline IgE and improvement in FEV_1 were found. Of the three outcomes studied, associations with the baseline biomarkers were strongest for FEV_1. This may be related in part to the nature of the measurement, being determined by a calibrated instrument, in contrast to exacerbations and asthma control, which involve some degree of subjectivity.

Subgroup analysis suggested that the association with FEV_1 was strongest in patients who were not on LTOCS at baseline for BEC, and patients with 1 or more allergies for BEC and FeNO, though the differences (i.e. interactions) were not statistically significant (section 7.5.4.2). Benefits in terms of FEV_1 following biologic treatment were similar in patients with either high (≥2) or low (0 or 1) exacerbations per year at baseline.

Asthma control

The probability of uncontrolled asthma at follow-up (adjusted for baseline asthma control) was lowest in patients with high baseline BEC for the anti-IL5/5R biologics and a similar (though non-significant) trend was seen for baseline FeNO (section 7.5.3). There was no apparent association between baseline BEC or FeNO and asthma control in the patients prescribed anti-IgE. There was also no association between asthma control at follow-up and baseline IgE for either class of biologics.

When asthma control was analysed by subgroups it appeared that patients who were not on LTOCS at baseline generally had better outcomes, though the difference was very small for patients with the highest levels of baseline BEC. The non-significant association between asthma control and baseline FeNO noted above was significant in biologic patients with ≥2 exacerbations at baseline (p=0.031) but not in patients with 0 or 1 exacerbations at baseline (p=0.230). This difference in the strength of association was statistically significant (p=0.028)

as assessed by the interaction between FeNO and the baseline exacerbation rate (section 7.5.4.3).

8.3 Objective 3

For biomarkers found to have significant associations with outcomes in objective 2, coefficients and statistical significance remained similar in the objective 3 analysis in both the single biomarker or multi-biomarker models (Appendix 4). Some differences were to be expected due to the reduced sample sizes in objective 3 resulting from the requirement for patients to have all three baseline biomarkers available. Coefficients for non-significant associations between biomarkers and outcomes were less stable across the objective 2 and objective 3 analyses, reflecting the fact that these associations were not strong and there was insufficient data to estimate the magnitude of these associations accurately, if any do exist.

Only the FEV_1 model was significantly improved by including multiple biomarkers rather than the best single biomarker (BEC) (p=0.029 for comparison of the BEC only vs mutiple biomarker model) (section 7.6.1). However, this appeared to have limited clinical significance, improving the overall variance in follow-up FEV_1 explained by the model by only 0.3%. For exacerbations and asthma control, including mutiple biomarkers did not lead to any statistically significant improvement in the predictive ability of the models compared to the best single biomarker. Similarly when compared in terms of the models' ability to predict the outcomes correctly, including multiple biomarkers rather than the best single biomarker did not appear to give any advantage of clinical importance.

9.0 Limitations

ISAR is a large dataset, collected from a wide range of countries and provides opportunities to discover important underlying trends even when between patient variability is high. Using data from such a large and diverse source creates challenges. Although ISAR processes have been developed and refined to standardise the data collected, it can be expected there are some differences between countries in the methods and completeness of data collection. Also, some of the data relies on patients' recollection of events and/or dates so it may not all be accurate. Despite the overall size of the ISAR dataset there were still only 428 patients prescribed anti-IL4 available to include in the study, so conclusions about this biologic class were less clear.

The study aimed to consider whether the effectiveness of different biologic classes could be predicted from biomarkers measured before starting biologics, and hence used to help select appropriate biologic treatments for patients. In this study we measured "effectiveness" by the change in outcomes from pre-biologic to post-biologic as there was no suitable control group. Hence, our design differs considerably from that used in most clinical trials. An apparent lack of association between effectiveness of the biologics and the biomarkers seen in this study may be because we could not observe the effect in a comparable untreated control group.

The ability of this study to assess associations between biomarkers and outcomes is also limited by the timing of the biomarker samples. Although we used highest pre-biologic **measurements** there was no control of when these samples were taken so they do not necessarily reflect the highest **levels** of these biomarkers that the patient had experienced. Of the three outcomes studied, FEV_1 was the most objective measure, being determined by a calibrated piece of equipment. Exacerbations and asthma control are partially subjective and may be influenced by the patient's expectations for the effect of the biologics, as well as the actual effect of the treatments. The fact that change in FEV_1 showed the clearest association with baseline biomarkers may be related to this difference in the nature of the outcomes.

Patients were not randomised to the different classes of biologics so some differences in profiles of clinical characteristics were evident at baseline. The subgroup analysis revealed some factors which may affect the strength of associations between baseline biomarkers and outcomes. Adjusting for other factors was problematic in these analyses because the data for some factors are incomplete so this would result in reduced and/or biased subsamples being included in the adjusted analyses. Also, other factors known to affect the outcomes may

themselves be correlated with the biomarkers that we were interested in, so adjusting for them could have masked the associations between the outcomes and the biomarkers that we were trying to study.

10.0 Conclusions

Most patients who were prescribed biologics included in this study had high levels of exacerbations and uncontrolled asthma at baseline but generally had improved outcomes after biologic treatments for all three of the outcomes studied (exacerbation rates, FEV_1 and uncontrolled asthma). There was, however, high variability between individual patients (see confidence intervals on graphs). This may be related to the wide range of durations of asthma prior to biologic initiation (median = 19.8 years; IQR 9.7-34.0) with the lung damage caused by longer durations being inherently more difficult to treat. The overall high level of improvement in outcomes following the biologic treatments might also go some way to explaining the relatively low associations between treatment effectiveness and the biomarkers that we observed, particularly for exacerbations and asthma control, since generally good improvements across the range of biomarker values were seen. The strongest association with the biomarkers seen was for improvement in FEV_1 which was greater with higher baseline levels of BEC and FeNO. This effect was observed for both patients with ≤1 exacerbations or ≥2 exacerbations per year at baseline, suggesting the biologics could be a useful treatment even in patients with low exacerbation rates. Baseline IgE was not strongly predictive of any of the outcomes.

Lack of associations between the biomarkers and decrease in exacerbations was probably also due to the selection criteria for patients to be started on biologics. This led to patients in this study having very homogeneous baseline levels of exacerbations across the range of baseline biomarkers.

The biomarkers BEC, FeNO and IgE were positively but only weakly correlated with each other. This might be seen as an opportunity to gain information about potential outcomes by using multiple biomarkers if the different biomarkers have different associations with the outcomes. However, as seen in objective 2, baseline BEC and FeNO provided broadly similar insight into patient outcomes, whilst baseline IgE provided very little. Although they showed similar associations with the outcomes, BEC may be a better biomarker to use than FeNO on the grounds of better reliability of the measurements and because it showed a marked change following initiation of treatment, particularly in the patients prescribed anti-IL5/5R. Using a combination of the biomarkers rather than the best single biomarker was only found to give a statistically significant improvement for predicting change in FEV_1. However, even this did not appear to be a clinically significant improvement. The real value of using multiple biomarkers

may be for predicting which patients will benefit most for compound outcomes (such as improved asthma control and reduced LTOCS use) but these were not investigated in this study.

11.0 Advisory Group

[redacted], 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
[redacted]	Argentina
[redacted]	Australia
[redacted]	Belgium
[redacted]	Bulgaria
[redacted]	Canada
[redacted]	Colombia
[redacted]	Denmark
[redacted]	Estonia
[redacted]	Finland
[redacted]	France
[redacted]	Germany
[redacted]	Greece

[redacted]	
[redacted]	India
[redacted]	Ireland
[redacted]	Italy
[redacted]	
[redacted]	
[redacted]	
[redacted]	Japan
[redacted]	
[redacted]	Kuwait
[redacted]	Mexico
[redacted]	Norway
[redacted]	Poland
[redacted]	Portugal
[redacted]	Saudi Arabia
[redacted]	Singapore
[redacted]	South Korea
[redacted]	Spain
[redacted]	
[redacted]	Sub-Saharan Africa
[redacted]	Sweden
[redacted]	Taiwan
[redacted]	
[redacted]	
[redacted]	UAE
[redacted]	UK
[redacted]	
[redacted]	
[redacted]	
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14.0 Appendices

14.1 Appendix 1: Marginal estimates from regression models

Marginal estimates of the outcomes at follow-up for different levels of biomarkers, as plotted in Figures 5, 6 & 7.

Outcome: Decrease in exacerbations vs BEC

Biologic	BEC	Outcome Estimate	[95% Conf.	Interval]
Anti-IgE	50	1.89	1.81	1.98
Anti-IL5	50	1.80	1.69	1.90
Anti-IL4	50	1.81	1.61	2.00
Anti-IgE	250	1.88	1.81	1.94
Anti-IL5	250	1.80	1.73	1.88
Anti-IL4	250	1.85	1.72	1.98
Anti-IgE	500	1.85	1.78	1.92
Anti-IL5	500	1.81	1.75	1.87
Anti-IL4	500	1.90	1.78	2.01
Anti-IgE	750	1.82	1.72	1.93
Anti-IL5	750	1.82	1.74	1.89
Anti-IL4	750	1.94	1.79	2.08
Anti-IgE	1000	1.79	1.62	1.96
Anti-IL5	1000	1.82	1.72	1.93
Anti-IL4	1000	1.97	1.79	2.15

Outcome: Decrease in exacerbations vs FeNO

Biologic	FeNO	Outcome Estimate	[95% Conf.	Interval]
Anti-IgE	5	1.78	1.63	1.93
Anti-IL5	5	1.85	1.74	1.97
Anti-IL4	5	2.03	1.91	2.16
Anti-IgE	25	1.81	1.71	1.91
Anti-IL5	25	1.83	1.74	1.92
Anti-IL4	25	1.98	1.85	2.10
Anti-IgE	50	1.85	1.76	1.94
Anti-IL5	50	1.81	1.74	1.89
Anti-IL4	50	1.88	1.74	2.02
Anti-IgE	75	1.88	1.76	2.01
Anti-IL5	75	1.79	1.69	1.88
Anti-IL4	75	1.74	1.51	1.97
Anti-IgE	100	1.91	1.75	2.08

Anti-IL5	100	1.76	1.62	1.90
Anti-IL4	100	1.54	1.08	2.00

Outcome: Decrease in exacerbations vs IgE

Biologic	IgE	Outcome Estimate	[95% Conf.	Interval]
Anti-IgE	50	1.84	1.75	1.94
Anti-IL5	50	1.86	1.79	1.93
Anti-IL4	50	1.83	1.64	2.01
Anti-IgE	200	1.84	1.76	1.91
Anti-IL5	200	1.83	1.76	1.90
Anti-IL4	200	1.82	1.65	1.99
Anti-IgE	400	1.83	1.76	1.90
Anti-IL5	400	1.78	1.70	1.87
Anti-IL4	400	1.81	1.62	2.01
Anti-IgE	600	1.82	1.72	1.91
Anti-IL5	600	1.73	1.60	1.86
Anti-IL4	600	1.81	1.55	2.06
Anti-IgE	800	1.81	1.66	1.95
Anti-IL5	800	1.67	1.47	1.87
Anti-IL4	800	1.80	1.47	2.13

Outcome: Improvement in FEV1 vs BEC

Biologic	BEC	Outcome Estimate	[95% Conf.	Interval]
Anti-IgE	50	-0.014	-0.069	0.041
Anti-IL5	50	-0.003	-0.055	0.050
Anti-IL4	50	0.097	-0.026	0.220
Anti-IgE	250	0.027	-0.014	0.067
Anti-IL5	250	0.043	0.004	0.083
Anti-IL4	250	0.129	0.040	0.218
Anti-IgE	500	0.077	0.034	0.120
Anti-IL5	500	0.101	0.070	0.132
Anti-IL4	500	0.169	0.090	0.249
Anti-IgE	750	0.128	0.062	0.194
Anti-IL5	750	0.158	0.122	0.195
Anti-IL4	750	0.210	0.096	0.323
Anti-IgE	1000	0.178	0.083	0.274
Anti-IL5	1000	0.216	0.164	0.268
Anti-IL4	1000	0.250	0.084	0.415

Outcome: Improvement in FEV1 vs FeNO

Biologic	FeNO	Outcome Estimate	[95% Conf.	Interval]
Anti-IgE	5	-0.021	-0.098	0.056
Anti-IL5	5	0.032	-0.024	0.089
Anti-IL4	5	0.128	-0.006	0.261
Anti-IgE	25	0.041	-0.015	0.098
Anti-IL5	25	0.069	0.026	0.112
Anti-IL4	25	0.148	0.049	0.247
Anti-IgE	50	0.119	0.056	0.182
Anti-IL5	50	0.115	0.080	0.151
Anti-IL4	50	0.173	0.078	0.269
Anti-IgE	75	0.196	0.100	0.293
Anti-IL5	75	0.161	0.117	0.206
Anti-IL4	75	0.199	0.063	0.335
Anti-IgE	100	0.274	0.135	0.413
Anti-IL5	100	0.207	0.144	0.270
Anti-IL4	100	0.224	0.030	0.419

Outcome: Improvement in FEV1 vs IgE

Biologic	IgE	Outcome Estimate	[95% Conf.	Interval]
Anti-IgE	50	0.036	-0.021	0.093
Anti-IL5	50	0.125	0.083	0.167
Anti-IL4	50	0.157	0.053	0.261
Anti-IgE	200	0.042	-0.003	0.087
Anti-IL5	200	0.126	0.092	0.161
Anti-IL4	200	0.179	0.087	0.270
Anti-IgE	400	0.050	0.010	0.091
Anti-IL5	400	0.128	0.086	0.170
Anti-IL4	400	0.208	0.090	0.325
Anti-IgE	600	0.058	0.005	0.111
Anti-IL5	600	0.130	0.068	0.192
Anti-IL4	600	0.237	0.067	0.407
Anti-IgE	800	0.067	-0.008	0.141
Anti-IL5	800	0.132	0.046	0.218
Anti-IL4	800	0.266	0.034	0.499

Outcome: Probability of uncontrolled asthma vs BEC

Biologic	BEC	Outcome Estimate	[95% Conf.	Interval]
Anti-IgE	50	0.354	0.282	0.427

Anti-IL5	50	0.419	0.357	0.481
Anti-IgE	250	0.347	0.295	0.399
Anti-IL5	250	0.379	0.334	0.423
Anti-IgE	500	0.339	0.284	0.393
Anti-IL5	500	0.330	0.296	0.364
Anti-IgE	750	0.330	0.248	0.413
Anti-IL5	750	0.284	0.242	0.326
Anti-IgE	1000	0.322	0.204	0.440
Anti-IL5	1000	0.242	0.186	0.298

Outcome: Probability of uncontrolled asthma vs FeNO

Biologic	FeNO	Outcome Estimate	[95% Conf.	Interval]
Anti-IgE	5	0.352	0.256	0.448
Anti-IL5	5	0.382	0.316	0.449
Anti-IgE	25	0.351	0.281	0.421
Anti-IL5	25	0.366	0.318	0.415
Anti-IgE	50	0.349	0.267	0.431
Anti-IL5	50	0.347	0.307	0.387
Anti-IgE	75	0.347	0.219	0.475
Anti-IL5	75	0.327	0.276	0.379
Anti-IgE	100	0.345	0.161	0.530
Anti-IL5	100	0.309	0.236	0.381

Outcome: Probability of uncontrolled asthma vs IgE

Biologic	IgE	Outcome Estimate	[95% Conf.	Interval]
Anti-IgE	50	0.314	0.245	0.383
Anti-IL5	50	0.340	0.294	0.386
Anti-IgE	200	0.319	0.265	0.373
Anti-IL5	200	0.339	0.301	0.377
Anti-IgE	400	0.326	0.279	0.374
Anti-IL5	400	0.338	0.293	0.382
Anti-IgE	600	0.334	0.273	0.395
Anti-IL5	600	0.336	0.271	0.401
Anti-IgE	800	0.341	0.255	0.427
Anti-IL5	800	0.335	0.245	0.424

14.2 Appendix 2: Statistical significance tests for comparisons of subgroups

The table below includes the results of statistical tests for associaitons between outcomes and biomarkers in each subgroup (as described in section 7.5.4). The results of interaction tests between the subgroups and the biomarker are also shown.

P-values for each subgroup are from a test of association between the relevant baseline biomarker and the outcome (adjusted for baseline level of the outcome).
P-values for interactions are for a comparison of the associations (i.e. slopes of the lines) for the two subgroups.

Exacerbations

Associations between baseline biomarkers and follow-up exacerbation rates, adjusted for baseline exacerbation rate. IRR - Incidence rate ratios.
IRR for BEC is per 1000 cells/µL; IRR for FeNO is per 100 ppb; IRR for IgE is per 1000 IU/mL

		BEC	FeNO	IgE
LTOCS as biologic initiation				
	Non-LTOCS	IRR = 0.963, N = 896, p = 0.881	IRR = 1.020, N = 565, p = 0.953	IRR = 1.290, N = 758, p = 0.325
	LTOCS	IRR = 1.090, N = 449, p = 0.729	IRR = 1.475, N = 315, p = 0.219	IRR = 1.790, N = 401, p = 0.108
	Interaction	p = 0.726	p = 0.430	p = 0.463
Exacerbation rates at baseline				
	0 or 1	Not applicable	Not applicable	Not applicable
	>=2	Not applicable	Not applicable	Not applicable
	Interaction	Not applicable	Not applicable	Not applicable
No or any allergies detected				
	No allergies	IRR = 1.031, N = 300, p = 0.946	IRR = 1.063, N = 228, p = 0.862	IRR = 1.194, N = 281, p = 0.715
	>=1 allergy	IRR = 1.013, N = 541, p = 0.950	IRR = 0.675, N = 382, p = 0.212	IRR = 1.291, N = 499, p = 0.383
	Interaction	p = 0.972	p = 0.339	p = 0.891
Age at asthma onset				
	<18 years	IRR = 0.747, N = 221, p = 0.485	IRR = 0.923, N = 154, p = 0.887	IRR = 0.864, N = 195, p = 0.728
	>= 18 years	IRR = 1.155, N = 522, p = 0.546	IRR = 1.015, N = 344, p = 0.952	IRR = 2.288, N = 456, p = 0.016
	Interaction	p = 0.364	p = 0.878	p = 0.074

FEV1

Associations between baseline biomarkers and follow-up FEV1, adjusted for baseline FEV1
Coeff – increase in FEV1 for a given increase in the baseline biomarker value.
Coeff for BEC is per 1000 cells/µL; coeff for FeNO is per 100 ppb; coeff for IgE is per 1000 IU/mL

		BEC	FeNO	IgE
LTOCS as biologic initiation				
	Non-LTOCS	Coeff = 0.249, N = 954, p < 0.001	Coeff = 0.222, N = 605, p < 0.001	Coeff = 0.015, N = 838, p = 0.787
	LTOCS	Coeff = 0.164, N = 456, p = 0.005	Coeff = 0.217, N = 324, p = 0.001	Coeff = -0.108, N = 404, p = 0.232
	Interaction	p = 0.249	p = 0.959	p = 0.246
Exacerbation rates at baseline				
	0 or 1	Coeff = 0.201, N = 610, p < 0.001	Coeff = 0.271, N = 403, p < 0.001	Coeff = 0.014, N = 544, p = 0.846
	>=2	Coeff = 0.216, N = 707, p < 0.001	Coeff = 0.143, N = 483, p = 0.017	Coeff = -0.008, N = 630, p = 0.901
	Interaction	p = 0.833	p = 0.143	p = 0.819
No or any allergies detected				
	No allergies	Coeff = 0.074, N = 295, p = 0.351	Coeff = 0.118, N = 223, p = 0.177	Coeff = -0.141, N = 275, p = 0.211
	>=1 allergy	Coeff = 0.234, N = 602, p < 0.001	Coeff = 0.265, N = 400, p < 0.001	Coeff = 0.075, N = 581, p = 0.271
	Interaction	p = 0.098	p = 0.184	p = 0.101
Age at asthma onset				
	<18 years	Coeff = 0.127, N = 245, p = 0.142	Coeff = 0.342, N = 173, p < 0.001	Coeff = 0.030, N = 230, p = 0.778
	>= 18 years	Coeff = 0.258, N = 621, p < 0.001	Coeff = 0.180, N = 375, p = 0.006	Coeff = -0.052, N = 544, p = 0.471
	Interaction	p = 0.204	p = 0.162	p = 0.526

Uncontrolled asthma

Associations between baseline biomarkers and odds of uncontrolled asthma at follow-up, adjusted for baseline uncontrolled asthma status. OR - Odds ratios.
OR for BEC is per 1000 cells/µL; OR for FeNO is per 100 ppb; OR for IgE is per 1000 IU/mL

		BEC	FeNO	IgE
LTOCS as biologic initiation				
	Non-LTOCS	OR = 0.756, N = 673, p = 0.334	OR = 0.632, N = 438, p = 0.210	OR = 1.205, N = 603, p = 0.560
	LTOCS	OR = 0.388, N = 388, p = 0.005	OR = 0.776, N = 287, p = 0.476	OR = 1.185, N = 349, p = 0.691
	Interaction	p = 0.131	p = 0.688	p = 0.975

Exacerbation rates at baseline				
	0 or 1	OR = 0.589, N = 330, p = 0.214	OR = 1.751, N = 223, p = 0.230	OR = 1.697, N = 295, p = 0.317
	>=2	OR = 0.524, N = 684, p = 0.014	OR = 0.509, N = 471, p = 0.031	OR = 1.027, N = 617, p = 0.929
	Interaction	p = 0.816	p = 0.028	p = 0.406
No or any allergies detected				
	No allergies	OR = 0.475, N = 208, p = 0.118	OR = 0.602, N = 163, p = 0.337	OR = 1.914, N = 201, p = 0.315
	>=1 allergy	OR = 1.027, N = 460, p = 0.935	OR =0.893, N = 332, p = 0.763	OR = 1.031, N = 454, p = 0.933
	Interaction	p = 0.181	p = 0.543	p = 0.402
Age at asthma onset				
	<18 years	OR = 0.430, N = 256, p = 0.043	OR = 0.756, N = 177, p = 0.559	OR = 1.178, N = 242, p = 0.725
	>= 18 years	OR = 0.664, N = 642, p = 0.165	OR = 0.668, N = 414, p = 0.257	OR = 1.000, N = 569, p = 1.000
	Interaction	p = 0.393	p = 0.835	p = 0.777

14.3 Appendix 3: Summary of statistics comparing the predictive abilities of single and multiple biomarker models to predict outcomes

Post-treatment outcome predicted		Predictors used				
		Baseline outcome only	Baseline outcome + BEC	Baseline outcome + FeNO	Baseline outcome + IgE	Baseline outcome + all biomarkers
FEV_1	Adjusted R^2	0.737	0.747*	0.743	0.736	0.750
	P-value[a] (comparison with all biomarkers model)		0.029			
Uncontrolled asthma	Pseudo R^2	0.055	0.062*	0.060	0.056	0.067
	% of outcomes correctly predicted	62	62	63	62	64
	P-value[a] (comparison with all biomarkers model)		0.464			
Exacerbations	Pseudo R^2	0.045	0.045	0.048	0.049*	0.054
	Mean absolute error in predicted rate/yr	0.62	0.62	0.61	0.62	0.60
	P-value[a] (comparison with all biomarkers model)				0.323	

* Best single biomarker for the outcome, based on adjusted R^2 (linear models) or pseudo R^2 (nonlinear models) which measure the relative ability of the models to predict the follow-up level of the outcome; [a] P-value for likelihood ratio test comparing the model including all biomarkers vs. the best single biomarker model. Clinical relevance of using models with multiple biomarkers was assessed qualitatively considering the proportion of total variance in FEV_1 explained (given by adjusted-R^2), percentage of follow-up outcomes correctly predicted (asthma control), and mean absolute error in the predicted follow-up exacerbation rate, for the single vs. all-biomarker models. BEC: blood eosinophil count; FEV_1: post-bronchodilator forced expiratory volume in one second; FeNO: fractional exhaled nitric oxide; IgE: Immunoglobulin E

14.4 Appendix 4: Regression coefficients for single and multiple biomarker models in objective 3

Regression coefficients for associatons between outcomes and baseline biomarkers in the objective 3 models. Note that the single biomarker models were equivalent to those in objective 2 but objective 3 included fewer patients due to the requirement for patients to have baseline data for all three biomarkers available.

Exacerbations

			BEC	FeNO	IgE
All biomarker model	anti-IgE	IRR	1.987	0.556	1.065
		95% CI	(0.69, 5.727)	(0.195, 1.587)	(0.334, 3.397)
		p-value	0.203	0.273	0.915
	anti-IL5	IRR	1.036	1.132	1.887
		95% CI	(0.622, 1.726)	(0.676, 1.894)	(1.027, 3.468)
		p-value	0.892	0.638	0.041
	anti-IL4	IRR	0.703	5.871	0.133
		95% CI	(0.124, 3.982)	(1.087, 31.719)	(0.007, 2.48)
		p-value	0.691	0.040	0.176
Single biomarker models	anti-IgE	IRR	1.630	0.689	1.057
		95% CI	(0.597, 4.452)	(0.254, 1.873)	(0.331, 3.369)
		p-value	0.341	0.466	0.926
	anti-IL5	IRR	1.089	1.137	1.890
		95% CI	(0.672, 1.766)	(0.698, 1.853)	(1.023, 3.492)
		p-value	0.729	0.605	0.042
	anti-IL4	IRR	1.231	3.441	0.324
		95% CI	(0.258, 5.866)	(0.83, 14.26)	(0.029, 3.606)
		p-value	0.794	0.088	0.359

IRR = Incidence rate ratio per 1000 cells/µL (BEC), per 100 ppb (FeNO), or per 1000 IU/mL (IgE)

FEV1

			BEC	FeNO	IgE
All biomarker model	anti-IgE	coeff	0.129	0.231	0.011
		95% CI	(-0.115, 0.373)	(0.009, 0.453)	(-0.201, 0.224)
		p-value	0.301	0.041	0.916
	anti-IL5	coeff	0.260	0.171	-0.009
		95% CI	(0.143, 0.377)	(0.055, 0.288)	(-0.164, 0.147)
		p-value	<0.001	0.004	0.914

	anti-IL4	coeff	0.392	-0.135	0.186
		95% CI	(0.011, 0.773)	(-0.482, 0.213)	(-0.18, 0.552)
		p-value	0.044	0.447	0.318
Single biomarker models	anti-IgE	coeff	0.225	0.276	0.039
		95% CI	(-0.001, 0.451)	(0.067, 0.485)	(-0.177, 0.256)
		p-value	0.051	0.010	0.721
	anti-IL5	coeff	0.293	0.222	0.016
		95% CI	(0.178, 0.409)	(0.107, 0.338)	(-0.144, 0.175)
		p-value	<0.001	<0.001	0.846
	anti-IL4	coeff	0.380	-0.018	0.196
		95% CI	(0.008, 0.753)	(-0.353, 0.318)	(-0.17, 0.562)
		p-value	0.046	0.918	0.293

coeff = increase in follow-up FEV1 per 1000 cells/µL (BEC), per 100 ppb (FeNO), or per 1000 IU/mL (IgE)

Asthma control

			BEC	FeNO	IgE
All biomarker model	anti-IgE	OR	1.563	1.269	0.580
		95% CI	(0.421, 5.795)	(0.361, 4.462)	(0.178, 1.893)
		p-value	0.504	0.710	0.367
	anti-IL5	OR	0.505	0.590	1.255
		95% CI	(0.256, 0.999)	(0.301, 1.157)	(0.554, 2.841)
		p-value	0.050	0.125	0.586
Single biomarker models	anti-IgE	OR	1.574	1.368	0.599
		95% CI	(0.438, 5.664)	(0.401, 4.669)	(0.185, 1.935)
		p-value	0.487	0.617	0.391
	anti-IL5	OR	0.466	0.533	1.124
		95% CI	(0.238, 0.912)	(0.275, 1.031)	(0.502, 2.515)
		p-value	0.026	0.061	0.776

OR = odds ratio for uncontrolled asthma per 1000 cells/µL (BEC), per 100 ppb (FeNO), or per 1000 IU/mL (IgE)

15.0 List of Tables

16.0 List of Figures