Study Protocol

Full study title: Characterising neutrophil serine protease activity in type 2 low asthma

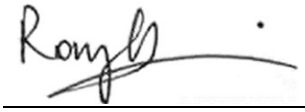
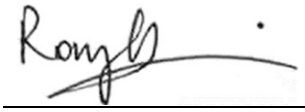
Short Title /Study Acronym	Neutrophil serine protease activity in type 2 low asthma
Sponsor	**University of Dundee**
Sponsor ID	Will be provided on application
Funder	Asthma + Lung UK
Chief Investigator Principal Investigator	Dr Rory Chan
IRAS Number	361386
Version Number and Date	V6.0 20/02/2026

PROTOCOL APPROVAL

Characterising neutrophil serine protease activity in type 2 low asthma

Signatures

The undersigned confirm that the following protocol has been agreed and approved by the Sponsor and that the Chief Investigator agrees to conduct the study in compliance with this approved protocol and will adhere to the principles of GCP, the Sponsor SOPs, and any other applicable regulatory requirements as may be amended from time to time.

Rory Chan		11/01/2026
Chief Investigator	Signature	Date
Rory Chan		11/01/2026
Individual Responsible for Statistical Review	Signature	Date

LIST OF ABBREVIATIONS

AE	Adverse Event
CI	Chief Investigator
CNORIS	Clinical Negligence and Other Risks Indemnity Scheme
CRF	Case Report Form
DMC	Data Monitoring Committee
GCP	Good Clinical Practice
ICF	Informed Consent Form
IF	Incidental Findings
ISF	Investigator Site File
PI	Principal Investigator
REC	Research Ethics Committee
SAE	Serious Adverse Event
SOP	Standard Operating Procedures
SMF	Study Master File
SMG	Study Management Group
SSC	Study Steering Committee

SUMMARY/SYNOPSIS

Study Title (including acronym)	**Characterising neutrophil serine protease activity in type 2 low asthma**	
Study Design	Single-centre, prospective observational study	
Study Population	Moderate to severe asthma	
Sample Size	120	
Planned study Period	April 2026 – October 2027 (18 months)	
Clinical phase duration	April 2026 – October 2027 (18 months)	
Follow up phase duration	None	
Primary	Objectives To determine whether sputum neutrophil elastase activity is increased in patients with T2-low asthma compared to T2-high asthma.	Outcome Measures Sputum neutrophil elastase activity
Secondary	Objectives To compare other neutrophil-derived proteins (proteinase-3, cathepsin-G, azurocidin-1, neutrophil extracellular trap markers) in patients with T2-low asthma versus T2-high asthma. To assess relationships between neutrophil protease activity and clinical outcomes such as symptom control, lung function, quality of life, and exacerbation history.	Outcome Measures Proteinase-3, cathepsin-G, azurocidin-1 and neutrophil extracellular trap levels/activity and fractional exhaled nitric oxide and peripheral blood eosinophils Asthma control questionnaire Spirometry Oscillometry Mini asthma quality of life questionnaire Exacerbation frequency
Inclusion Criteria	1) patients aged ≥18 years old with GINA defined moderate-to-severe asthma 2) taking a medium to high dose of ICS/LABA OR high dose ICS with another second line controller (BDP equivalent dose of ≥800µg) of step 4/5 GINA therapy 3) established diagnosis of persistent asthma ≥6 months according to GINA guidelines 4) ability to give informed consent 5) agreement for their GP to be made aware of study participation and to receive feedback as relevant to the participant's wellbeing 6) able to understand the study procedures and the risks involved. 7) good physical and mental status, determined on the basis of the medical history and a general clinical examination at screening	
Exclusion Criteria	1) patients who are taking biologics for asthma 2) patients who are taking maintenance oral corticosteroids 3) patients who have required a course of oral corticosteroids in the past month	

	4) any other respiratory diseases such as COPD and moderate to severe bronchiectasis which in the opinion of the investigator are clinically significant and may have an impact on the study outcomes. 5) any disorder that is not stable in the opinion of the Investigator 6) patients unable or unwilling to consent.

1. INTRODUCTION / BACKGROUND & RATIONALE

Type 2 (T2) high asthma is driven by type 2 helper (Th2) CD4+ T cells, which secrete inflammatory cytokines such as interleukins (IL) 4, 5, and 13.[1] These cytokines, in turn, promote systemic and airway inflammation, leading to increased production of commonly measured biomarkers such as immunoglobulin E (IgE), peripheral blood eosinophils (PBE), and fractional exhaled nitric oxide (FeNO).[1] T2 high asthma has garnered significant attention in recent years due to the development of biologics such as omalizumab, mepolizumab, benralizumab, dupilumab, and tezepelumab, which have shown markedly greater efficacy in treating this phenotype.[1,2] For instance, blocking IL5 with mepolizumab resulted in a 62% relative reduction in severe exacerbations for patients with PBE ≥300 cells/μL and FeNO ≥25ppb compared to placebo, whereas only an 18% relative reduction in exacerbations was observed in those with PBE <300 cells/μL and FeNO <25ppb.[3] In NHS Tayside, we have previously found that 81.5% of patients with moderate-to-severe asthma exhibit the T2 high phenotype (figure 1), defined by any combination of elevated total IgE, PBE, and FeNO, which is consistent with other studies. Conversely, only 12% of patients were identified as having the T2 low phenotype, characterised by total IgE <100 kU/L, PBE <150 cells/μL, and FeNO <25ppb.[4]

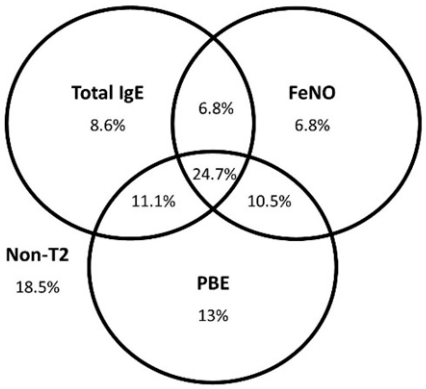


Figure 1 Venn diagram depicting the relative prevalence of type 2 phenotypes. Cut points used: PBE ≥300 cells/μl; FeNO ≥25ppb and total IgE ≥100 kU/L.[4] In this figure, the non-T2 phenotype refers to those with PBE <300 cells/μl; FeNO <25ppb and total IgE <100 kU/L.

T2 low asthma is typically characterised by the absence of T2 inflammatory biomarkers.[5] Although T2 low asthma is perhaps associated with better lung function,[4] individuals are usually less responsive to short acting bronchodilators which may contribute to symptom burden.[5] Airway neutrophilia can occur in T2 low asthma, although findings on this have been inconsistent. Additionally, neutrophilia in these patients can be influenced by oral corticosteroid treatment, which is known to prolong neutrophil survival.[6]

There is currently an unmet need for effective therapies in individuals with T2 low asthma. So far, three suboptimal therapeutic options have been explored. The first is the macrolide azithromycin, which was shown to reduce exacerbations by 34% in patients with non-eosinophilic asthma, defined by a PBE count <300 cells/μL.[7] However, the effect on "true" non-eosinophilic asthma (PBE <150 cells/μL) was not reported, nor was there any data on other T2 biomarkers such as FeNO or total IgE, which may have been elevated in some patients, potentially contributing to the observed efficacy of azithromycin. In a post hoc analysis of the AMAZES trial,[7] azithromycin 500mg three times weekly was shown to reduce sputum neutrophil extracellular traps after 12 months in asthma.[8]

The second option is tezepelumab, an anti-thymic stromal lymphopoietin (anti-TSLP) biologic. The greatest reduction in exacerbation frequency (77%, 95% CI: 67–84) was observed in patients with T2 high asthma (PBE ≥300 cells/μL and FeNO ≥25 ppb).[2] However, in patients with T2 low disease (both PBE <300 cells/μL and FeNO <25 ppb), the reduction in exacerbations was only borderline significant, at 29% (95% CI: 0–50). The wide 95% confidence intervals suggest considerable variability in response, raising questions about the efficacy of tezepelumab in specific individuals with T2 low asthma.[2] Furthermore, only patients with PBE <300 cells/μL were included, whereas the ideal PBE definition for T2 low asthma is <150 cells/μL. The third potential option, although primarily reserved for research purposes,

was bronchial thermoplasty which is a non-pharmacological intervention. Bronchial thermoplasty involves applying thermal energy via bronchoscopy to the airways to reduce bronchial smooth muscle mass and has been shown to improve asthma control.[9] Due to the relative procedural invasiveness, it has been proposed as an option for T2 low asthma, although in real life clinical practice this is rare.[10]

Neutrophils are recruited to sites of inflammation from the bone marrow as part of the immune response. At the site of inflammation, neutrophils engulf foreign material, such as bacteria by phagocytosis or undergo degranulation or neutrophil extracellular trap formation. In the latter two processes, neutrophil granules are released extracellularly. The cytotoxic and proteolytic granule contents include reactive oxygen species, neutrophil elastase, cathepsin G, Azurocidin-1 and proteinase 3. Airway serine protease activity can lead to mucus gland hyperplasia, smooth muscle cell proliferation, epithelial damage and impairment of defence against microbes.

There are currently no treatments which target neutrophilic inflammation in bronchiectasis. Characterisation of neutrophilic inflammation in bronchiectasis over the past decade, largely funded by Asthma and Lung UK grants to Prof Chalmers and Prof Shoemark, has led to the development of DPP1 inhibitors which are set to be licensed for bronchiectasis next year. These drugs reduce airway neutrophilic inflammation, reduce exacerbations by up to 60% and prevent lung function decline in bronchiectasis. Whether there is a potential role in asthma is unknown.

2 STUDY OBJECTIVES & OUTCOMES

Table 1: Primary Objectives and Outcome Measures

Primary Objective:	**Outcome Measure:**	**Timepoint of outcome measured**
To determine whether sputum neutrophil elastase activity is increased in patients with T2-low asthma compared to T2-high asthma.	Sputum neutrophil elastase activity	Visit 1

Table 2: Secondary Objectives and Outcome Measures

Secondary Objective:	**Outcome Measure:**	**Timepoint of outcome measured**
To compare other neutrophil-derived proteins (proteinase-3, cathepsin-G, azurocidin-1, neutrophil extracellular trap markers) in patients with T2-low asthma versus T2-high asthma.	Proteinase-3, cathepsin-G, azurocidin-1 and neutrophil extracellular trap levels/activity	Visit 1
To assess relationships between neutrophil protease activity and clinical outcomes such as, FeNO, BEC, symptom control, lung function, quality of life, and exacerbation history.	Asthma control questionnaire FeNO Blood eosinophil count Total blood IgE Spirometry Oscillometry Mini asthma quality of life questionnaire Exacerbation frequency	Visit 1

3 STUDY DESIGN

This is a single-centre, cross-sectional observational study in adults with moderate-to-severe asthma.

3.1 INTERVENTION

This is an observational diagnostic study; all participants undergo the same assessments and are subsequently classified into T2-low or T2-high asthma groups for comparison. No therapeutic intervention or randomisation is involved.

3.2 STUDY DESCRIPTION

Potential participants (adults with moderate-to-severe asthma under NHS Tayside care) are identified by their clinical care team. They receive a Patient Information Sheet and at least 24 hours to consider participation. In practice, many patients will be given several days to a week or more, depending on when it is convenient for them to return a decision. Written informed consent is obtained by trained study staff. Participants are assigned a pseudonymised study ID; personal identifiers are stored separately. When attending for their routine clinic appointment, patients will undergo routine clinical assessments including a blood test to look at eosinophils and total IgE, questionnaires to assess symptoms and quality of life, spirometry, oscillometry, and fractional exhaled nitric oxide (FeNO). The only test we propose to perform in addition to routine clinical practice is induced sputum (for neutrophil elastase activity and other protease/NET markers). The single visit will take approximately 1 hour. All clinical and laboratory data are recorded against pseudonymised IDs. Data will be stored securely on encrypted NHS/University servers with access limited to authorised staff. Samples are processed and analysed in research labs; results linked only via study IDs.

3.2 STUDY FLOWCHART

3.4 STUDY MATRIX

3.5 STUDY ASSESSMENTS

Spirometry and oscillometry measure lung function (airflow limitation, small airway resistance) and can be used to compare lung function between T2-low and T2-high asthma. These are standardised clinical tests (GINA/NHS/ERS guidelines) with high reproducibility when performed by trained staff. Fractional exhaled nitric oxide (FeNO) measures inflammation linked to type 2 asthma and is a biomarker for classifying participants into T2-high vs T2-low. FeNO is a non-invasive, point-of-care test, reproducible in clinical settings. Blood samples will be taken for full blood count (including eosinophil count) and total and specific IgE to phenotype patients (T2-high vs T2-low classification) and for exploratory biomarker analysis.

The Asthma Control Questionnaire (ACQ) is a clinically validated symptom control tool widely used internationally with scores ranging from 0 (well controlled) to 6 (worst control). The mini-Asthma Quality of Life Questionnaire (mini-AQLQ) is a validated quality of life tool with scores between 1 (severely impaired) to 7 (no impairment). Induced sputum will be collected and sent to research labs for processing and analysed for neutrophil elastase activity, proteinase-3, cathepsin-G, azurocidin-1, neutrophil extracellular trap (NET) markers.

3.6 STUDY SAFETY ASSESSMENTS

3.7 TISSUE

Induced sputum (airway sample) will be collected during the single study visit. Blood samples will be collected at the same visit. Both samples are collected once, during the participant's single study visit (~1 hour). Blood samples will be processed by the NHS laboratories as this will be part of routine clinical care. Induced sputum samples will be processed in University of

Dundee/NHS Tayside research laboratories. They will be labelled with pseudonymised study IDs (no personal identifiers). Residual samples will be stored securely for the duration of the study to complete planned analyses. After study completion, samples will be destroyed unless explicit additional consent is obtained for longer-term storage. No genetic testing is planned. No unspecified future analyses will be undertaken without separate participant consent.

3.8 INCIDENTAL FINDINGS

Clinically significant incidental findings will be shared with the participant's GP or consultant by the CI or Site PI, with the participant's consent.

3.9 STUDY POPULATION

The study population consists of adults with moderate-to-severe asthma, stratified into T2-low and T2-high phenotypes, recruited from NHS Tayside clinics.

3.10 NUMBER OF PARTICIPANTS

The study aims to recruit a total of 120 adults with moderate-to-severe asthma, comprising 40 individuals with T2-low asthma and 80 with T2-high asthma, in order to provide sufficient statistical power for group comparisons. Because this is a single-visit, observational study, each participant's involvement will last for about one hour during which clinical assessments, lung function testing, questionnaires, and biological sampling will take place. The risk of dropout is expected to be very low; however, if any participants withdraw prior to completing their visit, they will be replaced to ensure that the target sample size is achieved.

3.11 INCLUSION CRITERIA

1) patients aged ≥18 years old with GINA defined moderate-to-severe asthma.

2) taking a medium to high dose of ICS/LABA OR high dose ICS with another second line controller (BDP equivalent dose of ≥800µg) of step 4/5 GINA therapy.

3) established diagnosis of persistent asthma ≥6 months according to GINA guidelines.

4) ability to give informed consent.

5) agreement for their GP to be made aware of study participation and to receive feedback as relevant to the participant's wellbeing.

6) able to understand the study procedures and the risks involved.

7) good physical and mental status, determined on the basis of the medical history and a general clinical examination at screening.

3.12 EXCLUSION CRITERIA

1) patients who are taking biologics for asthma.
2) patients who are taking maintenance oral corticosteroids.
3) patients who have required a course of oral corticosteroids in the past month.
4) any other respiratory diseases such as COPD and moderate to severe bronchiectasis. which in the opinion of the investigator are clinically significant and may have an impact on the study outcomes.
5) any disorder that is not stable in the opinion of the Investigator.
6) patients unable or unwilling to consent.

Individuals will not be enrolled in the study if they are currently participating in the clinical phase of another interventional study or have done so within the past 30 days. Individuals who are in the follow-up phase of another interventional study, or who are enrolled in an observational study, may be co-enrolled if the Chief Investigators (CIs) of both studies agree that it is appropriate.

4 PARTICIPANT SELECTION AND ENROLMENT

4.1 IDENTIFYING PARTICIPANTS

Potential participants will be identified by their NHS clinical care teams at asthma clinics within NHS Tayside. Eligible individuals will be screened through review of medical records to confirm a diagnosis of moderate-to-severe asthma and potential T2-low or T2-high phenotype based on available biomarker data.

Participants will be approached in person during routine clinic visits or contacted directly by a member of their usual healthcare team (via telephone or letter) to introduce the study. Interested patients will be provided with a Patient Information Sheet (PIS) and given at least 24 hours to consider participation before providing written informed consent. Enrolment and the study visit will take place at Ninewells Hospital, Dundee, conducted by trained research staff.

4.2 CONSENTING PARTICIPANTS

Informed consent will be obtained by trained research staff, delegated by the Chief Investigator and listed on the study delegation log. Potential participants will be identified and provided with a Participant Information Sheet (PIS) either during a routine clinic visit or by post/email following referral from their NHS care team.

Participants will be given a minimum of 24 hours to review the PIS and consider their decision before being asked to provide written consent. In practise, most potential participants will have more than 24 hours to decide if they want to take part, no potential participant will be pressurised to make a quick decision. If, after this period, they wish to take part, written informed consent will be obtained in person at Ninewells Hospital, Dundee, immediately prior to the study visit.

Consent will cover participation in study procedures, use of pseudonymised clinical and laboratory data, and storage of biological samples for the duration of the study. Any optional consent for extended sample storage or future analyses will be sought separately, where relevant.

Where a participant requests to speak with a physician from the study team the consent process will not be completed until the participant has spoken to the physician and had all their questions answered to their satisfaction. The informed consent process will be conducted in compliance with TASC SOP: Obtaining Informed Consent from Potential Participants in Clinical Research

4.3 SCREENING FOR ELIGIBILITY

4.6 WITHDRAWAL PROCEDURES

Because this is a single-visit, cross-sectional study, participant follow-up is not required after the study visit. Therefore, the risk of loss to follow-up is very low. Participants will be tracked using a pseudonymised study ID assigned at enrolment, which links their clinical data, questionnaires, and biological samples. Identifiable information will be stored separately and securely, only accessible to authorised study staff. If a participant withdraws before completing the study visit, no data or samples will be collected, and their study ID will be retired. If a participant withdraws after completing the visit, they may request that their data and biological samples are withdrawn from the study. In this case: Any stored samples not yet analysed will be destroyed. Pseudonymised data already generated up to the point of withdrawal will normally be retained for scientific integrity, unless the participant specifically requests otherwise. Participants will be informed of these options during the consent process, in line with ethical and data protection regulations.

5 DATA COLLECTION & MANAGEMENT

5.1 DATA COLLECTION

Data will be collected during a single study visit lasting approximately one hour at Ninewells Hospital, Dundee. Trained research staff, delegated by the Chief Investigator and listed on the study delegation log, will be responsible for data collection, including clinical assessments (spirometry, oscillometry, FeNO), participant questionnaires (ACQ and mini-AQLQ), and biological sampling (blood and sputum).

Clinical data will be captured directly from validated hospital equipment, questionnaires will be completed on paper or electronic forms, and laboratory data will be entered into secure databases. All information will be recorded using pseudonymised study IDs, with identifiable information stored separately and securely. To ensure quality, data entry will be verified by a second researcher, supported by database validation checks, and monitored through periodic audits. Data will be stored on encrypted NHS and University of Dundee servers, with access limited to authorised personnel only; paper records such as consent forms will be kept in locked cabinets.

Portable diagnostic device outputs (e.g., from spirometers or FeNO meters) will be transferred to secure servers immediately after use, and no identifiable information will remain on these devices. The study will collect demographic, clinical, lung function, patient-reported, and laboratory health data, all of which are considered special category data under GDPR. No genetic data will be collected. Samples and related data will be retained only for the duration of the study and destroyed once analyses are complete, unless separate consent is obtained for longer-term storage. Anonymised, aggregate results may be shared with collaborators or in publications, but no identifiable data will ever be disclosed, and all procedures will comply with University of Dundee and NHS Tayside governance, as well as UK GDPR and the Data Protection Act 2018.

5.2 DATA MANAGEMENT SYSTEM

All data management procedures will be conducted in compliance with TASC (Tayside Academic Science Centre) Standard Operating Procedures (SOPs) for data management. If third-party systems are used for specific assays or analyses, their SOPs will be reviewed and followed where appropriate, in alignment with Sponsor requirements.

Data will be collected using a excel spreadsheet, aligned with the study protocol and case report form (CRF). The Chief Investigator (CI) holds overall responsibility for ensuring the accuracy and completeness of CRFs, with data queries resolved directly by the CI or a delegated member of the research team.

All study data will be stored securely on encrypted NHS and University of Dundee servers, with access restricted to authorised personnel only through role-based access controls. Any paper records (e.g., consent forms) will be stored in locked cabinets within secure NHS premises. Encryption and password protection will be applied for any local data storage, and no data will be stored on personal devices.

Data processing and entry will be performed by trained study staff listed on the delegation log, under the oversight of the CI. Data will be updated regularly to ensure completeness, with discrepancies or queries addressed promptly by the CI or delegated team members.

Where third-party laboratories or collaborators are involved in sample analyses, only pseudonymised data will be shared, and any third-party access will be regulated by data sharing agreements.

Only pseudonymised data will be used for analysis. Personal identifiers will be stored separately and securely and will never be shared outside NHS Tayside/University of Dundee. Ethical standards, UK GDPR, and the Data Protection Act 2018 will be strictly observed throughout data collection, processing, and storage.

Adequate IT infrastructure, encryption systems, and restricted server access are in place at NHS Tayside and the University of Dundee to ensure secure handling of study data. Only trained and authorised study staff will have access.

Final database locking will be conducted according to TASC SOPs. The Data Controller will be the University of Dundee (in partnership with NHS Tayside, as appropriate), while the Data Custodian will be the Chief Investigator.

6 STATISTICS AND DATA ANALYSIS

6.1 SAMPLE SIZE CALCULATION

In the CISCO study, summary statistics for NE concentration (µg/ml) in 76 Asthma patients were as follows: mean: 6.8; standard deviation 14.3; median 1.2; interquartile range: 0.6 to 4.0; range: 0.2 to 98.0. As the data were skewed, data were log10 transformed: mean: 0.28; standard deviation: 0.65; median: 0.09; IQR: -0.21 to 0.60; range: -0.67 to 1.99.

We aim to recruit 120 patients in total, with at least 40 of these in the T2-low group. A “moderate” effect is a standardised difference of 0.5 (Cohen (1988)). Assuming a standard deviation in each group of 0.65, this would be a log-difference of 0.325. This corresponds to NE levels being 2.1 times higher in the T2-low group vs T2-high. At the 1-sided 5% significance level, expecting NE levels to be higher in the T2-low group vs T2-high, 40 patients in the T2 low group versus 80 patients in the T2 high group would give approx. 82% power to detect a “moderate” difference.

Note that enrolment rates per T2 group will be monitored during the study. If it is possible to recruit more than 40 T2-low patients within the 120 patients, this would increase power. For example, 50 T2-low patients and 70 T2-high patients would provide 85% power.

6.2 PROPOSED DATA ANALYSES

The primary hypothesis is that NE activity differs between T2-low and T2-high groups. An initial unadjusted comparison will be performed using independent t-test (or Wilcoxon rank-sum if assumptions are violated), followed by a multivariable linear regression of NE (preferably log-transformed) on T2 group, adjusting for potential confounders including age, sex, BMI, smoking status, ICS dose, exacerbation history, and laboratory batch. Results will be reported as adjusted mean differences or ratios of geometric means with 95% confidence intervals and p-values. Secondary analyses will repeat this approach for other proteases and explore associations between protease activity and clinical outcomes using linear regression, with appropriate adjustments. Multiplicity will be addressed by controlling the false discovery rate at 5% for biomarker families.

Sensitivity analyses will include testing alternative definitions of T2-low asthma, handling missing data through multiple imputation if needed, assessing the impact of influential observations, and checking for nonlinear covariate effects. Exploratory subgroup analyses will examine interactions by smoking status and sex. Analyses will be conducted using SPSS, and all results will be reported with effect sizes, confidence intervals, exact p-values, and graphical summaries such as boxplots and adjusted marginal means plots.

6.3 MISSING DATA

Because this is a single-visit observational study, missingness is expected to be low. We will summarise patterns of missing data by variable and group (T2-low vs T2-high). Primary analyses will use available cases. If >5–10% data are missing for key outcomes or covariates, we will perform multiple imputation (including group, demographics, treatment step, lung function, FeNO, and outcomes in the imputation model), generate ≥20 imputations, complete-case and imputed results will be presented side-by-side as a sensitivity check. For skewed outcomes (e.g., protease activities) imputation will be performed on appropriately transformed (e.g., log) scales to respect distributional assumptions.

“Non-compliance” in this context primarily means incomplete procedure sets at the single visit (e.g., unable to produce sputum or uninterpretable spirometry). Participants with a valid T2 classification and a valid measurement of the analysis-specific outcome will be included in that analysis set; those lacking a required outcome will be excluded from that specific analysis but may contribute to others (e.g., contribute questionnaire analyses despite missing sputum). We

will document reasons for missing procedures (e.g., contraindication, participant choice, technical failure) and compare basic characteristics of participants with vs without the missing measure to assess potential bias.

If a participant withdraws before the visit, no data are retained. If withdrawal occurs after data/samples are collected, participants may request destruction of stored samples not yet analysed; pseudonymised data already generated are ordinarily retained to preserve scientific integrity unless the participant explicitly requests deletion, as outlined in the protocol's confidentiality and safety sections. All withdrawals will be logged with date and reason (if provided), and their disposition (data retained/destroyed; samples retained/destroyed) will be recorded. Analyses will report the number of withdrawals and their timing; where feasible, sensitivity analyses excluding withdrawn participants' data will be presented.
The Full Analysis Set comprises participants with valid T2 classification and a valid primary outcome (sputum NE activity). Per-analysis sets will be defined for secondary outcomes. Sensitivities will include (i) complete-case only, (ii) multiple-imputed datasets

Detailed algorithms (variable-level missingness thresholds, imputation specifications, and shell tables) will be finalised in the Statistical Analysis Plan (SAP) prior to database lock; any deviations will be documented in an SAP addendum and the final report.

6.4 TRANSFER OF DATA

Personal data will not be shared outside the University of Dundee and NHS Tayside except where required by law or regulation (e.g., audit or inspection by regulatory authorities) or with explicit participant consent. Research data may be shared in anonymised or pseudonymised form with approved collaborators for the purpose of scientific analysis, publication, or verification of results. No identifiable information will ever be shared with external collaborators.

When data are shared with collaborators or regulatory authorities, they will be transferred only by secure means such as encrypted email, password-protected files, or secure file transfer protocols (SFTP). Access will be restricted to authorised individuals, and all transfers will be logged.

The study does not plan to transfer identifiable data outside the UK/EU. Should anonymised or pseudonymised datasets need to be shared with collaborators based abroad, this will be done in compliance with the UK GDPR and Data Protection Act 2018. Where applicable, Standard Contractual Clauses (SCCs) or equivalent safeguards will be put in place for transfers to non-UK/EU countries.

All research data will be pseudonymised at the point of collection, with identifiers (e.g., name, date of birth, NHS number) stored separately in secure NHS systems. Only the study ID will be used in data analysis and shared datasets. Where feasible and appropriate, data will be fully anonymised prior to external sharing to minimise the risk of re-identification.

No third-party sub-processors outside the UK/EU are anticipated for this study. If future laboratory analyses or data processing involve external partners or subcontractors, their locations and data protection standards will be reviewed in advance, and data will only be shared under formal agreements ensuring GDPR compliance.

Only the minimum necessary data will be disclosed to authorised parties, and only under defined conditions (e.g., regulatory inspection, collaborative research agreement). No identifiable data will be included in publications or public datasets. Participants will be informed of data-sharing plans in the Participant Information Sheet, including the safeguards in place to protect their confidentiality.

7 STUDY MANAGEMENT AND OVERSIGHT ARRANGEMENTS

7.1 STUDY MANAGEMENT GROUP

The study will be co-ordinated by a Study Management Group (SMG), consisting of the grant holder Chief Investigator (CI), Principal Investigators (PIs) and clinical research assistants.

7.2 STUDY STEERING COMMITTEE

A Study Steering Committee (SC) will be established to oversee the conduct and progress of the study. The terms of reference of the SC are detailed in the S/TMF. Minutes of the SC will be maintained in the S/TMF.

7.3 DATA MONITORING COMMITTEE

An independent Data Monitoring Committee (DMC) will be established to oversee study progress. The terms of reference of the DMC are detailed in the S/TMF. Minutes of the DMC will be maintained in the S/TMF

7.4 INSPECTION OF RECORDS

The CI, PIs and all institutions involved in the study will permit study related monitoring, audits, and REC review. The CI agrees to allow the Sponsor or, representatives of the Sponsor, direct access to all study records and source documentation.

8 GOOD CLINICAL PRACTICE

8.1 ETHICAL CONDUCT OF THE STUDY

The study will be conducted in accordance with the principles of good clinical practice (GCP).

In addition to Sponsorship approval, a favorable ethical opinion will be obtained from the appropriate REC and appropriate NHS R&D approval(s) will be obtained prior to commencement of the study.

8.2 CONFIDENTIALITY AND DATA PROTECTION

The Chief Investigator (CI) and study staff will adhere to all applicable confidentiality and data protection laws, including the NHS Scotland Code of Practice on Protecting Participant Confidentiality or equivalent. All personal data will be securely stored, with access restricted to authorized staff. Personal data will only be used for study purposes and will not be shared without prior written consent from the Sponsor, except as required by regulatory bodies. If personal data must be transferred, a Data Transfer Agreement will be established. Results will be published without any identifiable personal information.

To mitigate data privacy and security risks, measures such as encryption, access controls, and regular monitoring will be implemented. Compliance with data protection regulations will be ensured throughout the study by the Data Protection Officer (DPO). Documentation such as Data Protection Impact Assessments (DPIA) and data processing agreements will be maintained for compliance verification.

8.3 INSURANCE AND INDEMNITY

The University of Dundee and Tayside Health Board are Co-Sponsoring the study.

Insurance – The University of Dundee holds Clinical Trials indemnity cover which covers the University's legal liability for harm caused to patients/participants.

Where the study involves University of Dundee staff undertaking clinical research on NHS patients, such staff will hold honorary contracts with Tayside Health Board which means they will have cover under Tayside's membership of the CNORIS scheme.

Indemnity The Co-Sponsors do not provide study participants with indemnity in relation to participation in the Study but have insurance for legal liability as described above.

9 ADVERSE EVENTS

9.1 DEFINITIONS

Adverse Event (AE)	Any untoward medical occurrence in a clinical research participant which does not necessarily have a causal relationship with study participation
Serious Adverse Event (SAE)	A serious adverse event is any untoward medical occurrence that: • results in death • is life threatening • requires hospitalisation or prolongation of existing hospitalisation • results in persistent or significant disability or incapacity • is a congenital anomaly or birth defect • Or is otherwise considered serious

9.2 RECORDING AND REPORTING AE

All SAEs will be recorded on the AE Log in the CRF and will be assessed for severity by the CI or delegate. SAEs will be recorded from the time a participant consents to join the study until the participant's last study visit.

The Investigator will make a clinical judgment as to whether or not an AE is of sufficient severity to require the participant's removal from the study. A participant may also voluntarily withdraw from treatment due to what he or she perceives as an intolerable AE. If either of these occurs, the participant should, if required, be offered an end of study assessment and be given appropriate care under medical supervision until symptoms cease, or the condition becomes stable. SAEs will be followed up until 30 days after participant's last visit.

The CI or delegate will ask about the occurrence of SAEs and hospitalisations at every visit during the study. SAEs which are both unexpected and related to study participation will be submitted on an HRA NCTIMP Safety Report form to the REC by the CI, within 15 days of becoming aware of the SAE, and copied to the Sponsor Research Governance Office.

Worsening of the condition under study will not be classed as an AE but will be defined as an outcome. Pre-specified outcome(s) will not be classed as an AE but as an outcome. Elective admissions and hospitalisations for treatment planned prior to randomisation, where appropriate, will not be considered as an AE. However, SAEs occurring during such hospitalisations will be recorded.

10 STUDY CONDUCT RESPONSIBILITIES

10.1 PROTOCOL AMENDMENTS, BREACHES

The Chief Investigator (CI) must obtain approval from the Sponsor, REC, and NHS R&D Office(s) before amending the protocol or study documents.

Any deviations from the approved protocol must be documented in the CRF, explained, and submitted to the Sponsor's Governance Office as a potential breach..

10.2 STUDY RECORD RETENTION

For non-CTIMP studies in the UK, study records must be retained for a minimum of 5 years after the completion of the study, in compliance with legal and regulatory requirements. Records should be securely stored, with access limited to authorized personnel. After the retention period, records should be safely destroyed, unless otherwise required for legal, regulatory, or ethical reasons.

At the University of Dundee, research data underlying publications should be retained for at least 10 years. This period aligns with the University's commitment to ensuring data availability for verification and future research purposes. It's important to store data securely using University-approved IT facilities and to manage data in compliance with institutional policies and legal requirements.

10.3 END OF STUDY

The end of study is defined as last patient last visit (LPLV)/database lock. The Sponsor, CI and/or the SC have the right at any time to terminate the study for clinical or administrative reasons.

The end of the study will be reported to the Sponsor and REC within 90 days, or 15 days if the study is terminated prematurely. The CI will ensure that any appropriate follow up is arranged for all participants.

A summary report of the study will be provided to the Sponsor and REC within 1 year of the end of the study.

11 REPORTING, PUBLICATIONS AND NOTIFICATION OF RESULTS

11.1 AUTHORSHIP POLICY

Ownership of the data arising from this study resides with the study team and their respective employers. On completion of the study, the study data will be analysed and tabulated, and a clinical study report will be prepared. Participants will be informed of the study results once the analysis is complete and the findings have been reviewed for accuracy and published in scientific outlets.

We expect this to be after the end of the study and once results are ready to share in a reliable and accessible format. Results will be shared in plain-language summaries, developed with input from our Patient and Public Involvement (PPI) group. These will be distributed via: (a) Direct email or post to participants who indicate they would like to receive them (b) The University of Dundee and Asthma + Lung UK websites (c) Newsletters, public talks, and patient group meetings. Summaries will be written in clear, non-technical language, highlighting what the study found, why it matters, and what the next steps will be. Participants will be asked at the time of consent if they would like to receive a copy of the study results, and their preferences will be respected.

11.2 PUBLICATION

The clinical study report will be used for publication and presentation at scientific meetings. Investigators have the right to publish orally or in writing the results of the study.

Summaries of results will also be made available to Investigators for dissemination within their clinical areas (where appropriate and according to their discretion).

12 REFERENCES

1. Chan R, RuiWen Kuo C, Lipworth B. Pragmatic Clinical Perspective on Biologics for Severe Refractory Type 2 Asthma. *J Allergy Clin Immunol Pract* 2020; **8**(10): 3363-70.
2. Chan R, Stewart K, Misirovs R, Lipworth BJ. Targeting Downstream Type 2 Cytokines or Upstream Epithelial Alarmins for Severe Asthma. *J Allergy Clin Immunol Pract* 2022; **10**(6): 1497-505.

3. Shrimanker R, Keene O, Hynes G, Wenzel S, Yancey S, Pavord ID. Prognostic and Predictive Value of Blood Eosinophil Count, Fractional Exhaled Nitric Oxide, and Their Combination in Severe Asthma: A Post Hoc Analysis. *Am J Respir Crit Care Med* 2019; **200**(10): 1308-12.
4. Chan R, Lipworth B. The triple type 2 signature confers more frequent exacerbations and worse midexpiratory flow in moderate to severe asthma. *J Allergy Clin Immunol Pract* 2023; **11**(9): 2926-8.e1.
5. Fitzpatrick AM, Chipps BE, Holguin F, Woodruff PG. T2-"Low" Asthma: Overview and Management Strategies. *J Allergy Clin Immunol Pract* 2020; **8**(2): 452-63.
6. Cox G. Glucocorticoid treatment inhibits apoptosis in human neutrophils. Separation of survival and activation outcomes. *J Immunol* 1995; **154**(9): 4719-25.
7. Gibson PG, Yang IA, Upham JW, et al. Effect of azithromycin on asthma exacerbations and quality of life in adults with persistent uncontrolled asthma (AMAZES): a randomised, double-blind, placebo-controlled trial. *Lancet* 2017; **390**(10095): 659-68.
8. Keir HR, Shoemark A, Dicker AJ, et al. Neutrophil extracellular traps, disease severity, and antibiotic response in bronchiectasis: an international, observational, multicohort study. *Lancet Respir Med* 2021; **9**(8): 873-84.
9. Wechsler ME, Laviolette M, Rubin AS, et al. Bronchial thermoplasty: Long-term safety and effectiveness in patients with severe persistent asthma. *J Allergy Clin Immunol* 2013; **132**(6): 1295-302.
10. Thomson NC. Recent Developments In Bronchial Thermoplasty For Severe Asthma. *J Asthma Allergy* 2019; **12**: 375-87.