AIRWAY REMODELLING IN RESPONSE TO NOVEL ASTHMA THERAPIES

Thesis submitted for the degree of

Doctor of Philosophy

at the University of Leicester

By

Richard Russell MBBS MRCP (UK)

Department of Respiratory Sciences

University of Leicester

March 2020

Abstract

Airway Remodelling in Response to Novel Asthma Therapies

Dr Richard Russell

Airway remodelling encompasses a range of structural changes seen in the airways of asthma patients, and can be assessed in bronchial biopsy samples. However, there is a paucity of data investigating remodelling responses to asthma therapies. The relationships between airways remodelling and airways inflammation are also not fully understood.

In this thesis I present an overview of asthma pathogenesis, including specific mechanisms underlying both airway inflammation and airway remodelling, before investigating the responses to two novel asthma therapies. I firstly examine the changes seen in airway inflammation and airway remodelling following 12 weeks of treatment with an anti-interleukin-13 antibody. Secondly, I investigate the remodelling responses to bronchial thermoplasty. Where remodelling changes are observed in response to therapy I investigate how this relates to clinical outcomes.

Inflammatory and remodelling responses to anti-interleukin-13 were not significantly different to placebo, despite reductions in exhaled nitric oxide and immunoglobulin-E demonstrating engagement with the target receptor. This established that inhibition of interleukin-13 in isolation does not lead to significant remodelling or inflammatory changes in moderate-to-severe asthma.

Bronchial thermoplasty led to significant improvements in airway remodelling (airway smooth muscle mass, reticular basement membrane thickness and epithelial integrity), although direct relationships between these changes and clinical improvements appear weak or absent. Data from a small number of patients suggests that improvements in epithelial integrity may be more important to clinical benefits than reductions in airway smooth muscle mass, but further investigation is needed.

This thesis contributed new data to the understanding of remodelling and inflammatory pathways in asthma. It also reports the largest study undertaken examining remodelling changes, and their relationships to clinical outcomes, in response to bronchial thermoplasty. Finally, it has provided new evidence of significant epithelial repair after thermoplasty, which may be a key contributor to clinical improvements.

Acknowledgements

The work presented in this thesis was undertaken as part of numerous teams and collaborations; the list of those who contributed being too long to cover here in its entirety. The help of these many unmentioned colleagues and friends is very much valued.

My first thanks go to my supervisor Professor Chris Brightling, for all of the time, insight, opportunity and inspiration he has afforded me over the last few years. And particularly for his flexibility and patience with my approach to deadlines. Thanks also to my second supervisor Professor Pete Bradding, for his encouragement and support along the way.

I am indebted to Latifa Chachi, for her patience and good humour while teaching me the ways of histology and immunohistochemistry. And to my predecessor in post, Rachid Berair, who set in place the foundations of the thermoplasty study.

My thanks go to everyone involved in the MESOS study, across the 15 recruiting centres and at AstraZeneca. Particular thanks are deserved by Sarah Parker, Debbie Parker, Simonetta Baldi, Gill Elliott, Hilary Marshall, Vijay Mistry, Patricia Nisa, Lauren Wilkes, Malak Baderkhan, Marcia Soares and Matt Richardson.

Thank you to the following investigators, and their local teams, for their contributions to the AirPROM thermoplasty study; Rekha Chaudhuri (Glasgow), Peter Howarth (Southampton), Adel Mansur (Birmingham), and Salman Siddiqui, Neil Martin and Rakesh Panchal (Leicester); and to the pooled thermoplasty study; Michel Aubier and Marina Pretolani (Paris, France), Pascal Chanez (Marseille, France), Michel Laviolette and Jamila Chakir (Quebec, Canada), Daiana Stolz and Desiree Schumann (Basel, Switzerland), Mario Castro (St. Louis, USA), and Peter Bonta (Amsterdam, Netherlands).

For adding plenty of extra enjoyment and cake to time at work, I thank all who have come and gone within the clinical fellows' office; in particular Wadah

Ibrahim, Sarah Diver, Leena George, Ahmed Yousuf, Raman Verma, Jo Lee, Cate Weston, and Christobelle White; and all of the wonderful research nurses and physiologists in the Leicester Respiratory BRC. Special thanks goes to Amisha Singapuri, my work sister, for helping with absolutely everything, from late night biopsy data QC and endless data queries, to recommendations for gelato.

My love and thanks to my Mum and Dad for their continued encouragement and guidance. The biggest thanks of all go to my wife Mel for her unfailing patience, support, and love, and for first giving me the belief that I could 'do research'; and to my son Julian, who arrived during the course of this PhD, and continues to be my ultimate inspiration.

Finally, I thank the patients who volunteered their time, energy, and all manner of bodily samples to the studies described in this thesis. Without these generous people none of this research would be possible.

Statement of work personally performed

The anti-interleukin-13 monoclonal antibody study (MESOS) was a multicentre trial, in which 79 patients were recruited from 15 centres in the UK, Denmark and Canada. Along with a research nurse I led the recruitment of the 8 subjects that Leicester contributed to the study population. My role included screening and consenting potential patients, and collecting data at each study visit (including clinical assessment and physical examination; measures of lung function and airway inflammation; blood, sputum, and bronchial biopsy collection; and computed tomography (CT) scans). I analysed all of the epithelial and basement membrane remodelling markers on baseline and follow-up biopsies for all 79 subjects (including measuring epithelial integrity and reticular basement membrane thickness, counting epithelial goblet cells, and performing stain intensity scoring and thresholding for MUC5AC, eCadherin, Involucrin, and cytokeratin-7 stained slides), amounting to 2880 data points. I contributed significantly to data analysis and interpretation for the study as a whole, and led the writing of the published manuscript, including designing the figures and tables. I also presented the results at the American Thoracic Society conference (May 2018).

The small bronchial thermoplasty study was undertaken as part of the AirPROM project, and was already recruiting when I began working on it. I co-ordinated the later stages of the study, including consenting patients and collecting study visit data for the Leicester patients (performing clinical assessments and examinations; measures of lung function and airway inflammation; blood and sputum collection; CT scans; and assisting in the collection of bronchial biopsies at bronchoscopy). I also co-ordinated thermoplasty treatment visits and assisted in delivering thermoplasty treatment for the Leicester patients. Additionally, I processed the data received from the other three collaborating research centres, including generating and processing data queries. I undertook all of the analysis on the bronchial biopsy samples obtained at all four centres (including measuring

structural areas, reticular basement membrane thickness, epithelial integrity, and myofibroblast counts). I undertook all of the data analysis, and have presented some of the data at the European Respiratory Society congress (September 2017).

For the larger thermoplasty pooled biopsy study I contributed to initial discussions with potential collaborators and undertook a survey of data available at each centre. Based on this survey I created an electronic data collection sheet tailored to the available information and liaised closely with contributing centres to centralise all of the data. I performed the biopsy analysis for the Leicester and Basel bronchial biopsy samples (area measurements, reticular basement membrane thickness and epithelial integrity). I analysed all of the data, and have presented some of the results at the European Respiratory Society congress (October 2019).

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Publications arising from this thesis

* denotes equal contribution of authors

Original research articles

- Chernyavsky IL*, **Russell RJ***, Saunders RM*, *et al*. In vitro, in silico and in vivo study challenges the impact of bronchial thermoplasty on acute airway smooth muscle mass loss. *Eur Respir J.* 2018 May;51(5). pii: 1701680
- **Russell RJ**, Chachi L, FitzGerald JM, *et al*. Effect of tralokinumab, an interleukin-13 neutralising monoclonal antibody, on eosinophilic airway inflammation in uncontrolled moderate-to-severe asthma (MESOS): a multicentre, double-blind, randomised, placebo-controlled phase 2 trial. *Lancet Respir Med.* 2018 Jul;6(7):499-510
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Prizes

- **Russell RJ**. Bronchial Thermoplasty: More to it than muscle? *East Midlands Thoracic Society*. Oct 2016. **Best Spoken Abstract**

Abbreviations

ACQ	Asthma control questionnaire
ACT	Asthma control test
AE	Adverse event
AHR	Airway hyper-responsiveness
AQLQ	Asthma quality of life questionnaire
ASM	Airway smooth muscle
BT	Bronchial thermoplasty
CRTH2	Chemoattractant receptor-homologous molecule expressed on Th2 cells
CT	Computed tomography
DP2	D prostanoid receptor 2
DPP4	Di-peptidyl pepitidase-4
ECP	Eosinophil cationic protein
FeNO	Fractional exhaled nitric oxide
FEV1	Forced expiratory volume in 1 second
FVC	Forced vital capacity
GM-CSF	Granulocyte-monocyte colony stimulating factor
ICS	Inhaled corticosteroids
IgE	Immunoglobulin-E
IL	Interleukin
ILC	Innate lymphoid type 2 cells
LABA	Long-acting beta-agonist
LLL	Left lower lobe
RBM	Reticular basement membrane

MBP	Major basic protein
MCID	Minimum clinical important difference
µg	Micrograms
µm	Micrometres
mg	Milligrams
ml	Millilitres
mm	Millimetres
OCS	Oral corticosteroids
PGD2	Prostaglandin D2
ppb	Parts per billion
Q2W	Every 2 weeks
RLL	Right lower lobe
RML	Right middle lobe
RV	Residual volume
SAE	Serious adverse event
SC	Subcutaneous
SD	Standard deviation
SEM	Standard error of the mean
SNP	Single-nucleotide polymorphism
TGF-β	Transforming growth factor β
Th	T-helper
TLC	Total lung capacity
TSLP	Thymic stromal lymphopoietin
ULs	Upper lobes

1 Introduction

1.1 Background and epidemiology of asthma

Asthma is a common respiratory condition, and affects an estimated 300 million people worldwide [1]. Approximately 20 million of these are moderately or severely disabled by the disease [2]. The prevalence of asthma is reported as between 1 and 18% of the population depending on the country surveyed, and is increasing in many regions [3]. An estimated 250,000 to 345,700 deaths are attributed to asthma worldwide every year [1, 4]. In the UK there are over 65,000 asthma related hospital admissions per year, with approximately 5.4 million people receiving asthma treatment [5].

Asthma is clinically characterised by a typical constellation of symptoms (cough, wheeze, shortness of breath and chest-tightness) in combination with evidence of expiratory airflow limitation [3]. Symptoms and airflow limitation can be extremely variable, both between patients and within an individual at different points in time [3].

The pharmacological treatment of asthma involves step-wise increases in the dose of current medications, or the addition of further medications, if asthma control is deemed to be inadequate on the current treatment regime. Therefore, the treatment of severe asthma often involves numerous medications targeting different mechanistic pathways [3, 6, 7]. Yet despite this approach, some patients continue to suffer persistent symptoms and frequent exacerbations, and are therefore diagnosed with 'difficult-to-treat' or 'refractory' asthma [3, 8]. These patients require a holistic multi-disciplinary approach to their management, which may involve specialist nurses, physiotherapists, medical psychologists, and speech and language therapists [7]. To ensure optimisation of treatment, it is increasingly clear that a detailed understanding of the underlying mechanisms of individual patients' disease is vital in directing their care. As additional new, and often

expensive, asthma treatments become available this personalised approach to treatment becomes ever more critical.

The heterogeneous nature of asthma gives rise to significant inter-patient variability in not only clinical characteristics, but also the type and degree of airway inflammation, airway hyper-responsiveness, and airway remodelling present [8, 9]. Various phenotype models have been developed, based on clinical characteristics [9, 10], but underlying disease mechanisms are not necessarily reflected in these observable features. Two patients with very different asthma phenotypes and severities may have the same underlying pathogenesis; while conversely, two patients with a similar clinical picture may have greatly contrasting disease mechanisms. Differentiating patients according to the underlying functional and pathological disease process, or endotype, may hold much greater utility in choosing the most effective treatment strategy [11, 12].

1.2 Asthma pathogenesis

The pathogenesis of asthma involves a complex web of interacting factors at all scales of the disease; from genetic, to cellular, to tissue, to organ. It is complicated further by the effects of environmental influences, pharmacotherapy and non-asthma-specific patient factors such as co-morbidity, lifestyle, and treatment adherence. Asthma pathogenesis can be considered in four principal domains; $T2^{HIGH}$ airway inflammation, $T2^{LOW}$ airway inflammation, airway hyper-responsiveness (AHR), and airway remodelling. Each of these elements spans the scales of disease described above, whilst also overlapping and impacting on one another. To further complicate the picture, these disease elements not only co-exist within an individual asthmatic, but their interactions over time, or in response to an exposure or intervention, are often variable.

Although far from complete, advances in our understanding of asthma disease mechanisms has led to the development of a range of targeted treatments, and

begins to offer opportunities for a precision medicine approach for the individual patient. At present the overlaps in our understanding of different endotype groups and the varied effects of therapy remain significant, but future progress in unpicking asthma pathogenesis will hopefully bring some clarity. In addition, a significant proportion of our current knowledge originates from animal and *in vitro* models, and therefore needs validating by *in vivo* human studies.

With the growth of biomarkers and a more detailed understanding of endotype profiles it should hopefully soon be possible to characterise an individual patient's disease in great detail and tailor their treatment to that which will give the most clinical benefit. This is critical in developing future precision medicine strategies for asthma, in a bid to reduce the burden of the disease to healthcare providers, and most importantly, to patients.

$T2^{HIGH}$ airway inflammation is the most commonly encountered inflammatory pattern in asthma [8, 13, 14], with predominantly eosinophilic inflammation and high levels of T2 cytokines such as interleukin [IL]-4, 5 and 13. Severe asthmatics with persistent T2-mediated inflammation are high risk for loss of asthma control and frequent severe asthma exacerbations [15]. Novel therapies targeting T2 cytokines, such as IL-5, have proven efficacious in reducing asthma exacerbations in patients with markers of $T2^{HIGH}$ disease, likely due to suppression of eosinophil number and activity [16-18]. $T2^{LOW}$ inflammation is characterised by primarily by non-eosinophilic T1 and T17 pathways, with or without neutrophilic inflammation and oxidative stress [8, 13, 14]. Interestingly, some asthmatics switch between T2/T17 phenotypes although these phenotypes rarely co-exist [19]. Airway hyper-responsiveness reflects increased or dysfunctional airway smooth muscle (ASM) contraction due to a range of direct and indirect stimuli [20]. Airway remodelling encompasses changes in the airway epithelium, leading to ciliary dysfunction and reduced barrier function; increased matrix deposition and ASM mass; and mast cell localisation to the ASM [21-23]. These differing disease components co-exist within the individual patient, but their interactions over time, or their response to intervention, are poorly understood.

The onset of asthma symptoms can occur at any age. Children who develop asthma during their early years may continue to suffer asthma as an adult, but a proportion will have full resolution of their symptoms during childhood. Contrastingly, some asthmatic adults have no symptoms in childhood and only develop the disease later in life. Factors in early life that increase an individual's risk of developing asthma, and influence whether asthma persists into adulthood, include female gender, smoke exposure, atopy, and the severity of childhood symptoms [24]. Regardless of the pattern of onset, both adults and children with asthma can have a relapsing and remitting nature to their disease at different times of their life; sometimes with significant time separating episodes of illness. Even during periods of symptom remission, a large proportion of patients still exhibit evidence of airway hyper-responsiveness and/or airway remodelling [24]. This highlights the discord often present between symptoms and disease activity, and emphasises the importance of ongoing treatment and monitoring, even during periods of disease stability, in order to minimise a patient's future risk from under-treated asthma. Importantly, asthma can be mild, moderate or severe [24] at the point of disease onset, and severe asthma is not necessarily the result of a gradual progression from mild, to moderate, to severe disease. The vast variability in disease onset and clinical course is evidence that asthma is not simply an outward manifestation of an entirely intrinsic process, but indicates that a range of interacting intrinsic and extrinsic factors are responsible for disease development and progress.

A genetic susceptibility to asthma has been known for many years, with strong family trends towards asthma and other atopic diseases. A number of large Genome-Wide Association Studies (GWAS) have been undertaken to identify specific sets of asthma-related genes, and to characterise links between the genetic and clinical characteristics of the disease.

Numerous gene associations for asthma have been identified, including single-nucleotide polymorphisms (SNPs) found on chromosome 2 (IL18R1 and IL1RL1), chromosome 5 (RAD50, IL4 and IL13 on the Th2 cytokine locus), chromosome 6

(HLA-DQ region of the major histocompatibility complex gene), chromosome 9 (flanking the IL33 gene), chromosome 15 (SMAD3) and chromosome 22 (IL2RB) [25-27]. The IL33 gene encodes for the production of the cytokine interleukin-33, which is present in airway epithelial cells, and particularly in damaged tissues [25]. Interleukin-33 stimulates production of Th2-associated inflammatory cytokines interleukin-4, interleukin-5, and interleukin-13, which play numerous important roles in asthma pathogenesis (discussed in detail below). The IL18R1 and IL1RL1 locus on chromosome 2 also appears to be functionally related to interleukin-33 activity, as IL1RL1 encodes for the ST2 receptor, to which IL-33 binds to exert its various pro-inflammatory effects [25]. SMAD3 and IL2RB may have regulatory roles in healing and repair, as well as T-cell function [25], and are therefore potentially important in airway remodelling.

Several SNPs on chromosome 17q21 show different associations between childhood onset asthma and adult onset asthma [25, 28]. One such gene, which shows strong correlations with the development of childhood asthma, is ORMDL3 [28], and ORM genes may have a role in airway inflammation, although this has not yet been shown in human subjects. The CDHR3 gene is also associated with the presence of asthma in children aged between two and six years old [29]. CDHR3 may be implicated in airway remodelling, and in particular with the regulation of epithelial integrity, and could therefore be important in airway responses to inhaled insults such as pathogens and pollutants [29]. Differential expression of the CDHR3 gene in young children highlights the early age at which remodelling changes in the airway can occur.

ORMDL3 and IL1RL1/IL18R1 have been validated as significant gene associations in a cohort of severe asthmatics, although no genes specific to severe asthma have so far been identified [30]. However, some of the gene SNPs described here correlate with the risk of hospitalisation due to severe asthma exacerbation [29], thereby potentially implicating genetic factors in the development of specific phenotype traits such as exacerbation susceptibility and resistance to therapy.

Correlations have also been identified between specific gene expression and patterns of lung function decline [31], although further work is needed in this area.

The future possibility of targeted gene therapies for asthma carries significant appeal, with the theoretical potential to treat, cure, or even prevent asthma in genetically susceptible individuals. However, despite the identification of genes associated with asthma, the presence of these genes has not proven particularly sensitive or specific as a predictor of asthma diagnosis in an individual patient, even when multiple genes are used in combination [25]. Nor have genetic differences been shown to be directly related to other important clinical markers such as serum immunoglobulin (Ig) E levels [25]. This illustrates that genetic factors cannot be considered in isolation, as they are only one of many important host and environmental interactions in an individual's asthma. Even if gene therapies do become a reality for asthma, other disease factors may limit their application and success.

1.2.1 $T2^{HIGH}$ inflammation

$T2^{HIGH}$-mediated eosinophilic disease, heavily consequent on T-cell polarisation by the transcription factor GATA3, is the dominant inflammatory profile across the asthma spectrum [8, 13, 14]. Sputum eosinophilia is a feature in up to 80% of corticosteroid naïve, and 50% of corticosteroid treated asthmatics [32, 33]. In addition to blood and/or tissue eosinophilia these patients also exhibit high levels of T2 cytokines such as interleukins IL-4, IL-5 and IL-13 [8, 13, 14], which are critical to the regulation of eosinophilic inflammation.

$T2^{HIGH}$ eosinophilic inflammation is most commonly associated with atopy, and allergic asthma accounts for most cases in children and around half of cases in adults. The hallmark of atopic and allergic disease is a raised level of serum Immunoglobulin (Ig) E, although the presence of high levels of IgE in isolation is neither a good predictive marker for the development of asthma, nor whether T2-mediated inflammation is the dominant disease profile in patients who have asthma [34]. Sputum eosinophilia has been shown to be a more robust marker of

T2-mediated disease, and can be reliably reproduced in the short term [35, 36]. However, the inflammatory profile of some asthmatics can vary greatly over time. For example, in a study of 995 asthma patients, 31% of subjects were found to have an intermittent eosinophilia compared to 22% with a persistent eosinophilia [14].

There is some evidence to suggest that almost all asthmatics have an element of $T2^{HIGH}$ disease, even in the absence of an obviously demonstrable eosinophilia. Firstly, reduction of T2-inflammation suppressing corticosteroids in apparently non-eosinophilic asthmatics leads to the emergence of detectable eosinophil levels in most cases [37]. Secondly, eosinophils are cleared from the lung by macrophages, and the rate of clearance can be determined by measurement of the amount of eosinophil proteins in airway macrophages. In apparently non-eosinophilic patients, examination of eosinophil proteins in airway macrophages confirms the presence of eosinophil clearance processes, suggesting eosinophilic inflammation which is being controlled by therapy [37].

Conversely, some patients have high sputum eosinophil counts but low levels of airway macrophage eosinophil clearance, suggesting treatment resistance due to either macrophage dysfunction or inadequate quantities of steroid reaching the airways [37]. Those severe asthmatics with persistent $T2^{HIGH}$ inflammation and a significant sputum eosinophilia are more likely to suffer from uncontrolled asthma, and have a high risk of asthma exacerbation [15]. Similarly, serum periostin is another marker of T2 activity, and higher periostin levels are associated with a greater exacerbation frequency [38]. The use of a composite biomarker score derived from blood eosinophil, serum periostin, and exhaled nitric oxide values to wean corticosteroid therapy is currently being evaluated as part of the RASP-UK clinical trial [39, 40].

As described above, the eosinophil is central to $T2^{HIGH}$ inflammation. Eosinophil function is largely regulated by a complex immune response involving numerous stimulatory and inhibitory inflammatory cytokines and effector cells. OX40 and its

ligand OX40L, and epithelial derived thymic stromal lymphopoietin (TSLP), are early stimulators of T2-mediated inflammatory processes. They promote both the innate and adaptive immune response, and the activity of both TSLP and OX40L is elevated in response to inhaled allergens [41]. Following allergen exposure TSLP originating from the epithelium directly stimulates mast cell activity and triggers maturation of immature dendritic cells. Mature dendritic cells produce OX40L and migrate into lymph nodes. Here they cause differentiation of naïve CD4+ T cells into inflammatory Th2 cells (with subsequent promotion of T2 inflammatory cytokines such as interleukins as described below), and blockade of the TSLP/OX40 axis has been shown to reduce Th2 related inflammation [41].

OX40L inhibition may potentially be disease modifying and prevent the development of allergic asthma in early life, as it could interrupt the allergic sensitisation process. However, this hypothesis is difficult to test as it would require identifying and treating susceptible children (such as those with childhood wheeze), and as such it has not been investigated. When used in adults with established asthma anti-OX40L therapy does reduce serum IgE and sputum eosinophils but has no effect on allergen-induced airway responses [42], presumably because the sensitisation process has already occurred. TSLP has a role in both allergic sensitisation and also ongoing T2 inflammation, and so anti-TSLP treatment appears more promising. Treatment with a human anti-TSLP monoclonal antibody reduces blood and sputum eosinophil levels and lowers exhaled nitric oxide, as well as improving lung function measures after allergen challenge [43]. Thus anti-TSLP treatment holds the potential to be both disease modifying and disease controlling, and further clinical trials are currently ongoing.

Th2 cells release inflammatory cytokines and activate a series of downstream processes which play key roles in eosinophilic inflammation [34]. Eosinophils develop in the bone marrow from pluripotent CD34+ progenitor stem cells, which differentiate into eosinophils under the influence of granulocyte-monocyte colony stimulating factor (GM-CSF) and interleukin (IL)-3 in the early stages, and IL-5 in the later stages [44]. CD34+ cells bearing receptors for IL-3 and IL-5 have been

shown to be present in higher numbers in the bone marrow of subjects with asthma in response to an allergen challenge [45, 46], suggesting an acute phase response in the bone marrow to produce and develop additional eosinophils following allergic stimulus. The continued influence of IL-5 stimulates release of mature eosinophils from the bone marrow into circulating blood.

Once in the circulation eosinophils remain relatively inactive until they are primed. Priming increases their responsiveness to chemotaxis, degranulation and cytokine production and is also mediated by cytokines IL-3, IL-5 and GM-CSF [47, 48]. Once primed, eosinophils move into the tissues under the influence of cytokines including IL-4, IL-5 and IL-13 [49]. This process first requires the eosinophil to adhere to the vascular endothelium, from where they can migrate into the surrounding tissues. Interactions between integrins on the surface of eosinophils and adhesion receptors on the surface of vascular endothelium (such as P-selectin/P-selectin glycoprotein ligand-1 and VCAM-1 ligand) are critical to this process [50, 51]. P-selectin and VCAM-1 are upregulated by IL-4 and IL-13, again highlighting the critical role that cytokines IL-4, IL-5 and IL-13 play at several stages of eosinophil development and function [48, 52, 53].

Once adherence to the vascular endothelium has occurred, eosinophils are recruited into the lung mucosa under influence from the eotaxin family of chemokines (CCL11, CCL24 and CCL26) [54]. These chemokines are upregulated following allergen exposure, and act on the CCR3 chemokine receptor expressed on the eosinophil cell surface [54]. In the lung tissue eosinophil survival is prolonged by IL-5 and GM-CSF produced locally [55].

The eosinophil itself regulates several aspects of the asthma response. Specific basic proteins released by eosinophils located in the lung tissue cause damage to the bronchial epithelium (Major Basic Protein - MBP, Eosinophil Cationic Protein – ECP, Eosinophilic Peroxidase – EPO, Eosinophil-derived Neurotoxin – EDN), and MBP can also induce airway hyper-responsiveness (see below) [55]. Eosinophils are also a source of cysteinyl leukotrienes (along with basophils, mast cells,

macrophages and myeloid dendritic cells) [55, 56], which act primarily to cause bronchoconstriction, especially in asthmatics who are sensitive to aspirin [56].

D prostanoid receptor 2 (DP2), also called chemoattractant receptor-homologous molecule expressed on Th2 cells (CRTH2) receptor, has been implicated in allergic disease [57], and there is an increased level of DP2 positive inflammatory cells in patients with allergy and asthma [57]. Activated mast cells produce Prostaglandin D2 (PGD2), which acts as a DP2 agonist [58], and elevated levels of PGD2 have been found in bronchoalveolar lavage samples from patients with asthma [57]. Furthermore, bronchial biopsy samples from patients with severe asthma have shown that DP2 positive cells are more frequent in the airway submucosa (particularly DP2 positive T cells), although less frequent in the bronchial epithelium, compared to healthy controls [57]. Activation of the DP2 receptor on Th2 cells has been shown to increase production of IL-2, IL-4, IL-5 and IL-13 [57], and promote eosinophil production, migration, recruitment into the lung tissue, and survival. For this reason the DP2 receptor is of interest as a therapeutic target, and a phase 2 study of a DP2 receptor antagonist showed a significant reduction in sputum eosinophil count in asthmatics with a baseline sputum eosinophilia compared to placebo [59]. Results from phase III trials are expected soon.

In the absence of allergy, eosinophilic inflammation can still arise in response to epithelial damage from inhaled pollutants and microbes. The epithelial 'alarmins' IL-25, IL-33 and TSLP are cytokines involved in the early stages of the inflammatory process. They are typically released in response to airway epithelial damage, and promote a T2 immune response even when allergy/atopy is not present.

Expression of IL-25 is greater in the tissues of patients with asthma, and IL-25 appears to be implicated in viral exacerbations of asthma. Asthmatic bronchial epithelial cells show a significantly heightened response in IL-25 production following infection with rhinovirus, and the magnitude of this response is also related to the degree of allergy within the patient [60]. Increased levels of IL-25 in response to rhinovirus infection are also associated with increases in IL-4, IL-5 and

IL-13, suggesting that IL-25 mediated responses in bronchial epithelial cells result in release of T2 inflammatory cytokines and upregulation of the T2 response [60]. In support of this, blocking IL-25 has been shown to reduce inflammation, airway hyper-responsiveness and T2 cytokine (IL-5 and IL-13) production [60].

IL-33 is released from necrotic epithelial cells, probably in response to injury from allergens, but also from microbes and airborne pollutants. It binds to the ST2 receptor present on a wide range of effector cells, and exhibits a number of functions, including stimulating innate lymphoid type 2 cells (ILC2s) and Th2 cells to upregulate secretion of IL-4, IL-5 and IL-13, thereby promoting eosinophil adhesion and survival [61]. IL-33/ST2 binding on mast cells, macrophages and basophils also upregulates secretion of inflammatory cytokines [61]. In addition, IL-33 promotes the maturation of CD34+ cells into mast cells, and stimulates CD34+ progenitor cells to secrete IL-5, IL-6, IL-13, CXCL8, CCL1 and CCL17, resulting in a heightened allergic response [61]. Anti-IL-33 therapy is in phase 2 development.

In addition to allergen stimulation of the epithelium, TSLP is also upregulated in response to mechanical epithelial injury, viruses and pro-inflammatory cytokines [62]. As described above, TSLP promotes T2 pathways through interactions with epithelial dendritic cells, but it also appears to exert a number of other direct functions including promotion of eosinophil survival and adhesion [62].

ILC2s have been implicated in non-atopic eosinophilic asthma. These recently identified cells are thought to be key mediators in the production of T2 cytokines and other mediators of tissue growth, inflammation and repair [63]. For example, mast cell derived PGD2 interacts with ILC2s at the CRTH2 receptor to promote cytokine production [63]. Significantly higher amounts of ILC2s are present in the peripheral blood of asthmatics, suggesting that they play an important role in the pathogenesis of the disease [63], although evidence for their specific function in this role is relatively limited at present, partly due to practical difficulties in isolating specific ILC populations for study.

Advances in the understanding of $T2^{HIGH}$ inflammation have led to the development of several new treatments in asthma. Monoclonal antibodies against IL-4α subunit receptor, IL-5, and IL-5α receptor have all shown clinical effectiveness in reducing eosinophilic inflammation. Blocking the α-subunit of the IL-4 receptor effectively inhibits both IL-4 and IL-13 activity, and studies using an anti-IL-4α monoclonal antibody (dupilumab) have shown an improvement in lung function, asthma control and reduction in asthma exacerbation frequency, when used to treat moderate to severe asthmatics with a peripheral blood eosinophilia [64, 65]. Similarly, binding IL-13 with the monoclonal antibodies lebrikizumab or tralokinumab has led to improvements in lung function in phase II studies [66, 67]. Anti-IL-5 therapy (mepolizumab, reslizumab) and anti-IL-5R is now licensed for use in severe eosinophilic asthma following trial evidence confirming reductions in both exacerbation rate and eosinophil levels when given to selected eosinophilic asthmatics with frequent exacerbations [17, 18, 68-72]. Mepolizumab has also been shown to allow reduction of maintenance oral corticosteroid dose without loss of asthma control for patients taking oral corticosteroids [16]. Inhibition of the upstream 'alarmins' IL-25, IL-33 and TSLP may potentially be an even more effective therapeutic approach, by simultaneously reducing multiple downstream cytokines such as IL-4, IL-5, and IL-13, with promising results from anti-TSLP in a phase II trial [73]. Phase III trial evidence is not yet available.

1.2.2 $T2^{LOW}$ inflammation

$T2^{LOW}$ inflammation is mediated predominantly by non-eosinophilic T1 and T17 pathways, with or without neutrophilic inflammation and oxidative stress [8, 13, 14]. Some asthmatics switch between T2 and T17 inflammatory profiles, although T2 and T17 inflammation rarely occurs simultaneously [19]. Patients with isolated $T2^{LOW}$ neutrophilic airway inflammation (and absence of T2 cytokines) are more likely to have non-atopic late-onset asthma and an impaired response to inhaled corticosteroid treatment [13], although significant variation in clinical presentation can exist. Suppression of T2 inflammation appears to upregulate T17 immunity and increases T1/T17 cytokines [19]; therefore some neutrophilic

asthma may be iatrogenic, occurring as a consequence of T2-suppressing asthma therapies such as corticosteroids. This persistent neutrophilic inflammation, with or without co-existent T2 inflammation, may be an important contributor in asthma that is unresponsive or poorly responsive to steroids.

In addition to the ILC2 cells implicated in $T2^{HIGH}$ inflammation, ILC precursor cells can alternatively differentiate into ILC3 cells under the influence of transcription factors such as RORγT [63]. ILC3 cells are important regulators of T17 inflammation, producing IL-17A, IL-17F, IL-22, GM-CSF and tissue necrosis factor (TNF) [63].

One suggested mechanism for neutrophilic airway inflammation is bacterial colonisation and the presence of bronchiectasis. However, a study of patients with severe asthma undergoing CT scans (most often for suspected concurrent bronchiectasis) found similar levels of sputum neutrophils in patients with and without CT defined bronchiectasis [74], suggesting a neutrophilic sputum profile is not necessarily linked to the development of bronchiectasis, and that other factors are contributory.

The concept of bacterial colonisation and secondary inflammation (independent of the presence of bronchiectasis) could explain the lack of efficacy of anti-neutrophil treatments (summarised in [75]) and the suggestion of some efficacy from antibiotic therapy for $T2^{LOW}$ inflammation. Neutrophil function in asthmatics is impaired compared to health which could lead to increased susceptibility to infection and asthma exacerbation [76]. Allergic inflammation has been shown to increase susceptibility for an acute infection with the common respiratory pathogen Haemophilus influenzae to develop into chronic infection or colonisation [77]. H. influenzae colonised asthmatics have increased levels of IL-17 with subsequent recruitment of additional neutrophils to the airway [77]. Despite increased neutrophil numbers in the airway of these patients, treatment with steroids appeared to further increase the bacterial load, suggesting

traditional asthma therapy may actually worsen this situation where neutrophils are abundant in the airway but seemingly dysfunctional [77].

Treatment with the macrolide antibiotic azithromycin at low dose (250mg three times weekly) for 6 months reduces exacerbation frequency only in non-eosinophilic asthmatics; possibly suggesting that bacterial colonisation in $T2^{LOW}$ patients is a significant contributing factor to their disease behaviour and exacerbation risk [78]. A more recent, large study using a higher dose (500mg three times weekly for 48 weeks) showed a reduced exacerbation rate and improved quality of life in an uncontrolled asthma population taking inhaled corticosteroids regardless of inflammatory profile [79]. However, it is not clear whether this clinical benefit is related to the anti-bacterial or anti-inflammatory properties of macrolide antibiotics. In contrast, treatment with a 3-day course of azithromycin in response to an asthma exacerbation does not achieve any significant benefit in symptoms, quality of life or lung function during recovery from the exacerbation [80], although this was not a selected $T2^{LOW}$ population.

T17 cells produce the cytokines IL-17A, IL-17F and IL-22, which in turn promote the release of a range of other pro-inflammatory cytokines and chemokines; predominantly from neutrophils, but also from epithelial and vascular endothelial cells, fibroblasts, and eosinophils [81]. These include IL-6, GM-CSF, CXCL10 and CXCL8. CXCL8 is a potent neutrophil chemokine, suggesting a role for T17 cytokines in neutrophilic airway inflammation, although this has not been conclusively proven [81]. IL-17A expression in the bronchial submucosa is elevated in mild-moderate asthma compared to healthy non-asthmatics, although this is not the case in severe asthma [81]. IL-17F expression in the bronchial submucosa is increased in all severities of asthma when compared to healthy controls [81]. It is not known whether the lack of increased expression of IL-17A in severe asthma is truly reflective of pathology, or is indicative of the effects of high-dose corticosteroids taken in these patients on the T17 pathway. Indeed, previous data shows that systemic corticosteroids can reduce IL-17 expression [81]. Clinical trials of monoclonal antibodies that target IL-17 have yielded disappointing results,

possibly due to these studies not pre-selecting a $T2^{LOW}$ population [82]. Blocking IL-23 may hold greater promise as a treatment for $T2^{LOW}$ asthma as it is upstream of IL-17 in the inflammatory cascade, and results of phase II trials of anti-IL-23 treatment are awaited.

Although mixed T2 and T1/17 inflammation do not often occur together, and the patient population with a mixed granulocytic sputum profile is relatively small, they present a group whose asthma management remains a significant challenge. Interventions able to target both $T2^{HIGH}$ and $T2^{LOW}$ inflammation may be helpful in treating this group, but none are currently available.

As described above, genuine isolated $T2^{LOW}$ inflammation may be much less common than currently thought. Most apparently $T2^{LOW}$ patients have an element of $T2^{HIGH}$ disease, which may be being masked by corticosteroid therapy. Therefore, it is important to consider that persistent symptoms in seemingly $T2^{LOW}$ corticosteroid-treated patients may be related to other asthma and non-asthma mechanisms and factors.

1.2.3 Airway hyper-responsiveness

Airway hyper-responsiveness (AHR) reflects increased/dysfunctional airway smooth muscle (ASM) contraction due to a range of direct or indirect stimuli [20]. AHR is augmented by $T2^{HIGH}$ and $T2^{LOW}$ inflammation. Asthmatics who do not obviously exhibit either $T2^{HIGH}$ or $T2^{LOW}$ inflammatory profiles (i.e. paucigranulocytic profile in sputum) usually have less severe asthma and less frequent exacerbations. However, this group still have asthma characterised primarily by airway hyper-responsiveness, and may exhibit significant bronchospastic symptoms, even in the absence of detectable airways inflammation. It is therefore important to remember that AHR can be a feature in any asthmatic patient, regardless of their underlying inflammatory profile.

Mast-cell infiltration into the airway smooth muscle is increased in asthma, when compared to both healthy controls and to patients with eosinophilic bronchitis

[83, 84]. This contrast between asthma and eosinophilic bronchitis is important given that the degree of eosinophilic inflammation is similar in both conditions [84], with the presence or not of AHR being the important physiological difference between the two diseases. Degree of ASM mast cell infiltration also correlates with bronchial reactivity as measured by methacholine PC20 [84]. Together these findings suggest that mast cells in airway smooth muscle play a specific role in AHR, through release of mediators including histamine, PGD2 and cysteinyl leukotrienes, which can directly induce contraction of the ASM, with consequent AHR [83, 84]. Mast cells located in the ASM bundle also release cytokines IL-4 and IL-13, which is not seen in health or in eosinophilic bronchitis [85], again suggesting that mast cells play a specific role in asthma and AHR that is not seen in eosinophilic bronchitis. Recruitment of mast cells to the airway smooth muscle appears to be primarily mediated by CXCL10 expressed on ASM cells interacting with CXCR3 expressed on the surface of mast cells [83]. CXCL10 and CXCR3 show markedly increased expression in smooth muscle cells and mast cells located in the region of the ASM of asthmatics [83]. Mast cells located in the airway ASM also differentiate under the influence of ASM-derived extracellular matrix proteins to become fibroblastic, and the prevalence of such altered mast cells in the ASM is related to the degree of AHR [86].

Stimuli such as oxidative stress and inhaled environmental pollutants can also increase AHR. ASM exhibits increased responsivity in association with higher levels of oxidative stress related to increased Nicotinamide Adenine Dinucleotide Phosphate (NADPH) oxidase 4 (NOX4) expression [22]. Epithelial derived IL-33 also contributes towards maintenance of airway hyper-responsiveness during allergen challenge [61]. Damage of the airway also promotes release of nuclear-located high mobility box-1 protein (HMGB1). This HMGB1 amplifies ASM hyper-contractility via activation of Toll-like receptor-4 rather than the receptor for advanced glycation end-products (RAGE) [87].

1.2.4 Airway remodelling

Airway remodelling encompasses various structural changes in the airway including: epithelial changes; mucous-gland hyperplasia; thickening of the subepithelial collagen layer (reticular basement membrane); increased submucosal matrix deposition; hypertrophy and hyperplasia of ASM; and mast cell localisation and degranulation in the ASM bundle [21, 22, 88].

CT imaging diagnosed bronchial wall thickening and bronchiectasis are common in the severe asthma population [74]. Prevalence of bronchiectasis is significantly higher in severe asthmatics who have smoked compared to those who have not, suggesting that environmental exposures in susceptible patients leads to a greater risk of developing airway wall changes such as bronchiectasis [74]. However, it is not possible to say definitively whether bronchiectasis is a part of the natural progression of severe asthma, or whether bronchiectasis in severe asthma instead reflects a separate co-morbid entity contributing to the difficulties in treatment [74].

Macro-level CT-derived measures of airway remodelling are associated with micro-level remodelling changes on bronchial biopsy samples. ASM mass and epithelial thickness measured on bronchial biopsies both predict cross-sectional bronchial luminal area and airway wall area on CT [89]. Therefore increased ASM mass and epithelial thickness could account for some of the bronchial wall thickening seen on CT scans of severe asthmatics.

Bronchial biopsy samples from mild, moderate and severe asthmatics, and from healthy individuals, show that the degree of epithelial hyperplasia and metaplasia significantly increases in line with severity of asthma [57]. Changes in the structure and integrity of the epithelial barrier results in ciliary dysfunction, with reduced ciliary beat frequency and increased dyskinetic and immotile cilia [23]. Cilial dysfunction is associated with important clinical features such as reduced lung function and AHR, although not with sputum eosinophilia, suggesting it is not simply related to airways inflammation, but more likely a result of a complex of

factors including exposure to inhaled irritants and bacterial infection [23]. Altered cilial function contributes to impaired sputum clearance from the airways and mucous plugging, which are features of severe asthma and often found in fatal asthma exacerbations. Epithelial damage also leads to reduced barrier function with a subsequent increase in susceptibility to inhaled pathogens, allergens and pollutants, which in turn triggers the inflammatory cascades presented above.

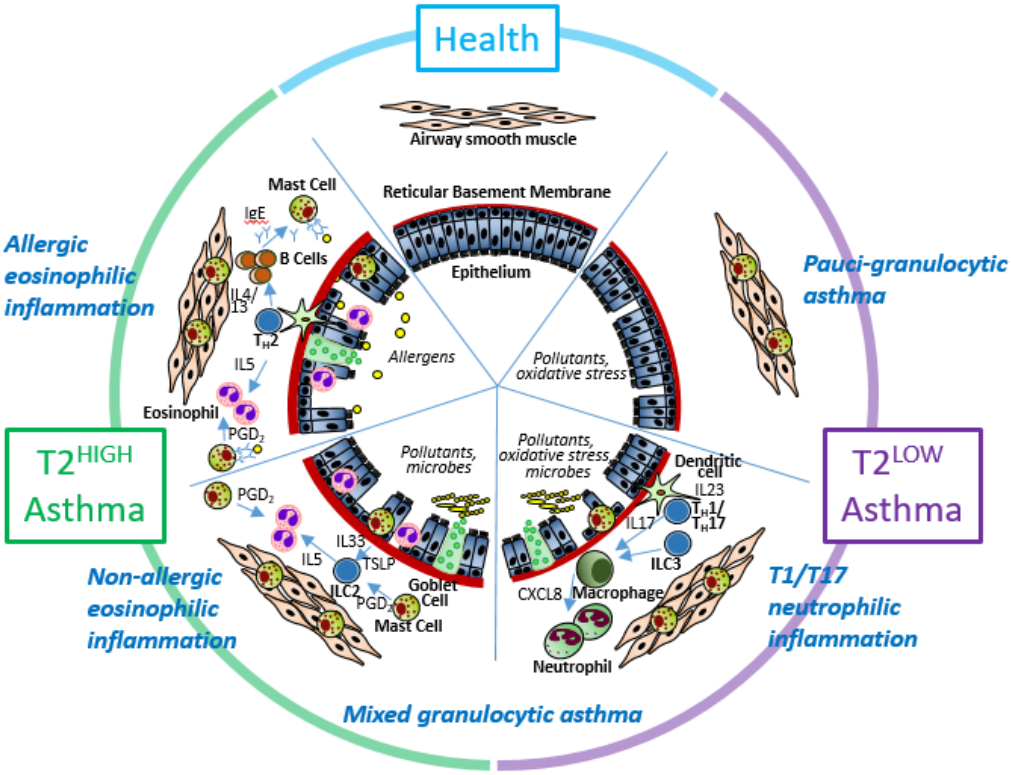
Bronchial thermoplasty has been shown to reduce some features of airway remodelling seen in severe asthma by the bronchoscopic application of radiofrequency thermal energy to the airway wall [90-94]. Large trials have also shown improvements in asthma symptoms and exacerbation rates, but no change in lung function [95]. This is discussed in more detail below.

A small number of recent studies of pharmacological asthma therapies have examined the effect of treatment on airway remodelling. *In vitro* studies have shown that the presence of DP2 in asthmatic airways increases goblet cell formation and epithelial metaplasia [57]. Placebo-controlled clinical trial data also showed that 12 weeks of treatment with fevipiprant (a DP2 antagonist) significantly reduces airway smooth muscle mass [96, 97]. Mechanistically this appears to be the result of the combined effects of reduced airway inflammation and a direct effect from fevipiprant of the ASM itself via reduced recruitment of myofibroblasts and fibrocytes [96, 97]. Treatment with the calcium channel blocker gallopamil for 12 months also led to reductions in airway smooth muscle mass and reticular basement membrane thickness, but changes were not significantly different to those observed with placebo, which questions any true effect [98].

Dynamic mechanical forces on the lung, and in particular the airway epithelium, can cause significant inflammatory stress independent of the $T2^{HIGH}$ or $T2^{LOW}$ mechanisms described above. These forces originate from a range of actions including breathing (particularly deep inspiration), coughing, and airway smooth muscle contraction with bronchoconstriction [99]. Mechanical strain and

compressive stress *in vitro* inhibits epithelial repair in response to injury, increases the release of pro-fibrotic cytokines such as transforming growth factor (TGF)-β2 and endothelin, increases production of reactive oxygen species (ROS) and resultant oxidative stress, and down-regulates prostaglandin E2 synthesis [99].

Figure 1.1: Asthma immunopathology: key mechanisms and pathological features of $T2^{HIGH}$ and $T2^{LOW}$ asthma compared to health



Representation of the key biological mechanisms and pathological features seen typically in $T2^{HIGH}$ (green) and $T2^{LOW}$ (purple) asthma, compared to health (blue).

1.2.5 Extra-thoracic factors

A range of extra-thoracic factors also influence and interact with asthma disease mechanisms. Asthma is often accompanied by allergic rhinitis (over 80% of asthmatics) and/or nasal polyps (4%) [100]. Atopy alone does not explain the

association between asthma and allergic rhinitis, as rhinitis is frequently seen in non-atopic asthmatics, with the presence of nasal symptoms associated with more severe asthma [100]. Even in the absence of asthma, patients with upper respiratory tract disease have evidence of lower airways inflammation and remodelling, although to a lesser degree than those with asthma [100]. This supports the concept of the upper and lower airways being intrinsically linked through a shared epithelium, and nasal challenge testing increases the presence of eosinophil progenitor cells in the bone marrow in the same way as lower airway stimulation [100]. However, some of the relationship between upper and lower airway disease may be as a consequence of physical factors, as rhinitis and polyps cause nasal congestion that leads to increased mouth breathing. This increases the exposure of the lower airways to allergens and pathogens, as mouth-inspired air is not filtered by the nose [100].

Asthmatics who are obese often present as a specific phenotype, with a lesser degree of eosinophilic inflammation and reduced sensitivity to corticosteroid therapy [10]. Although the mechanisms are not entirely understood, obesity may influence asthma through numerous factors including mechanical (such as extra-thoracic lung volume restriction), inflammatory (such as increased inflammatory cytokines and proteins associated with obesity and the metabolic syndrome), genetic, or obesity related co-morbidities (such as obstructive sleep apnoea and gastro-oesophageal reflux disease) [100, 101]. There is also an increase in adipose cells within the large airways of obese subjects, which is associated with increased airway wall thickness and inflammatory cell prevalence [102]. It has also been shown that obese asthmatics recruit more eosinophils to the airway tissue than non-obese asthmatics, despite having a lower degree of sputum eosinophils [103], highlighting altered inflammatory cell distribution in obesity, with possible influences on disease behaviour. Regardless of the underlying mechanisms involved, weight loss improves asthma symptoms, exacerbation rates and lung function [100].

Smoking is common among asthmatics, with up to 50% of asthmatics in Western Countries being current or former smokers [104]. Asthmatics who smoke have an increased burden of symptoms, and a higher risk of asthma exacerbation [104]. Smoking asthmatics have an attenuated response to corticosteroids, possibly related to increased neutrophilic airway inflammation, increased oxidative stress, and an altered glucocorticoid receptor balance associated with smoking [104]. Cessation of smoking reduces sputum neutrophil levels in non-eosinophilic asthmatics, and may subsequently lead to improved corticosteroid sensitivity [75]. Smoke exposure in non-asthmatics is associated with increased levels of eosinophils, macrophages, and CD8+ lymphocytes and mast cells in the airways, as well as pro-inflammatory mediators such as IL-8 [104]. However, in the asthmatic airway, where an altered inflammatory profile already exists, the effect of smoke exposure is less clear. Studies have identified contrasting changes in the airway inflammatory profile in smoking asthmatics, although some of these differences could be accounted for by variations in study design and population [104]. There is even some evidence in rats to suggest that smoking may suppress eosinophilic inflammation, however the mechanism for this is poorly understood [104]. Smoking also has airway remodelling effects, with epithelial hyperplasia and increased goblet cells seen in the airways of smoking asthmatics, although these changes appear to be mostly reversible following cessation of smoking [104].

1.3 Treatment approach for asthma

The principles of asthma treatment are relatively standardised across the various treatment guidelines, and involve stepping up or down asthma medication depending on asthma control [3, 6, 7]. The step on which a newly diagnosed asthma patient commences treatment depends on the severity of their disease at presentation [3]. Patients presenting with mild intermittent symptoms are suitable to start at a lower step of treatment, in contrast to patients presenting

with a life-threatening asthma exacerbation, who should commence treatment at a higher step.

In recent years there has been a move away from the singular use of 'as needed' short-acting beta-agonists (SABA) for mild asthma, as the use of bronchodilators without inhaled corticosteroids is associated with worse outcomes, and patients with infrequent or mild symptoms are still at risk of severe or fatal exacerbations [105]. Treatment with low-dose inhaled corticosteroids (ICS) is now the preferred option, which may be most easily delivered in a combined ICS/formoterol inhaler taken as needed, such that symptomatic episodes are treated with both corticosteroid and bronchodilator medications ('step 1') [3]. The corticosteroid helps to reduce the risk of increasing inflammation leading to an exacerbation, while the bronchodilator improves symptoms acutely [106].

Most asthmatics have frequent or persistent symptoms at the time of diagnosis, and therefore 'step 1' treatment is not appropriate. These patients should commence regular ICS treatment from diagnosis. If symptoms remain uncontrolled then the dose of ICS is increased, and additional controller medications are added in sequence, until asthma control is achieved. Long-acting beta-agonists (LABA), leukotriene-receptor antagonists (LTRA), long-acting muscarinic antagonists (LAMA) and theophylline can be added alongside an increasing dose of ICS ('steps 2-4')[3].

'Step 5' is the highest level of therapy, and is used in severe asthmatics who remain uncontrolled despite high-dose ICS and additional asthma controller medications. Treatments such as monoclonal antibodies targeting immunoglobulin (Ig)-E, interleukin (IL)-5 or the IL-5 receptor (summarised in table 1.1), and/or maintenance oral corticosteroids, are added in order to gain asthma control. Anti-IgE therapy is effective in reducing asthma exacerbations in atopic patients [107], but reports of lung function effects are mixed. Anti-IL-5 therapy attenuates eosinophilic inflammation and leads to a significant reduction in asthma exacerbations in eosinophilic patients, although with only limited effects on lung

function [17, 68, 69]. These patients may also be suitable for bronchial thermoplasty treatment.

Throughout all treatment steps it is of paramount importance to consider and address issues such correct inhaler technique, medication adherence, smoking cessation, as well as minimising environmental and occupational exposures, managing co-morbid conditions, and addressing healthcare beliefs [3].

There is ongoing need for new treatments options for those patients who are not eligible for, or who do not respond to, existing ‘step 5’ treatment options.

Table 1.1: Key phase 3 studies of currently licensed monoclonal antibody treatments in asthma

Target	Therapy	Study	Study design	Key patient selection criteria	Key outcomes (primary outcome in bold)
IL-5	Mepolizumab	Ortega 2014 (MENSA) [17]	75mg IV vs 100mg SC vs placebo Q4W 32 weeks n=576	Adults and children age 12-82 years. ≥2 exacerbations in last year. Blood eosinophils ≥150 cells/μL at screening or ≥300 cells/μL in last year.	**↓ Exacerbation rate 47 % (IV) & 53% (SC)** ↓ Exacerbations requiring ED/hospitalisation ↑ FEV1 ↓ SGRQ, ↓ ACQ
		Bel 2014 (SIRIUS) [16]	100mg SC vs placebo Q4W 20 weeks n=135	Age 16-74 years. 5-35 mg/day prednisone or equivalent for >6 months. Blood eosinophils ≥150 cells/μL at screening or ≥300 cells/μL in last year.	**↓ Oral corticosteroid dose (50% dose reduction)** ↓ Exacerbation rate ↓ ACQ, ↓ SGRQ ↔ FEV1
		Chupp 2017 (MUSCA) [108]	100mg SC vs placebo Q4W 24 weeks n=556	Adults and children >12 years. ≥2 exacerbations in last year. Blood eosinophils ≥150/μl at screening or ≥300/μl in last 12 months.	**↓ SGRQ (7.7 points)** ↓ ACQ ↑ FEV1 ↓ Exacerbation rate, ↓ Exacerbations requiring ED/hospitalisation
	Reslizumab	Castro 2015 [18]	3mg/kg IV vs placebo Q4W 52 weeks n=953	Adults and children aged 12-75 years. ≥1 exacerbation in last year. Blood eosinophils ≥400 cells/μL at screening.	**↓ Exacerbation rate 54%** ↔ Exacerbations requiring ED/hospitalisation ↑ FEV1 ↑ AQLQ, ↓ ACQ ↔ SABA use
		Bjermer 2016 [109]	0.3mg/kg vs 3mg/kg IV vs placebo Q4W 16 weeks n=315	Adults and children age 12-75 years. Blood eosinophils ≥400 cells/μL at screening.	**↑ FEV1 (~140 mL)** ↓ SABA use ↓ ACQ, ↑ AQLQ
		Corren 2016 [110]	3mg/kg IV vs placebo Q4W 16 weeks n=492	Adults age 18-65 years. ACQ ≥1.5.	**↔ FEV1 in overall population** (↑ FEV1 (270 mL) in baseline eosinophil ≥400 cells/μL group) ↓ ACQ ↔ SABA use
IL-5Rα	Benralizumab	Bleecker 2016 (SIROCCO) [70]	30mg SC vs placebo Q4W or Q8W 48 weeks n=1205	Adults and children age 12-75 years. ≥2 exacerbations in last year.	**↓ Exacerbations 45% (Q4W) & 51% (Q8W) in eosinophil ≥300 cells/μL group** ↓ Exacerbations requiring ED/hospitalisation in Q8W eosinophils ≥300 cells/μL group ↑ FEV1 in eosinophil ≥300 cells/μL group ↓ Asthma Symptom Score, ↓ ACQ, ↑ AQLQ in Q8W eosinophils ≥300 cells/μL group
		Fitzgerald 2016 (CALIMA) [72]	30mg SC vs placebo Q4W or Q8W 56 weeks n=1306	Adults and children age 12-75 years. ≥2 exacerbations in last year.	**↓ Exacerbations 36% (Q4W) & 28% (Q8W) in eosinophils ≥300 cells/μL group** ↓ Exacerbations 36% (Q4W) & 40% (Q8W) in eosinophils <300 cells/μL group ↔ Exacerbations requiring ED/hospitalisations ↑ FEV1 in eosinophils ≥300 cells/μL group ↓ Asthma Symptom Score, ↑ AQLQ in Q8W eosinophils ≥300 cells/μL group ↓ ACQ in eosinophils ≥300 cells/μL group

Target	Therapy	Study	Study design	Key patient selection criteria	Key outcomes (primary outcome in bold)
		Nair 2017 (ZONDA) [111]	30mg SC vs placebo Q4W or Q8W 28 weeks n=220	Adults up to 75 years. 7.5-40mg/day prednisolone or equivalent for > 6 months. Blood eosinophils ≥150 cells/µL at screening.	**↓ Oral corticosteroid dose (75% dose reduction)** ↓ Exacerbation rate ↓ Exacerbation requiring ED/hospitalisation in Q8W group ↔ FEV1 ↓ ACQ, ↑ AQLQ in Q8W group
IL-4Rα	Dupilumab	Castro 2018 (LIBERTY QUEST) [65]	200mg vs 300mg SC vs placebo Q2W 52 weeks n=1902	Adults and children ≥12 years. ≥1 exacerbation in last year. ACQ ≥1.5.	**↓ Exacerbations 48% (200mg) & 46% (300mg)** (↓ Exacerbations ~40% in eosinophils ≥150-300 cells/µL) (↓ Exacerbations ~67% in eosinophils ≥300 cells/µL) ↑ FEV1
		Rabe 2018 (LIBERTY VENTURE) [112]	300mg SC vs placebo Q2W 24 weeks n=210	Adults and children ≥12 years. 5-35 mg/day prednisone or equivalent for ≥6 months.	**↓ Oral corticosteroids dose (70% dose reduction)** ↓ Exacerbation rate ↑ FEV1 ↓ ACQ
IgE	Omalizumab	Solèr 2001 [113]	Dose based on weight and total IgE vs placebo 28 weeks n=546	Adults and children age 12 75 years. Positive skin prick test to common aeroallergen. Serum total IgE 30-700 IU/ml.	**↓ Exacerbation rate** ↓ ICS dose, ↓ SABA use ↓ Asthma Symptom Score ↑ FEV1
		Busse 2001 [114]	Dose based on weight and total IgE vs placebo 28 weeks n=525	Adults and children age 12-75 years. Positive skin prick test to common aeroallergen. Serum total IgE 30-700 IU/ml.	**↓ Exacerbation rate** ↓ ICS dose, ↓ SABA use ↓ Asthma Symptom Score ↑ FEV1
		Holgate 2004 [115]	Dose based on weight and total IgE vs placebo 32 weeks n=246	Adults and children age 12-75 years. Positive skin prick test to common aeroallergen. Serum total IgE 30-700 IU/ml. ≥1000 µg/day fluticasone.	**↓ ICS dose** ↔ Exacerbation rate ↑ AQLQ, ↓ Asthma Symptom Score ↓ SABA use
		Humbert 2005 (INNOVATE) [116]	Dose based on weight and total IgE vs placebo 28 weeks n=419	Adults and children age 12-75 years. Positive skin prick test to common aeroallergen. Serum total IgE 30-700 IU/ml. ≥2 exacerbations or ≥1 hospitalisation in last year. GINA step 4 or 5 treatment.	**↓ Exacerbation rate 26%** ↓ Exacerbations requiring ED/hospitalisation ↑ AQLQ, ↓ Asthma Symptom Score ↑ FEV1 ↔ SABA use

IL = interleukin. mg = milligrams. IV = intravenous. SC = subcutaneous. Q4W = every 4 weeks. Q8W = every 8 weeks. µL = microlitre. ED = emergency department. FEV1 = forced expiratory volume in 1 second. SGRQ = St George's Respiratory Questionnaire (decrease indicates improvement). ACQ = Asthma Control Questionnaire (decrease indicates improvement). kg = kilogram. AQLQ = Asthma Quality of Life Score (increase indicates improvement). mL = millilitres. SABA = short acting beta agonist. IgE = immunoglobulin-E. IU = international units. ICS = inhaled corticosteroid. µg = micrograms. GINA = Global Initiative for Asthma.

1.4 Anti-interleukin-13 therapy

Interleukin (IL)-13 is an archetypal type-2 cytokine, and is centrally implicated in asthma pathogenesis, as described in detail above. IL-13 is therefore an attractive target for novel monoclonal antibody therapy, and anti-IL-13 monoclonal antibody therapies, such as tralokinumab (AstraZeneca) and Lebrikizumab (Roche), have been developed and tested for use in asthma.

In animal models IL-13 has been shown to promote airway hyper-responsiveness (AHR), smooth muscle proliferation and mucus production [117-119]. IL-13 upregulates the production of eotaxin chemokines (which act as chemo-attractant molecules on CCR3 receptors expressed on the eosinophil cell surface) [54, 119, 120] and is thought to upregulate vascular adhesion receptors (such as P-selectin) [53], resulting in the trafficking of eosinophils from circulating blood into the lung tissue [117, 121]. IL-13 also augments eosinophil survival and activity, and the production of CCR3 chemokines in the bronchial epithelium and airway smooth muscle [122]. Therefore, inhibiting IL-13 may lead to reduced eosinophilic airway inflammation and reduced airway hyper-responsiveness. Whether inhibiting IL-13 results in decreased bronchial tissue eosinophils has not previously been investigated. However, reducing eosinophil migration from the vascular space to the lung tissue may lead to a consequent increase in eosinophil numbers retained in the peripheral blood, an effect which has been observed in previous phase II studies of tralokinumab [66, 123].

A 2015 phase II study of tralokinumab in patients with severe uncontrolled asthma did not show a significant overall reduction in asthma exacerbation rates, but subgroup analysis did show trends towards exacerbation reduction in those patients not receiving oral corticosteroids, with the greatest FEV1 reversibility, and with higher levels of the blood biomarkers periostin or di-peptidyl pepitidase-4 (DPP4) [66]. These results suggested that tralokinumab may benefit a selected sub-population of asthmatics, and prompted further phase II and III trials to

investigate this. One such phase II trial (MESOS [124]) is reported in chapter three of this thesis. Two large phase III trials (published together as STRATOS 1 and 2 [125]) which ran concurrently with the MESOS study did not consistently identify significant reductions in asthma exacerbation rates compared to placebo over 52 weeks. STRATOS 1 showed only a 7% reduction overall in annual asthma exacerbations rates compared to placebo, but did identify that participants with an exhaled nitric oxide level of 37 parts per billion (ppb) or greater ('FeNO-high') had a significant exacerbation reduction of 44% compared to placebo. However, this was not replicated in STRATOS 2, where FeNO-high participants had only a 15.8% reduction in annual exacerbations. The other investigated biomarkers (blood eosinophils, DPP4, total IgE, and periostin) did not show any outcome-predictive relationship in participants receiving tralokinumab every two weeks, and were therefore not investigated any further. Taken together, the STRATOS studies did not meet their endpoint of a significant reduction in annual exacerbation rate, although did demonstrate small improvements in lung function (FEV1) and asthma control questionnaire scores, which were greatest in FeNO-high participants [125].

Lebrikizumab is another neutralising IL-13 monoclonal antibody. Pooled data from two replicate phase IIb studies shows a trend towards exacerbation rate reduction in patients with high levels of periostin, blood eosinophils and FeNO. However, these studies were stopped early due to detection of a host-cell protein impurity in all patients who had received Lebrikizumab [38]. Phase III studies did not show consistent reductions in asthma exacerbation rates, even in patients with high levels of the biomarkers identified in phase II [67]. The safety profile of lebrikizumab over 52 weeks in these phase III trials of over 2000 asthmatics was comparable to placebo, suggesting the host-cell impurity was not clinically significant [67].

The effects of anti-IL-13 antibodies may be limited by the overlapping effects of IL-4, as these cytokines share a common receptor target. Dupilumab is a monoclonal antibody which targets the alpha subunit of the IL-4 receptor, and is therefore able

to block both the IL-4 and IL-13 pathway [126]. Phase II and III studies of dupilumab found significant reductions in exacerbation rates in uncontrolled asthma compared to placebo [64, 65]. Whether dupilumab affects tissue eosinophil counts is unknown.

IL-13 inhibition has consistently shown an effect on FEV1, with clinically and statistically significant increases observed compared to placebo. However, IL-5 therapy has not shown any consistent effect on lung function, and there is very limited evidence of any significant effect on features of remodelling [127]. Therefore it may be that the beneficial effects of IL-13 inhibition occur as a result of remodelling changes in the airway wall and bronchial epithelium. IL-13 neutralisation appears to lead to increases in peripheral blood eosinophil count [66, 123], likely due to inhibition of eosinophil–endothelial adhesion as described above [48], but does not reduce asthma exacerbations. In contrast, IL-5 neutralisation or IL-5 receptor blockade leads to significant reductions in exacerbations, with marked reductions in blood and sputum eosinophil counts and, to a lesser extent, eosinophil count in the bronchial submucosa [128, 129]. This could suggest that reducing exacerbations is dependent on reducing eosinophilic inflammation. However, dupilumab also reduces exacerbations despite a transient increase in peripheral blood eosinophil count in some patients [65], so this relationship may not be as directly causal as first appears. Whether inhibition of IL-13 affects bronchial or sputum eosinophil counts, or features of remodelling is unknown, and is investigated in chapter 3 of this thesis.

1.5 Bronchial thermoplasty

Bronchial thermoplasty (BT) is a non-pharmacological treatment for severe asthma, whereby heat energy is applied to the airway wall in an attempt to reverse airway remodelling changes seen due to asthma (described in detail above). During thermoplasty treatment a specially designed catheter is delivered via a

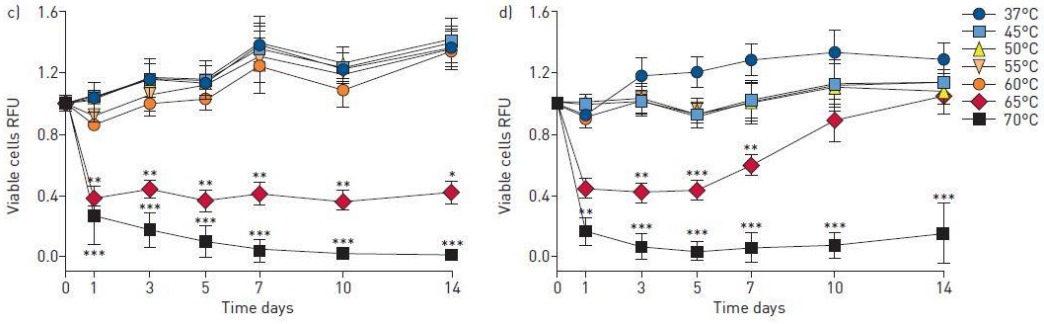
bronchoscope to the target airway. The catheter consists of a 'basket' of 4 wires, which expands to make contact with the internal airway wall at four points spaced evenly around the circumference of the airway. During treatment the catheter is positioned as distal as possible in the target airway and deployed. Radiofrequency energy is then passed through the wires of the catheter basket, heating the airway wall to 65°C for 10 seconds. This process is termed an 'activation'. The catheter basket is then closed, withdrawn proximally a short distance along the airway, and redeployed. A further activation is then delivered. This is repeated along the full length of the target airway. Each of the airways within a lung lobe is treated in this way during a single treatment session. Typically, airways in the range of 3mm to 10mm in diameter can be treated. The complete thermoplasty treatment involves three separate bronchoscopy sessions, spaced apart by approximately 3 weeks. Usually the right lower lobe (RLL) is treated in the first session; the left lower lobe (LLL) in the second session; and both of the upper lobes in the third session. The right middle lobe is not treated due to the risk of lobar collapse, termed 'right middle lobe syndrome' [130].

Bronchial thermoplasty first showed potential benefit as a treatment for asthma in studies of dogs. Danek *et al*. showed that dogs treated with bronchial thermoplasty had a reduction in airway smooth muscle mass, and an improvement in airways hyper-responsiveness [131]. This study also demonstrated that thermoplasty must heat the airway to 65°C in order to elicit the benefits in terms of remodelling and airways hyper-responsiveness. When 55°C was used there was no significant benefit, and 75°C elicited an even greater response than 65°C, although the magnitude of additional gains was small. Beneficial effects were still observed at the end of the 3 year follow-up period, with airways hyper-responsiveness and reduced airway smooth muscle mass still a feature at the end of the study compared to baseline. At three years there was no evidence of any regeneration of airway smooth muscle. Importantly, the changes seen on post-mortem histology samples were distributed around the entire circumference of the treated airway, and not limited to the area immediately in contact with the

thermoplasty basket wire. The study also demonstrated a significant correlation between improvement in airways hyper-responsiveness and reduction in airways smooth muscle mass. However, this correlation used data from several treatment temperatures, and by virtue of increasing temperature leading to increased benefits in both hyper-responsiveness and remodelling, this correlation was perhaps inherent in the data, and not necessarily proof of a causative link between reduced airways smooth muscle mass and reduced airway hyper-responsiveness.

The temperature threshold of 65°C required for airway smooth muscle mass reduction was also shown by Chernyavsky *et al*. [132]. Exposing airway smooth muscle cells *in vitro* to heated media at a range of temperatures showed a reduction in viable smooth muscle cells only at temperatures of 65°C and above (figure 1.2). No effect was seen at temperatures below this. 65°C also appeared to be the optimum temperature for affecting regeneration of human epithelial cells *in vitro*, with an initial fall in viable cells only at temperatures above this threshold, followed by regrowth not seen at even higher temperatures (figure 1.2) [132].

Figure 1.2: Airway smooth muscle and epithelial cell responses *in vitro* following exposure to medium at a range of temperatures



Proportion of viable airway smooth muscle cells (left panel) and epithelial cells (right panel) remaining after exposure to heated media at a range of temperatures for 10 seconds, compared to baseline. Reproduced from Chernyavsky *et al.* [132].

1.5.1 Thermoplasty clinical studies in humans

The first study of bronchial thermoplasty in humans was undertaken in nine patients awaiting lung resection for lung cancer. The area of the lung planned for resection was treated with thermoplasty up to 3 weeks prior to removal. The study showed that the procedure was safe and well tolerated over this short follow-up period. Histological examination of the resected lung showed reduced airway smooth muscle mass, as had been shown in previous animal studies [133].

Cox *et al*. subsequently used thermoplasty to treat 16 patients with asthma for the first time, although selected patients with stable mild-to-moderate disease, in stark contrast to those whom receive the treatment in current clinical practice. In this mild-to-moderate patient group thermoplasty was safe and well tolerated, with the reported treatment related adverse effects deemed to be consistent with any bronchoscopic procedure [134]. The study showed clinical benefits in terms of reduced airway hyper-responsiveness, and improvements in symptoms (recorded in patient diaries), and peak expiratory flow values up to 12 weeks after treatment. A small improvement in pre-bronchodilator FEV1 was observed at 12 weeks and 1 year follow up, but this was not maintained at 2 years after treatment [134].

Several subsequent randomised trials have shown improvements in clinical outcomes following treatment with bronchial thermoplasty. The Research in Severe Asthma (RISA) group undertook a small randomised trial investigating the effects of thermoplasty in severe symptomatic asthmatics [135]. 15 patients were randomised to bronchial thermoplasty, and 17 to usual care (unblinded). They demonstrated that patients with severe asthma undergoing bronchial thermoplasty had an initial increase in adverse events, including hospitalisation due to respiratory symptoms, during the treatment phase, but after the initial treatment period the rate of adverse events was no different to the usual care group. At 22 and 52 weeks after treatment there was a significant reduction in rescue medication use, and improvements in asthma control questionnaire (ACQ) and asthma quality of life questionnaire (AQLQ) scores, compared to usual care.

There was also a transient improvement in pre-bronchodilator FEV1 seen at 22 weeks, although this was not maintained at 52 weeks. Interestingly, there was no observed difference in airways hyper-responsiveness as assessed by methacholine challenge testing, in contrast to earlier studies, perhaps suggesting that this effect was attenuated in patients with more severe asthma [135].

The Asthma Intervention Research (AIR) Trial Study Group undertook a larger study of 112 moderate-to-severe asthmatics receiving inhaled corticosteroids and long acting beta-agonists (LABA), who suffered deteriorating asthma control following withdrawal of LABA treatment. Patients were randomised to bronchial thermoplasty or usual care (unblinded). Again there was an observed increase in adverse events immediately after treatment, but these were then similar between thermoplasty treated patients and control patients from 6 weeks to 12 months after treatment. Following thermoplasty there was a significant reduction in the number of exacerbations compared to baseline when LABA was again withdrawn for 2 weeks at 3 and 12 months after treatment. This exacerbation reduction was not observed in the control group. The study also showed significant improvements from baseline in morning peak expiratory flow rate, AQLQ, ACQ, rescue medication use, symptom scores, and proportion of symptom free days at 12 months compared to the control group. Once again there was no observed effect on airway hyper-responsiveness or FEV1 [136].

In the largest randomised trial of thermoplasty, published in 2010, Castro and the AIR2 group randomised 190 severe asthmatics to thermoplasty, and 98 to sham treatment, in an attempt to blind patients to their treatment allocation [95]. AQLQ improved by more than double the 0.5 point minimum clinically important difference (MCID) with both thermoplasty and sham treatment, although the magnitude of improvement was slightly greater in the thermoplasty group (mean improvement 1.35 with thermoplasty and 1.10 with sham procedure). This study also showed that asthmatics undergoing bronchial thermoplasty were more likely to have a respiratory adverse event (including hospitalisation) during the treatment period compared to those undergoing sham procedures, suggesting

additional adverse events risk related to thermoplasty itself in addition to the risk from standard bronchoscopy. In the post-treatment period thermoplasty treated patients experienced significantly fewer asthma exacerbations, emergency department visits, and days missed from work or school compared to patients undergoing sham procedure [95]. These findings were pivotal to bronchial thermoplasty obtaining approval for clinical use in severe asthma patients.

Follow-up data extending to five years for 162 of the 190 thermoplasty-treated AIR2 patients found that the reduction in exacerbations compared to baseline was maintained over five years of follow-up. When compared to the 12 months before treatment, patients had an average reduction in exacerbations of 44%, and a reduction in emergency room visits of 78% over the 5 year follow-up period [137]. A further study extending to at least 10 years of follow-up is currently in progress.

1.5.2 Thermoplasty biopsy-assessed remodelling studies

A number of uncontrolled observational studies have investigated the effects of airway remodelling in asthma patients undergoing bronchial thermoplasty. In 2014 Pretolani *et al*. published a study detailing biopsy changes seen in response to thermoplasty in 10 patients with uncontrolled asthma despite high-dose inhaled corticosteroids, and a history of at least 3 exacerbations in the preceding 12 months [92]. They demonstrated a reduction in mean airways smooth muscle mass from 20.25% at baseline to 7.28% at 3 months after completion of treatment ($p=<0.0001$). Interestingly, this study also demonstrated a reduction in airways smooth muscle mass in the untreated right middle lobe, although to a lesser degree than demonstrated in the other 4 lobes (relative ASM reduction 48.7% in right middle lobe, 58.1-78.5% in the remaining 4 lobes). CT scans obtained in these patients one day after a thermoplasty treatment session demonstrated consolidation and ground glass changes in not only the treated lobe (7 out of 7 patients) but also in the untreated middle lobe (5 out of 7 patients). The reasons for the effect on an untreated area of the lung remain unclear.

In 2015, Denner *et al*. showed a significant reduction in smooth muscle actin expression on bronchial biopsies taken 6 weeks after bronchial thermoplasty compared to pre-treatment in 11 severe uncontrolled asthmatics (mean (SEM) expression 38 (±5)% at baseline to 16 (±5)% at 6 weeks; p=<0.001) [91]. They also demonstrated altered cell proportions and cytokine concentrations in samples collected at bronchoalveolar lavage. Of particular note, there was a significant decrease in concentrations of transforming growth factor β_1 (TGF β_1), which is implicated in various inflammatory and remodelling pathways in severe asthma, and an increase in the cytokine tumour-necrosis-factor-related apoptosis-inducing ligand (TRAIL), which is implicated in apoptosis of several cell types. A significant decrease in lavage eosinophil count, from 4% at baseline to 1% at 3 and 6 weeks post-thermoplasty, was also seen (p=<0.01), accompanied by a reduction in the eosinophil attractant Regulated upon Activation, Normal T-cell Expressed and Secreted (RANTES)/CCL5 cytokine. No change in lavage macrophage or lymphocyte count was observed. There was also no observed change in key asthma cytokines IL-4, -5, -13 and -17, possibly related to the use of peri-procedural high dose steroids.

Also in 2015, Chakir *et al*. published a study investigating the effects of thermoplasty on not only airway smooth muscle mass, but also on reticular basement membrane thickness, in 17 uncontrolled asthmatics [90]. They showed a reduction in mean (SEM) airway smooth muscle mass from 12.9 (±1.2) % to 4.6 (±0.8) % at 3 weeks and 5.3 (±1.3) % at 6 weeks after thermoplasty (p=<0.0001). There was a direct relationship between the amount of airway smooth muscle at baseline and the reduction observed in airway smooth muscle mass, suggesting that potentially the patients who may gain most benefit from the treatment are those with the most airway smooth muscle remodelling at baseline. Reticular basement membrane thickness also decreased from 6.8 (±0.3) µm to 4.3 (±0.2) µm at 3 weeks and 4.4 (±0.4) µm at 8 weeks (p=<0.0001). However, while all 17 patients had biopsies at baseline and 3 weeks after treatment (mean), only 9 patients underwent further biopsy at 8 weeks after treatment. 9 of these 17

patients were subsequently followed up over a longer time period (mean 34.2 (range 27-48) months) and underwent further bronchial biopsies [94]. This showed that the reduction in airway smooth muscle mass and reticular basement membrane thickness appeared to be sustained over a longer follow-up period.

In 2017, Pretolani *et al*. again showed a significant reduction in airway smooth muscle mass from 19.7% at baseline to 5.2%, and reticular basement membrane thickness from 4.4 µm at baseline to 3.9 µm at 3 months, following thermoplasty treatment [93]. This study also investigated a range of additional remodelling features. It showed significant reductions in both submucosal and airway smooth muscle-associated nerve fibres, and epithelial neuroendocrine cells. There were no significant changes seen in subepithelial mucous glands number, eosinophil and neutrophil counts, the density of blood and lymphatic vessels, epithelial repair measures, or goblet cells in response to thermoplasty. This study also attempted to correlate biopsy changes with clinical outcomes. Improvements in asthma control test (ACT) scores and asthma quality of life (AQLQ) scores correlated with improvement in airway smooth muscle mass, reticular basement membrane thickness and numbers of neuroendocrine cells. Improvements in exacerbation frequency were correlated with airway smooth muscle mass, submucosal and ASM-associated nerve fibres, and number of neuroendocrine cells. No relationship between remodelling improvements and lung function was observed. Although this study was small (n=15), the presence of correlations between biopsy and clinical outcomes suggests that this relationship does exist, despite previous biopsy studies being unable to show this. It also highlights the relationship between outcomes and other previously unmeasured biopsy changes such as nerve fibres, suggesting that perhaps the benefit of thermoplasty arises from the combination of reductions in not only the mass of smooth muscle, but also the stimulation and function of smooth muscle cells.

Overall, these previous studies have consistently shown approximately a 50–80% relative loss of airway smooth muscle mass, and small but significant reduction in reticular basement membrane thickness (table 1.2 and figure 1.3) [90-94]. One

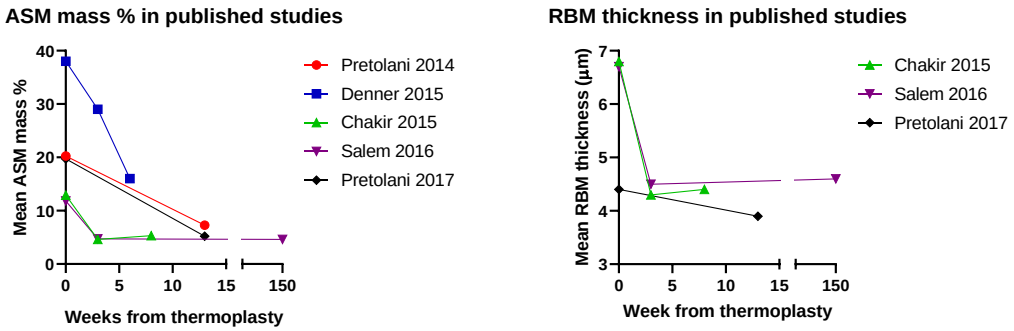
study has also identified relationships between remodelling improvements and important clinical outcomes such as exacerbation frequency and asthma symptoms, although this has not been replicated in other studies [93]. No consistent improvements in measures of lung function have been shown across these smaller biopsy studies and larger clinical trial of thermoplasty. This is perhaps in contrary to the mechanism by which bronchial thermoplasty is thought to lead to clinical improvement. If reducing airway smooth muscle hypertrophy is the primary driver in clinical responses to thermoplasty, then one might expect to see this reflected in improvements in spirometric volumes, bronchodilator reversibility, or bronchial provocation testing results, but these have not consistently been shown in humans. The most striking clinical improvement across the breadth of thermoplasty studies is a large and sustained reduction in asthma exacerbation events, which are typically inflammatory in nature rather than directly related to airway smooth muscle mass. Whether other unidentified effects of thermoplasty affect either airways inflammation directly, or downregulate an individual's susceptibility to factors which provoke inflammatory events such as airway allergens and pathogens, remains to be seen. Little work has been done investigating changes in the epithelium in response to thermoplasty, which provides not only the physical barrier against inhaled allergens and pathogens, but also plays a key role in the inflammatory response to such stimuli. The question also remains as to how important the observed changes in the untreated right middle lobe are, and the mechanisms by which these occur. Transmission of heat into the middle lobe from the surrounding treated lung areas has been suggested as a possible explanation, although a systemic response to thermoplasty leading to uniform lung changes should not be discounted.

Table 1.2: Remodelling changes demonstrated in previous thermoplasty studies

Study	n	Outcome measure	Baseline	3 weeks	6 weeks	8 weeks	13 weeks	150 weeks	p-value
Pretolani 2014 [92]	10	ASM mass % ^	20.25 (4.12)	-	-	-	7.28 (3.2)	-	<0.0001
Denner 2015 [91]	11	ASM mass % ~	38 (±5)	29 (±4)	16 (±5)	-	-	-	<0.001 (6 weeks)
Chakir 2015 [90]	17	ASM mass % ~	12.9 (±1.2)	4.6 (±0.8)	-	5.3 (±1.3)	-	-	<0.0001 (3 weeks)
		RBM thickness µm ~	6.8 (±0.3)	4.3 (±0.2)	-	4.4 (±0.4)	-	-	<0.0001
Salem 2016 [94]	9	ASM mass % ~	11.8 (±1.24)	4.7 (±0.95)	-	-	-	4.6 (±1.05)	0.0002 (150 weeks)
		RBM thickness µm ~	6.7 (±0.4)	4.5 (±0.5)	-	-	-	4.6 (±0.5)	0.003 (150 weeks)
Pretolani 2017 [93]	15	ASM mass % #	19.7 (16.2-21.8)	-	-	-	5.2 (3.7-9.8)	-	<0.001
		RBM thickness µm	4.4	-	-	-	3.9	-	0.02
		Submucosal nerve fibres ‰ #	1.0 (0.7-1.3)	-	-	-	0.3 (0.1-0.5)	-	<0.001
		ASM-associated nerve fibres /mm^2 #	452.6 (196.0-811.2)	-	-	-	62.7 (0.0-230.3)	-	0.02
		Epithelial neuroendocrine cells/mm^2 #	4.9 (0.3-14.1)	-	-	-	Not stated	-	0.04

^Values shown are mean (SD). ~ Values are mean (SEM). # Values are median (IQR). Mean follow-up time for group rounded to nearest whole week. Note: The 9 patients in the Salem 2016 study also formed part of the 17 patients in the Chakir 2015 study.

Figure 1.3: Summary data reported in previous thermoplasty biopsy studies



Summary of results for airway smooth muscle mass (left panel) and reticular basement membrane thickness (right panel) in previous thermoplasty remodelling studies. All values are means, except for Pretolani 2017 which is median. ASM = airway smooth muscle. RBM = reticular basement membrane. µm = micrometres.

1.5.3 Limitations of the existing thermoplasty evidence

Although the evidence described above was sufficient for bronchial thermoplasty to gain approval for clinical use, and provided some insight into its mechanism, there are significant limitations in the existing evidence. With the exception of the AIR2 trial [95], every other study into the effects of treatment has been unblinded, with no true placebo for comparison. In some ways this is inherent in the nature of the treatment, but does limit how much one can reliably take from the evidence. The AIR2 trial attempted to overcome this and implement blinding by randomising a third of their subjects to sham procedures. However, despite this a large number of subjects were able to accurately guess their treatment allocation after undergoing the procedure [95]. Comparing the effects of thermoplasty to a sham procedure also raised questions of its own, primarily due to the very large 'placebo effect' seen in the sham group, with an improvement in asthma quality of life questionnaire (AQLQ) scores of 1.1 from baseline, which is more than double the minimum clinically important difference of 0.5, and only a marginally less than was seen with thermoplasty treatment (AQLQ improvement from baseline of 1.35) [95]. The reason for such a large improvement in the placebo

group is unclear, although improvement due to participation in a clinical study is well recognised and may relate to factors such as improved adherence to existing medications and a feeling of reassurance due to being closely monitored throughout the study. It may also be attributable to 'placebo effect' bias in sham-treated subjects who may have believed they were receiving a novel treatment which had garnered significant clinician and media enthusiasm and optimism. It should also be noted that the most striking benefits of thermoplasty compared to sham in this study were related to exacerbation reduction, which was not the primary outcome of the study [95].

Another possible confounder contributing to the large effect of the sham procedure may be factors related to the procedure itself. Thermoplasty treatment protocols include a short course of high dose oral corticosteroids in the few days immediately before, and after, each thermoplasty procedure (typically 50mg Prednisolone on the three days before, the day of, and one day after each procedure). Some of the benefits seen in clinical or biopsy outcomes may be as a direct or indirect consequence of this.

Another limitation relates to the selection of subjects in the larger clinical trials. Mostly, these have been selected as those having relatively few asthma exacerbations in the preceding 12 months. For example, in the AIR2 trial subjects with 4 or more courses of oral corticosteroids, 3 or more hospital admissions for asthma, or 3 or more respiratory tract infections in the preceding 12 months were excluded. Although understandable in the context of a clinical trial, these exclusion criteria mean that the study population does not necessarily reflect the patient population that thermoplasty is currently being used to treat; that is, patients who typically have severe asthma and frequent exacerbations. Whether the results from these larger clinical trials in less severe patients are generalizable to the more severe 'real-world' thermoplasty population is not certain.

The biopsy studies are also limited by their small sample sizes, ranging between 9 and 17 subjects (see table 1.2). It is also worth noting that there is crossover in the

patients within these published biopsy studies. For example, 9 of the patients reported in Salem 2016 [94] were also included in the 17 patients comprising the Chakir 2015 [90] study. A graphical representation of summary data from these studies is shown in figure 1.3, and makes this crossover quite visually apparent. This highlights just how few individual patients have contributed biopsies to the existing published pool of evidence. Small study sizes have limited investigation into the relationships between clinical outcomes and biopsy changes. Although Pretolani *et al*. did find some such relationships in their 2017 study of 15 patients [93], these have not been further demonstrated in other studies. No study has recruited a control population alongside a thermoplasty group to compare biopsy changes between those receiving thermoplasty and those having matching asthma management but without thermoplasty. All reported effects are therefore in comparison to baseline data, rather than a control group, which limits the reliability of the findings. Alongside this, although it is not described in the published text, the authors of the first remodelling study (Pretolani *et al*., 2014 [92]) preselected patients with the highest degree of airway smooth muscle mass for inclusion in the study. There is therefore a risk that some of the published improvement in smooth muscle mass can be explained by 'regression to the mean', potentially including the effect seen in the untreated right middle lobe in this study.

Despite these limitations, the published data has been very consistent in relation to the effect thermoplasty has on features of remodelling (see table 1.2). The clinical benefits of bronchial thermoplasty are believed to primarily result from improvements in airway remodelling. In this respect, thermoplasty is the only treatment currently licensed in asthma to have shown consistent improvements in remodelling in humans. Therefore, it is potentially applicable to asthma patients across the full spectrum of asthma phenotypes, including those with predominant $T2^{HIGH}$ and $T2^{LOW}$ airways inflammation. Whether a particular phenotype population gain the most benefit from thermoplasty, and if any clinical features or biomarkers exists to identify these patients is unknown.

1.6 Hypothesis and aims

As described above, the two treatments investigated in this thesis both potentially exhibit significant remodelling effects via contrasting mechanisms. IL-13 inhibition consistently leads to improvements in FEV1. In contrast, anti-IL-5 has shown only small effects on lung function, along with limited effects on airway remodelling [127, 138]. It therefore follows that the FEV1 improvements seen with anti-IL-13 may be a result of airway remodelling improvements. In contrast to this, bronchial thermoplasty has consistently shown large reductions in airway smooth muscle mass, but no effect on lung function. If a remodelling effect is demonstrated with anti-IL-13 treatment, then it will be interesting to compare the different mechanisms involved with IL-13 immune-mediated processes with the physical insult that most likely underpins the remodelling changes seen in response to thermoplasty. Anti-IL-13 also has a modest effect on exacerbation rates, whereas thermoplasty leads to significant exacerbation reductions. How these exacerbation responses relate to remodelling effects also needs further investigation. Therefore, I intend to investigate these anti-IL-13 and thermoplasty treatments in greater mechanistic detail, to attempt to further understand the interactions between remodelling, lung function and exacerbations. I will give particular attention to changes in the airway epithelium, as anti-IL-13 and thermoplasty could influence this particular feature of remodelling significantly.

The central hypothesis of this thesis is that airway remodelling changes, and in particular the changes seen in the epithelium, are keys determinants of the clinical responses to novel asthma treatments. The specific hypotheses I aimed to address are:

- That treatment with anti-interleukin-13 leads to improvements in biopsy assessed markers of epithelial health, alongside reductions in airway inflammation.
- That biopsy assessed structural remodelling changes correlate with baseline clinical asthma characteristics in patients with severe asthma.

- That significant structural repair in the epithelium occurs in response to bronchial thermoplasty, alongside improvement in airways smooth muscle mass and reticular basement membrane thickness.
- That improvements in epithelial integrity following bronchial thermoplasty relate to improvements in clinical outcomes.

In order to address the hypothesis I aimed to undertake the following:

- Investigate the effect of 12 weeks of treatment with the anti-interleukin-13 monoclonal antibody tralokinumab on measures of airways inflammation and airways remodelling in asthma.
- Investigate the relationship between baseline biopsy and clinical characteristics in severe asthmatics undergoing bronchial thermoplasty.
- Investigate the effect of bronchial thermoplasty on key measures of airway remodelling, including a detailed focus on changes within the epithelium.
- Investigate the relationship between improvements in airways remodelling, and improvements in key clinical outcomes, in response to treatment with bronchial thermoplasty.

2 Methods and materials

2.1 Ethics

Anti-IL-13 monoclonal antibody study (MESOS): Independent ethics committee approval was obtained at all participating centres and all participants provided written informed consent. The trial was registered with ClinicalTrials.gov (NCT02449473) and EudraCT (2015-000857-19).

Bronchial thermoplasty study (AirPROM): Undertaken as part of the Airway Disease Predicting Outcomes through Patient Specific Computational Modelling (AirPROM) project, and was approved by the Leicestershire Research Ethics Committee (REC 13/EM/0068). Informed written consent was obtained from all subjects.

Pooled thermoplasty study: All contributing centres had previously obtained written consent from all participants in respect to the ongoing sharing and use of their data and samples for research related to the mechanisms of thermoplasty. All patient data was anonymised at the host centre before being centralised.

2.2 Funding

The anti-IL-13 trial (MESOS) was funded by AstraZeneca and supported by the NIHR Respiratory Translational Research Collaboration and NIHR Biomedical Research Centres.

The AirPROM thermoplasty study was supported by the AirPROM 7th EU Framework grant 270194 and by the NIHR Leicester Biomedical Research Centre.

The pooled thermoplasty study was supported by the NIHR Leicester Biomedical Research Centre, although involved minimal costs.

2.3 Subjects

2.3.1 Anti-interleukin-13 monoclonal antibody therapy in asthma (MESOS)

The MESOS trial was a phase II multicentre randomised placebo controlled trial, investigating the effect of 12 weeks of treatment with tralokinumab, an anti-IL-13 monoclonal antibody, compared to placebo [124]. 79 patients were recruited from 15 centres in the UK, Denmark and Canada (table 3.2).

Participants were aged between 18 and 75 years, with a physician-diagnosed history of moderate or severe asthma for at least 12 months. They were required to be receiving at least 250 micrograms per day of inhaled fluticasone propionate or equivalent, but not be receiving either oral corticosteroids or biological therapy. They were never smokers, or ex-smokers with a smoking history of less than 10 pack-years (pack-years = (number of cigarettes smoked per day/20) x number of years smoked). Participants also had to exhibit evidence of bronchodilator reversibility (at least 200ml and 12% improvement in forced expiratory volume in 1 second (FEV1) following administration of bronchodilator), and evidence of uncontrolled asthma (asthma control questionnaire (ACQ-6) score of at least 1.5). They were required to have had 3 or fewer asthma exacerbations requiring treatment with oral corticosteroids in the preceding 12 months, and be exacerbation free for 6 weeks before recruitment. Subjects were excluded if they had a history of a life-threatening asthma exacerbation requiring treatment in the intensive care setting, were pregnant or breastfeeding, or had any other significant or unstable medical condition that may have affected their safety during the study period. For a full list of the inclusion and exclusion criteria see table 3.1.

2.3.2 Bronchial thermoplasty study (AirPROM)

The bronchial thermoplasty study was a multicentre observational study in severe asthma patients undergoing thermoplasty as part of their clinical care, as guided

by their local asthma team. 33 subjects were recruited from four UK specialist asthma centres (see table 4.1).

Included patients were aged over 18, with a physician diagnosis of asthma currently receiving GINA step 3-5 therapy. They were required to be free of lower respiratory tract infection for at least 4 weeks prior to recruitment. Patients with serious co-morbidities such as active cancer, emphysema, clinically significant bronchiectasis, or blood-borne viruses were excluded.

2.3.3 Thermoplasty pooled analysis

The thermoplasty pooled analysis study was a multicentre observational study in severe asthma patients undergoing bronchial thermoplasty as part of their clinical care, as guided by their local asthma team. 119 patients were recruited from 8 asthma centres in Europe and North America (table 5.2). Specific inclusion and exclusion criteria were set by each recruiting centre according to their own clinical and research protocols.

2.4 Questionnaires

2.4.1 Asthma Control Questionnaire-6

The asthma control questionnaire-6 (ACQ6) is a shortened version of the full ACQ7, which omits the measurement of forced expiratory volume in 1 second (FEV1) used in the full version. It assesses asthma control by scoring six measures (night time waking, symptoms on waking, activity limitation, shortness of breath, wheezing, and short acting beta-agonist use). Each measure is scored between 0 (totally controlled) and 6 (severely uncontrolled) by the patient. The overall ACQ score calculated as the mean of the six scores, which are equally weighted. A score of ≤0.75 indicates well controlled asthma, between 0.75 and ≤1.5 indicates partially controlled asthma, and above 1.5 indicates uncontrolled asthma [139]. A change of 0.5 points is considered clinically significant.

2.4.2 Asthma Quality of Life Questionnaire

The asthma quality of life questionnaire (AQLQ) is designed to assess asthma related quality of life, and comprises 32 questions in 4 domains (symptoms, environmental stimuli, activity limitation, and emotional function). Each question is scored between 7 (not impaired at all) and 1 (severely impaired). The overall AQLQ score is the mean of all 32 question responses [140]. A change of 0.5 points is considered clinically significant [140].

2.4.3 Sino-Nasal Outcomes Test-20 (SNOT-20)

The sino-nasal outcomes test (SNOT-20) is a questionnaire designed to assess the impact of rhinosinusitis on quality of life. It comprises 20 questions across five subgroups (nasal symptoms, paranasal symptoms, sleep-related symptoms, social impairment, and emotional impairment). Patients score their experiences over the preceding two weeks between 0 (no problem) and 5 (most serious problem).

2.5 Lung function

2.5.1 Spirometry

Spirometry was performed according to American Thoracic Society/European Respiratory Society (ATS/ERS) guidelines [141], and repeated 20 minutes after inhalation of 400 µg salbutamol, given via a metered-dose inhaler and valved-spacer device, to derive pre- and post-bronchodilator (BD) values. Spirometry was undertaken between 6am and 11am for consistency, due to daily variability in these measures. The Global Lung Function Initiative (GLI) equations were used to calculate predicted normal values for each subject. Forced expiratory volume in 1 second (FEV1) percent of predicted is calculated as:

(measured FEV1/predicted normal FEV1) x 100.

FEV1 reversibility reflects the degree of improvement in airways obstruction in response to bronchodilator treatment, and is calculated as:

(post-BD FEV1 – pre-BD FEV1) x (100/pre-BD FEV1).

2.5.2 Body plethysmography

Whole body plethysmography was performed according to ATS/ERS guidelines [142, 143], using existing equipment at each site. Total lung capacity (TLC), residual volume (RV), vital capacity (VC), inspiratory capacity (IC) and functional residual capacity (FRC) will be recorded.

2.5.3 Multiple breath washout

Multiple breath washout assesses the small airways by measuring inert gas clearance over a series of tidal breaths. Patients breathe normally at tidal volume through a pneumotachometer connected to a gas analyser and mouthpiece, which continuously measures the concentrations of the inert gas during expiration. The test comprises two phases. During phase 1 ('wash-in'), the gas is inhaled by the patient, until the analyser detects a plateau in expired breath gas concentration, confirming maximal lung distribution. In phase 2 ('wash-out'), gas is no longer inhaled and the analyser measures the decreasing concentration of gas in each expired breath alongside the lung turnover (calculated as the cumulative expired volume divided by functional residual capacity). Ventilation heterogeneity is calculated from these data.

2.5.4 Impulse oscillometry

Impulse oscillometry is a non-invasive test designed to assess small airway physiology. The patient breathes through a mouthpiece that includes a loudspeaker capable of generating soundwaves in the 5-20Hz frequency range. Sound waves are superimposed over the patient's breathing via the mouthpiece, and delivered to the airways. The apparatus detects the airwave response, which is then used to calculate various components of the resistance to breathing within

the airways. Oscillometry was performed at 5Hz and 20Hz, and used to calculate airway resistance and reactance.

2.5.5 Airway hyper-responsiveness (methacholine PC20)

Bronchial provocation testing to methacholine was performed using the tidal breathing method as previously described [144]. Methacholine was inhaled via nebuliser in a step-wise dose concentration increase to a maximum concentration of 16 mg/mL, with forced expiratory volume in 1 second measures after each concentration. Once the concentration of methacholine eliciting a 20% decrease in FEV1 was reached the test was stopped. The methacholine provocation concentration required to cause this decrease was determined by linear interpolation of the log transformed plotted FEV1 response.

2.6 Fractional exhaled Nitric Oxide

Fractional exhaled nitric oxide (FeNO) concentration was measured using a NIOX MINO® device (Aerocrine AB, Solna, Sweden), as previously described [145]. Measurements were taken prior to undertaking spirometry. Using the standard single exhalation method, the subject exhales through the electrochemical sensor which detects the number of nitric oxide particles in the exhaled breath, and expresses this in parts per billion.

2.7 Blood sampling

Blood samples were collected from peripheral veins using standard methods. Samples were processed as per relevant protocols in either a central or local laboratory, depending on the type of test.

2.8 Computed tomography (CT) imaging

2.8.1 Obtaining CT images

Volumetric whole lung scans were obtained at full inspiration (total lung capacity) and full expiration (residual volume). All participants were coached in the breath-holding techniques, and practiced breath-holding immediately prior to scanning. All participants were scanned 10–60 minutes after receiving 400 µg salbutamol via a metered dose inhaler and valved spacer device. Images were obtained using Siemens, Philips or GE scanners, and reconstructed with overlapping slices of thickness <1 mm, utilising B35f (Siemens), B (Philips), and STANDARD (GE) kernels. Post-processing was performed using the VIDA Apollo software (VIDA Diagnostics, Iowa, United States).

2.8.2 Analysis of CT images

Quantitative CT parameters included large airway morphometry (measured in mm^2): lumen area (LA), wall area (WA), and percentage wall area (WA%=100 x (WA/(WA+LA))). When calculating the average change in morphometry parameters for airways in the same generation, the airways were assigned to generations 3–5 (G3–G5) according to (RB, right bronchus; LB, left bronchus) as follows:

G3: RB1, RB2, RB3, RB4+5, RB6, LB1+2, LB3, LB4+5, LB6.

G4: RB1a, RB1b, RB4, RB5, RB7, LB1, LB2, LB4, LB5, LB8, LB9, LB10.

G5: RB1a1, RB1a2, RB1b1, RB1b2, RB4a, RB4b, RB8, RB9, RB10, LB1a, LB1b, LB10a, LB10b.

Air trapping index was calculated as the fraction of the lung with density less than –856 Hounsfield units [HU] in the expiratory scan.

All morphometry measures were corrected for body surface area and expressed in mm^2/m^2:

(√ ((height (cm) x weight (kg))/3600)).

In addition, a fully automated lung density analysis was performed using software from Imbio (Imbio, Minnesota, United States) to calculate parametric response mapping parameters, describing the fraction of the lung with functional small airways disease, emphysema, and normal tissue.

2.9 Bronchoscopy and endobronchial biopsy

2.9.1 Obtaining biopsy samples

All bronchoscopies were performed by blinded senior clinicians in accordance with published guidelines [146] and clinical standards of care at the individual site.

In the MESOS study, all bronchoscopy staff were blinded to treatment allocation. Biopsies were taken from 2 separate sites, with up to 3 samples from lobar and up to 3 samples from sub-segmental carinae.

In the thermoplasty studies, blinding of bronchoscopy staff was not possible due to the design of the study, where biopsy samples were obtained during the same procedure when thermoplasty treatment was delivered. Up to a maximum of 6 biopsies were obtained from segmental and subsegmental carinae in the right upper lobe (for baseline samples) during the first thermoplasty procedure, and subsequently the right lower lobe (which had been treated in the first thermoplasty procedure) for follow-up biopsies.

2.9.2 Preparation of biopsy samples

Biopsy specimens were fixed with 10% neutral buffered formalin at room temperature for 24 hours, and treated with 70% ethanol. Specimens were embedded in paraffin wax for analysis. In the MESOS study, all samples were sent to a central lab (Covance, UK/Canada) for paraffin-processing and embedding. In the thermoplasty studies this was done locally. Paraffin blocks were then cut and

stained for the relevant markers. Initially, four-µm sections of each specimen were stained with Haematoxylin & Eosin, and assessed for quality control purposes, and to confirm the integrity of key tissue elements needed for subsequent immunohistochemistry staining and analysis. In the MESOS study, all cutting and staining was undertaken at Leicester Respiratory Biomedical Research Centre. For the thermoplasty studies, cutting and staining was undertaken at the lead laboratory for the individual contributing centre or study. A list of stains and markers used in the MESOS study is shown in table 2.1.

Although all antibodies were validated by the manufacturer for immunohistochemistry purposes, further in-house antibody validation was undertaken for the MESOS biopsy samples, using known positive and negative control tissues, in order to ensure confidence and reproducibility with the staining procedure. All immunostaining steps were performed using the Autostainer Link 48 (Dako, UK) with appropriate isotype controls.

Stained sections were scanned to digital image using a Carl Zeiss Imager Z2 microscope and AxioCam HRc digital camera (Carl Zeiss, Germany).

For the MESOS study 9280 sections were cut from 884 paraffin blocks. 8396 sections were stained, and 7689 slides were scanned to digital image.

Table 2.1: Immunohistochemical stains used for bronchial biopsy analysis

Inflammatory cells	Mesenchymal cells and vessels	Epithelium	Matrix	Remodelling activation markers
MBP (Monosan, NL) MON6008-1	α-SMA (Dako, UK) IR611	MUC5AC (Dako, UK) IR661	Tenascin (Abcam, UK) ab86182	Periostin (Abcam, UK) ab14041
CD3 (Dako, UK) IR503	CD34 (Dako, UK) M7165	Involucrin (Abcam, UK) ab68	Collagen IV (Dako, UK) M0785	TGF-β (Abcam, UK) ab66043
CD4 (Leica, UK) NCL-L-CD4-368	Collagen I (Abcam, UK) ab34710	Cytokeratin 7 (Dako, UK) IR619	Lumican (Abcam, UK) ab168348	Caspase 3 (Abcam, UK) ab2171
CD8 (Dako, UK) IR623	Endothelium (USBio, USA) E2292-02A	eCadherin (Dako, UK) M3612	Fibronectin (Dako, UK) Q0149	
Neutrophil Elastase (Dako, UK) M0752		CC16 (Hycult Bio, NL) HM2178		
Mast cell Tryptase (Dako, UK) IR640		P63 (Dako, UK) M7317		
Mast cell Chymase (Abcam, UK) ab2377				
Macrophage CD68 (Dako, UK) IR609				

CC16 = club cell secretory protein. CD = cluster of differentiation. MBP = major basic protein. MUC5AC = mucin-5AC. SMA = smooth muscle actin. TGF-β = transforming growth factor-beta. Stain, manufacturer and product code listed.

2.9.3 Analysis of biopsy samples

Immunohistochemical analysis of biopsy samples obtained pre- and post-treatment was performed using ZEN Pro 2012 (Carl Zeiss AG, Germany) and ICY (Institut Pasteur, France) software. In the MESOS study I measured and recorded approximately 2200 data points related to epithelial health and function. These outcomes included; semi-quantitative scoring and stain thresholding of slides stained for MUC5AC (mucin marker), Involucrin (epithelial metaplasia marker), Cytokeratin-7 (epithelial metaplasia marker) and e-Cadherin (cell adhesion

marker); cell counting on MUC5AC and Periodic acid–Schiff (PAS) slides; and quantitative measurements of epithelial integrity and reticular basement membrane thickness. The additional MESOS biopsy outcomes were measured by a colleague, and included inflammatory cell counts for eosinophils (this was the primary outcome of the study), mast cells, neutrophils, macrophages, and CD3+ T cells performed on the corresponding stained areas, and expressed as cells/mm^2 of the respective tissue area.

Stained slides were scored semi-quantitatively, which involves evaluating each biopsy section and grading the intensity of the staining on a four-point scale (0 = no staining, 1 = mild, 2 = moderate, 3 = strong). Stain intensity was then also assessed quantitatively using thresholding techniques, which involves manually setting thresholds for hue, light and saturation in the Zen Pro software. These are set using the most intensely stained sections as judged by the semi-quantitative method described above. Adjustments are made to the upper and lower limits of hue, light and saturation in order that positive stained areas on the section are included within the thresholds, but no un-stained structures. These threshold values are then tested against negative controls to ensure that the set thresholds are adequately selective for stained areas only. Once hue, light and saturation thresholds have been confirmed they are applied to the region of interest (such as the epithelium) to give a percentage value denoting how much of the region of interest is positively stained for that particular marker. This method was also applied to other structural regions of the biopsy, and using a range of other stains, as listed in table 2.1.

Reticular basement membrane thickness was measured 50 times on each section, approximately 20 micrometres apart. The mean of the 50 measurements is taken as the mean reticular basement membrane thickness for that particular section. Adequate samples must include at least 1mm of basement membrane (i.e. 50 x 20 micrometres). This method is validated by Sullivan et al [147].

Epithelial integrity was expressed as the percent of the total basement membrane length associated with healthy intact epithelium. This is done by measuring lengths of intact, damaged and denuded epithelium, and expressing each of these as a percentage of the total basement membrane length. In this way the proportion of intact, damaged and denuded epithelium can each be assessed.

Mucin-containing goblet cells in the epithelium were counted using MUC5AC stained sections, and expressed as positively stained cells per mm^2 of epithelium. PAS staining was also used to count positively stained cells containing mucin or glycogens in the epithelium.

All measurements and cellular counts were performed by one observer, blinded to participant identification, treatment allocation, and study visit, on two non-continuous tissue sections 30 µm apart in the same biopsy block (MESOS), or a single tissue section (thermoplasty).

2.10 Sputum induction and analysis

2.10.1 Collection of specimens

Sputum induction and processing was performed as previously described [148]. After confirming baseline forced expiratory volume in 1 second (FEV1) was at least 60% of predicted, and administration of 200 micrograms of salbutamol, participants inhaled 7ml of 3% saline delivered by ultrasonic nebuliser. Participants then blew their nose, rinsed their mouth and swallowed some water, before expectorating sputum into a specimen container. If an additional sample was needed then the process was repeated after re-checking FEV1. If the FEV1 had dropped by less than 10% from baseline then 4% saline was used, and subsequently 5% if required and providing FEV1 remained <10% lower than baseline. If FEV1 reduced by 10 to 20% then the same concentration of saline was

repeated. If FEV1 dropped by more than 20% then the procedure was discontinued.

2.10.2 Analysis of specimens

Differential inflammatory cell counts were recorded by a blinded observer. A total of 400 non-squamous cells were counted on cytospins stained with Rapidiff stain. Counts were expressed as a percentage of the total cells.

2.11 Statistical analysis

For the anti-IL-13 study (chapter 3), statistical analysis was performed using SAS 9·4 (Cary, North Carolina, United States) and R (Lucent Technologies, New Jersey, United States). The primary and secondary outcomes were analysed using geometric means, which allowed log-transformation of the data and dampened the skewing effect of extreme outlying data points. Biopsy specimens taken after 12 weeks of treatment with tralokinumab or placebo were assessed and compared with those taken before treatment. Changes from baseline to end of treatment were calculated as effect ratios for each of tralokinumab and placebo separately. The between-group results were then expressed as treatment effect ratios comparing the effects ratio for tralokinumab against the effect ratio for placebo. Therefore, a treatment effect ratio of 1.0 signifies no difference in effect between the two treatment groups. A treatment effect ratio for a given parameter of >1.0 indicates a greater increase, and <1.0 a greater decrease, in the tralokinumab group compared to the placebo group. Log transformed data were used for the primary and secondary analyses as these variables were known to have a log-normal distribution. The within-participant change for the primary outcome was analysed using analysis of covariance, including at least baseline values and treatment as covariates. Where the change from baseline for an individual participant was zero, the value was replaced by half the smallest change observed in the population to allow for the statistical analysis described above. The

secondary outcomes were performed using log transformed data with a mixed model for repeated measures, including at least baseline values, treatment, and treatment-by-visit interaction as covariates. The model included a treatment-time interaction to allow the treatment effect to change for each visit. The effect ratio of the geometric mean at Week 12 compared with baseline, and 95% confidence intervals (CI) are reported. P-values are presented for all outcomes. Exploratory analyses for change in FeNO, blood IgE, pre- and post-bronchodilator FEV1, FVC and submucosal CD3+ T cells were undertaken as per primary and secondary outcomes as these were log-normally distributed. Other exploratory endpoints were analysed as absolute change within and between treatment groups. Analysis of covariance or mixed model for repeat measures were applied to exploratory endpoints that were available either at baseline and Week 12, or at baseline and Weeks 6 and 12, respectively. Corrections were not made for multiplicity, and nominal significance for exploratory outcomes is reported. No imputation was done for missing data in these analyses. Subgroup analyses were performed in participants defined by baseline FeNO concentration (< or ≥37 ppb). FeNO has been identified as a potential predictor of tralokinumab response in the STRATOS 1 trial, following demonstration of enhanced efficacy in FeNO high (≥37 ppb) participants.

The sample size, based on the primary outcome, assumed a standard deviation of the treatment group log values of 1·62 and 1·82 for tralokinumab and placebo. It was therefore estimated that 31 participants per treatment group would be needed to achieve ≥80% power to detect a 3·5-fold difference between treatment groups, using a two sided test at the 5% significance level. With these assumptions, a 2·4-fold difference would be the smallest change required to yield a significant result. It was predicted that a proportion of participants would withdraw prematurely or produce poor quality biopsies, and therefore the target sample size was 40 participants per treatment arm.

In the AirPROM thermoplasty study (chapter 4), clinical outcomes were analysed using GraphPad Prism version 8.1.2 (San Diego, USA), using parametric (paired t

test) and non-parametric (Wilcoxon matched-pairs signed rank test) tests as appropriate, dependant on whether the variable was normally distributed. Assuming a mean ± standard deviation ASM mass of 25 ± 15% [89], n=14 subjects were required to observe an absolute reduction of 10% ASM mass using a one-tailed paired test with 80% power at the significance level of 0.05 (post-hoc calculation). Features of remodelling on baseline and follow-up biopsies were compared using paired t-tests. Relationships between clinical outcomes and biopsy changes, and different features of biopsy changes, were tested using Pearson's test. A p-value of <0.05 was considered statistically significant.

In the pooled thermoplasty analysis study (chapter 5), absolute change from baseline for biopsy outcomes was determined by subtracting baseline values from follow-up results. Relative change from baseline was determined by dividing the absolute change by the baseline values and expressed as a percentage. As some patients had more than one set of biopsies taken at a range of time-points within the first 12 months after thermoplasty treatment, the mean change from baseline for first two biopsy time-points was calculated and used for the correlations with clinical outcomes.

Clinical outcomes were analysed using GraphPad Prism version 8.1.2 (San Diego, USA), using paired t-tests. Features of remodelling on baseline and follow-up biopsies were compared using paired t-tests. Relationships between clinical outcomes and biopsy changes, and different features of biopsy changes, were tested using the Pearson's correlation coefficient. Responder/non-responder group analysis was undertaken using unpaired t-tests. Both ACQ6 and AQLQ have a minimum clinically important difference of 0.5, but sham thermoplasty has been shown to effect an AQLQ improvement of 1.1 in a population of moderate to severe asthmatics [95]. However, the results of my study described in chapter 4 showed treatment responses of 0.51, in a severe asthma population reflective of that which is included in this pooled study. Therefore responder analysis was undertaken using thresholds of ≥0.5 to <1.0 (termed 'responder') and ≥1.0 (termed 'super-responder') improvement in ACQ or AQLQ, where sufficient data

was available. For improvement in exacerbation rate after thermoplasty, the relative annual exacerbation rate in the 12 months after treatment was calculated compared to the preceding 12 months, and expressed as a percent. 'Responders' were defined as those with a post-treatment exacerbation rate of ≤50% of that at baseline (i.e. a ≥50% relative reduction), approximately in keeping with the exacerbation rate reduction seen with most licensed biological agents. 'Super-responder' was arbitrarily defined as those with a post-treatment exacerbation rate of ≤25% of baseline (i.e. a ≥75% relative reduction). A p-value of <0.05 was considered statistically significant.

3 Anti-interleukin-13 monoclonal antibody treatment for moderate-to-severe asthma (MESOS)

3.1 Introduction

Asthma is characterised by the symptoms and variable airflow obstruction associated with persistent airway inflammation and remodelling [3, 8, 149]. It is a heterogeneous condition with respect to clinical features and inflammatory profile [3], although most people with asthma have type-2 mediated immunity (deemed T helper 2 [Th2]-high) with eosinophilic inflammation [8, 149, 150]. This phenotype occurs in up to 80% of corticosteroid-naïve and 50% of corticosteroid-treated people with asthma [151].

Interleukin (IL)-13, an archetypal type-2 cytokine, is implicated in asthma pathogenesis and has been reported to play an important role in airway inflammation, airway hyper responsiveness, smooth muscle proliferation, and sputum production in preclinical animal studies and *in vitro* [117-119, 122]. IL-13 upregulates the production of eotaxin chemokines in the bronchial epithelium and airway smooth muscle (which act as chemo-attractant molecules on CCR3 receptors expressed on the eosinophil cell surface) [54, 119, 120, 122] and is thought to induce release of vascular adhesion molecules such as P-selectin [53]. This results in increased eosinophil adhesion to the endothelium, with subsequent eosinophil trafficking from the blood into the lung tissue [117, 121]. In addition, IL-13 augments eosinophil survival and activation [122]. Therefore, inhibiting IL-13 may lead to reduced eosinophilic airway inflammation and reduced airway hyper-responsiveness, making IL-13 an attractive target for novel asthma therapy.

Phase 2 and 3 studies of tralokinumab (AstraZeneca), a human monoclonal antibody that potently and specifically neutralises IL-13, have reported improvements in lung function, as measured by spirometry, with modest or no impact upon asthma exacerbations [66, 123, 125], contrasting with biologics

targeting IL-5 [17, 18] or its receptor [70, 72]. IL-13 neutralisation consistently leads to increases in peripheral blood eosinophil count [66, 123], likely due to inhibition of eosinophil–endothelial adhesion [48]. Conversely, IL-5 neutralisation or disruption of IL-5 signalling via IL-5 receptor blockade has resulted in marked reductions in blood and sputum eosinophil counts and, to a lesser extent, eosinophil count in the bronchial submucosa [128, 129]. Whether inhibition of IL-13 affects bronchial, or sputum eosinophil counts, has not previously been investigated.

We hypothesised that treatment with tralokinumab would have an effect on airway eosinophilic infiltration, blood and sputum eosinophil concentrations, eosinophil activation and airway remodelling. To test our hypothesis, we undertook MESOS, a phase 2, multicentre, randomised, double-blind, parallel-group, placebo-controlled, 12-week trial of tralokinumab in participants with inadequately controlled moderate to severe asthma. We analysed the change from baseline to Week 12 in bronchial, blood, and sputum eosinophil counts, fractional exhaled nitric oxide (FeNO) and total blood immunoglobulin (Ig) E concentrations, airway physiology, and other measures of airway inflammation and remodelling assessed by bronchial biopsies and quantitative computed tomography (CT).

3.2 Methods

3.2.1 Participants

Participants were recruited from 15 centres in the United Kingdom, Denmark, and Canada (table 3.2). This was a complex study that required centres with appropriate capabilities and willing participants to undertake all measurements required for the study endpoints. The centres reflected a federation of national networks that worked together to deliver the study. Participants were aged 18–75 years with a documented history of physician-diagnosed asthma for ≥12

months, requiring treatment with inhaled corticosteroids (ICS; ≥250 μg/day fluticasone propionate or equivalent) at a stable dose with or without other asthma controller medications. Participants receiving regular systemic corticosteroids or biologics were excluded. Current smokers and past-smokers of >10 pack-years, and participants with clinically significant co morbidities were also excluded. All participants were required to be exacerbation free for ≥6 weeks prior to enrolment, and to have had no more than three asthma exacerbations requiring treatment with oral corticosteroids in the preceding 12 months. Furthermore, all participants had post-bronchodilator forced expiratory volume in 1 second (FEV1) reversibility of ≥12% and 200 mL, and evidence of uncontrolled asthma (defined by an asthma control questionnaire [ACQ]-6 score ≥1·5) during the run-in phase. A full list of inclusion and exclusion criteria is provided in table 3.1.

Table 3.1: Inclusion and exclusion criteria

Inclusion criteria	
1	Informed consent prior to undertaking any study procedures
2	Female or male, aged 18 to 75 (inclusive)
3	Use of highly effective contraception in women of childbearing potential, from enrolment until 16 weeks after the last dose of investigational product, and a negative pregnancy test prior to enrolment
4	Weight ≥40 and <150 kg at enrolment
5	Documented physician-diagnosis asthma for at least 12 months, requiring treatment with ≥250µg fluticasone propionate or equivalent for at least 6 months and at stable dose for at least 1 month and during the run-in period
6	Stable dose of any additional asthma controller medications for at least 1 month and during the run-in period
7	Morning pre-bronchodilator FEV1 of >50% of predicted normal value, and at least 1 litre
8	Post-bronchodilator reversibility in FEV1 of ≥12% and ≥200ml
9	Acceptable inhaler, peak flow meter, and spirometry techniques
10	Minimum of 70% compliance with usual asthma medications and eDiary between visits 1 and 2
11	Asthma Control Questionnaire-6 (ACQ6) score of ≥1.5
12	Successful baseline bronchial biopsy procedure
Exclusion criteria	
1	History of interstitial lung disease, chronic obstructive pulmonary disease, or other clinically significant lung disease other than asthma which may compromise either the safety of the subject or study end point assessments
2	Significant physical or mental illness that is not stable, which could compromise the safety of the subject, influence the findings of the study, or impede the subject's ability to complete the entire duration of the study
3	Known history of allergy or reaction to any component of the investigational product formulation
4	History of anaphylaxis following any biological therapy
5	Helminth parasite infection diagnosed within 6 months prior to enrolment that has not been treated, or has failed to respond to treatment
6	Clinically significant infection, including upper and lower respiratory tract infection, requiring antibiotic or antiviral therapy within 30 days of enrolment or within the run-in period
7	Tuberculosis treatment in the preceding 12 months
8	Clinically significant finding on physical examination, vital signs, electrocardiogram (ECG) , CT scan, blood tests, or urinalysis during enrolment or run-in which may compromise the safety of the subject, the results of the study, or the subject's ability to complete the entire duration of the study
9	Chronic alcohol or drug abuse within 12 months of enrolment, or a condition associated with poor compliance
10	Positive hepatitis B or C serology
11	Known primary immunodeficiency including human immunodeficiency virus (HIV)
12	Current tobacco smoking (must have stopped for at least 3 months prior to enrolment) or a history of tobacco smoking for >10 pack-years
13	History of cancer, with the exception of; basal cell carcinoma of the skin, localised squamous cell carcinoma of the skin or carcinoma of the cervix in situ, which is in remission and curative therapy was completed at least 12 months ago; or any malignancy which is in remission and curative therapy was completed at least 5 years ago
14	Use of immunosuppressive medication within 3 months of enrolment

15	Chronic use of oral corticosteroids
16	Unable to safely undergo flexible fibre-optic bronchoscopy
17	Asthma exacerbation requiring hospitalisation or treatment with oral corticosteroids within 6 weeks of enrolment, or more than 3 asthma exacerbations requiring oral corticosteroid treatment in the preceding 12 months, or history of intubation or intensive care treatment for asthma
18	Receipt of immunoglobulin or blood products within 30 days prior to enrolment
19	Receipt of any biological agent within 4 months or 5 half-lives prior to enrolment
20	Receipt of live attenuated vaccines within 30 days prior to enrolment or during the study period, or receipt of inactive/killed vaccines within 5 days prior to a dosing visit
21	Receipt of any investigational non-biological agent with 30 days or 5 half-lives prior to enrolment
22	Previous receipt of tralokinumab
23	New allergen immunotherapy or change in existing immunotherapy within 30 days prior to enrolment, or receipt of any immunotherapy within 5 days of a dosing visit
24	Current use of oral or ophthalmic non-selective β-adrenergic antagonist
25	Current use of five-lipoxygenase inhibitors or roflumilast
26	Previously undergone bronchial thermoplasty
27	Major surgery within 8 weeks prior to enrolment, or planned inpatient surgery or hospitalisation during the study period
28	Alanine aminotransferase (ALT) or aspartate aminotransferase (AST) level ≥2.5 times the upper limit of normal at enrolment
29	Currently pregnant or breast-feeding
30	Previous randomisation in the present study
31	Concurrent enrolment in another clinical study where the subject is receiving an investigational product
32	Involvement in the planning and/or conduct of the study (applies to both AstraZeneca staff and/or staff at study sites)
33	Employee of a study site, or any other any other individual directly involved with the planning on conduct of the study, or immediate family members of such individuals

Kg = kilograms, µg = micrograms, FEV1 = forced expiratory volume in 1 second, ml = millilitres, CT = computer tomography, pack-years calculated as: (number of cigarettes smoked per day/20) x number of years smoked.

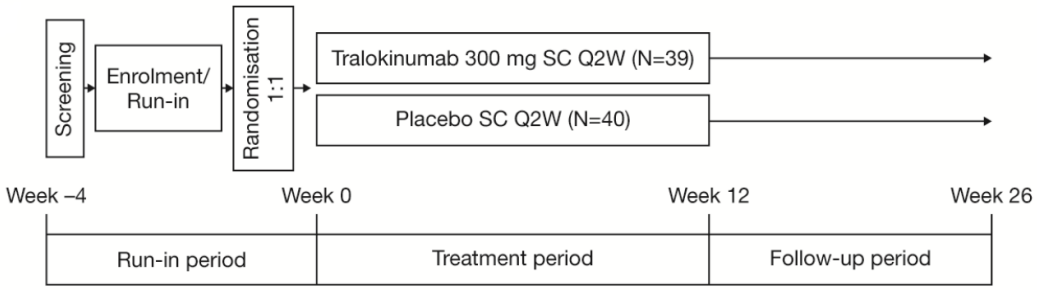
The trial was conducted in accordance with the Declaration of Helsinki and the International Conference on Harmonisation Guidance for Good Clinical Practice. Independent ethics committee approval was obtained at all participating centres and all participants provided written informed consent. The trial was registered with ClinicalTrials.gov (NCT02449473) and EudraCT (2015-000857-19).

3.2.2 Study design

MESOS was a phase 2, randomised, double-blind, parallel-group, placebo controlled, 12-week trial. The study design is summarised in figure 3.1. A four-week run-in period (to ensure participant eligibility, asthma stability, and

adequate compliance with trial procedures such as symptom diary completion and asthma treatment adherence) was followed by randomisation to 12 weeks of treatment with either tralokinumab 300 mg or placebo, administered subcutaneously (SC) every 2 weeks (Q2W) in addition to standard-of-care treatment. Assessments including fibre-optic bronchoscopy with biopsies and brushings, thoracic CT, blood, sputum and urine sampling, and lung function measures, which were performed prior to treatment initiation and at the end of the treatment period. The immunohistochemical stains used for bronchial biopsy analysis are shown in table 2.1. A four week follow-up period was then undertaken (extended to 14 weeks for women of child bearing potential). Participants also completed electronic symptom questionnaires and performed home electronic peak flow measurements twice per day during the study period. Further detail of the assessments performed is included in chapter 2.

Figure 3.1: Study design



Mg = milligrams, SC = subcutaneous, Q2W = every 2 weeks.

Criteria for withdrawal from the trial were defined *a priori*, and included withdrawal of consent, pregnancy, and the occurrence of an adverse event (AE) where continued exposure to treatment could be detrimental to the participant.

3.2.3 Randomisation and masking

Participants were randomised 1:1 to receive tralokinumab 300 mg SC Q2W or placebo by an interactive web or voice response system. Participants, site staff and investigators, and sponsor personnel remained blinded to treatment allocation until trial completion and the database had been locked. Unblinding of treatment allocation was not required for any participant.

3.2.4 Outcomes

The primary outcome was change from baseline to Week 12 in airway submucosal eosinophils per mm^2 of the lamina propria (determined by bronchial biopsy). Secondary outcomes were change from baseline to Week 12 in eosinophil count and eosinophil cationic protein (ECP) concentration, measured in blood and sputum.

Exploratory outcomes included change from baseline to Week 12 in FeNO concentration, total blood IgE concentration, daily asthma symptom score, ACQ6 score, sino-nasal outcome test (SNOT)-20 score, and airway physiology measured by: spirometry (determined by pre- and post-bronchodilator FEV1, forced vital capacity (FVC), and forced expiratory flow of 25–75% of the FVC (FEF25–75)); airwave oscillometry (Tremoflo (Thorasys Thoracic Medical Systems, Montreal, Canada), determined by R5–R20 and reactance area); lung volume (evaluated by body box-determined total lung capacity (TLC) and residual volume (RV)), and airway hyper responsiveness (evaluated by the methacholine provocation concentration required to cause a 20% decrease in FEV1 (PC20)). Other exploratory outcomes measured were change from baseline to Week 12 in sputum differential cell count, airway inflammation and remodelling (determined via bronchial biopsy to evaluate cell count per mm^2 of the lamina propria), lamina reticularis, and reticular basement membrane (RBM) thickness, intensity determined by percentage change in thresholding, epithelial integrity, and airway smooth muscle area. Changes in airway lumen and wall dimension in airway generations 3–5, air-trapping, and parametric response mapping parameters were

assessed by quantitative CT using analysis software from VIDA Diagnostics (Coralville, Iowa, United States) and Imbio (Minnesota, Minneapolis, United States) [152-154].

AEs, including serious AEs (SAEs) and AEs leading to discontinuation, were recorded from the receipt of informed consent to the end of the follow-up period. The study did not include a data safety monitoring board as it was of a short duration and had a small number of participants.

3.2.5 Statistical analysis

Statistical analysis was performed using SAS 9·4 (Cary, North Carolina, United States) and R (Lucent Technologies, New Jersey, United States). The primary and secondary outcomes were analysed using geometric means, which allowed log-transformation of the data and dampened the skewing effect of extreme outlying data points. The effect ratio at Week 12 compared with baseline was calculated for the tralokinumab and placebo arms; the between-group treatment effect ratio was also calculated. Log transformed data were used for the primary and secondary analyses as these variables were known to have a log-normal distribution. The within-participant change for the primary outcome was calculated using analysis of covariance, including at least baseline values and treatment as covariates. Where the change from baseline for an individual participant was zero, the value was replaced by half the smallest change observed in the population, to allow for statistical analysis as described above. The secondary outcomes were performed using log transformed data with a mixed model for repeated measures, including at least baseline values, treatment, and treatment-by-visit interaction as covariates. The model included a treatment-time interaction to allow the treatment effect to change for each visit. The effect ratio of the geometric mean at Week 12 compared with baseline, and 95% confidence intervals (CI) are reported. P-values are presented for all outcomes. Exploratory analyses for change in FeNO, blood IgE, pre- and post-bronchodilator FEV1, FVC and submucosal CD3+ T cells were undertaken as per primary and secondary

outcomes as these were log-normally distributed. Other exploratory endpoints were analysed as absolute change within and between treatment groups. Analysis of covariance or mixed model for repeat measures were applied to exploratory endpoints that were available either at baseline and Week 12, or at baseline and Weeks 6 and 12, respectively. Corrections were not made for multiplicity, and nominal significance for exploratory outcomes is reported. No imputation was done for missing data in these analyses. Subgroup analyses were performed in participants defined by baseline FeNO concentration (< or ≥37 ppb). FeNO has been identified as a potential predictor of tralokinumab response in the STRATOS 1 trial, following demonstration of enhanced efficacy in FeNO high (≥37 ppb) participants.

The sample size, based on the primary outcome, assumed a standard deviation of the treatment group log values of 1·62 and 1·82 for tralokinumab and placebo. It was therefore estimated that 31 participants per treatment group would be needed to achieve ≥80% power to detect a 3·5-fold difference between treatment groups, using a two sided test at the 5% significance level. With these assumptions, a 2·4-fold difference would be the smallest change required to yield a significant result. It was predicted that a proportion of participants would withdraw prematurely or produce poor quality biopsies, and therefore the target sample size was 40 participants per treatment arm.

3.3 Results

Between 25th September 2015 and 21st June 2017, a total of 224 participants were enrolled and screened for inclusion, with 172 entering the four week run-in period (figure 3.2). Of these, 88 participants did not meet eligibility criteria and five withdrew consent. The most common reasons for failing to meet eligibility criteria were; FEV_1 bronchodilator reversibility of less than the required 12% and 200ml, and ACQ6 score of less than the required 1.5 (57% and 17% of excluded

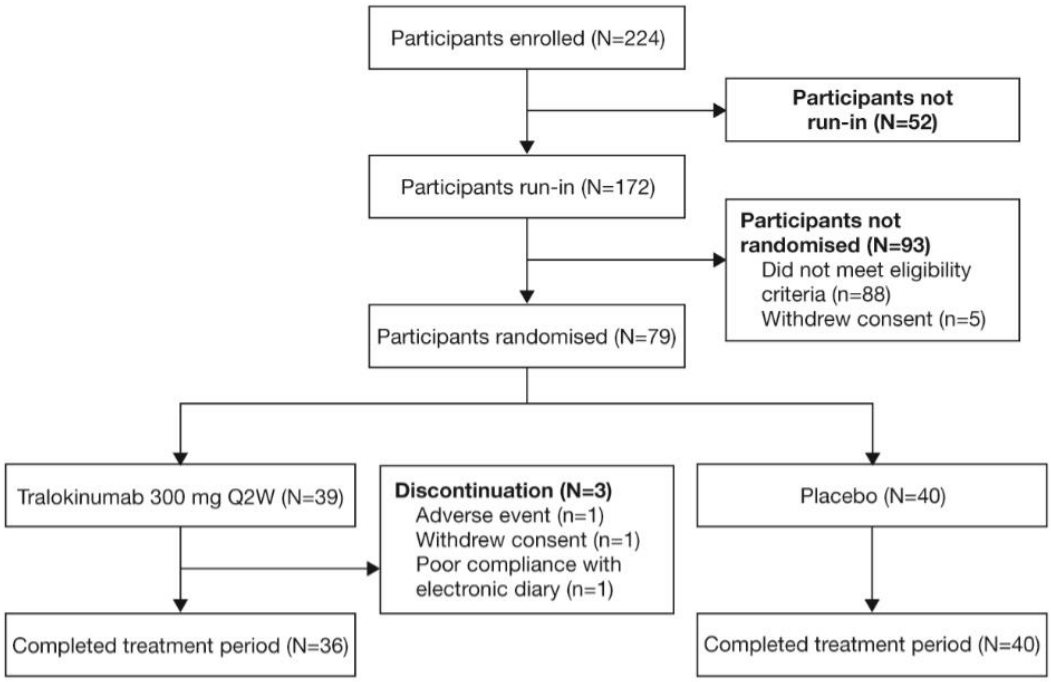
participants respectively). Eligible participants were subsequently randomised to receive tralokinumab (n=39) or placebo (n=40). Compliance to treatment with tralokinumab was high (table 3.3) and all participants that completed the study successfully underwent baseline and end of treatment bronchoscopy. The biopsies obtained were of sufficient quality for analysis. A representative photomicrograph of a bronchial biopsy stained for major basic protein-positive eosinophils is shown in figure 3.3A. Adequate paired sputum samples were obtained in only 16 participants that received tralokinumab and 17 that received placebo. This was primarily due to some centres being unable to obtain samples of sufficient quantity, despite each centre receiving sputum induction training.

Table 3.2: Participating trial sites and recruitment status

Country	**Lead investigator**	**Participants enrolled (n)**	**Participants randomised (n)**
Canada	Dr. J Mark FitzGerald	8	5
Canada	Dr. Michel Laviolette	8	3
Canada	Dr. Ronald Olivenstein	6	3
Denmark	Læge Vibeke Backer	27	7
Denmark	Læge Ingrid Titlestad	14	4
Denmark	Dr. Tina Skjold	4	4
Denmark	Læge Charlotte Suppli Ulrik	18	4
Denmark	Læge Carl Nielsen	14	4
United Kingdom	Dr. Brian Leaker	31	16
United Kingdom	Dr. Sukh David Singh	43	12
United Kingdom	Prof. Chris E Brightling	22	8
United Kingdom	Dr. Lorcan McGarvey	22	4
United Kingdom	Dr. Timothy Harrison	6	3
United Kingdom	Dr. Rekha Chaudhuri	3	2
United Kingdom	Dr. Peter Howarth	2	0

Number (n) of participants enrolled and subsequently randomised at each of the 15 study centres.

Figure 3.2: Participant disposition



Q2W = every 2 weeks.

Table 3.3: Number of study drug doses received

Doses received, n (%)	Placebo (n=40)	Tralokinumab (n=39)
6	39 (97·5)	34 (87·2)
5	40 (100)	36 (92·3)
4	40 (100)	37 (94·9)
3	40 (100)	39 (100)
2	40 (100)	39 (100)
1	40 (100)	39 (100)

A participant was considered to have received a dose of study treatment on a given occasion, whether they received a full or partial dose.

Baseline demographics and clinical characteristics (table 3.4) and baseline sputum, bronchial biopsy (table 3.5), ACQ6, FeNO concentration, physiological, and CT parameters (table 3.6) were similar for those participants receiving tralokinumab versus placebo.

Table 3.4: Baseline demographics and clinical characteristics

	Tralokinumab 300mg Q2W (N=39)	**Placebo Q2W (N=40)**	**p-value**
Age, years, mean (SD)	47·1 (14·2)	50·1 (14·2)	0.35
Sex, n (%)			0.50
Male	16 (41·0)	20 (50·0)	
Female	23 (59·0)	20 (50·0)	
Race, n (%)			0.16
White	34 (87·2)	39 (97·5)	
Black	2 (5·1)	1 (2·5)	
Asian	3 (7·7)	0 (0·0)	
Body Mass Index, kg/m^2, mean (SD)	28·42 (5·68)	27·80 (5·51)	0.62
Smoking status, n (%)			>0.99
Never	25 (64·1)	25 (62·5)	
Former	14 (35·9)	15 (37·5)	
Atopy (Phadiatop, n [%])			0.50
Positive	29 (74·4)	25 (62·5)	
Negative	9 (23·1)	14 (35·0)	
Not done	1 (2·6)	1 (2·5)	
Asthma exacerbations in last 12 months, n (%)			0.58
0	25 (64·1)	26 (65·0)	
1	8 (20·5)	11 (27·5)	
2	5 (12·8)	3 (7·5)	
3	1 (2·6)	0 (0·0)	
ICS dose, n (%)			0.99
Low	12 (30·8)	12 (30·0)	
Medium	10 (25·6)	10 (25·0)	
High	17 (43·6)	18 (45·0)	
Other asthma medications, n (%)			
LABA	32 (82·1)	34 (85·0)	0.77
LAMA	1 (2·6)	7 (17·5)	0.06
LTRA	6 (15·4)	4 (10·0)	0.52
Xanthine	2 (5·1)	0 (0·0)	0.24

ICS = inhaled corticosteroid. LABA = long-acting beta-agonist. LAMA = long-acting muscarinic receptor antagonist. LTRA = leukotriene receptor antagonist. SD = standard deviation. Q2W = every 2 weeks. p-values are t-tests for age and BMI, and Fisher's exact (2 variables) or chi-square (>2 variables) for all other characteristics.

Tralokinumab did not significantly alter bronchial eosinophil count compared with placebo at Week 12 (treatment effect ratio [95% CI]: 1·43 [0·63, 3·27], P=0·39) (table 3.5 and figure 3.3B). Nor did tralokinumab significantly change blood and sputum eosinophil counts (treatment effect ratio [95% CI]: 1·21 [1·00, 1·48]; P=0·055, and 0·57 [0·06, 6·00]; P=0·63, respectively; table 3.5 and figures 3.3C and 3.3D), or blood and sputum ECP concentrations (treatment effect ratio [95% CI]: 1·11 [0·88, 1·40]; P=0·38, and 0·49 [0·20, 1·20]; P=0·11, respectively), compared

with placebo (table 3.5 and figures 3.3E and 3.3F). However, there was a numerical increase in blood and bronchial eosinophil counts, and blood ECP concentration, in contrast to a numerical decrease in sputum eosinophil count and ECP concentration in tralokinumab- versus placebo-treated participants.

FeNO concentration and total blood IgE were significantly reduced in tralokinumab treated participants compared with placebo (table 3.6 and figures 3.4A and 3.4B). ACQ6 score improved substantially from baseline in participants who received tralokinumab or placebo but was not significantly different between treatment groups (table 3.6 and figure 3.4C). Mean pre-bronchodilator FEV1 increased numerically in those treated with tralokinumab versus placebo, but the between-group effect was not significant (table 3.6 and figure 3.4D). There was no difference in post-bronchodilator FEV1 or airway hyper-responsiveness between treatment groups (table 3.6). Small airway resistance heterogeneity (R5–R20) and reactance measures from airwave oscillometry were numerically improved in those receiving tralokinumab versus placebo (table 3.6). There were small improvements observed in airway lumen area determined by CT, which were statistically significant for generation 3 airways, and small numerical improvements in air-trapping indices in tralokinumab treated participants versus placebo (table 3.6).

Table 3.5: Bronchial biopsy airway inflammation and remodelling and sputum cell differentials

	Baseline values		Week 12 values		Change from baseline to Week 12			
	Tralokinumab 300 mg Q2W	Placebo Q2W	Tralokinumab 300 mg Q2W	Placebo Q2W	Tralokinumab 300 mg Q2W	Placebo Q2W	Treatment difference (95% CI)	*P*-value
Eosinophilic inflammation								
Eosinophils/mm² lamina propria#	40 [55]	31 [35]	56 [57]	38 [44]	1·42	0·99	1·43 (0·63, 3·27)	0·39
Blood eosinophil count (×10⁹/L)#	0.30 [0·19]	0·27 [0·14]	0·37 [0·27]	0·26 [0·15]	1·11	0·91	1·21 (1·00, 1·48)	0·055
Sputum eosinophils (10⁶/g) (n=16 vs 17)#	0.51 [1·02]	0·50 [1·34]	0·22 [0·28]	0·16 [0·20]	0·27	0·46	0·57 (0·06, 6·00)	0·63
Blood ECP (µg/L) (n=24 vs 28)#	20 [23]	23 [19]	21 [12]	22 [20]	1·05	0·95	1·11 (0·88, 1·40)	0·38
Sputum ECP (µg/L) (n=16 vs 17)#	120 [165]	148 [208]	131 [179]	202 [313]	0·75	1·54	0·49 (0·20, 1·20)	0·11
Inflammatory cells/mm² lamina propria								
CD3⁺ T cells#	193 [113]	201 [117]	258 [162]	254 [189]	1·24	1·16	1·06 (0·74, 1·52)	0·73
Neutrophils	57 [43]	75 [54]	66 [31]	77 [44]	–1 [7]	9 [6]	–10 (–28, 8)	0·28
Macrophages	81 [39]	87 [53]	96 [52]	87 [56]	13 [9]	2 [8]	11 (–14, 35)	0·39
Mast cells	34 [32]	34 [31]	41 [36]	23 [20]	4 [5]	–13 [4]	18 (5, 30)	0·0069
Tissue remodelling in bronchial biopsies								
RBM thickness (µm)	7·4 [1·9]	8·2 [2·5]	6·9 [2·2]	7·0 [1·7]	–0·7 [0·3]	–0·9 [0·3]	0·2 (–0·7, 1·0)	0·73
Periostin (%)	25 [19]	23 [19]	21 [17]	18 [15]	–5 [2]	–5 [2]	1 (–6, 7)	0·83
Intact epithelium (%)	32 [21]	33 [17]	35 [22]	31 [20]	1 [4]	–2 [3]	3 (–7, 13)	0·51
Partially intact epithelium (%)	45 [16]	48 [15]	43 [18]	48 [18]	–3 [3]	1 [3]	–4 (–12, 4)	0·35
Denuded epithelium (%)	24 [18]	20 [16]	22 [23]	21 [21]	2 [4]	1 [4]	1 (–10, 11)	0·87
Epithelial MUC5AC (%)	12 [8]	12 [7]	12 [11]	13 [9]	–1 [2]	1 [1]	–2 (–6, 3)	0·40
Airway Smooth Muscle Area (%)	12 [7]	16 [9]	11 [6]	10 [8]	–3 [1]	–4 [1]	1 (–2, 5)	0·47
Collagen Type IV (%)	11 [6]	12 [8]	12 [8]	13 [9]	1 [1]	1 [1]	0 (–4, 3)	0·79
Fibronectin (%)	14 [11]	16 [12]	16 [11]	23 [14]	2 [2]	8 [2]	–6 (–12, 0)	0·041
Tenascin (%)	14 [14]	13 [11]	14 [18]	19 [16]	1 [3]	6 [3]	–6 (–13, 2)	0·13
TGF-β (%)	9·7 [6·7]	11·1 [5·8]	7·4 [8·3]	10·3 [9·3]	–3·2 [1·4]	–0·5 [1·4]	–2·6 (–6·6, 1·3)	0·19
TGF-β⁺ cells/mm² lamina propria	113 [67]	117 [80]	111 [55]	128 [98]	–4 [14]	13 [13]	–18 (–56, 20)	0·35
Sputum (n=16 tralokinumab vs 17 placebo)								
Eosinophils (%)	11·0 [14·5]	8·1 [17·8]	10·2 [16·2]	8·2 [13·9]	–1·3 [3·1]	–3·5 [3·0]	2·2 (–6·5, 10·8)	0·61
Macrophages (%)	36 [22]	34 [22]	23 [19]	34 [23]	–10 [5]	–1 [5]	–9 (–24, 6)	0·24
Neutrophils (%)	47 [23]	53 [25]	54 [29]	52 [26]	5 [7]	4 [7]	0 (–19, 20)	0·96

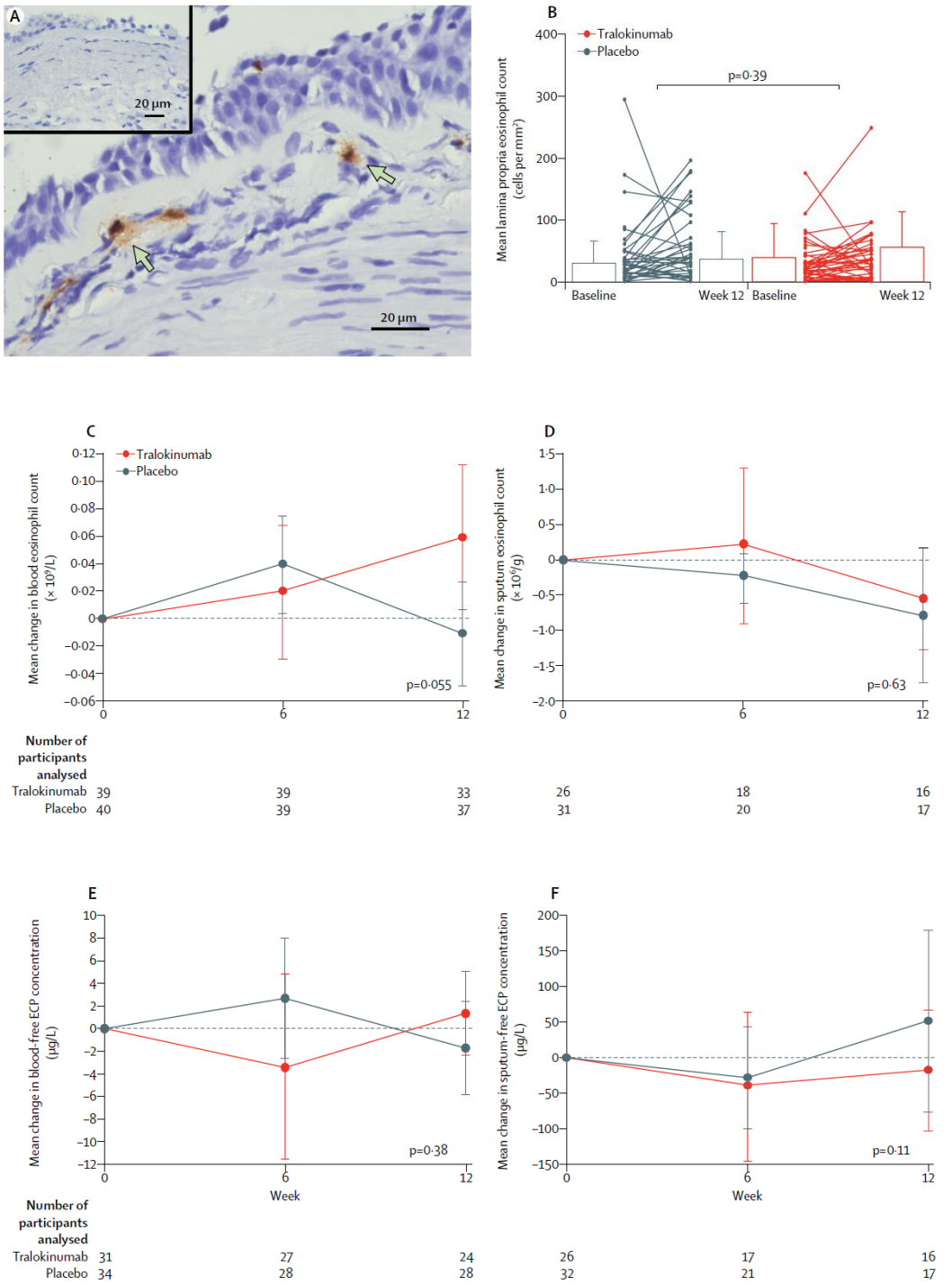
Baseline and post-treatment values are given as arithmetic mean [SD]; change from baseline to Week 12 in each treatment group are presented as LS mean [SE] unless otherwise stated. #Change from baseline to Week 12 in each treatment group are presented as LS geometric mean ratios. Nominal P-values are given for all exploratory endpoints. CI = confidence interval. ECP = eosinophil cationic protein. LS = least squares. MUC5AC = mucin-5AC. Q2W = every 2 weeks. RBM = reticular basement membrane. SD = standard deviation. TGF-β = transforming growth factor-beta.

Table 3.6: Outcome measures at baseline and post-treatment in the full analysis set population

	Baseline values		Week 12 values		Change from baseline to Week 12			
	Tralokinumab 300 mg Q2W N=36	**Placebo Q2W N=40**	**Tralokinumab 300 mg Q2W**	**Placebo Q2W**	**Tralokinumab 300 mg Q2W**	**Placebo Q2W**	**Treatment difference (95% CI)**	***P*-value**
Asthma control, symptoms, FeNO and IgE								
ACQ6 score	2·24 [0·83]	2·12 [0·86]	1·27 [0·86]	1·28 [0·93]	–0·96 [0·14]	–0·87 [0·14]	–0·08 (–0·47,0·31)	0·67
FeNO (ppb)[#]	39·54 [30·05]	32·23 [24·82]	25·42 [18·48]	29·70 [19·98]	0·69	0·89	0·78 (0·63, 0·96)	0·023
Blood total IgE (IU/mL)[#]	534 [798]	420 [778]	345 [404]	445 [796]	0·81	0·94	0·86 (0·77, 0·97)	0·014
Lung function								
FEV_1 pre-bronchodilator (L)[#]	2·46 [0·79]	2·37 [0·62]	2·57 [0·83]	2·43 [0·62]	1·05	1·03	1·02 (0·97, 1·08)	0·47
FEV_1 post-bronchodilator (L)[#]	2·75 [0·80]	2·67 [0·67]	2·76 [0·86]	2·62 [0·66]	1·01	1·00	1·00 (0·96, 1·05)	0·91
FVC pre-bronchodilator (L)[#]	3·74 [1·08]	3·73 [0·91]	3·83 [1·11]	3·77 [0·92]	1·02	1·01	1·01 (0·97, 1·05)	0·56
FEF_{25-75} pre-bronchodilator (L/s)	1·51 [0·78]	1·36 [0·70]	1·68 [0·89]	1·38 [0·62]	0·19 [0·07]	0·01 [0·07]	0·18 (–0.01, 0·37)	0·067
FEF_{25-75} post-bronchodilator (L/s)	1·88 [0·89]	1·71 [0·80]	1·94 [1·03]	1·68 [0·78]	0·06 [0·06]	–0·03 [0·06]	0·09 (–0·08, 0·26)	0·28
RV post-bronchodilator (L) (n=26 vs 29)	2·00 [0·75]	2·16 [0·74]	2·08 [0·71]	2·16 [0·81]	0·07 [0·08]	0·00 [0·07]	0·07 (–0·15, 0·28)	0·53
TLC post-bronchodilator (L) (n=26 vs 29)	5·94 [1·42]	6·15 [1·35]	6·04 [1·28]	6·09 [1·29]	0·06 [0·07]	–0·04 [0·06]	0·10 (–0·08, 0·29)	0·28
Methacholine $PC_{20}FEV_1$ (mg/mL) (n=20 vs 19)	3·00 [5·08]	5·02 [6·40]	3·93 [6·08]	5·12 [6·10]	0·08 [1·24]	0·68 [1·27]	–0·60 (–4·25, 3·06)	0·74
Airwave oscillometry								
R5–R20 (kPa s/L)	0·16 [0·16]	0·14 [0·14]	0·13 [0·12]	0·14 [0·12]	–0·04 [0·02]	–0·01 [0·01]	–0·03 (–0·07, 0·01)	0·19
AX (kPa/L)	2·75 [3·12]	2·64 [2·16]	2·38 [2·52]	2·40 [2·52]	–0·48 [0·38]	–0·32 [0·36]	–0·16 (–1·21, 0·89)	0·76
Quantitative CT parameters								
Generation 3 luminal area / BSA (mm^2/m^2)	16·9 [5·5]	15·3 [4·4]	17·2 [5·4]	14·6 [3·7]	0·4 [0·5]	–1·0 [0·5]	1·4 (0·1, 2·7)	0·042
Generation 4 luminal area / BSA (mm^2/m^2)	9·9 [2·7]	9·1 [2·1]	9·9 [2·4]	9·1 [1·7]	0·1 [0·2]	–0·2 [0·2]	0·3 (–0·4, 1·0)	0·33
Generation 5 luminal area / BSA (mm^2/m^2)	7·2 [2·3]	7·1 [1·5]	7·9 [2·0]	7·2 [1·6]	0·2 [0·2]	–0·1 [0·2]	0·3 (–0·4, 0·9)	0·44
Air-trapping index <–856 HU (%) (n=33 vs 36)	11·65 [13·22]	11·14 [10·04]	11·28 [10·49]	10·85 [10·93]	–1·28 [1·23]	–0·80 [1·18]	–0·48 (–3·89, 2·92)	0·78
PRM fSAD (%) (n=34 vs 36)	8·9 [14·7]	7·1 [8·8]	7·9 [10.0]	7·3 [10·6]	–1·0 [1·4]	–0·7 [1·3]	–0·4 (–4·2, 3·4)	0·85

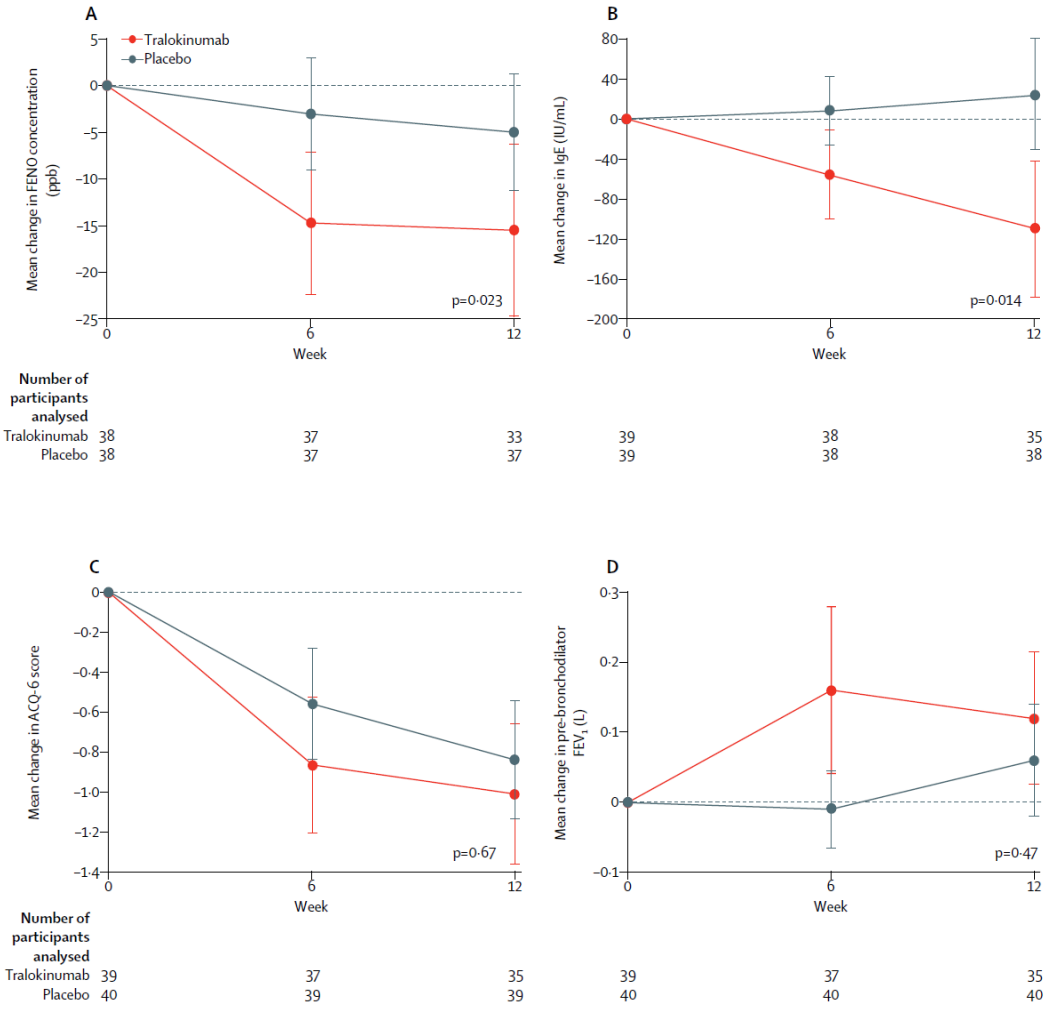
Baseline and post-treatment values are given as arithmetic mean [SD]; change from baseline to Week 12 in each treatment group are presented as LS mean [SE] unless otherwise stated. [#]Change from baseline to Week 12 in each treatment group are presented as LS geometric mean ratios. All P-values are nominal. ACQ6 = asthma control questionnaire-6. AX = reactance area. BSA = body surface area. CI = confidence interval. HU = Hounsfield unit. FeNO = fractional exhaled nitric oxide. $FEF_{25–75}$ = forced expiratory flow of 25–75% of the FVC. FEV_1 = forced expiratory volume in 1 second. fSAD = functional small airways disease. FVC = forced vital capacity. IgE = immunoglobulin E. LS = least squares. $PC_{20}FEV_1$ = methacholine provocation concentration required to cause a 20% decrease in FEV_1. PRM = parametric response mapping. Q2W = every 2 weeks. RV = residual volume. SD = standard deviation. TLC = total lung capacity.

Figure 3.3: Eosinophilic inflammation outcomes



Representative photomicrograph of a bronchial biopsy stained for MBP-positive eosinophils with isotype control as inset (A); lamina propria eosinophil count at baseline and Week 12 (B); change from baseline (absolute difference [95% confidence intervals]) in eosinophil count in blood (C) and sputum (D), and ECP concentration in blood (E) and sputum (F), at Week 6 and Week 12. P-values refer to differences between treatment groups in LS geometric mean ratio with respect to change from the baseline visit. CI = confidence interval. ECP = eosinophil cationic protein. LS = least squares. MBP = major basic protein. Q2W = every 2 weeks. SD = standard deviation.

Figure 3.4: Exploratory outcomes: FeNO (A), IgE (B), ACQ6 (C) and FEV1 (D)



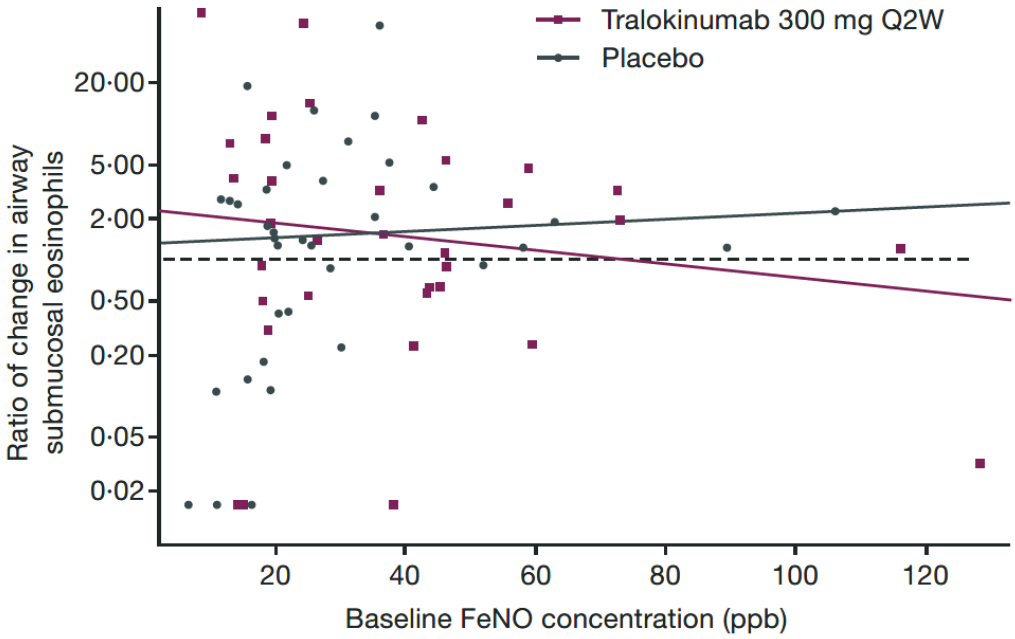
Change from baseline (absolute difference [95% confidence intervals]) in FeNO concentration (A), IgE concentration (B), ACQ-6 (C), and pre-bronchodilator FEV_1 (D), at Week 6 and Week 12. P-values are nominal and refer to differences between treatment groups in LS geometric mean ratio with respect to change from the baseline visit. ACQ6 = asthma control questionnaire-6. BD = bronchodilator. CI = confidence interval. FeNO = fractional exhaled nitric oxide. FEV_1 = forced expiratory volume in 1 second. ppb = parts per billion. LS = least squares. Q2W = every 2 weeks. SD = standard deviation.

In bronchial biopsies, there was no observed difference between tralokinumab- and placebo treated participants in inflammatory cell (T cell, neutrophil, and macrophage) counts, features of remodelling (airway smooth muscle mass, RBM thickening, epithelial integrity, goblet cell count, and epithelial expression of mucin 5AC, involucrin, cytokeratin-7 and eCadherin) and expression in the lamina propria of periostin, transforming growth factor-beta (TGF-β), type IV collagen,

and tenascin (table 3.5). There was a statistically significant difference in the mast cell number and fibronectin deposition between those receiving tralokinumab versus placebo, which was a consequence of changes following receipt of placebo rather than tralokinumab, and therefore unlikely to be an effect of treatment (table 3.5).

Subgroup analyses in FeNO-high (≥37 ppb) and FeNO-low (<37 ppb) participants did not demonstrate a significant difference between treatment groups in eosinophilic inflammation in bronchial biopsies. Furthermore, there was no correlation between baseline FeNO concentration and change in bronchial submucosal eosinophil count (figure 3.5).

Figure 3.5: Scatterplot of baseline FeNO concentration vs change in bronchial biopsy eosinophil count in participants treated with tralokinumab or placebo



FeNO = fractional exhaled nitric oxide. ppb = parts per billion. mg = milligrams. Q2W = every 2 weeks.

The incidence of AEs was similar between treatment groups. One participant withdrew from treatment in the tralokinumab group because of an injection-site reaction. There were no deaths in either treatment group. One SAE of asthma was reported in a participant receiving placebo. The most frequently reported AEs were upper respiratory tract viral infection and headache. There were numerically fewer participants reporting these AEs in tralokinumab treated participants compared with placebo (8 versus 17 for upper respiratory tract viral infection; 2 versus 9 for headache). AEs considered related to study drug administration by the investigator and injection-site reactions occurred more frequently in participants treated with tralokinumab than placebo.

3.4 Discussion

In participants with moderate to severe asthma inadequately controlled despite treatment with inhaled corticosteroids, the anti–IL-13 monoclonal antibody tralokinumab did not significantly reduce eosinophilic airway inflammation compared with placebo. Consistent with previous studies, tralokinumab-treated participants demonstrated a numerical increase in blood eosinophil count [66] with a concomitant increase in blood ECP concentration, and a hitherto unreported numerical increase in bronchial eosinophil count compared to placebo. Combined with a numerical decrease in sputum eosinophil count, this suggests that IL-13 neutralisation might promote eosinophil retention in blood and bronchial submucosa, although none of these trends were statistically significant.

Importantly, tralokinumab did significantly reduce FeNO and blood IgE concentrations versus placebo, confirming a biological effect of anti-IL-13 therapy. This is in keeping with previous studies of IL-13 neutralisation [67, 155]. Furthermore, the magnitude of effect upon FeNO was similar to previous reports describing the effect of inhaled corticosteroids [156, 157] and greater than the effect of treatment with oral corticosteroids [158]. Observational studies have

demonstrated that FeNO concentration and eosinophilic airway inflammation are correlated, with both biomarkers reduced with corticosteroid therapy. However, the regulation of FeNO concentration and eosinophilic inflammation have been shown to be independent in studies of IL-5 antagonists where sputum and bronchial eosinophil counts were reduced without an effect on FeNO concentration [149]. In keeping with this view, baseline FeNO concentration was not significantly correlated to change from baseline to Week 12 in bronchial eosinophil count. Periostin and dipeptidyl peptidase-4 (DPP-4) have also been proposed as biomarkers of the IL-13 axis [66]. Submucosal periostin concentration decreased in response to treatment with tralokinumab and placebo, with no difference between groups. Serum periostin is weakly responsive to tralokinumab (a 17% reduction in concentration from baseline to Week 52 was demonstrated in STRATOS 1 [125]). This was in contrast to a 26% reduction in FeNO concentration, which is similar to the magnitude of effect seen here in the MESOS study. Similarly, DPP-4 concentration did not significantly decrease in response to tralokinumab in STRATOS 1 [125]. Participants with increased IL-13 activity (determined by concentrations of serum periostin or DPP-4 above the baseline median) were not identified as responder groups to tralokinumab in STRATOS 1 [125], and thus were not measured in MESOS. In contrast, FeNO-high participants demonstrated increased clinical efficacy in STRATOS 1 [125]. Taken together, these data suggest periostin and DPP-4 are not responsive biomarkers of IL-13 neutralisation.

In this trial, participants treated with tralokinumab did not experience significant improvements in lung function or asthma control versus placebo. However, there were numerical improvements in pre-bronchodilator FEV1 (as observed in previous studies [66, 123]) in addition to associated numerical improvements in small airway resistance. Similarly, there were small improvements in CT determined airway geometry. Such findings suggest that there might be small effects upon airway luminal dilatation in response to tralokinumab, which is consistent with previous results [154].

Some clinical benefits in response to tralokinumab have been observed in phase 2 and 3 studies [66, 125]. Neutralisation of IL-13 is associated with improvements in lung function with small effects on symptoms and exacerbation frequency [66]. In this mechanistic trial, tralokinumab did not significantly affect eosinophilic inflammation, suggesting that a clinical response to tralokinumab is unlikely to be mediated via attenuation of eosinophilic airway inflammation. Thus, reported improvements in lung function are probably due to an alternative mechanism that is independent of inflammation. IL-13 directly affects airway smooth muscle [159, 160] and thus IL-13 neutralisation may affect airway smooth muscle tone, leading to bronchodilation with reduced small airway resistance and improved FEV1. Importantly, we did not observe a change in airway hyper-responsiveness although this measurement was only undertaken in a subgroup of participants. Post-bronchodilator FEV1 was also similar between participants treated with tralokinumab versus placebo. Thus, in contrast to previously reported *in vitro* studies [153, 154], our findings do not support the view that IL-13 neutralisation attenuates the bronchoconstrictor effect of methacholine, nor does it promote response to treatment with beta-agonists.

The impact upon exacerbation frequency following IL-13 neutralisation is small in response to treatment with tralokinumab [66, 125] and lebrikizumab [67]. One plausible explanation from the MESOS trial for the limited impact upon exacerbations of IL-13 neutralisation is the lack of effect upon eosinophilic inflammation. These therapies reduce FeNO concentration, suggesting that FeNO reduction in isolation is not sufficient to impact upon exacerbation frequency. In contrast, other biologic therapies targeting Th2 pathways do reduce exacerbation frequency. Neutralisation of IL-5 (e.g. by mepolizumab) or inhibition of its receptor (by benralizumab) has been demonstrated to reduce exacerbation frequency by approximately 50% with concomitant blood, bronchial submucosal, and sputum eosinophil count reductions [17, 70, 72, 129], without affecting FeNO concentration [68, 71]. The anti-thymic stromal lymphopoietin agent (TSLP) tezepelumab also demonstrated a substantial reduction in asthma exacerbations

with a similar impact upon blood cell-count frequencies [73]. It is not yet known whether targeting TSLP reduces bronchial submucosal eosinophil count. Inhibition of IL-4R (by dupilumab) blocks both IL-4 and IL-13 signalling and, like IL-13 neutralisation, is associated with increases in blood eosinophil count, albeit transiently. However, dupilumab has been shown to have marked effects on exacerbation frequency [65, 161], and therefore a reduction in the blood eosinophil count is not necessary to lead to a reduction in exacerbation frequency. It is unknown if treatment with dupilumab reduces bronchial submucosal eosinophil count.

In addition to a lack of effect upon eosinophilic inflammation there was no significant effect on other inflammatory cell counts in sputum or bronchial biopsies in participants receiving tralokinumab versus placebo, except for bronchial biopsy mast cell number, which did not change in response to tralokinumab treatment, but did decrease following receipt of placebo. Beyond airway inflammation, we considered the effects of IL-13 neutralisation on airway remodelling. In preclinical studies, IL-13 has been implicated in epithelial differentiation via promotion of goblet cell hyperplasia, activating the release of TGF-β with consequential downstream effects on airway matrix protein composition [162]. Here, we did not observe effects in response to tralokinumab versus placebo on the epithelial integrity or matrix deposition, and there was no impact on RBM thickening or matrix composition except for an increase in fibronectin observed in placebo-treated participants. Taken together, these findings suggest that isolated IL-13 neutralisation has a limited effect on airway remodelling, perhaps due to interacting or competing pathways in the real-world asthma patient.

Our study has a number of potential limitations. The most striking and consistent feature of this trial is the lack of impact of tralokinumab on airway inflammation, airway remodelling, and clinical outcomes. One possible limitation in our study is that tralokinumab did not sufficiently neutralise IL-13 in the airway. However, this is unlikely, given that tralokinumab treatment reduced the concentrations of FeNO

(which is released by the epithelium in response to induction of nitric oxide synthase by IL-13 [151]) and total blood IgE. Therefore, the lack of effect of tralokinumab on airway inflammation and remodelling is unlikely to be attributable to failure of target engagement, as tralokinumab had clearly exerted a biological effect on the bronchial epithelium. The participants in this trial had less severe asthma than those typically included in phase 3 pivotal trials of biologics, including tralokinumab [149]. We therefore cannot exclude the possibility that tralokinumab might have had additional effects on airway inflammation in participants with more severe disease. However, in our trial, the reduction in FeNO concentration and clinical outcome responses are comparable with other studies of tralokinumab in participants with more severe asthma [66], making this less likely.

Another possible limitation to explain the lack of anti-inflammatory effect is that the trial was insufficient in duration (12 weeks) or underpowered to determine a treatment effect. However, previous studies with small molecule inhibitors and biologics, which did show an airway anti-inflammatory effect, were of similar duration [59, 149]. Moreover, the pivotal phase 3 trials of tralokinumab did not identify any beneficial effects at Week 52 that were not observed following 12 weeks of treatment [125]. It is therefore unlikely that a longer study would have identified major effects on airway remodelling, although this possibility cannot be discounted. Critically, the baseline airway inflammation and epithelial damage was comparable to previous reports [59], and therefore the trial design was appropriate to observe important changes in these outcomes. The trial was a technical success, with bronchoscopy well tolerated in this group of participants with moderate to severe asthma. All participants that completed the study provided bronchial biopsies of adequate size and quality to undertake the comprehensive analysis before and after receipt of tralokinumab or placebo. Therefore, we are confident the study was adequately powered.

Another major limitation of our study was that, in contrast to the high success rate for obtaining bronchial biopsies, the number of participants able to produce

adequate sputum samples was low, and therefore the changes in sputum cell counts should be interpreted with caution. All participants were treated with inhaled corticosteroids, and it is possible that the IL-13 axis is sensitive to corticosteroids [163]. Therefore, our ability to observe additional effects with tralokinumab might be limited in this population. Notwithstanding this shortcoming, the target population for biologic therapies is currently patients with moderate to severe asthma because of greater clinical need in this group [3]. Thus, even though we cannot exclude a possible effect in mild disease, we are confident that tralokinumab does not substantially affect airway inflammation or remodelling in participants with moderate-to-severe asthma.

In conclusion, in this 12-week trial, tralokinumab did not significantly affect either eosinophilic airway inflammation or airway remodelling compared to placebo, but did reduce FeNO and IgE concentrations. Benefits in lung function observed in previous studies, and small improvements in markers of both large and small airway function observed here are independent of eosinophilic inflammation and might be a consequence of effects of IL-13 directly on airway smooth muscle.

4 Effects of bronchial thermoplasty on clinical, physiological, inflammatory and remodelling measures of asthma

4.1 Introduction

Bronchial thermoplasty is a non-pharmacological treatment for severe asthma. Treatment involves the use of heat energy applied to the airway wall, in an attempt to reverse the airway remodelling changes seen as a consequence of asthma (described in detail in chapter 1). During bronchoscopy a specially designed thermoplasty catheter is deployed in the target airway and expanded so that the catheter wires come into contact with the airway wall. Radiofrequency thermal energy is used to heat the airway wall to 65°C for 10 seconds [130]. The catheter is then moved a short distance proximally along the airway, and the heating process is repeated. All of the accessible airways in the target lung lobe are treated in this manner. The full treatment course is delivered over three treatment sessions, with the right lower lobe (RLL) being treated in the first, the left lower lobe (LLL) in the second, and both upper lobes in the third. Treatment sessions are generally undertaken at approximately 3 week intervals.

The primary target of thermoplasty is the airway smooth muscle (ASM); a key contributor to airway remodelling, particularly in severe asthma [130, 164-168]. Studies in dogs demonstrated a reduction in airway hyper-responsiveness and altered ASM histological appearance following thermoplasty [131]. Subsequent clinical trials showed improvement in quality of life and reduced frequency of severe exacerbations in those receiving thermoplasty versus a sham procedure, but found no significant difference in lung function as a result of the treatment [95, 134, 135]. In uncontrolled observational studies thermoplasty has been associated with approximately 50–80% relative loss of ASM mass measured on bronchial biopsies [90-94], typically obtained 1 to 3 months after completion of the thermoplasty treatment. Effects have also been demonstrated on reticular basement membrane thickness [90, 93, 94], but no beneficial effect has been

demonstrated on the airway epithelium. Myofibroblasts are present in increased numbers in the lamina propria of asthma subjects compared to controls, and traffic to sites of injury where they promote wound repair [169-171]. Whether they play a role in remodelling repair following thermoplasty has not been investigated.

4.1.1 Hypothesis and aims

The aim of the study is to examine the changes seen in airway composition following thermoplasty treatment, in an attempt to gain a better understanding of the mechanism of action, and to investigate the relationship between clinical and remodelling responses to treatment. The clinical outcomes investigated included exacerbation frequency, asthma symptoms, asthma control, lung function, and sputum differential cell counts. The biopsy-determined remodelling outcomes include airway smooth muscle mass, reticular basement membrane thickness, epithelial integrity, epithelial area, epithelial thickness, and myofibroblast count.

The hypothesis was that thermoplasty treatment would lead to significant improvements in important features of airways remodelling, and that these changes would relate to clinical outcomes. Specifically, I hypothesised that the airway epithelium, which is in direct contact with the thermoplasty catheter during treatment, would show evidence of significant repair, and that due to the important barrier function that the epithelium plays, these changes would be associated with improved clinical improvements.

4.2 Methods

This observational study investigated severe asthma patients referred for bronchial thermoplasty by their responsible asthma team. All subjects had severe asthma as defined by ATS/ERS guidelines [8], and were undergoing bronchial

thermoplasty treatment as part of their clinical care at one of four participating UK specialist asthma centres (Leicester, Glasgow, Southampton and Birmingham). 33 patients were recruited, including 8 from Leicester.

Prior to undergoing bronchial thermoplasty, patients were approached by their own severe asthma team to take part in the study. Those who agreed gave written informed consent to undergo additional visits and assessments beyond those necessary for standard thermoplasty treatment. Consented participants underwent clinical evaluation prior to thermoplasty treatment, and then approximately 6 weeks and 6 months after the final thermoplasty treatment session. Demographic data was collected, including age, sex, age of asthma diagnosis, smoking history, body mass index, annual exacerbation rate, and medications. Clinical assessments undertaken at baseline and follow-up included questionnaires of asthma control (asthma control questionnaire-6, ACQ6) and asthma quality of life/symptoms (asthma quality of life questionnaire, AQLQ), blood tests, lung function testing including plethysmography and transfer factors, airwave oscillometry, and sputum analysis.

Thermoplasty was performed as per manufacturer's guidelines over three treatment sessions in the following order: right lower lobe (RLL); left lower lobe (LLL), and both right and left upper lobes (RUL, LUL). The right middle lobe was not treated due to the risk of airway collapse and 'right middle lobe syndrome' [172]. Two to five bronchial biopsies were obtained from segmental and subsegmental airways in the untreated RUL during the first procedure (baseline). Subsequently, follow-up biopsies were obtained during the second and third treatment sessions from the RLL, which had been treated in procedure one. It was recommended that each treatment session be separated by approximately 3-4 weeks. Therefore, follow-up biopsies were obtained from the RLL at two time points, approximately 3-4 weeks and 6-8 weeks after treatment.

Bronchial biopsies were embedded in paraffin. Four-micrometre sections were cut and stained with Haematoxylin and Eosin (H&E) or alpha-smooth muscle actin

(aSMA) (clone 1A4, Dako, UK). Assessment of biopsies was blinded in relation to participant and visit number to avoid potential bias. Analysis was performed using the image analysis platforms ZEN Pro 2012 (Carl Zeiss AG, Germany) and ICY (Institut Pasteur, France). Airway smooth muscle mass ('mass' used synonymously with 'area', consistent with previous publications in the field) and epithelial area were determined as percentages of the total biopsy structural area. Epithelial integrity was assessed by measuring the length of intact, damaged and denuded epithelium as a percentage of the total reticular basement membrane length. Reticular basement membrane thickness was measured as the mean of 50 thickness measurements spaced approximately 20 micrometres apart. Myofibroblasts, identified as isolated aSMA staining cells in the lamina propria that were neither located as part of the ASM-bundle, nor as vascular smooth muscle cells adjacent to vessels, were counted and expressed as cells per mm^2 of lamina propria.

Clinical outcomes were analysed using GraphPad Prism version 8.1.2 (San Diego, USA), using parametric (paired t-test) and non-parametric (Wilcoxon matched-pairs signed rank test) tests as appropriate, depending whether the variable was normally distributed or not. Assuming a mean ± standard deviation ASM mass of 25 ± 15% [89], n=14 subjects were required to observe an absolute reduction of 10% ASM mass using a one-tailed paired test with 80% power at the significance level of 0.05 (post-hoc calculation). Features of remodelling on baseline and follow-up biopsies were compared using paired t-tests. Relationships between clinical outcomes and biopsy changes, and different features of biopsy changes, were tested using Pearson's correlation coefficient. A p-value of <0.05 was considered statistically significant.

This study was undertaken within the Airway Disease Predicting Outcomes through Patient Specific Computational Modelling (AirPROM) project. Ethical approval was granted by the Leicestershire Research Ethics Committee (REC 13/EM/0068).

4.3 Results

33 patients were recruited from the four participating asthma centres (table 4.1). Not all of the 33 recruited participants agreed to undergo bronchial biopsies, and not all biopsy samples obtained were of sufficient quality for analysis. Therefore, adequate matched pre- and post-thermoplasty biopsies were available for only 14 to 16 participants, depending on the outcome measure.

Table 4.1: Study centres and recruitment status

Centre	Lead investigator	Participants enrolled (n)
Glasgow	Dr. Rekha Chaudhuri	11
Southampton	Dr. Peter Howarth	9
Leicester	Prof. Chris Brightling	8
Birmingham	Dr. Adel Mansur	5

Baseline clinical characteristics of the 33 participants are as shown in table 4.2. Participants were reflective of a typical severe asthma clinic cohort, with a mean age of 44.9, mean body mass index of 30.4, and being 70% female. Mean time since asthma diagnosis was 24.7 years, although this ranged widely from 1 to 52 years. 10 participants were past smokers and 1 was currently smoking. Mean smoking history of those who were current or ex-smokers was 6.8 pack-years. Approximately half of subjects were atopic and mean IgE values were raised at 810.9, although only two participants were currently receiving anti-IgE monoclonal antibody treatment. 17 participants were receiving treatment with regular oral corticosteroids, with a mean daily dose of 19.1mg (range 5 to 50mg). All were prescribed inhaled corticosteroids, with a mean daily dose of 2116 micrograms (range 800 to 4000 micrograms).

Table 4.2: Baseline clinical characteristics

Characteristic (n=33)	**Value**
Age (years)*	44.9 (13.5)
Sex:	
Female	23 (70%)
Male	10 (30%)
BMI (kg/m^2)*	30.4 (7.4)
Time since asthma diagnosis (years)*	24.7 (14.7)
Smoking status:	
Current	1 (3%)
Ex-smoker	10 (30%)
Never	22 (67%)
Smoking history (pack-years) [n=11 current or ex-smokers]*	6.8 (6.3)
Evidence of atopy	17 (52%)
Total IgE (kU/L) [n=9]*	810.9 (1663.0)
Asthma exacerbations in last 12 months (n)*	4.8 (3.2)
Asthma related hospital admissions in last 12 months (n)*	1.4 (2.0)
GINA treatment step:	
Step 5	19 (58%)
Step 4	14 (42%)
Prescribed oral corticosteroids (OCS)	17 (52%)
OCS dose (mg/day) [n=17 taking OCS]*	19.1 (12.3)
Prescribed anti-IgE	2 (6%)
Prescribed inhaled corticosteroids (ICS)	33 (100%)
Prescribed ICS/LABA as MART	2 (6%)
ICS dose (mg, BDP equivalent)*	2116 (982)
Prescribed Xanthine	17 (52%)
Prescribed LTRA	24 (73%)
Prescribed LAMA	17 (52%)
Prescribed LABA	33 (100%)
Prescribed SABA	32 (97%)

Values shown are number (percent), unless stated. *Denotes mean (standard deviation). Pack-years = (number of cigarettes smoked per day/20) x number of years smoked. BMI = Body Mass Index. IgE = Immunoglobulin E. GINA = Global Initiative for Asthma. OCS = oral corticosteroids. ICS = inhaled corticosteroids. LABA = long-acting beta-agonist. MART = maintenance and reliever therapy. BDP = beclomethasone dipropionate. LTRA = leukotriene receptor antagonist. LAMA = long-acting muscarinic antagonist. SABA = short-acting beta-agonist.

Mean number of total thermoplasty activations was 113.4 (mean RLL = 30.8, LLL = 33.0, upper lobes = 49.7). Clinical outcome responses to thermoplasty are summarised in table 4.3. There was a significant improvement in both ACQ6 (mean (SD) 3.1 (1.4) at baseline to 2.7 (1.5) at 6 weeks; p=0.03, figure 4.1A) and AQLQ (3.7 (1.5) at baseline and 4.0 (1.6) at 6 weeks; p=0.03; figure 4.1B) 6 weeks after completion of thermoplasty treatment. The mean improvement for both ACQ6 and AQLQ scores was 0.51, which exceeds the minimum clinical important

difference (MCID). However, this improvement was not maintained at 6 months. The largest component of the improvement in AQLQ scores was in the 'symptoms' and 'environment' domains.

Annual exacerbation rate improved significantly from a mean (SD) of 4.8 (3.2) at baseline to 1.8 (1.3) at 12 months (p=0.03; figure 4.2A), although data at 12 months were only available for 9 participants.

At 6 weeks and 6 months after thermoplasty, sputum eosinophils were significantly raised compared to baseline (median (IQR) 1.9 (0.4-6.9) at baseline to 4.8 (0.9-29.4) at 6 weeks; p=0.02, and 6.5 (1.8-13.3) at 6 months; p=0.02; figure 4.2B). Sputum lymphocytes also decreased at 6 weeks (p=0.03), but this reduction was not maintained at 6 months. However, it should be noted that the majority of participants had a lymphocyte count of 0.0% at all three time-points (as reflected in the median value of 0.0 at each time point), and so the interpretation of whether this change is significant clinically is limited. Sputum neutrophils and epithelial cell levels did not change after treatment. There was no change in blood eosinophils.

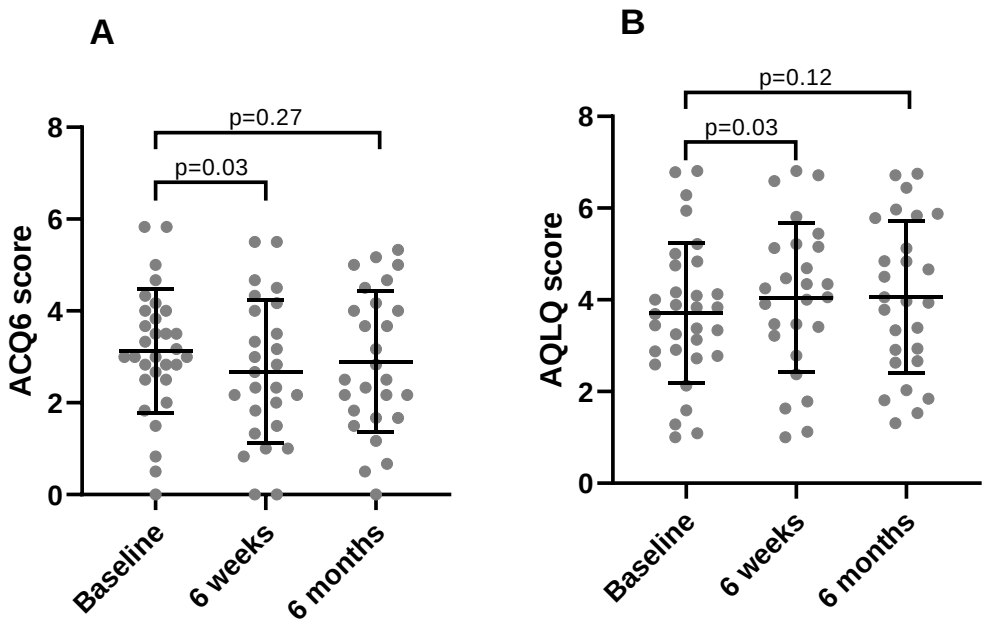
There was no change in pre-bronchodilator spirometry. There was a small but statistically significant reduction in post-bronchodilator forced vital capacity at 6 months (mean (SD) reduction 6.6 (13.6) %; p=0.02; figure 4.3A). The trend for all pre- and post-bronchodilator lung volumes on spirometry was towards a small reduction in lung function. There was a small but statistically significant increase in RV/TLC ratio at 6 months (mean (SD) 36.7 (10.7) % at baseline to 39.2 (11.9) % at 6 months; p=0.04; figure 4.3B), suggesting a higher degree of air trapping after thermoplasty. DLCO also showed a small but significant deterioration at 6 months (mean (SD) 90 (15.2) % predicted at baseline to 86.2 (17.0) at 6 months; p=0.03; figure 4.3C), with no change in KCO. There was no significant change in airways resistance (R5-R20) or reactance (AX) as measured by oscillometry.

Table 4.3: Clinical outcome responses at 6 weeks and 6 months after thermoplasty

Outcome	Baseline		6 weeks				6 months			
	Value	n	Value	n	Change from baseline	p-value	Value	n	Change from baseline	p-value
ACQ6	3.1 (1.4)	31	2.7 (1.5)	26	-0.51 (1.12)	**0.03**	2.9 (1.5)	27	-0.29 (1.22)	0.27
AQLQ Total	3.7 (1.5)	31	4.0 (1.6)	26	0.51 (1.11)	**0.03**	4.1 (1.7)	27	0.38 (1.21)	0.12
AQLQ symptoms	3.6 (1.5)	31	4.0 (1.7)	26	0.60 (1.26)	**0.02**	3.9 (1.7)	27	0.38 (1.43)	0.19
AQLQ environment	3.9 (1.8)	31	4.3 (2.0)	26	0.52 (1.04)	**0.02**	4.2 (1.8)	27	0.41 (1.32)	0.12
AQLQ activity	3.8 (1.6)	31	4.1 (1.6)	26	0.47 (1.18)	0.06	4.0 (1.6)	27	0.26 (1.23)	0.29
AQLQ emotions	3.7 (1.9)	31	3.9 (1.9)	26	0.37 (1.36)	0.19	4.1 (2.0)	27	0.45 (1.52)	0.14
Pre-BD FEV1 % predicted	70.1 (19.5)	29	64.0 (20.9)	22	-3.1 (11.2)	0.22	64.8 (19.4)	20	-0.2 (17.9)	0.96
Pre-BD FVC % predicted	94.9 (17.6)	29	88.8 (18.2)	22	-4.9 (16.5)	0.19	86.9 (18.8)	20	-5.9 (17.4)	0.15
Pre-BD FEV1/FVC ratio	62.5 (13.8)	29	59.9 (13.2)	22	-0.1 (7.1)	0.93	62.1 (11.2)	20	3.1 (8.9)	0.14
Post-BD FEV1 % predicted	79.3 (17.9)	32	75.7 (18.7)	26	-3.2 (11.5)	0.17	75.9 (19.3)	27	-4.8 (13.6)	0.08
FEV1 % reversibility	22.5 (17.2)	28	20.6 (19.2)	22	1.3 (19.4)	0.75	21.8 (26.9)	20	-8.0 (24.3)	0.18
Post-BD FVC % predicted	101.7 (18.8)	32	98.7 (16.7)	26	-3.1 (12.8)	0.23	97.1 (19.7)	27	-6.6 (13.6)	**0.02**
Post-BD FEV1/FVC ratio	66.3 (12.4)	32	64.1 (11.5)	26	-1.5 (8.5)	0.38	65.3 (9.4)	27	-0.8 (8.7)	0.64
RV/TLC %	36.7 (10.7)	31	38.7 (11.8)	22	2.5 (9.4)	0.23	39.2 (11.9)	19	3.4 (6.5)	**0.04**
DlCO % predicted	90.0 (15.2)	32	84.9 (16.4)	22	-2.5 (12.0)	0.34	86.2 (17.0)	18	-4.2 (7.5)	**0.03**
KCO % predicted	103.8 (16.2)	32	103.3 (17.5)	22	-1.6 (8.8)	0.41	100.5 (19.1)	18	-2.2 (11.1)	0.42
R5-R20 (kPa x s/L)	0.23 (0.18)	19	0.82 (2.0)	13	0.63 (2.09)	0.32	0.34 (0.26)	12	0.10 (0.23)	0.17
Reactance area, AX (kPa/L)	2.5 (2.2)	19	2.8 (2.5)	13	0.1 (1.7)	0.86	3.9 (3.3)	12	1.2 (3.4)	0.25
Sputum neuts % #	59.0 (44.8-72.8)	18	46.5 (25.9-63.1)	13	-7.7 (13.6)	0.09^	43.0 (25.0-86.3)	11	-8.3 (24.2)	0.38^
Sputum eos % #	1.9 (0.4-6.9)	18	4.8 (0.9-29.4)	13	10.2 (17.6)	**0.02^**	6.5 (1.8-13.3)	11	10.2 (17.5)	**0.02^**
Sputum epithelial cell % #	1.3 (0.7-5.6)	18	1.0 (0.3-2.9)	13	-1.9 (5.2)	0.38^	1.8 (1.0-7.0)	11	0.8 (7.4)	0.73^
Sputum lymphocyte % #	0.0 (0.0-0.7)	18	0.0 (0.0-0.0)	13	-0.4 (0.6)	**0.03^**	0.0 (0.0-0.3)	11	-0.4 (0.6)	0.09^
Blood eos, $x10^{-9}$/L #	0.2 (0.1-0.5)	22	0.3 (0.1-0.5)	9	0.10 (0.11)	0.08^	0.2 (0.1-0.8)	13	0.04 (0.40)	0.74^
	Baseline						**12 months ‡**			
Annual exacerbations	4.8 (3.2)	33					1.8 (1.3)	9	**-2.8 (3.3)**	**0.03^**

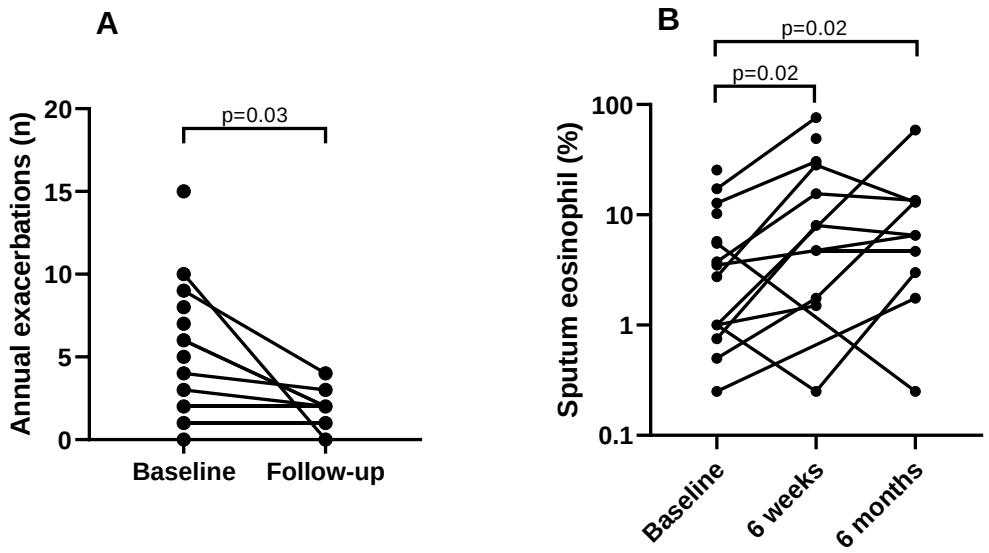
Paired t-test reported unless stated. Wilcoxon signed-rank test (non-parametric) denoted by ^. Mean (SD) shown except where marked. # denotes median (interquartile range) for measurements known to be not normally distributed. BD = Bronchodilator. FEV1 = Forced Expiratory Volume in 1 second. FVC = Forced Vital Capacity. ACQ6 = Asthma Control Questionnaire (6). AQLQ = Asthma Quality of Life Questionnaire. ‡ Exacerbations reported at 12 months.

Figure 4.1: ACQ6 (A) and AQLQ (B) responses to thermoplasty



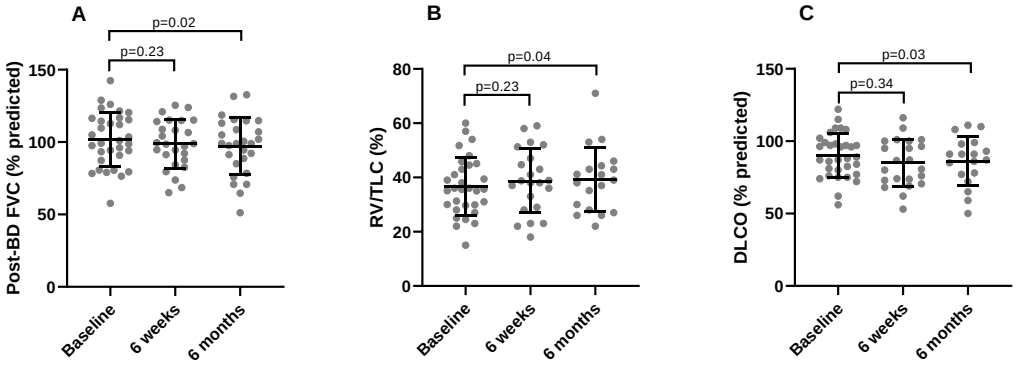
Mean (SD) and individual values for ACQ6 (A) and AQLQ (B) shown at baseline, 6 weeks and 6 months after completion of thermoplasty treatment. P-value reported is paired t-test. ACQ6 = asthma control questionnaire-6. AQLQ = asthma quality of life questionnaire.

Figure 4.2: Exacerbation rate (A) and sputum eosinophil (B) responses to thermoplasty



Individual values for annual asthma exacerbation rate (A) at baseline and 12 months after completion of thermoplasty treatment. Individual values for sputum eosinophil count (B) shown at baseline, 6 weeks and 6 months after completion of thermoplasty treatment (log10 scale). Exacerbation rate p-value reported is paired t-test. Sputum eosinophil p-value reported is Wilcoxon signed-rank test.

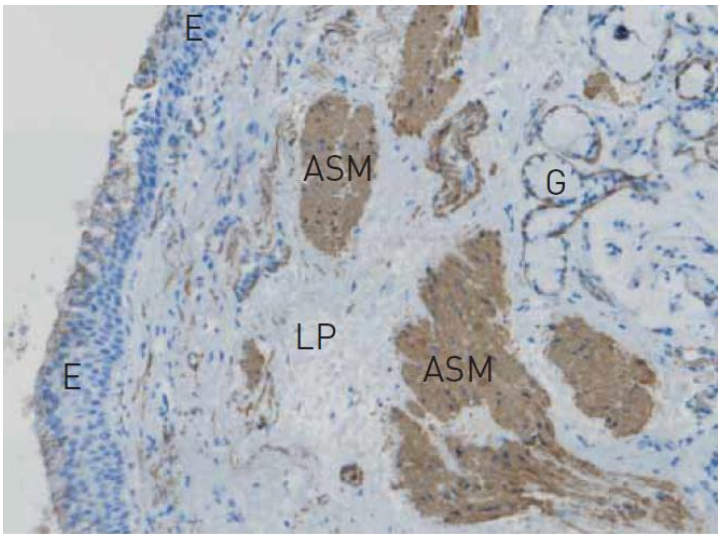
Figure 4.3: Statistically significant lung function responses to thermoplasty



Mean (SD) and individual values for post-bronchodilator forced vital capacity (A), residual volume/total lung capacity ratio (B), and diffusing capacity of lung for carbon monoxide (C) shown at baseline, 6 weeks and 6 months after completion of thermoplasty treatment. P-value reported is paired t-test. BD = bronchodilator. FVC = forced vital capacity. RV = residual volume. TLC = total lung capacity. DLCO = diffusing capacity of lung for carbon monoxide.

Biopsies were collected during thermoplasty procedures as detailed above. An example micrograph stained for alpha-smooth muscle actin is shown in figure 4.4. For a number of reasons, such as local service provision and variable patient recovery times, the time between thermoplasty procedures varied greatly between participants. Mean (range) interval between baseline and follow-up biopsies was 39 (14-91) days for follow-up 1, and 85 (35-182) days for follow-up 2. Therefore, in the following results and discussion follow-up 1 will be referred to as '6 weeks' and follow-up 2 as '12 weeks', based on the mean number of days between biopsies described here.

Figure 4.4: Example micrograph of bronchial biopsy stained for alpha-smooth muscle actin



Example endobronchial biopsy stained for α-smooth muscle actin. ASM = airway smooth muscle. E = epithelium. LP = lamina propria. G = gland.

Mean (SD) airway smooth muscle mass reduced from 13.5 (8.9) % to 7.6 (6.9) % at 6 weeks (p=0.05), and 8.1 (5.7) % at 12 weeks (p=n.s) (table 4.4 and figure 4.5A). Median relative reduction from baseline was 58% and 60% at 6 weeks and 12 weeks respectively. The reduction at 12 weeks was not statistically significant, although had a large median relative reduction due largely to one participant who

had low airway smooth muscle mass at baseline and a significant worsening at 12 weeks. Reticular basement membrane thickness reduced numerically, although this did not reach statistical significance. Mean (SD) thickness was 7.3 (1.9) µm at baseline, 6.8 (3.2) µm at 6 weeks (p=n.s.), and 6.3 (2.1) µm at 12 weeks (p=n.s) (table 4.4 and figure 4.5B).

There were significant improvements in all measures of epithelial health. Epithelial integrity improved from 28.7 (19.1) % at baseline to 44.1 (18.7) % at 6 weeks (p=0.004). At 12 weeks mean epithelial integrity remained improved from baseline at 42.1 (26.9) %, although this did not quite reach statistical significance (p=0.06) (table 4.4 and figure 4.5D). The improvement in intact epithelium was accompanied by a reduction in damaged epithelium, which was significant at 6 weeks (p=0.03; table 4.4 and figure 4.6A). However, there was no significant change in the percent of denuded epithelium. Epithelial area showed significant improvements at 12 weeks (p=0.05), although was not significant at 6 weeks (table 4.4 and figure 4.6B). Mean epithelial thickness improved from 13.1 (7.8) µm at baseline, to 27.5 (18.8) µm at 6 weeks (p=0.004), and 33.5 (37.3) at 12 weeks (p=n.s) (table 4.4 and figure 4.6C).

Epithelial integrity and mean epithelial thickness showed large and statistically significant improvements at 6 weeks. However, although the mean values remained approximately static from 6 weeks to 12 weeks, the results at 12 weeks were not significant when compared to baseline. This appeared to be largely due to a broad range of responses at 12 weeks after treatment (shown by large standard deviations at 12 weeks, and demonstrated visually in figure 4.5D).

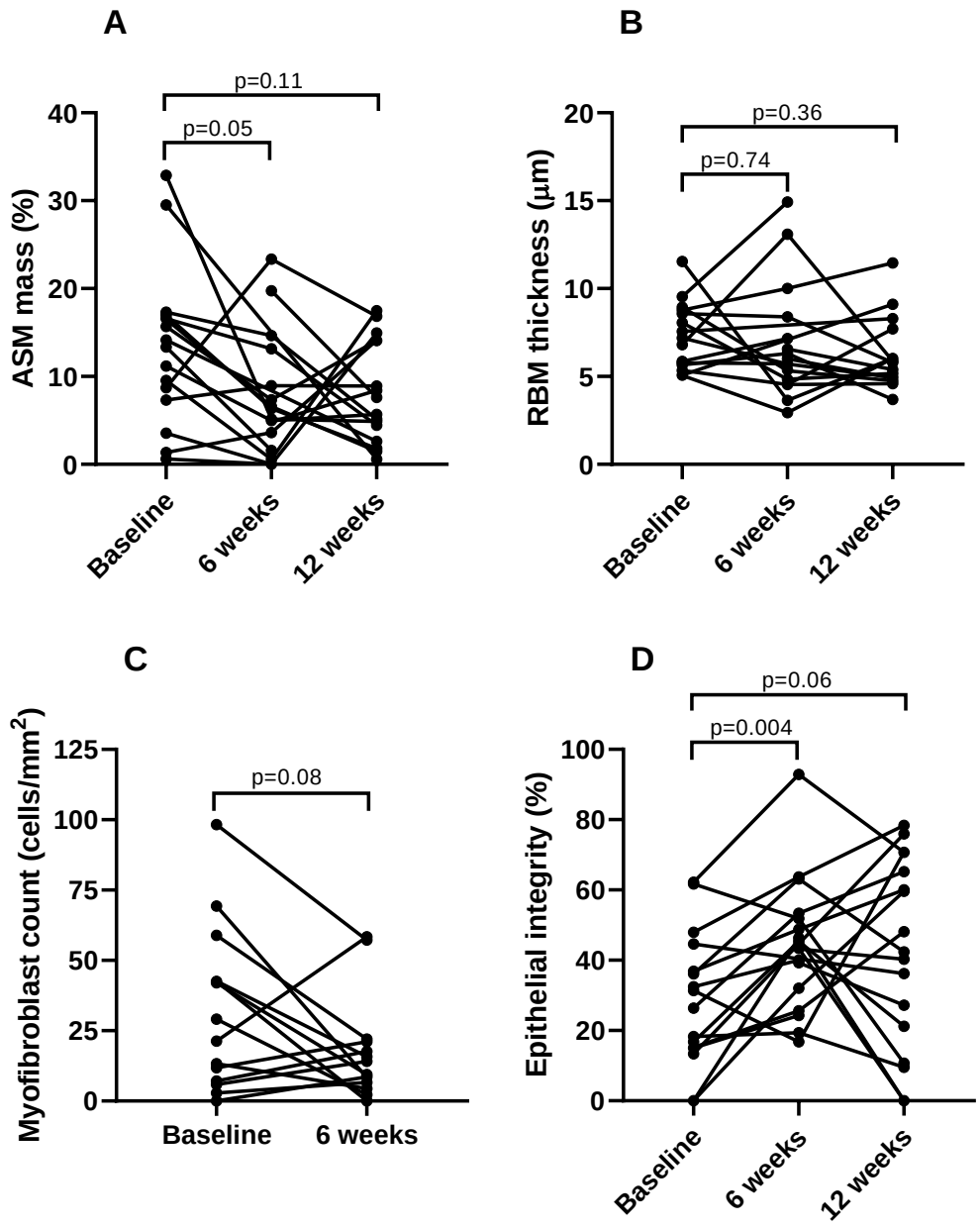
Subepithelial myofibroblasts reduced numerically from baseline to 6 weeks, although this reduction was not significant (31.8 (28.9) cells/mm^2 to 17.6 (18.3) cells/mm^2; p=0.08; figure 4.5C).

Table 4.4: Bronchial biopsy remodelling changes following thermoplasty

	Baseline		Follow-up 1 (6 weeks)				Follow-up 2 (12 weeks)			
Outcome	**Value**	**n**	**Value**	**n**	**Change from baseline**	**p-value**	**Value**	**n**	**Change from baseline**	**p-value**
Airway smooth muscle mass (%)	13.5 (8.9)	16	7.6 (6.9)	16	-5.4 (9.4)	**0.05**	8.1 (5.7)	16	-6.3 (13.6)	0.11
Reticular basement membrane thickness (µm)	7.3 (1.9)	15	6.8 (3.2)	17	-0.3 (3.7)	0.74	6.3 (2.1)	15	-0.8 (3.0)	0.36
Intact epithelium (%)	28.7 (19.1)	16	44.1 (18.7)	17	16.0 (17.1)	**0.004**	42.1 (26.9)	17	17.0 (30.3)	0.06
Damaged epithelium (%)	52.8 (22.4)	16	43.9 (17.3)	17	-10.0 (15.7)	**0.03**	39.0 (17.6)	17	-14.7 (28.2)	0.07
Denuded epithelium (%)	18.6 (20.1)	16	12.0 (9.3)	17	-5.9 (17.6)	0.23	18.9 (22.1)	17	-2.3 (15.0)	0.57
Epithelium area (%)	7.0 (5.9)	16	9.6 (5.2)	16	2.9 (7.0)	0.15	10.4 (7.8)	16	4.9 (8.2)	**0.05**
Epithelium thickness (µm)	13.1 (7.8)	16	27.5 (18.8)	16	16.4 (17.4)	**0.004**	33.5 (37.3)	16	21.1 (42.3)	0.09
Myofibroblast count (cells/ mm^2)	31.8 (28.9)	14	17.6 (18.3)	14	-14.2 (27.8)	0.08	ND	ND		ND

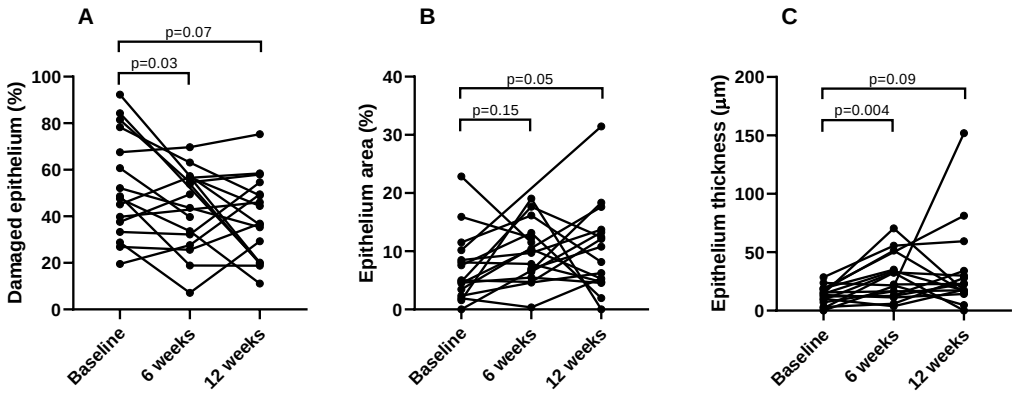
Mean (standard deviation) reported. µm = micrometres. mm = millimetres. ND = not done. P-value reported is paired t-test.

Figure 4.5: ASM (A), RBM (B), myofibroblast (C) and epithelial integrity (D) responses to thermoplasty



Individual values for airway smooth muscle mass (A), reticular basement membrane thickness (B), myofibroblast count (C), and epithelial integrity (D) shown at baseline, 6 weeks and 12 weeks after completion of thermoplasty treatment. P-value reported is paired t-test. ASM = airway smooth muscle. RBM = reticular basement membrane.

Figure 4.6: Damaged epithelium (A), epithelium area (B) and epithelium thickness (C) responses to thermoplasty



Individual values for damaged epithelium (A), epithelium area (B), and epithelium thickness (C) shown at baseline, 6 weeks and 12 weeks after completion of thermoplasty treatment. P-value reported is paired t-test. µm = micrometres.

Correlations between biopsy-assessed remodelling features at baseline, and in response to thermoplasty, were undertaken to explore the relationship between the different components of remodelling. For correlation purposes the mean change in biopsy measures from baseline to week 6 and baseline to week 12 was calculated and used as a composite 'post-thermoplasty' value. There were no correlations seen between baseline airway smooth muscle mass, reticular basement membrane thickness, and measures of epithelial integrity. Baseline myofibroblast count was directly correlated with epithelial integrity at both baseline (r=0.64, p=0.01) and post-thermoplasty (r=0.57, p=0.03). However, there was no relationship between baseline numbers of myofibroblasts and the change in epithelial integrity from baseline to post-treatment. There were no direct correlations between responses in airway smooth muscle mass, reticular basement membrane thickness, epithelial measures or myofibroblasts in response to thermoplasty. Changes in the different measures of epithelial health did closely correlate with each other, as would be expected.

Relationships between baseline clinical characteristics and baseline features of remodelling were investigated to establish if baseline remodelling predicted poor asthma outcomes pre-treatment in this patient group (table 4.5 and figure 4.7). Baseline epithelial thickness had a weak positive correlation with baseline ACQ6 (r=0.54, p=0.04), although the direction of this relationship is contrary to that which would be expected, with higher epithelial thickness associated with worse asthma control. Baseline epithelial integrity was positively correlated with years since asthma diagnosis (r=0.57, p=0.02), showing that participants who had been diagnosed with asthma for the longest had better epithelial integrity. Baseline epithelial area was positively correlated with the dose of inhaled corticosteroids (r=0.62, p=0.01). No other clinically relevant correlations were found between baseline remodelling on biopsies and baseline clinical features.

Baseline remodelling features were also tested for correlations against relevant clinical responses to thermoplasty, to investigate if there were any remodelling features that predicted clinical response to treatment. The following correlations were identified comparing baseline biopsy remodelling features and change in clinical outcomes at 6 weeks (table 4.5 and figure 4.8): epithelial thickness and RV/TLC (r=0.74, p=0.006); reticular basement membrane thickness and ACQ6 (r=0.62, p=0.02), and R5-R20 (r=0.88, p=0.05). No relevant significant correlation was seen between remodelling features and change in clinical outcomes at 6 months.

Correlations between changes in remodelling features and changes in clinical outcomes were also investigated, to further explore the mechanism by which thermoplasty leads to clinical benefit (table 4.5 and figure 4.9). Change in airway smooth muscle mass was correlated with R5-R20 at both 6 weeks (r=-0.99, p=0.007) and 6 months (r=-0.90, p=0.04); however the direction of this relationship was the opposite of expected, with decreased smooth muscle mass correlating with increased airways resistance. It should be noted that only 5 participants had matched pre- and post-thermoplasty data for both biopsies and oscillometry. Change in epithelial area correlated with both R5-R20 (r=0.93,

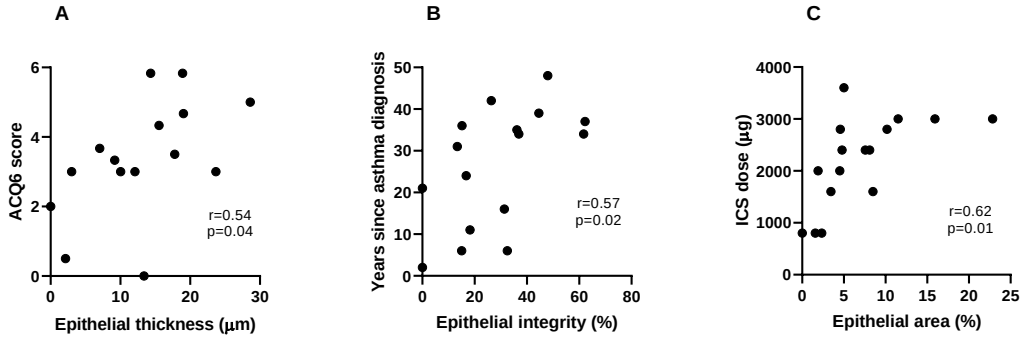
p=0.02) and AX (r=0.99, p=0.005) responses at 6 months. Again, these relationships were converse to expected, and included only 5 participants. Change in reticular basement membrane thickness was correlated with change in ACQ6 at 6 months (r=-0.65, p=0.03); this negative relationship suggests that reductions in basement membrane thickness are related to worsening asthma control, again contrary to that which would be expected. Reticular basement membrane thickness also showed an association with AQLQ scores at 6 months, although this did not quite reach significance (p=0.06), and again the result was contrary to the expected direction. Epithelial thickness showed a weak positive correlation with the number of thermoplasty activations in the right lower lobe (i.e. the lobe treated first, and from where follow-up biopsies were subsequently obtained) (r=0.54, p=0.03) suggesting greater epithelial repair with higher numbers of thermoplasty activations. However, no other relationships between the number of thermoplasty activations and remodelling changes were seen, including in the other measures of epithelial repair.

Table 4.5: Selected statistically significant correlations between biopsy remodelling features and clinical outcomes

Remodelling feature	Clinical outcome	Time point	Pearson r	p-value	Relationship in expected direction?
BASELINE REMODELLING FEATURE vs BASELINE CLINICAL OUTCOMES					
Epithelial thickness	ACQ6	Baseline	0.54	**0.04**	No. Suggests ACQ6 worse with thicker epithelium
Epithelial integrity	Years since asthma diagnosis	Baseline	0.57	**0.02**	N/A
Epithelial area	ICS dose	Baseline	0.62	**0.01**	N/A
BASELINE REMODELLING FEATURE vs CHANGE IN CLINICAL OUTCOMES					
Epithelial thickness	Change in RV/TLC	6 weeks	0.74	**0.006**	No. Suggests higher epithelial thickness at baseline associated with worsening gas trapping after BT
RBM thickness	Change in ACQ6	6 weeks	0.62	**0.02**	Yes. Suggests thinner RBM at baseline associated with better ACQ6 response to BT
RBM thickness	Change in R5-R20	6 weeks	0.88	**0.05**	Yes. Suggests thinner RBM at baseline associated with improvement in airway resistance after BT
CHANGE IN REMODELLING FEATURES vs CHANGE IN CLINICAL OUTCOMES					
ASM mass	Change in R5-R20	6 weeks	-0.99	**0.007**	No. Suggests increase in ASM associated with improved airways resistance
ASM mass	Change in R5-R20	6 months	-0.90	**0.04**	No. Suggests increase in ASM associated with improved airways resistance
RBM thickness	Change in ACQ6	6 months	-0.65	**0.03**	No. Suggests reduction in RBM thickness associated with worse ACQ6 outcomes
Epithelial area	Change in R5-R20	6 months	0.93	**0.02**	No. Suggests smallest improvement in epithelial area associated with improved airways resistance
Epithelial area	Change in AX	6 months	0.99	**0.005**	No. Suggests smallest improvement in epithelial area associated with improved airway compliance
Epithelial thickness	Number of activations in RLL	N/A	0.54	**0.03**	Yes. Suggests more treatment activations leads larger change in epithelial thickness

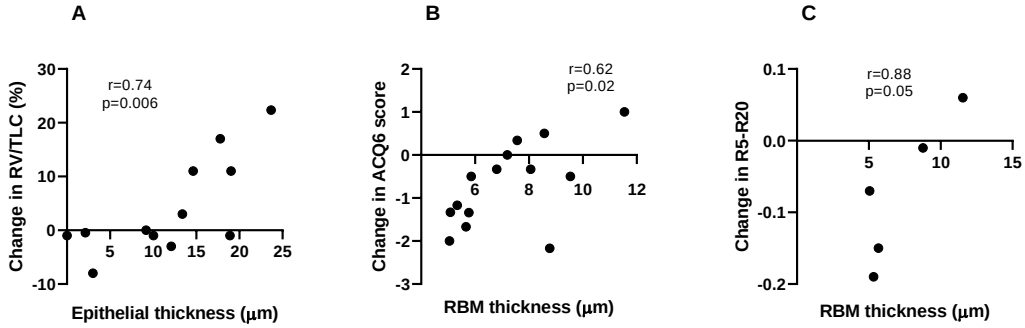
Note n=5 for oscillometry (R5-R20 and AX) results. BT = bronchial thermoplasty. RBM = reticular basement membrane. ASM = airway smooth muscle. ICS = inhaled corticosteroids. RV = residual volume. TLC = total lung capacity. R5-R20 = airways resistance. AX = airways reactance. RLL = right lower lobe.

Figure 4.7: Correlations of baseline remodelling features vs baseline clinical features



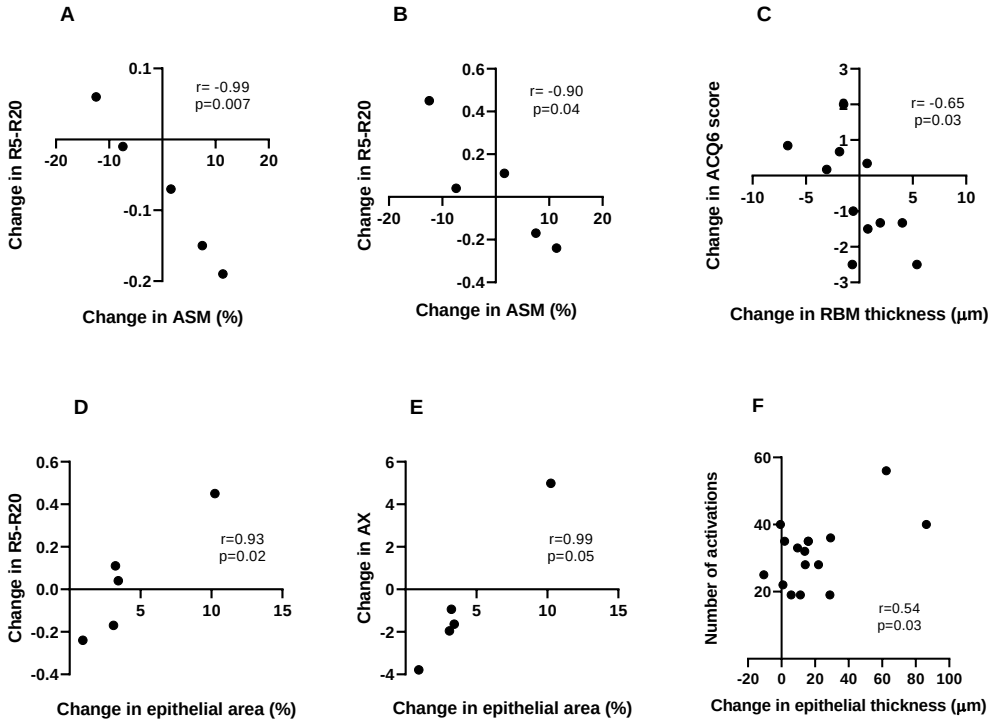
Statistically significant correlations between biopsy remodelling features and clinical features at baseline; epithelial thickness vs ACQ6 score (A), epithelial integrity vs years since asthma diagnosis (B), and epithelial area vs inhaled corticosteroid dose (C). r and p-values shown are Pearson's correlations. ACQ6 = asthma control questionnaire-6. μm = micrometres. ICS = inhaled corticosteroids. μg = micrograms.

Figure 4.8: Correlations of baseline remodelling features vs clinical responses to thermoplasty



Statistically significant correlations between baseline biopsy remodelling features and clinical responses to thermoplasty at 6 weeks; baseline epithelial thickness vs change in RV/TLC (A), baseline RBM thickness vs change in ACQ6 score (B), and baseline RBM thickness vs change in R5-R20 (C). r and p-values shown are Pearson's correlations. RV = residual volume. TLC = total lung capacity. ACQ6 = asthma control questionnaire-6. RBM = reticular basement membrane. μm = micrometres. R5-R20 = airways resistance.

Figure 4.9: Correlations of remodelling responses vs clinical responses to thermoplasty



Statistically significant correlations between biopsy remodelling responses and clinical responses to thermoplasty; change in ASM vs change in R5-R20 at 6 weeks (A), change in ASM vs change in R5-R20 at 6 months (B), change in RBM vs change in ACQ6 at 6 months (C), change in epithelial area vs change in R5-R20 at 6 months (D), change in epithelial area vs change in AX at 6 months (E), change in epithelial thickness vs number of thermoplasty activations (F). r and p-values shown are Pearson's correlations. R5-R20 = airways resistance. ASM – airway smooth muscle. ACQ6 = asthma control questionnaire-6. RBM = reticular basement membrane. µm = micrometres. AX = airways reactance.

Due to fewer participants than expected having paired pre- and post-thermoplasty data for both biopsy and clinical outcomes, the planned responder group analysis was not undertaken.

4.4 Discussion

In this study we aimed to examine the clinical and remodelling responses to bronchial thermoplasty, and explore the relationships between them.

The improvement at 6 weeks in ACQ and AQLQ, although meeting thresholds for both clinical and statistical significance was numerically less than half of the AQLQ improvement reported after thermoplasty or sham procedure at 6-12 months in the largest clinical study of thermoplasty responses [95]. Moreover, the improvements observed here were not maintained at 6 months. This could be due to our cohort having more severe asthma than those previously investigated, as demonstrated by a higher proportion requiring maintenance oral corticosteroids, and at a higher mean dose, in our population. Asthma symptom burden was also higher at baseline in our cohort (mean AQLQ 3.7 in our population, compared to 4.3 reported in *Castro 2010* [95]). This perhaps suggests that the sustained symptomatic benefit following thermoplasty is reduced in more severe disease, possibly due to other ongoing contributors to symptoms such as uncontrolled inflammation and extra-thoracic factors. It is also notable that these questionnaire based assessments improved as early as 6 weeks in our study, despite the previously reported increase in exacerbations during and immediately after the treatment period.

Only small effects were seen on measures of lung function, consistent with previously published data [95, 135, 136], but those changes were consistently in the direction of worsening of lung function, with a reduction in post-bronchodilator FVC, increased gas trapping as demonstrated by an increase in RV/TLC, and worsening of DLCO. Although these changes were small, and may not be clinically meaningful, it is of concern that thermoplasty treatment may worsen lung function impairment in this population of severe asthmatics. The deterioration in post-bronchodilator FVC and RV/TLC suggest increased gas trapping after treatment. However, an increase in small airways gas trapping was not supported by oscillometry measured R5-R20, which was unchanged after

treatment (although this data was restricted to only 12 patients with paired oscillometry results).

Sputum eosinophils increased significantly after treatment, and this increase was sustained at 6 months. At baseline, median eosinophil level was below the 2% cut-off considered the upper limit of normal. This increased to 4.8% and 6.5% at 6 weeks and 6 months respectively. This rise was despite participants receiving 3 short courses of high dose oral corticosteroids during the treatment phase, as per treatment protocol. This suggests that thermoplasty itself promotes an eosinophilic inflammatory response in the airway, although it should be noted that this result contradicts published data showing a reduction in eosinophil in bronchoalveolar lavage samples after thermoplasty [91]. It is also of note that, although eosinophil levels increased significantly in the sputum, there was no change in levels in the blood. Migration of eosinophils into the lung and sputum is regulated by IL-4, -5, and -13, and it may be that during thermoplasty the heating insult to epithelial cells in the airway wall promotes activity of these cytokines. IL-33 and TSLP upregulate T2 inflammation cytokines, and are released from necrotic epithelial cells as would likely be seen immediately following thermoplasty [61, 62]. However, one previous study has shown that the presence of T2 cytokines is not altered in bronchoalveolar lavage samples taken 3 and 6 weeks after thermoplasty [91]. The mechanism of this increased sputum eosinophil count is therefore unclear, but may perhaps contribute to the increased prevalence of asthma adverse events seen during, and shortly after, thermoplasty. It is interesting that asthma control and asthma symptom scores improved significantly despite this rise in sputum eosinophils, although it is known that there is discord between asthma symptoms and inflammation in many asthma patients [10]. It is also of interest that sputum eosinophils significantly increased, while asthma exacerbations significantly decreased, as exacerbations are typically mediated by an inflammatory event. However, it is not known whether thermoplasty alters the character of asthma exacerbations, or whether the remodelling benefits of thermoplasty somehow offset some of the adverse effects

of increased sputum eosinophil levels. Further investigation to characterise in detail the exacerbations that occur before and after thermoplasty treatment may answer this question.

As there was significant inter-patient variability in timings of thermoplasty procedures there was some overlap in the timings of biopsies classified as follow-up 1 (6 weeks) and follow-up 2 (12 weeks). It was decided to keep biopsies grouped by visit/procedure, rather than time, to eliminate possible confounders such as the cumulative effect of oral corticosteroids given with each procedure, and possible effects of each procedure on neighbouring lobes (as suggested as an explanation for the previously noted right middle lobe effect [92]). Due to relatively small participant numbers, grouping biopsies by time from first procedure, rather than by procedure number was not feasible. However, one assumes that, due to the nature of the procedure and this patient group, the variability in procedure intervals was likely also the case in other published thermoplasty biopsy trials, which used similar methods.

The baseline value and magnitude of improvement in airway smooth muscle mass (relative median reduction of 58% and 60% at 6 weeks and 12 weeks respectively) were consistent with previously published data [90, 92-94]. The improvement seen at 12 weeks was not statistically significant, owing primarily to small participant numbers with paired biopsies, as well as the skewing effect of one participant who had a very large relative increase in smooth muscle mass (relative increase of 950%, suggesting this particular baseline biopsy was not truly representative) compared to the rest of the cohort. The reduction in reticular basement membrane thickness was less than is reported elsewhere. Baseline values were consistent with previously reported data [90], but the magnitude of reduction in this study was less than previously shown.

There were significant improvements in markers of epithelial health, which has not previously been reported in the literature. Intact epithelium improved significantly after thermoplasty, and was accompanied by a significant reduction

in damaged epithelium, although no change in denuded epithelium. This suggest that in this acute time-frame (6 weeks), the improvement may be due to regeneration of epithelium which already has some epithelial cells present, rather than establishment of regrowth in areas of completely denuded basement membrane. Whether completely denuded epithelium has the capacity to regenerate a healthy epithelium, and the length of time this would be seen over, is not known. Detailed characterisation of epithelial recovery in areas that are damaged and denuded before treatment would require a larger study with biopsies taken at regular time intervals over a longer period.

Baseline myofibroblast numbers correlated with both pre- and post-treatment epithelial integrity. However, there was no relationship between baseline numbers of myofibroblasts and the change in epithelial integrity from baseline to post-treatment. This is likely explained by the observed effect that, with a small number of exceptions, the change from baseline to post-treatment epithelial integrity was centred closely to the mean (i.e. regardless of myofibroblast number or epithelial integrity at baseline, most participants had an improvement in epithelial integrity of approximately 16-17%). This suggests that the presence of increased numbers of myofibroblasts in the lamina propria leads to better epithelial integrity, but that the improvement seen after thermoplasty is not dependent on having more or less myofibroblasts. It may be that there is a 'ceiling effect' in the rate at which myofibroblasts contribute to epithelial repair over the short time periods used in this study. Comparing epithelial repair to baseline myofibroblasts over a longer follow-up period may reveal a relationship that could not be demonstrated over 6 weeks. Whether other mechanisms involved in tissue repair are affected by thermoplasty, beyond those related to myofibroblast function, is unknown.

Correlations between remodelling and clinical features were investigated, although this was limited by small participant numbers with matched biopsy and clinical data. Baseline biopsy assessed epithelial integrity and epithelial area correlated with number of years since asthma diagnosis and inhaled corticosteroid

dose respectively; years since diagnosis and inhaled corticosteroid dose were not themselves related. The relationship between epithelium area and inhaled corticosteroid dose suggests that a higher dose is beneficial in terms of epithelial maintenance or repair, although this single time-point observation is not conclusive. Previously published data in this area is limited, concerned mostly with mild asthma, and conflicting in its results [173]. The relationship between epithelial integrity and time since asthma diagnosis may also be related to the observed link between inhaled corticosteroid dose and epithelial health. One interpretation could be that those asthmatics who have been diagnosed for the shortest time may have not yet had their inhaled therapy fully optimised, and are therefore on an insufficient dose of corticosteroids, for an insufficient time, to see improvements in epithelial health. Alternatively, those diagnosed more recently may have had undiagnosed asthma for a long time, leading to deterioration in epithelial health while not prescribed asthma treatment. The correlation between epithelial thickness and ACQ6 at baseline suggests that higher levels of epithelial are associated with worse asthma control. This seems counter-intuitive, but could also be a function of the relationship described here between epithelial thickness and inhaled corticosteroid dose. Those patients with poor asthma control are more likely to have their inhaled therapy increased, and if an effect of inhaled corticosteroids is a regrowth of epithelium, then this could explain the correlation between ACQ6 and epithelium. However, in our data set there was no relationship between inhaled corticosteroid dose and ACQ6 at baseline, which therefore does not support this explanation.

Current understanding of how thermoplasty is beneficial clinically centres on reductions in airway smooth muscle mass. What is apparent from this study is that airway smooth muscle mass does not appear to correlate well to any clinical outcome. Although there was a relationship shown between change in smooth muscle mass and change in R5-R20 on oscillometry, data was only available for 5 participants in this correlation, so caution is required when interpreting this result. The correlation with R5-R20 was also in the opposite direction to the expected

relationship; namely, that decreasing airway smooth muscle mass would reduce small airways gas trapping through reducing small airway smooth muscle mediated constriction (although this theory has not been proven). A small study has previously shown patients with a good clinical response to thermoplasty exhibit a greater reduction in R5-R20, compared to poor responders [174], although this study saw no significant difference in oscillometry from baseline to post-treatment overall.

Airway smooth muscle did not relate to any other clinical outcome measure at baseline or in response to thermoplasty. This raises significant questions as to whether improvements in airway smooth muscle mass are clinically important to patient outcomes after thermoplasty treatment, or whether other remodelling changes or mechanisms are responsible.

There are a greater number of correlations between epithelial measures and clinical outcomes; however, as shown in table 4.5, these are all in the opposite direction to those expected for thermoplasty treatment, and seem to suggest that those with thicker epithelium at baseline, or greatest improvement in epithelium markers following treatment, are associated with poorer clinical outcomes, in relation to gas trapping (RV/TLC), small airways resistance (R5-R20), and airway compliance (AX). However, these correlations are obtained from few participant numbers (n=5 for oscillometry data) and larger studies are required to investigate this further.

The reduction in reticular basement membrane thickness observed in this study was smaller than previously published and not statistically significant [90]. However, basement membrane thickness was related to clinical outcomes. Participants with a lesser degree of basement membrane thickening at baseline had better ACQ6 response to treatment. This was also true of AQLQ response, although the p-value (0.06) did not reach significance. However, change in basement membrane thickness was negatively correlated with ACQ6 response, suggesting that the greatest improvements in basement membrane thickening

after thermoplasty were associated with a deterioration in asthma control. These two correlations seem contradictory, and therefore interpretation of whether reticular basement membrane thickness relates directly to clinical outcomes such as asthma control remains unclear, and requires clarification in larger studies.

One possible mechanism for the remodelling effects of thermoplasty considered in this study relates to the role of myofibroblasts. Fibroblasts stain positive with aSMA, and are associated with the airway smooth muscle bundle [86], are able to traffic to sites of injury, differentiate into myofibroblasts, and contribute to tissue repair [169-171]. We therefore considered whether the observed reduction in airways smooth muscle mass, and increase in epithelial integrity, could be an effect of fibroblast migration from the smooth muscle bundle to assist in epithelial repair. There was an association between myofibroblasts and epithelial health at baseline, confirming that these cells likely play a role in epithelial repair processes in severe asthma. However, although there was a numerical decrease in myofibroblasts in the lamina propria after thermoplasty, this was not statistically significant, and myofibroblasts did not directly correlate with changes in airway smooth muscle mass or epithelial integrity. On balance, therefore, this proposed mechanism seems unlikely. However, there may be a role for myofibroblasts in response to thermoplasty, as described above, that this limited dataset is not able to show over the short follow-up period of 6 weeks. Further investigation is again needed.

4.4.1 Limitations

The main limitation of this study was the small participant numbers with matched pre- and post-thermoplasty complete data sets. Although 33 participants were enrolled, the biopsy outcomes included only 14-16 matched sets of results. This was sufficient for assessing airway smooth muscle mass changes (as per our sample size calculation), but the study may have been underpowered to detect changes in other remodelling features. This small population size also led to significant limitations in comparing biopsy and clinical outcomes, as the 14-16

participants with match biopsy data had numerous data gaps in their clinical outcome data. For some of the outcomes explored (such as biopsy changes vs oscillometry) there was data for only 5 participants. Additionally, many of the identified correlations are difficult to explain, and are in the opposite direction to that which you would expect them to be. It is therefore difficult to draw any firm conclusions from these correlations, especially in light of small sample size. The small numbers of participants with a complete data set meant that the planned responder analysis could not be undertaken. Due to these factors, the correlations described in this study should be viewed as exploratory, and further investigation with a large clinical study, to include biopsy analysis, is needed.

This was a 'real-world' study with no randomisation, and significant flexibility was given to the treating centre to manage participants through their thermoplasty treatment as per their own protocols and team decisions. This inevitably led to issues with data collection and consistency (for example, participants not withholding their inhalers prior to spirometry, meaning pre-bronchodilator results were not valid), and also with significant variability between patient participation (such as seen in the diverse time intervals between thermoplasty procedures). The variation in participant processes likely limits how reliable the results of this study are. To negate this effect larger studies would be needed.

Another limitation relates to biopsy sample quality. As this was a 'real-world' study the priority for the treating team at each thermoplasty session was to deliver the treatment. Collecting the biopsy samples was a secondary concern. Therefore, in several cases the number of biopsy samples obtained was only one or two, as it was deemed that further samples could not be collected safely. The size and quality of the biopsy samples was also often poor. Consequently, some samples had to be eliminated from the sample cohort. Also, in some instances only a limited number of slides could be cut from the tissue block due to size and tissue quality. In many cases a second good-quality sample block was not available. Accordingly, it was decided to cut only one slide for each marker from the biopsy block deemed highest quality at each biopsy visit. Assessing only one slide in this

way, as opposed to taking a mean value from several slides, could potentially skew results if that particular tissue sample was not representative of the overall airway wall structure. However, our mean values for airway smooth muscle and reticular basement membrane thickness [90], and the responses to thermoplasty [90, 92-94], were consistent with previously published data, suggesting that this was not a significantly limiting factor.

Baseline biopsies were obtained from the right upper lobe, and follow-up biopsies from the right lower lobe. It is therefore possible that some of the changes demonstrated are due to variability in remodelling features between lung lobes. Previous examination of thermoplasty responses in all lung lobes showed that the right upper lobe did have a numerically higher mean airway smooth muscle mass at baseline compared to the right lower lobe (approximately 25% for RUL vs 17% for RLL) [92]. However, both absolute values and relative reduction following thermoplasty was not significantly difference between lobe [92], and therefore our methods can be viewed as a reliable representation of thermoplasty effects. We also observed a high degree of inter-patient variability in the ASM response to BT. However, despite these factors the observed overall magnitude of ASM mass reduction was similar to previous reports, giving confidence that the observed changes are genuine.

A possible contribution from the peri-procedure prednisolone upon remodelling cannot be excluded, although existing evidence of the effects of prednisolone on the epithelium are inconsistent, and no effects on the ASM mass have been reported [21].

4.4.2 Summary and future study

In summary, this study shows that severe asthma patients treated with bronchial thermoplasty have significant clinical improvements in asthma control questionnaire scores, and asthma quality of life questionnaire scores at 6 weeks, but this effect was not maintained at 6 months. Our observed improvement in asthma quality of life scores was less than half that previously published [95],

although our cohort of patient had more severe disease. We also observed a significant reduction in annual exacerbation rate, consistent with previous data [95]. At 6 months after treatment, some of the clinical benefit was lost, and there were consistent trends (some statistically significant) towards small deteriorations in several markers of lung function. We also observed an acute increase in sputum eosinophil counts following treatment.

Biopsy-measured airway smooth muscle mass significantly reduced after thermoplasty, consistent with previous data [90-94], but our observed effect on reticular basement membrane thickness was smaller than previously demonstrated and not statistically significant [90, 93, 94]. We did observe significant improvements in epithelial integrity, and other measures of epithelial health, which has not previously been shown.

Baseline epithelial health appeared to be related to baseline numbers of myofibroblasts, consistent with their role in tissue repair, although myofibroblast numbers did not affect the response to thermoplasty in the epithelium. Other features of remodelling on biopsies neither correlated at baseline, nor in their response to thermoplasty, suggesting that responses to treatment are heterogeneous.

Correlations between biopsy and clinical responses to treatment were limited by missing data, and were often contradictory of the expected relationship based on the accepted relevant mechanisms, and should therefore be interpreted with significant caution.

Further study into the relationship between the different remodelling responses to thermoplasty may help clarify the mechanism, although this will require greater patient numbers undergoing bronchial biopsy, which is currently not common clinical practice. With greater patient numbers, the clinical improvements seen after thermoplasty could also be explored in more details including their relationship with remodelling changes. Characterisation of exacerbations before and after thermoplasty may help clarify the range of responses seen, and explain

the mechanism by which thermoplasty exerts this effect. A larger prospective study would be the most ideal way to explore remodelling changes and how they relate to future clinical outcomes, although the logistical challenges are considerable, as shown in this study. A large meta-analysis or pooled study of existing data would be a useful alternative.

5 Remodelling and clinical effects of bronchial thermoplasty in a large pooled population

5.1 Introduction

As described in detail in chapters 1 and 4 of this thesis, thermoplasty is a licensed treatment for severe asthma. While the clinical benefits of thermoplasty are established, primarily from a large sham-controlled randomised study (described in detail in chapter 1) [95], the mechanisms by which this clinical benefit occurs is still unclear. Established clinical benefits are an increase in asthma quality of life score (AQLQ), and exacerbation reduction, but no consistent effect on lung function has been shown [95, 135, 136]. Biopsy assessed remodelling effects are established in several small studies, with the greatest weight of evidence for reductions in airways smooth muscle mass and reticular basement membrane thickness in response to thermoplasty [90-94]. However, these studies have small study populations (n=9 to 17). There is also some overlap in subjects between these studies, as discussed in the introduction to this thesis, and so existing published biopsy data comes from as few as 43 subjects across five studies. Furthermore, only one study (9 patients) has investigated the persistence of remodelling changes over a longer timeframe, and showed that these appear to be maintained beyond 3 months [94]. No published data exists beyond that described in chapter 4 of this thesis showing an effect on airway epithelial repair. Only one study (n=15) has shown correlation between biopsy-assessed remodelling changes and clinical outcomes, with relationships shown between remodelling changes and asthma control scores, asthma exacerbations, and emergency department visits at 12 months.

The bronchial thermoplasty study described in chapter 4 of this thesis was significantly limited by a small sample size and missing data, but confirmed similar reductions in airway smooth muscle mass as previously identified [90-94]. However, the reduction in reticular basement membrane thickness in the chapter

4 study was small and not statistically significant, in contrast to three previous studies (combined n=32 individual subjects) [90, 93, 94]. The improvements in epithelial integrity have not been previously reported. Unfortunately, detailed correlations between clinical changes and biopsy changes were not possible due to study size.

Thermoplasty is a relatively uncommon asthma therapy, and so research centres working individually to collect biopsy data typically only have small data sets. This pooled analysis was therefore undertaken to compile a large dataset of severe asthma patients who had already undergone bronchial thermoplasty, and had clinical and biopsy remodelling data available, in an attempt to explore the persistence of biopsy changes over time, further determine the epithelial response to treatment, and correlate clinical outcomes with biopsy changes. This included data from several asthma research centres around the world, some of which had already individually been published. It aimed therefore to address the limitations of sample size identified in the previous studies, including that in chapter 4.

5.2 Methods

5.2.1 Study design and participants

Asthma centres which had published data related to biopsy remodelling responses to thermoplasty, or were known to be collecting biopsies before and after thermoplasty, were approached to contribute to this retrospective pooled analysis. All 8 centres approached (located in Europe and North America, see table 5.2) agreed to participate. A screening questionnaire was sent to each centre to collect information regarding available clinical and biopsy data, and to identify the number of subjects in each centre's cohort.

Using the information from the screening questionnaire, the biopsy and clinical outcomes with the most available data across the centres (selected as those with data for at least 50 subjects) were selected. A data collection sheet was created and distributed to each centre for them to populate. This included: clinical parameters such as exacerbation frequency and asthma questionnaires; physiological parameters such as lung function; blood parameters such as eosinophil count; and biopsy parameters (see table 5.1 for complete list of data collected). Completed data sheets were returned to the lead centre (Leicester, United Kingdom), where the data was assimilated into a common database for analysis.

Table 5.1: Data collected from each centre, as determined by screening questionnaire

Outcome	**Baseline**	**Follow-up***
Age	√	
Sex	√	
Height	√	
Weight	√	
Body mass index	√	
Smoking status	√	
Smoking history	√	
GINA treatment step	√	
Atopy	√	
Blood eosinophil count	√	
Asthma medication	√	√
Oral corticosteroid dose	√	√
Inhaled corticosteroid dose	√	√
Annual exacerbations	√	√
Asthma related hospital admissions	√	√
ACQ6 / ACT	√	√
AQLQ	√	√
Pre-BD spirometry	√	√
Post-BD spirometry	√	√
BD reversibility	√	√
Airway smooth muscle mass %	√	√
Reticular basement membrane thickness	√	√
Epithelial integrity	√	√
Thermoplasty activations		√

* If data from more than one follow-up visit was available then all data was collected. GINA = Global Initiative for Asthma. ACQ6 = asthma control questionnaire-6. ACT = asthma control test. AQLQ = asthma quality of life questionnaire. BD = bronchodilator.

5.2.2 Randomisation and masking

As this was an observational real-world study there was no randomisation of subjects, and no placebo or sham procedure. Investigators, including those assessing biopsy samples, were blinded to participant identity, and whether biopsy samples were pre or post-thermoplasty treatment.

5.2.3 Procedures

All patients underwent bronchial thermoplasty as part of the management of their severe asthma. Each centre applied their own patient selection criteria, in the absence of recognised selection criteria guidelines for bronchial thermoplasty. Patients had all been reviewed in a specialist severe asthma service, discussed at an asthma multidisciplinary team meeting, and had had the rest of their asthma management optimised prior to thermoplasty. Patients were consented locally for biopsies to be collected during thermoplasty treatment for use in future research.

Bronchial thermoplasty was delivered in the approved standardised method using the Alair catheter system (Boston Scientific, Marlborough, Massachusetts, USA), delivered to the target airway by flexible bronchoscope. Specifics of the bronchoscopy procedures were performed as per the standard operating procedure at each centre. Patients underwent three thermoplasty sessions, treating each of the target lobes in sequence: right lower lobe in session one; left lower lobe in session two; and both upper lobes in session three. Each treatment session was spaced apart by approximately 2-4 weeks to allow sufficient recovery time. In a small number of instances the second or third procedure was undertaken at a longer interval for patient safety reasons, such as an asthma exacerbation in the period between procedures.

Biopsies were obtained at a range of time points, depending on local research protocols: baseline samples were collected on all subjects, either shortly before starting thermoplasty treatment or during the first thermoplasty procedure; and follow-up samples were collected either during the second and third thermoplasty

procedures or at an additional follow-up bronchoscopy. Biopsies were taken from a different lobe to that being treated at each thermoplasty procedure. In the majority of cases the baseline samples were collected from the left lower lobe or one of the upper lobes during the first thermoplasty procedure, as the right lower lobe was being treated. Follow-up samples were taken from the previously treated right lower lobe during thermoplasty session two and three, or at subsequent bronchoscopies done specifically to obtain biopsy tissue.

5.2.4 Outcomes

Baseline and follow-up assessments were performed as per local clinical and research protocols. Body mass index (BMI) was calculated from height and weight as kg/m^2. Smoking status was defined as current, ex-smoker, or never. Smoking history (where participant was a current or ex-smoker) was recorded in pack-years. GINA treatment step was calculated based on baseline medications. Annual asthma exacerbations was defined as the number of exacerbations in the 12 months preceding thermoplasty treatment, where exacerbation was defined as an worsening of asthma control requiring a course of oral corticosteroids (or an increase from baseline dose if on regular oral corticosteroids) for at least 3 days. Asthma related hospital admission were recorded as the number in the 12 months preceding thermoplasty treatment. Asthma control was assessed using a verified questionnaire, such as asthma control questionnaire (ACQ) or asthma control test (ACT). Spirometry was performed pre- and post-bronchodilator as per standardised methods, and bronchodilator reversibility was calculated from the results.

Bronchial biopsy samples were collected from segmental and subsegmental carinae. Samples were stored, processed, cut and stained as per local standard operating procedures. Analysis was primarily undertaken on sections stained for haematoxylin and eosin (H&E) and alpha-smooth muscle actin (aSMA). Number of sections analysed, and number of analysts, varied at each centre as per their

research protocols. All analysts were blinded to patient identity, characteristics and timing of biopsies.

Data related to each thermoplasty procedure, including date, lobe(s) treated, and number of thermoplasty activations was recorded. Biopsy related outcomes were airway smooth muscle mass percent, reticular basement membrane thickness, and epithelial integrity, as previously described in chapters 2 and 4. Briefly: airway smooth muscle mass area was measured and expressed as a percentage of the total biopsy structural area; reticular basement membrane thickness was expressed in micrometres as the mean of 50 measurements taken approximately 20µm apart; and epithelial integrity was calculated by measuring the length of the reticular basement membrane with intact epithelium, and expressed as a percentage of the total reticular basement membrane length. Biopsy outcomes were measured before and after thermoplasty. Existing data was used in most instances. In a small number of cases additional biopsy analysis was undertaken on samples which had been collected but not yet analysed. The screening questionnaire sent to centres described standard methods for analysing and recording the biopsy outcomes, and all participating centres were able to provide data in this standardised format.

5.2.5 Statistical analysis

Absolute change from baseline for biopsy outcomes was determined by subtracting baseline values from follow-up results. As some patients had more than one set of biopsies taken at a range of time-points within the first 12 months after thermoplasty treatment, the mean change from baseline for these samples was calculated and used for the correlations against clinical outcomes.

Clinical and biopsy outcomes were analysed using GraphPad Prism version 8.1.2 (San Diego, USA), using paired t-tests. Relationships between clinical outcomes and biopsy changes, and different features of biopsy changes, were tested using Pearson's correlation coefficient. Responder/non-responder group analysis was undertaken using unpaired t-tests. Both ACQ6 and AQLQ have a minimum

clinically important difference of 0.5, but sham thermoplasty has been shown to effect an AQLQ improvement of 1.1 in a population of moderate to severe asthmatics [95]. However, the results described in chapter 4 showed mean treatment responses of 0.51, in a severe asthma population reflective of that which is described in this pooled study. Therefore, responder analysis was undertaken using thresholds of both ≥0.5 to <1.0 (termed 'responder') and ≥1.0 (termed 'super-responder') improvement in ACQ or AQLQ. For improvement in exacerbation rate after thermoplasty, the relative annual exacerbation rate in the 12 months after treatment was calculated compared to the preceding 12 months, and expressed as a percent. 'Responders' were defined as those with a post-treatment exacerbation rate of >25 to ≤50% of that at baseline, approximately in keeping with the exacerbation rate reduction seen with most licensed biological agents. 'Super-responder' was arbitrarily defined as those with a post-treatment exacerbation rate of ≤25% of baseline. Where there was insufficient data to analyse 'responders' and 'super-responders' separately, these were grouped together as 'responders'. A p-value of <0.05 was considered statistically significant.

5.3 Results

Biopsy and clinical data was available for 119 participants. Paired pre- and post-thermoplasty biopsy data were available as follows: 119 for airway smooth muscle mass; 55 for reticular basement membrane thickness; and 36 for epithelial integrity. The number of paired epithelium integrity results was lower than anticipated due to one centre reporting having this data but only being able to provide follow-up data, with no matched baseline data for comparison. The number of participants included from each contributing centre is shown in table 5.2.

5.3.1 Baseline characteristics

In this real-world study patients were selected based on the individual asthma centre's clinical criteria for thermoplasty treatment. No specific inclusion or exclusion criteria were applied at the stage of pooling the data, except that participants must have paired pre-and post-thermoplasty biopsy results. Those without paired samples were excluded from the analysis.

Baseline clinical characteristics are as shown in table 5.2. Mean age was 49.5 years, and 55% of participants were female; mean BMI was elevated at 28.8; 47 (39%) participants had a history of tobacco smoking (3 current smokers, 44 ex-smokers). The mean smoking history was 17.4 pack-years (range 0.2-100) for current and ex-smoking participants. 72 (61%) had a history of atopy. 73 (61%) were prescribed oral corticosteroids (prednisolone), and the mean dose for those on oral corticosteroids was 23.5mg (range 5-80mg). All participants were prescribed inhaled corticosteroids, and the mean dose was 1558 µg (fluticasone equivalent). 47% were prescribed leukotriene receptor antagonists, and 10% were prescribed anti-IgE therapy. In the 12 months prior to thermoplasty treatment the mean number of asthma exacerbations was 5.8, and mean number of asthma related hospital admissions was 1.5. Mean blood eosinophil count was 0.24 $x10^{-9}$/L, with the majority of participants below the UK eosinophil licensing thresholds for anti-IL5 therapy (0.3 $x10^{-9}$/L). Baseline asthma control test (ACT) and asthma control questionnaire (ACQ6) scores were 7.7 and 3.0 respectively, indicating poor asthma control at baseline. Mean asthma quality of life questionnaire (AQLQ) score was 3.2. Mean pre-bronchodilator FEV1 (forced expiratory volume in 1 second) was impaired at 67.4% of predicted, and there was a mean of 14.8% reversibility of FEV1 in response to bronchodilator. Despite this degree of reversibility, post-bronchodilator FEV1/FVC ratio was 0.66, indicating a degree of persistent airway obstruction. The mean cumulative number of thermoplasty activations administered for all three procedures was 176.5.

5.3.2 Consistency of baseline characteristics between contributing centres

Mean prednisolone dose in participants from Basel was significantly lower at 13.9mg compared to the overall study population dose of 23.5mg. Inhaled corticosteroid dose was significantly higher in the Leicester participants (2356µg) compared to the overall population (1558µg). Mean exacerbation in the previous 12 months was higher in the Paris cohort (10.0), and lower in the Quebec and Basel cohorts (2.3 and 2.8, respectively) when compared to the overall population mean of 5.8. Mean asthma control test score was higher in those recruited from Marseille, but this only related to 6 participants. Asthma quality of life questionnaire scores were significantly lower in the Paris cohort (2.1), and higher in the Quebec and Amsterdam cohorts (5.6 and 4.5, respectively) compared to the overall population (3.2). Both pre- and post-bronchodilator lung function was significantly worse in the Marseille cohort, and better in the Amsterdam cohort, compared to the overall study population.

Overall the breakdown of characteristics suggest differences in patient selection for each centre. Participants from Paris seemingly had more severe disease, with a higher rate of exacerbation and worse AQLQ scores. Participants from Marseille had markedly worse lung function. Participants from Amsterdam, Quebec and Basel appear to be less severe than the dataset average, based on the combined characteristics of lower oral corticosteroid dose, lower exacerbation frequency, better AQLQ scores, and better lung function. However, despite these variations between the cohorts from different centres, the overall picture of the study population was of asthmatics with uncontrolled symptoms, poor asthma control, frequent exacerbations, and impaired lung function. It was therefore deemed sufficiently reflective of the severe asthma population and suitable to be combined into a single study population.

Total number of thermoplasty activations was significantly lower in the Leicester cohort (114.4), and higher in the Quebec cohort (225.9), compared to the overall

population (176.5). This suggests some variability between centres in relation to how the thermoplasty procedure is carried out.

Summary baseline characteristics for each centre, and for the overall study population, is shown in table 5.2.

Table 5.2: Baseline clinical characteristics (overall study population and by centre)

Characteristic	Leicester # (n=16)	Paris (n=39)	Marseille (n=6)	Quebec (n=20)	Amsterdam (n=18)	Basel (n=20)	Overall (n=119)
Age, years	50.9 (13.2)	48.5 (10.8)	51.5 (9.6)	48.5 (11.4)	44.7 (13.2)	54.7 (15.7)	**49.5 (12.6)**
Female sex, n (%)	10 (63%)	19 (49%)	1 (17%)	10 (50%)	14 (78%)	12 (60%)	**66 (55%)**
BMI, kg/m^2	30.9 (7.9)	30.0 (6.8)	26.4 (4.8)	28.9 (5.9)	28.2 (4.9)	28.1 (5.9)	**28.8 (6.3)**
Current/ex-smoker, n (%)	4 (25%)	13 (33%)	5 (83%)	8 (40%)	7 (39%)	10 (50%)	**47 (39%)**
Smoking history, pack-years ^	8.9 (8.2)	15.6 (14.6)	10.4 (8.8)	20.6 (14.5)	6.7 (4.3)	32.0 (28.4)	**17.4 (18.1)**
History of atopy, n (%)	7 (44%)	24 (62%)	5 (83%)	15 (75%)	11 (61%)	10 (50%)	**72 (61%)**
On prednisolone, n (%)	9 (56%)	33 (85%)	5 (83%)	6 (30%)	6 (33%)	14 (70%)	**73 (61%)**
Prednisolone dose, mg ~	23.3 (13.2)	29.2 (15.5)	34.0 (26.3)	17.1 (9.3)	12.4 (6.1)	13.9 (7.3)*	**23.5 (15.4)**
ICS dose, µg	2356 (856)*	ND	1000 (500)	1368 (663)	1236 (615)	ND	**1558 (843)**
On LTRA, n (%)	10 (63%)	ND	0 (0%)	10 (50%)	8 (44%)	ND	**28 (47%)**
On Anti-IgE, n (%)	1 (6%)	0 (0%)	0 (0%)	5 (25%)	2 (11%)	4 (20%)	**12 (10%)**
Annual asthma exacerbations, n	4.1 (2.6)	10.0 (5.5)*	6.0 (3.3)	2.3 (2.8)*	5.2 (5.7)	2.8 (1.9)*	**5.8 (5.3)**
Annual asthma related hospital admissions, n	0.9 (2.0)	2.2 (3.1)	0.0 (0.0)	ND	ND	1.1 (1.1)	**1.5 (2.5)**
Blood eosinophils, $x10^{-9}/L$	0.22 (0.21)	0.28 (0.31)	0.18 (0.10)	ND	0.22 (0.19)	0.19 (0.22)	**0.24 (0.25)**
ACT, score	ND	7.2 (2.5)	11.0 (3.8)*	ND	ND	ND	**7.7 (2.9)**
ACQ6, score	3.4 (1.7)	ND	ND	ND	2.6 (0.7)	ND	**3.0 (1.3)**
AQLQ, score	3.2 (1.7)	2.1 (0.8)*	3.4 (1.1)	5.6 (0.8)*	4.5 (1.0)*	ND	**3.2 (1.6)**
Pre-BD FEV1, % predicted	66.5 (18.9)	64.5 (19.4)	37.9 (13.6)*	66.6 (18.9)	88.6 (25.4)*	61.1 (18.9)	**67.4 (22.5)**
Pre-BD FVC, % predicted	96.3 (21.7)	86.7 (14.4)	60.2 (14.4)*	82.0 (18.7)	101.2 (23.4)*	89.8 (15.8)	**89.3 (19.4)**
Pre-BD FEV1/FVC, ratio	0.58 (0.15)	0.60 (0.14)	0.52 (0.12)	0.64 (0.10)	0.74 (0.09)*	0.56 (0.16)	**0.62 (0.14)**
Post-BD FEV1, % predicted	75.1 (18.9)	68.8 (19.5)	47.8 (15.3)*	72.3 (16.2)	101.2 (21.5)*	63.2 (20.5)	**73.7 (23.1)**
Post-BD FVC, % predicted	101.3 (22.2)	93.2 (14.6)	69.8 (18.4)*	85.6 (14.3)	109.4 (18.2)*	93.1 (15.5)	**94.4 (18.9)**
Post-BD FEV1/FVC, ratio	0.63 (0.12)	0.61 (0.14)	0.58 (0.17)	0.67 (0.09)	0.78 (0.09)*	0.56 (0.18)	**0.66 (0.15)**
FEV1 reversibility, %	20.5 (12.2)	10.9 (13.2)	18.4 (9.2)	13.3 (13.1)	20.3 (37.7)	13.5 (9.8)	**14.8 (19.5)**
Total thermoplasty activations, n	114.4 (28.0)*	175.6 (37.7)	194 (66.2)	225.9 (53.4)*	183.8 (28.2)	166.6 (33.8)	**176.5 (49.8)**

Leicester includes samples from Glasgow and Southampton (part of same study). Values shown are mean (standard deviation) unless otherwise specified. ^Smoking history given as mean value for those who smoked. ~Prednisolone dose given as mean of those taking prednisolone. Kg/m^2 = kilograms per metres squared. Pack-year = (number of cigarettes smoked per day/20) x number of years smoked. mg = milligrams. µg = micrograms. LTRA = leukotriene receptor antagonist. IgE = immunoglobulin-E. L = litre. ACT = asthma control test. ACQ6 = asthma control questionnaire-6. AQLQ = asthma quality of life questionnaire. BD = bronchodilator. FEV1 = forced expiratory volume in 1 second. FVC = forced vital capacity. *denotes unpaired t-test p-value <0.05 when comparing the individual centre value to the overall study population value

5.3.3 Baseline biopsy remodelling features

Table 5.3 shows the baseline biopsy characteristics of participants at each centre and as a whole study population. At baseline, airway smooth muscle mass was 15.1% of total biopsy area. The value for the Paris cohort was significantly higher at 18.7%, and Basel was lower at 10.0%. Mean baseline reticular basement membrane thickness was 6.4µm, and epithelial integrity was 33.8%. The mean value for airway smooth muscle mass and reticular basement membrane thickness were consistent with previously published studies [90, 92-94]. Data was only available from 3 of the cohorts for reticular basement membrane thickness, and 2 of the cohorts for epithelial integrity.

Table 5.3: Baseline biopsy remodelling features (overall study population and by centre)

	Leicester # (n=16)	**Paris (n=39)**	**Marseille (n=6)**	**Quebec (n=20)**	**Amsterdam (n=18)**	**Basel (n=20)**	**Overall (n=119)**
ASM mass, %	13.5 (8.9)	18.7 (5.1)*	14.8 (2.4)	12.7 (5.4)	17.2 (6.7)	10.0 (6.3)*	**15.1 (6.8)**
RBM thickness, µm	7.3 (1.9)	ND	ND	6.4 (1.6)	ND	5.7 (3.4)	**6.4 (2.5)**
Epithelial integrity, %	28.7 (19.1)	ND	ND	ND	ND	37.9 (23.8)	**33.8 (22.1)**

Leicester includes samples from Glasgow and Southampton (part of same study). Values shown are mean (standard deviation). ASM = airway smooth muscle mass. RBM = reticular basement membrane. µm = micrometres. *denotes unpaired t-test p-value <0.05 when comparing the individual centre to the overall study population.

5.3.4 Clinical and remodelling correlations at baseline

Table 5.4 summarises the correlations between clinical features and biopsy remodelling features at baseline. Airway smooth muscle showed a weak correlation with smoking history (r= -0.23, p=0.02; figure 5.1A), suggesting that smokers with the greatest smoking history had lower baseline levels of airway

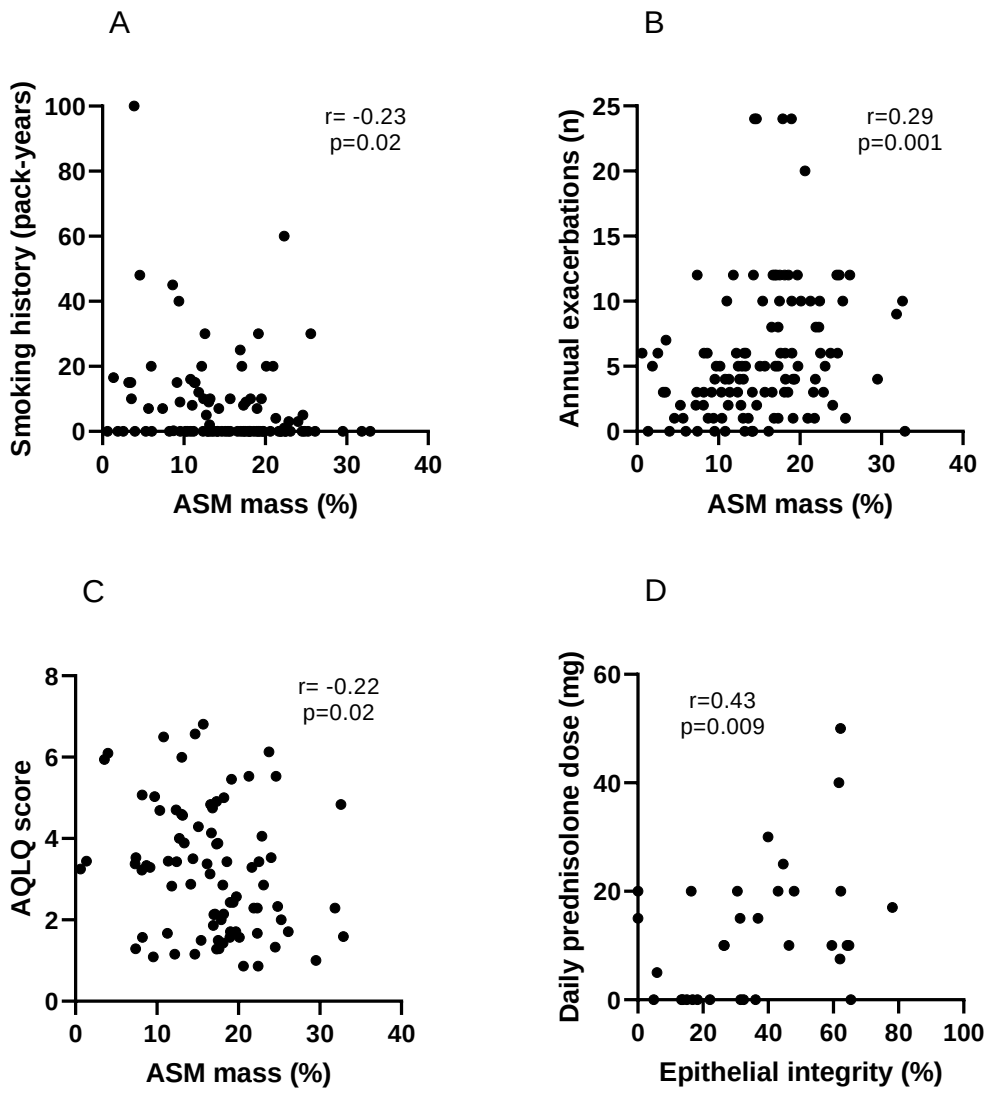
smooth muscle mass. Airway smooth muscle mass was also weakly correlated with exacerbation rate (r=0.29, p=0.001; figure 5.1B), showing increased exacerbation rates with increasing airway smooth muscle mass. There was also a relationship between airway smooth muscle mass and asthma quality of life scores (r= -0.25, p=0.02; figure 5.1C), suggesting worsening asthma symptoms at baseline with increasing airway smooth muscle mass. Epithelial integrity was correlated with baseline prednisolone dose (r=0.43, p=0.009; figure 5.1D), suggesting that higher levels of oral corticosteroids relate to better epithelial health prior to thermoplasty treatment. There were no correlations between reticular basement membrane thickness and clinical features at baseline.

Table 5.4: Baseline clinical and biopsy correlations

Baseline clinical characteristic	Baseline ASM mass	Baseline RBM thickness	Baseline epithelial integrity
Age	n.s	n.s	n.s
BMI	n.s	n.s	n.s
Smoking history	r= -0.23 **p=0.02**	n.s	n.s
Prednisolone dose	n.s	n.s	r=0.43 **p=0.009**
ICS dose	n.s	n.s	n.s
Exacerbations	r= 0.29 **p= 0.001**	n.s	n.s
Hospitalisations	n.s	n.s	n.s
Blood eosinophils	n.s	n.s	n.s
ACT	n.s	(too few pairs)	(too few pairs)
ACQ6	n.s	n.s	n.s
AQLQ	r= -0.25 **p=0.02**	n.s	n.s
Pre-BD FEV1	n.s	n.s	n.s
Pre-BD FVC	n.s	n.s	n.s
Pre-BD FEV1/FVC	n.s	n.s	n.s
Post-BD FEV1	n.s	n.s	n.s
Post-BD FVC	n.s	n.s	n.s
Post-BD FEV1/FVC	n.s	n.s	n.s
BD reversibility	n.s	n.s	n.s

Pearson's correlation coefficients and p-values. ASM = airway smooth muscle. RBM = reticular basement membrane. BMI = body mass index. ICS = inhaled corticosteroid. ACT = asthma control test. ACQ6 = asthma control questionnaire-6. AQLQ = asthma quality of life questionnaire. BD = bronchodilator. FEV1 = forced expiratory volume in 1 second. FVC = forced vital capacity. n.s = p-value >0.05.

Figure 5.1: Baseline remodelling features vs baseline clinical features



Statistically significant correlations between remodelling features on bronchial biopsies at baseline and clinical data at baseline for; airway smooth muscle mass vs smoking history (A) airway smooth muscle mass vs annual exacerbation rate (B), airway smooth muscle mass vs asthma quality of life score (C), and epithelial integrity vs daily prednisolone dose (D). ASM = airway smooth muscle. AQLQ = asthma quality of life score. mg = milligrams. Pearson's correlation coefficient and p-value reported.

There were no correlations identified between the three assessed features of airway remodelling at baseline ($p>0.05$ for Pearson's correlation in each case).

5.3.5 Clinical response to bronchial thermoplasty

The clinical responses to thermoplasty are summarised in table 5.5, taken at two time-points, approximately 1 to 3 months (mean 85 days), and 6 to 12 months (mean 317 days) after treatment. The range in follow-up time-points was a result of variations in visit scheduling between each of the contributing centres.

Mean (SD) annual asthma exacerbation rate decreased significantly from 5.8 (5.3) to 1.2 (1.7) following thermoplasty (p=<0.0001; figure 5.2A), which represents a 79% relative reduction from baseline. Annual asthma related hospital admissions also decreased from 1.5 (2.5) to 0.3 (0.7) (p=0.0004; figure 5.2B) (80% relative reduction).

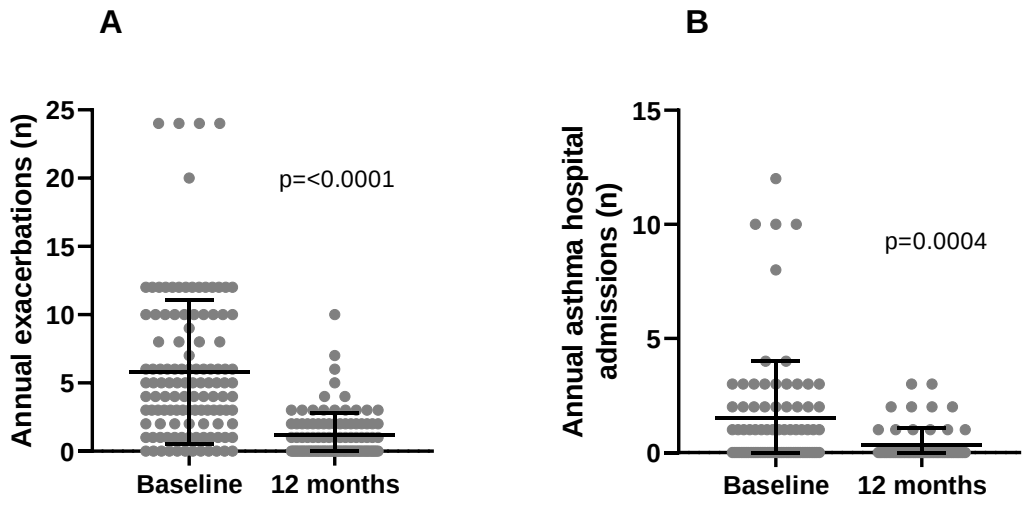
Asthma control significantly improved as determined by both the asthma control test (ACT) and asthma control questionnaire (ACQ6) within 1-3 months, and the improvement was maintained to 6-12 months. Asthma control test scores improved from 7.7 (2.9) at baseline, to 15.0 (5.4) at 1-3 months (p=<0.0001), and 15.2 (6.2) at 6-12 months (p=<0.0001) (figure 5.3A). 77% and 75% achieved the minimum clinically important difference of 3 points at 1-3 months and 6-12 months respectively. Asthma control questionnaire scores improved from 3.0 (1.3) at baseline to 2.8 (1.7) at 1-3 months (p=0.02), and 2.4 (1.3) at 6-12 months (p=0.01) (figure 5.3B). 50% and 57% achieved the minimum clinically important difference of -0.5 points at 1-3 months and 6-12 months respectively. Asthma quality of life scores (AQLQ) significantly improved from 3.2 (1.6) at baseline to 3.8 (1.5) at 1-3 months (p=<0.0001), and 4.4 (1.7) at 6-12 months (p=<0.0001) (figure 5.3C). 72% and 64% achieved the minimum clinically important difference at 1-3 months and 6-12 months respectively. Pre- and post-bronchodilator spirometry, and bronchodilator reversibility, were unchanged following thermoplasty treatment (see table 5.5).

Table 5.5: Clinical responses to thermoplasty

Characteristic	Baseline		1-3 months				6-12 months			
	Value	n	Value	n	Change from baseline	p-value	Value	n	Change from baseline	p-value
Annual exacerbations, n	5.8 (5.3)	117	-	-	-	-	1.2 (1.7)	99	-5.0 (5.5)	**<0.0001**
Annual hospitalisations, n	1.5 (2.5)	79	-	-	-	-	0.3 (0.7)	61	-1.3 (2.8)	**0.0004**
ACT, score	7.7 (2.9)	45	15.0 (5.4)	44	7.2 (4.8)	**<0.0001**	15.2 (6.2)	43	7.5 (5.4)	**<0.0001**
ACT % meeting MCID, n (%)	-	-	34 (77%)	44	-	-	33 (75%)	43	-	-
ACQ6, score	3.0 (1.3)	33	2.8 (1.7)	15	-0.7 (1.0)	**0.02**	2.4 (1.3)	31	-0.6 (1.2)	**0.01**
ACQ6 % meeting MCID, n (%)	-	-	7 (50%)	14	-	-	17 (57%)	30	-	-
AQLQ, score	3.2 (1.6)	84	3.8 (1.5)	59	1.3 (1.5)	**<0.0001**	4.4 (1.7)	75	1.3 (1.5)	**<0.0001**
AQLQ % meeting MCID, n (%)	-	-	41 (72%)	57	-	-	46 (64%)	72	-	-
Pre-BD FEV1, % predicted	67.4 (22.5)	108	62.5 (17.9)	73	-0.3 (15.2)	0.87	68.9 (23.4)	74	-0.1 (13.6)	0.96
Pre-BD FVC, % predicted	89.3 (19.4)	108	88.0 (17.0)	72	-0.5 (16.3)	0.80	89.6 (21.2)	74	-0.1 (14.8)	0.96
Pre-BD FEV1/FVC	0.62 (0.14)	108	0.60 (0.13)	72	0.01 (0.11)	0.28	0.63 (0.13)	74	0.01 (0.13)	0.50
Post-BD FEV1, % predicted	73.7 (23.1)	111	68.7 (17.9)	76	0.2 (11.6)	0.86	75.4 (23.3)	86	-0.9 (11.4)	0.46
Post-BD FVC, % predicted	94.4 (18.9)	111	89.8 (22.9)	75	-5.2 (20.7)	0.04	94.0 (19.9)	86	-0.7 (14.2)	0.64
Post-BD FEV1/FVC	0.64 (0.15)	111	0.63 (0.18)	73	0.03 (0.16)	0.16	0.66 (0.13)	86	-0.01 (0.10)	0.41
BD reversibility, %	14.8 (19.5)	100	12.2 (18.0)	70	-1.1 (18.9)	0.64	14.4 (23.2)	70	-2.3 (16.4)	0.26

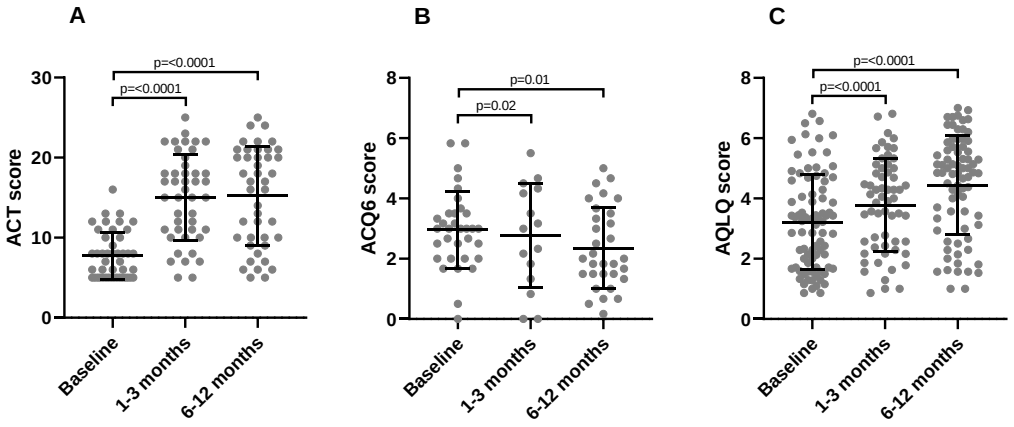
Data shown is mean (standard deviation) unless otherwise stated. ACT = asthma control test. ACQ6 = asthma control questionnaire-6. AQLQ = asthma quality of life score. BD = bronchodilator. FEV1 = forced expiratory volume in 1 second. FVC = forced vital capacity. MCID = minimum clinically important difference. MCID for ACT = 3 points [175]. MCID for ACQ6 = -0.5 points [176]. MCID for AQLQ = 0.5 [140]. p-values for changes from baseline are paired t-tests.

Figure 5.2: Exacerbation rate (A) and hospital admission rate (B) responses to thermoplasty



Annual exacerbation rate (A) and annual asthma-related hospitalisation rate (B) at baseline and 12 months after completing thermoplasty treatment. p-value is paired t-test.

Figure 5.3: ACT (A), ACQ6 (B) and AQLQ (C) responses to thermoplasty



Asthma control test (A), asthma control questionnaire (B) and asthma quality of life (C) scores at baseline, 1-3 months, and 6-12 months after completing thermoplasty treatment. p-value is paired t-test as shown. ACT = asthma control test. ACQ6 = asthma control questionnaire-6. AQLQ = asthma quality of life questionnaire.

The number of participants reaching minimum clinically important difference thresholds for asthma control test, asthma control questionnaire, asthma quality of life questionnaire, and annual exacerbations rates are shown in table 5.6. This shows that in each domain, the participants who achieved a clinically meaningful improvement generally reached double the minimum clinically important difference. Therefore, there appears to be a somewhat dichotomous response, of either no clinical improvement or a very substantial one, with a small proportion of patients in between.

Table 5.6: Minimum clinically important difference thresholds

Outcome	Group	n	%
ACT	Non-responder	10	23
	Responder (1≤ response <2 x MCID)	9	21
	Super responder (response ≥2 x MCID)	24	56
ACQ6	Non-responder	10	37
	Responder (1≤ response <2 x MCID)	3	11
	Super responder (response ≥2 x MCID)	14	52
AQLQ	Non-responder	26	36
	Responder (1≤ response <2 x MCID)	5	7
	Super responder (response ≥2 x MCID)	41	57
Exacerbations	Non-responder	14	16
	Responder *	13	15
	Super responder *	62	70

ACT = asthma control test (MCID = 3 points). ACQ6 = asthma control questionnaire-6 (MCID = -0.5 points). AQLQ = asthma quality of life questionnaire (MCID = 0.5 points). * For exacerbations the MCID thresholds were defined as a <50% reduction for 'non-responder', a ≥50% to <75% reduction for 'responder', and a ≥75% reduction for 'super-responder'.

5.3.6 Biopsy assessed remodelling response to bronchial thermoplasty

Table 5.7 summarises the biopsy remodelling changes observed in response to thermoplasty. Mean (SD) airway smooth muscle mass reduced from 15.1% (6.8) at baseline to 6.7% (4.1) after thermoplasty (p=<0.0001). Reticular basement membrane thickness reduced from 6.4µm (2.5) at baseline to 5.5µm (2.1) after thermoplasty (p=0.02). Epithelial integrity improved from 33.8% (22.1) at baseline to 43.9% (17.6) after thermoplasty (p=0.02).

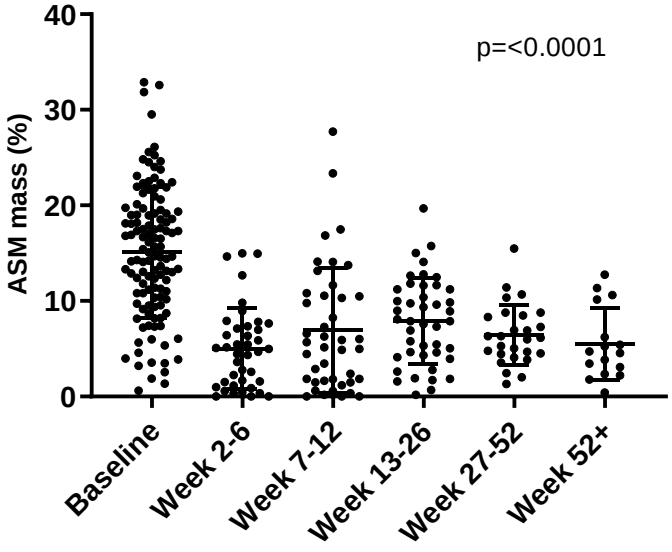
Table 5.7: Biopsy remodelling response to thermoplasty

	Baseline		Post-thermoplasty			
Remodelling feature	**Value**	**n**	**Value**	**n**	**Change from baseline**	**p-value**
ASM mass, %	15.1 (6.8)	119	6.7 (4.1)	119	-8.4 (7.7)	**<0.0001**
RBM thickness, µm	6.4 (2.5)	55	5.5 (2.1)	56	-0.9 (2.8)	**0.02**
Epithelial Integrity, %	33.8 (22.1)	36	43.9 (17.6)	36	10.1 (25.4)	**0.02**

ASM = airway smooth muscle. RBM = reticular basement membrane. µm = micrometres. Post-thermoplasty values stated are the mean of the earliest two follow-up biopsies. p-value stated is paired t-test.

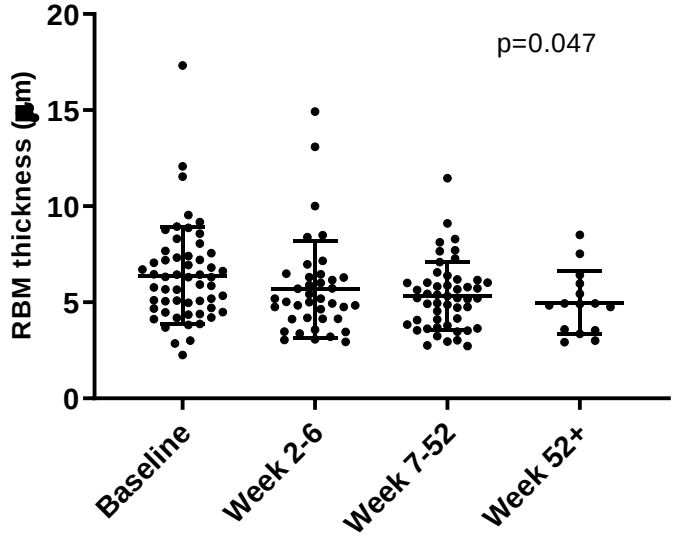
Figures 5.4, 5.5 and 5.6 show all biopsy sample results grouped by time after completion of thermoplasty treatment. Airway smooth muscle mass decreases significantly within 6 weeks of treatment, and this improvement is maintained beyond 1 year from treatment (ANOVA p=<0.0001; figure 5.4). Reticular basement membrane thickness also decreases within 6 weeks, and the effect is maintained beyond 1 year (ANOVA p=0.047; figure 5.5). The effect on reticular basement membrane thickness is small but statistically significant, and appears to continue to improve slightly at each time period after treatment. Epithelial integrity improves within 6 weeks of treatment (p=0.09; n.s) and the effect is maintained to 6 months (p=0.033); however this is only statistically significant at the latter time-point (figure 5.6).

Figure 5.4: Airway smooth muscle mass response to thermoplasty



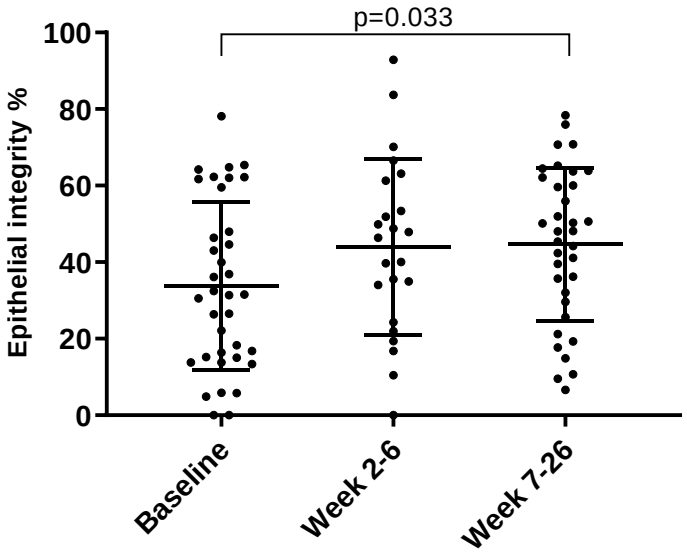
Airway smooth muscle mass on bronchial biopsy samples at baseline and specified time points after thermoplasty treatment. p-value is one-way ANOVA. ASM = airway smooth muscle.

Figure 5.5: Reticular basement membrane response to thermoplasty



Reticular basement membrane thickness on bronchial biopsy samples at baseline and specified time points after thermoplasty treatment. p-value is one-way ANOVA. RBM = reticular basement membrane. µm = micrometres.

Figure 5.6: Epithelial integrity response to thermoplasty



Epithelial integrity on bronchial biopsy samples at baseline and specified time points after thermoplasty treatment. p-value is paired t-test for baseline to 6-12 months.

5.3.7 Correlations between clinical and biopsy remodelling outcomes

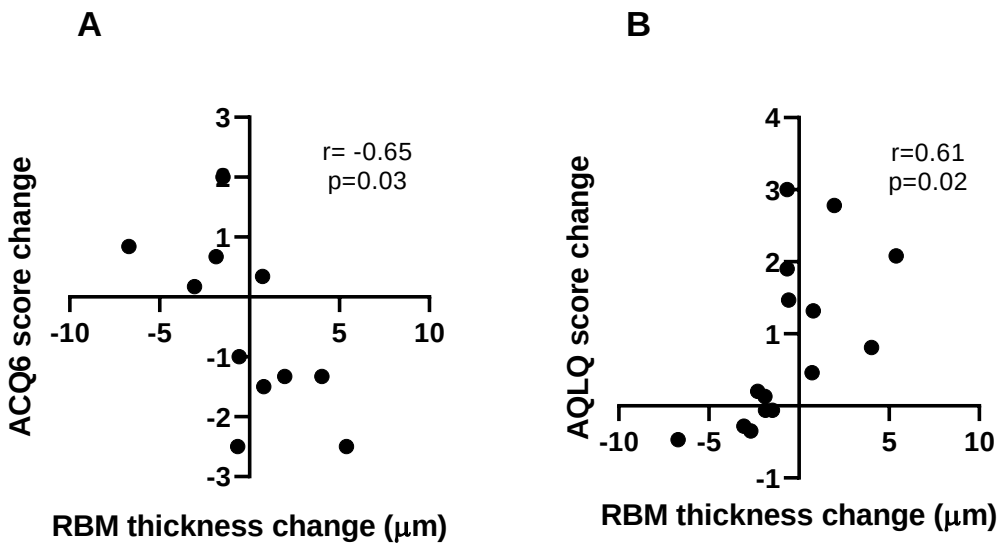
Table 5.8 summarises correlations between clinical outcomes and biopsy remodelling outcomes. The only direct correlations identified were between change in reticular basement membrane thickness and changes in asthma control questionnaire (ACQ6) score (r= -0.65, p=0.03; figure 5.7A), and asthma quality of life questionnaire score (AQLQ) (r= 0.61, p=0.02; figure 5.7B). The correlations identified here are in the opposite direction to that which would be expected, suggesting that a decrease in reticular basement membrane thickness is associated with worsening outcomes on both ACQ6 and AQLQ. However, these correlations are for a small number of participants (n=11 and n=15 respectively), and come from an individual centre (Leicester; discussed in detail in chapter 4).

Table 5.8: Clinical response and biopsy remodelling response correlations

	ASM mass change		RBM thickness change		Epithelial integrity change	
	Correlation	**n**	**Correlation**	**n**	**Correlation**	**n**
Exacerbation rate	r=0.14 p=0.18	89	r=0.19 p=0.32	30	r= -0.12 p=0.65	16
ACT	r= -0.21 p=0.17	43	Too few pairs	0	Too few pairs	0
ACQ6	r= -0.27 p=0.14	30	r= -0.65 **p=0.03**	11	r= -0.34 p=0.27	12
AQLQ	r=0.009 p=0.94	72	r=0.61 **p=0.02**	15	r=0.009 p=0.98	12
Pre-BD FEV1	r= -0.03 p=0.77	89	r=0.22 p=0.23	32	r= -0.05 p=0.81	27
Pre-BD FVC	r= -0.05 p= 0.62	89	r=0.04 p=0.83	32	r= -0.02 p=0.92	27
Pre-BD FEV1/FVC	r= -0.04 p=0.70	89	r=0.28 p=0.12	32	r= -0.006 p=0.97	27
Post-BD FEV1	r= -0.17 p=0.10	98	r=0.15 p=0.34	41	r=0.24 p=0.24	26
Post-BD FVC	r= -0.09 p=0.39	98	r=0.06 p=0.69	41	r=0.06 p=0.79	26
Post-BD FEV1/FVC	r= -0.04 p=0.71	98	r=0.07 p=0.67	41	r=0.20 p=0.33	26
BD reversibility	r= -0.11 p=0.32	78	r=0.09 p=0.67	24	r= -0.09 p=0.70	21
Total thermoplasty activations	r= -0.07 p=0.45	119	r= -0.13 p=0.36	55	r= -0.28 p=0.10	36

Absolute change used for biopsy changes due to some 0 values at baseline meaning relative change could not be calculated, and some extremely relative change values skewing the data significantly. Relative reduction used for exacerbations. ASM = airway smooth muscle. RBM = reticular basement membrane. ACT = asthma control test. ACQ6 = asthma control questionnaire-6. AQLQ = asthma quality of life questionnaire. BD = bronchodilator. FEV1 = forced expiratory volume in 1 second. FVC = forced vital capacity. Pearson's correlation coefficient and p-value reported.

Figure 5.7: RBM thickness response vs ACQ6 (A) and AQLQ (B) responses



Statistically significant correlations between change in reticular basement membrane thickness and asthma control questionnaire (A) and asthma quality of life questionnaire (B) scores in response to thermoplasty. p-value is Pearson's correlation. RBM = reticular basement membrane. ACQ6 = asthma control questionnaire-6. AQLQ = asthma quality of life questionnaire. μm = micrometres.

Although table 5.8 highlights the lack of robust direct correlations between clinical outcomes and biopsy remodelling changes, a responder group analysis was undertaken using pre-specified minimum clinically important differences, to determine if an indirect relationship between remodelling improvements and clinical improvements existed.

Table 5.9 and figure 5.8 show the responder group analysis for airway smooth muscle mass change. There was no significant difference, either numerically or statistically, between the reduction in airway smooth muscle mass between responders and non-responders in each of the clinical domains. As the asthma control test and asthma control questionnaire assess the same clinical feature (asthma control), and no participant had data for both outcomes, these results were combined to create larger groups for analysis, including a 'super-responder'

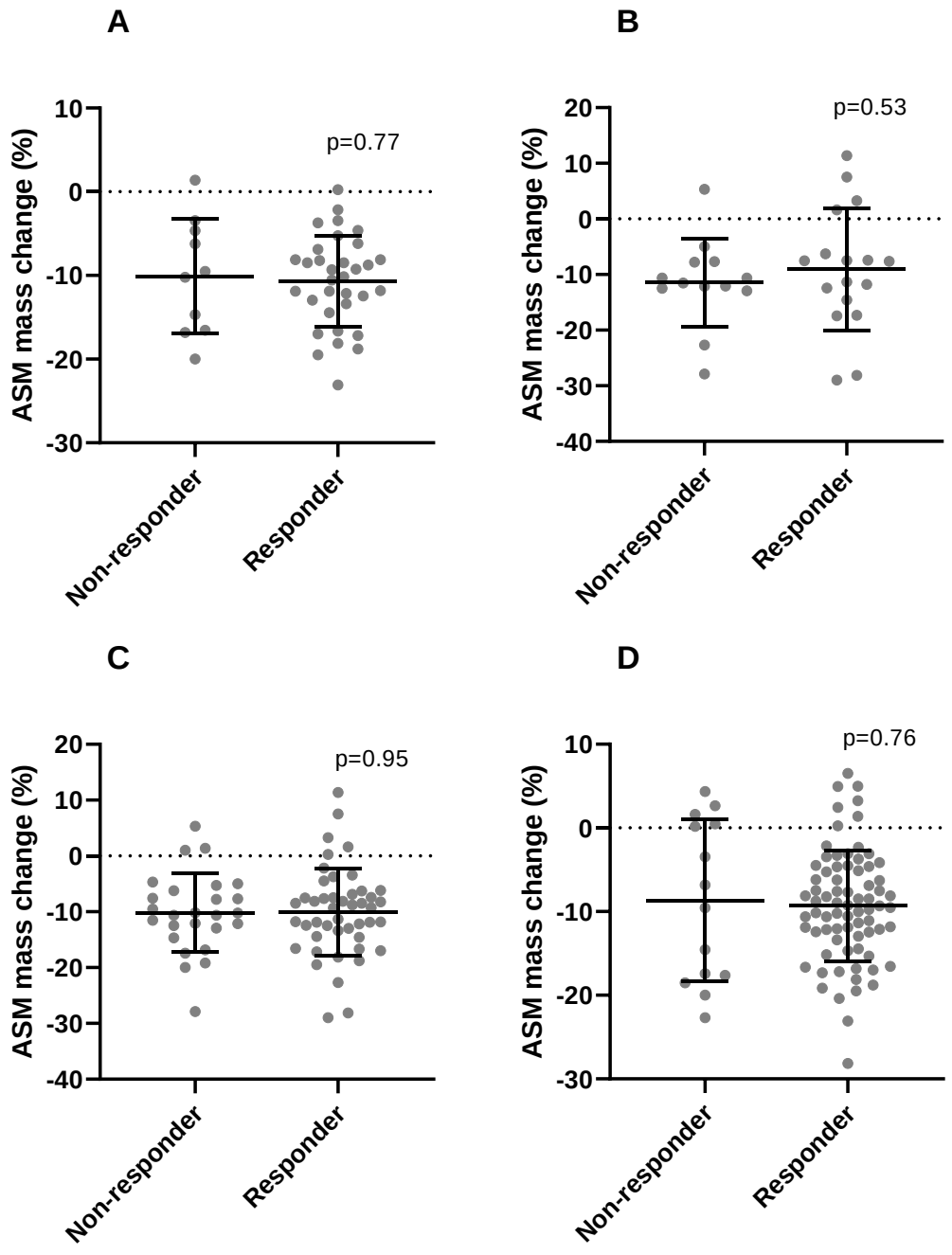
group with double the MCID. This combined group also showed no quantitative relationship to reductions in airway smooth muscle mass.

Table 5.9: Clinical responder groups and airway smooth muscle mass

Outcome	Group	n	Change in ASM	p-value
ACT	Non-responder	10	-10.1 (6.9)	
	Responder	33	-10.7 (5.4)	0.77
ACQ6	Non-responder	13	-11.4 (7.9)	
	Responder	17	-9.1 (11.0)	0.53
AQLQ	Non-responder	26	-10.2 (7.1)	
	Responder	46	-10.1 (7.9)	0.95
Exacerbations	Non-responder *	14	-8.7 (9.6)	
	Responder *	75	-9.3 (6.6)	0.76
Combined ACT/ACQ6	Non-responder	23	-10.8 (7.4)	
	Responder (1≤ response <2 x MCID)	12	-11.4 (7.4)	0.81
	Super responder (response ≥2 x MCID)	38	-9.7 (7.8)	0.60

Mean (SD) change in airway smooth muscle mass shown. * For exacerbations the MCID thresholds were defined as a <50% reduction for 'non-responder', and a ≥50% reduction for 'responder'. ASM = airway smooth muscle. ACT = asthma control test. ACQ6 = asthma control questionnaire-6. AQLQ = asthma quality of life score. MCID = minimum clinically important difference. p-values are unpaired t-tests comparing responder groups to non-responder groups.

Figure 5.8: ASM change in responder and non-responder clinical outcome groups: ACT (A), ACQ (B), AQLQ (C) and exacerbation rate (D).



Change in airway smooth muscle mass in responder and non-responder groups for asthma control test (A), asthma control questionnaire score (B), asthma quality of life questionnaire score (C), and annual exacerbation rate (D) following thermoplasty. Non-responder defined as those participants with a change of less than the minimum clinically important difference, and responder as those with a change greater than the minimum clinically important difference. For exacerbations the MCID thresholds were defined as a <50% reduction for 'non-responder', and a ≥50% reduction for 'responder'. ASM = airway smooth muscle mass. Reported p-values are paired t tests.

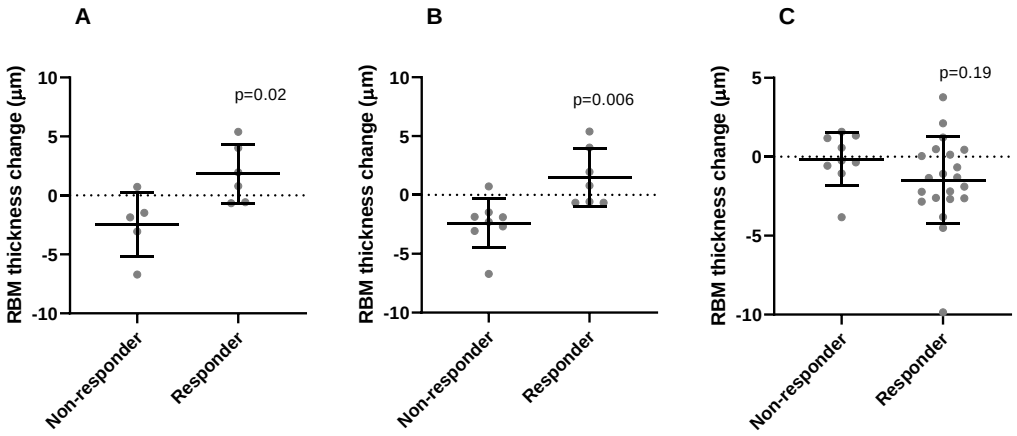
Responder group analysis for reticular basement membrane thickness is shown in table 5.10 and figure 5.9. The significant relationship with asthma control questionnaire score and asthma quality of life questionnaire scores is again demonstrated (p=0.02 and p=0.006 respectively). Again, this relationship is in the contrary direction to that which would be expected, as described in detail in chapter 4 of this thesis. There was no relationship with exacerbation rate response.

Table 5.10: Clinical responder groups and reticular basement membrane thickness

Outcome	**Group**	**n**	**Change in RBM**	**p-value**
ACQ6	Non-responder	5	-2.5 (2.7)	
	Responder	6	1.8 (2.5)	**0.02**
AQLQ	Non-responder	8	-2.4 (2.1)	
	Responder	7	1.5 (2.4)	**0.006**
Exacerbations	Non-responder *	9	-0.15 (1.7)	
	Responder *	21	-1.5 (2.8)	0.19

Mean (SD) change in reticular basement membrane thickness shown. * For exacerbations the MCID thresholds were defined as a <50% reduction for 'non-responder', and a ≥50% reduction for 'responder'. RBM = reticular basement membrane. ACQ6 = asthma control questionnaire-6. AQLQ = asthma quality of life questionnaire. p-values are unpaired t-tests comparing responder groups to non-responder groups.

Figure 5.9: RBM change in responder and non-responder clinical outcome groups: ACQ (A), AQLQ (B) and exacerbation rate (C).



Change in reticular basement membrane thickness in responder and non-responder groups for asthma control questionnaire score (A), asthma quality of life questionnaire score (B), and annual exacerbation rate (C) following thermoplasty. Non-responder defined as those participants with a change of less than the minimum clinically important difference, and responder as those with a change greater than the minimum clinically important difference. For exacerbations the MCID thresholds were defined as a <50% reduction for 'non-responder', and a ≥50% reduction for 'responder'. RBM = reticular basement membrane thickness. µm = micrometres. p-values are paired t-tests.

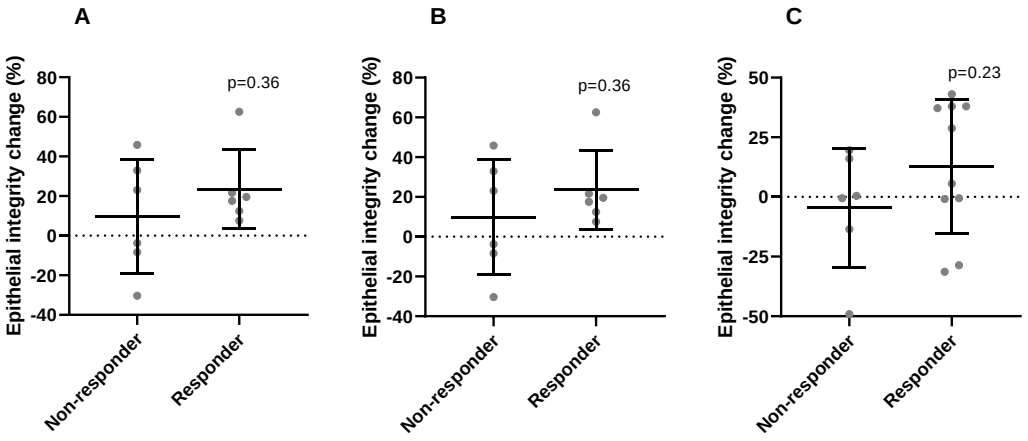
Responder group analysis for epithelial integrity is shown in table 5.11 and figure 5.10. Greater numerical improvements in epithelial integrity were seen in responder groups compared to non-responder groups in all clinical outcomes assessed. However, these were not statistically significant, likely due to small group numbers (n=6 to 10).

Table 5.11: Clinical responder groups and epithelial integrity

Outcome	Group	n	Change in epithelial integrity	p-value
ACQ6	Non-responder	6	9.9 (28.8)	
	Responder	6	23.6 (19.8)	0.36
AQLQ	Non-responder	6	9.9 (28.8)	
	Responder	6	23.6 (19.8)	0.36
Exacerbations	Non-responder	6	-4.5 (25.0)	
	Responder	10	12.9 (28.1)	0.23

Mean (SD) change in epithelial integrity shown. For exacerbations the MCID thresholds were defined as a <50% reduction for 'non-responder', and a ≥50% reduction for 'responder'. RBM = reticular basement membrane. ACQ6 = asthma control questionnaire. AQLQ = asthma quality of life score. p-values are unpaired t-tests comparing responder groups to non-responder groups.

Figure 5.10: Epithelial integrity change in responder and non-responder clinical outcome groups: ACQ (A), AQLQ (B) and exacerbation rate (C).



Change in epithelial integrity in responder and non-responder groups for asthma control questionnaire score (A), asthma quality of life questionnaire score (B), and annual exacerbation rate (C) following thermoplasty. Non-responder defined as those participants with a change of less than the minimum clinically important difference, and responder as those with a change greater than the minimum clinically important difference. . For exacerbations the MCID thresholds were defined as a <50% reduction for 'non-responder', and a ≥50% reduction for 'responder'. p-values are paired t-tests.

5.3.8 Effect of smoking on thermoplasty response

As there was a correlation between baseline airway smooth muscle mass and smoking history, the relationship between smoking and response to thermoplasty was further investigated. There was no direct correlation between airway smooth

muscle response to thermoplasty and smoking history. Comparisons were made between non-smokers and current or ex-smokers with a smoking history of more than 10 pack-years (this measure was chosen as being indicative of a clinically relevant smoking history). The results are summarised in table 5.12. There was a significant difference in the mean reduction in airway smooth muscle mass between non-smokers (-9.9%) and smokers (-4.8%) (p=0.003). Improvements in reticular basement membrane thickness and epithelial integrity were also numerically higher in non-smokers compared to smokers, although these differences did not reach significance. There were no statistically significant differences in clinical outcomes between smokers and non-smokers, except in asthma control questionnaire scores, although the smoker group for this outcome only included 2 participants and therefore the result should not be considered as representative. Non-smokers had a numerically greater reduction in exacerbation rates in the 12 months after thermoplasty compared to smokers of 10 pack-years or more (ratio of exacerbations compared to baseline of 0.2 in non-smokers, and 0.43 in smokers; p=0.06).

Table 5.12: Remodelling responses in non-smokers and smokers of >10 pack-years

	Non-smokers		**Smokers**		
	Value	**n**	**Value**	**n**	**p-value**
ASM change, %	-9.9 (7.5)	72	-4.8 (7.0)	28	**0.003**
RBM change, µm	-1.3 (3.2)	33	-0.1 (1.8)	16	0.17
Epithelial integrity change, %	12.2 (26.2)	22	4.9 (27.4)	11	0.46
ACT change, score	7.0 (5.4)	27	8.9 (6.0)	9	0.38
ACQ6 change, score	-0.3 (1.2)	20	-2.9 (0.6)	2	**0.01**
AQLQ change, score	1.2 (1.5)	49	2.0 (1.3)	9	0.13
Exacerbation ratio compared to baseline	0.20 (0.35)	52	0.43 (0.69)	20	0.06

ASM = airway smooth muscle. RBM = reticular basement membrane. µm = micrometres. ACT = asthma control test. ACQ6 = asthma control questionnaire-6. AQLQ = asthma quality of life questionnaire. Smokers defined as current smokers or ex-smokers of ≥10 pack-years. p-values are unpaired t-test.

5.4 Discussion

5.4.1 Summary of findings

This is the largest study investigating biopsy remodelling responses to bronchial thermoplasty, and included a population of 119 patients which were representative of a typical severe asthma population. Bronchial thermoplasty led to clinically and statistically significant improvements in asthma exacerbation and hospitalisation rates, asthma control scores, and asthma quality of life scores, consistent with previous data [95]. Compared to pre-treatment rates, the mean relative reduction in annual exacerbation was 74%, which is comparable or greater than that seen with biologics agents targeting the T2 inflammatory pathway [17, 18, 65]. Unlike T2 biologics, the improvement in clinical outcomes after thermoplasty was unrelated to blood eosinophil levels, and therefore may be applicable to a broader asthma population. The mechanism by which thermoplasty leads to such a large reduction in exacerbation rates is not clear. This study had no control group, and participants were not blinded to treatment. Therefore it is possible that the improvement could be related to a 'placebo' effect, due to factors such as unconscious bias, improved adherence with regular asthma medication during assessments for thermoplasty, and increased clinician contact and education. However, placebo effects are more typically observed in endpoints such as ACT, ACQ, AQLQ and FEV1. The ACT, ACQ, and AQLQ improvements seen here after thermoplasty were statistically significant, but clinically modest, compared to the large reduction in exacerbations. There was no effect on FEV1. These findings suggest that the placebo effect is unlikely to explain the observed exacerbation reduction. Other possible explanations could relate to the effects of thermoplasty upon airway nerves, changes in the lung microbiome, altered susceptibility to inhaled insults due to altered barrier function, and reduced ASM contractility during an exacerbation. One small study has demonstrated a correlation between reduction in airway nerve numbers, and improvements in asthma exacerbation rates and asthma control test scores [93]. However, the details of this mechanism remain unclear.

Benefits in terms of asthma control and asthma symptoms were seen as early as 1-3 months after treatment. This is somewhat unexpected given the previously shown increased incidence of asthma adverse events in the first 3 months after treatment [95]. In the present study, the rapid improvement may arise from bias related to lack of blinding procedures. There was consistently no effect on measures of lung function, consistent with most of the previously published data [95, 135, 136]. It is noteworthy that participants appeared to diverge in their response to thermoplasty into either 'non-responders' with asthma control and asthma symptom questionnaire scores of less than the minimum clinically important difference (MCID), and exacerbation reduction of less than 50%; or 'super-responders' with improvements in these outcomes of at least double the MCID. However, this study was unable to identify factors at baseline that predicted response to treatment. This would require a large, prospective study.

In current and ex-smokers we observed a negative correlation between the magnitude of smoking history and baseline levels of airway smooth muscle mass. Some of the participants included in this study had extensive smoking histories (up to 100 pack years), and therefore may have had an element of chronic obstructive pulmonary disease (COPD) in addition to asthma. This correlation could therefore be explained by concurrent COPD contributing to the individual's uncontrolled symptoms, and thus their decision to go ahead with thermoplasty. In COPD the increase in airway smooth muscle mass is predominantly seen in the small airways, rather than in the large airways as with asthma [177], and is therefore not accounted for in the bronchial biopsy samples assessed in this study. Nor would it be expected to improve in response to thermoplasty. However, there was no direct correlation found between changes in airway smooth muscle mass and smoking history, suggesting that even those with a greater smoking history and lower baseline airway smooth muscle can gain at least some benefit from thermoplasty.

To further explore the relationship between airway smooth muscle and smoking history, the thermoplasty response of non-smokers and smokers was compared.

While smoking status made no statistically significant difference to clinical outcomes, there appeared to be a relationship between smoking and remodelling responses to thermoplasty. In smokers the mean improvements in all three measures of biopsy remodelling were less than 50% of that seen in non-smokers, although this only reached statistical significance for airway smooth muscle mass (partly due to smaller sample sizes for the other outcomes). Some of these findings may reflect 'regression to the mean', in that non-smokers had a larger airway smooth muscle mass at baseline, and therefore more capacity for reduction after treatment. However, the differences in response between non-smokers and smokers was consistent across all biopsy measurements, and this is suggestive of a relationship. Therefore it appears that non-smokers gain greatest benefit in terms of remodelling response compared to smokers with more than a 10 pack-year smoking history. However, this does not appear to translate into improved clinical response, which were consistent between smokers and non-smokers, and is arguably more important to the patient.

At baseline there was a weak correlation between airway smooth muscle mass and asthma exacerbation frequency, suggesting that smooth muscle mass is either a marker of disease severity, or a factor driving exacerbations. There were no other correlations with baseline measures of asthma control, which therefore supports the latter as a possible relationship. The mechanism for this may relate to the pro-inflammatory function of airway smooth muscle, rather than its airway contractile function [178], especially as we saw no effect on lung function. It may also be that some reported (and treated) exacerbations relate to episodes of airway smooth muscle mediated bronchospasm, rather than true inflammatory events. In this way, it could be expected that a higher level of airway smooth muscle in the airway wall would lead to more frequent or more severe episodes of bronchoconstriction which may be treated as an exacerbation. The bronchoconstricting and pro-inflammatory roles of airway smooth muscle cells likely also explain the relationship seen with asthma symptoms, as those with

increased airway smooth muscle are likely also those affected by more airways constriction and inflammation.

The relationship seen at baseline between epithelial integrity and the dose of oral corticosteroids could be explained by the anti-inflammatory effect of corticosteroids reducing the inflammatory burden in the airway epithelium, which may then allow the healing and regeneration process to occur more freely. However, there is no published data to support this theory, and the data from this study only provides evidence of a correlative, not necessarily causative, relationship.

Interestingly, there were no direct correlations between baseline features of remodelling, showing that the degree of abnormality is not uniform across the difference morphological features of remodelling. This suggests that there may be different phenotypes of asthma patients with regards to their predominant remodelling changes. For example, some patients may be primarily affected by increased airway smooth muscle mass, whereas others may be primarily affected by decreased epithelial integrity. Potential driving mechanisms for this inter-patient variation and stability over time, the effect this may have on clinical features, and whether treatment approaches which target the individual's remodelling features can produce greater benefits would need to be investigated in large longitudinal biopsy studies.

Airway smooth muscle mass and reticular basement membrane thickness reduce rapidly following thermoplasty, and the improvement is maintained beyond 1 year after treatment. Epithelial integrity also improves significantly after thermoplasty until at least 6 months after treatment. The data for these findings is statistically robust, and shows consistency at every time point assessed after treatment. This offers reassurance that the remodelling effects of thermoplasty are sustained for significant periods of time. However, to provide evidence of this over several years would require follow-up biopsies of the patients included in this cohort, and may be difficult to undertake. Consideration should be given to approaching these

patients for longer term evaluation in the same way that is currently being undertaken to assess the clinical benefits of thermoplasty beyond 10 years after treatment.

The observed improvements in airway remodelling parameters are not consistently or directly correlated with improvements in clinical outcomes. Although airway smooth muscle consistently reduces after thermoplasty treatment, there is no statistical or numerical difference in airway smooth muscle mass reduction between clinical responders and non-responders. This goes against the commonly held theory as to the mechanism of action by which thermoplasty improves clinical outcomes. The consistent lack of effect on lung function, in both this study and others, also contradicts the previously proposed importance of airway smooth muscle mass to clinical outcomes. However, although no direct correlation was identified between airway smooth muscle reduction and clinical outcomes, the vast majority of participants did improve in both domains; therefore it is not possible to rule out a causative relationship.

Although correlations were seen in reticular basement membrane thickness against asthma control questionnaire and asthma quality of life questionnaire scores, these suggested that a decrease in RBM was actually associated with worse clinical outcomes, contrary to current thinking. However, this result should be interpreted with caution as there were only a small number of participants contributing data for this analysis. Further study of this is warranted, and if a relationship does exist then investigation into the mechanism of this should be undertaken, as RBM reduction may transpire to be a limiting factor to the clinical benefits seen following thermoplasty.

Epithelial integrity analysis was also undertaken in this study; unfortunately, the numbers of participants with data for this outcome was small which impedes the interpretation of the results. Nevertheless, there were non-significant trends suggesting that improvements in epithelial integrity were related to improvements in asthma control, asthma quality of life and exacerbation

frequency. This seems plausible, as a more effective epithelial barrier against inhaled pathogens and allergens would likely lead to improved asthma outcomes. Additionally, a more intact epithelium likely has more effective ciliary function and sputum clearance, which may reduce the risk of exacerbations related to bacterial infection. Further investigation of the relationship between epithelial changes following thermoplasty is certainly warranted.

No relationship was seen between the number of thermoplasty activations administered and clinical or remodelling responses to treatment. This perhaps suggests a ceiling effect of treatment with regard to the number of activations needed to achieve therapeutic benefit.

A strength of this study was its 'real-world' population of patients, and that the baseline demographics and characteristics were representative of a typical severe asthma clinic cohort. Therefore the results shown here can be considered applicable to all patients receiving bronchial thermoplasty in clinical practice.

Baseline airway smooth muscle mass was significantly higher in the Paris cohort, and lower in the Basel cohort, compared to the mean for the whole study population. This may reflect the observation that baseline clinical characteristics suggested that the Paris population had more severe disease, and the Basel population less severe disease. This relationship is strengthened by the baseline correlations showing a weak, but statistically significant relationships between increased airway smooth muscle mass and both increased exacerbation frequency and lower (i.e. worse) asthma quality of life scores. However, none of the other markers of disease severity correlated with airway smooth muscle mass at baseline. Minor variations in the way that airway smooth muscle mass was measured between analysts at the sites could also explain this difference.

5.4.2 Significance of findings

This is the first study undertaken examining biopsy remodelling responses to thermoplasty treatment in a large patient population. Previous studies have all

had less than 20 participants [90-94]. This study has a larger participant cohort than all of the existing published studies added together, in relation to biopsy remodelling data. Higher participant numbers allowed further investigation into clinical and remodelling effects, and importantly, the relationships between the two. This study adds significant weight to the existing small body of evidence showing reductions in airway smooth muscle mass and reticular basement membrane thickness following thermoplasty treatment.

This is the first study to plot the timescale of biopsy assessed remodelling changes in such detail, and finds that significant improvements are seen within the first 6 weeks after treatment, and then remain at this improved level beyond 1 year. It is also important to note that this study clearly shows that, although airway smooth muscle mass consistently decreases after thermoplasty treatment, this reduction does not show a clear relationship with clinical outcomes. This finding questions the widely held belief that airway smooth muscle mass reduction is the primary mechanism leading to clinical benefit following bronchial thermoplasty.

This is also the first study to investigate in detail the changes in epithelial integrity in response to bronchial thermoplasty. Although epithelial outcomes are limited by smaller patient numbers than the other biopsy outcomes, the data presented here suggests a possible important role for epithelial repair in relation to improvements in important clinical outcomes such as asthma control, asthma symptoms, and exacerbation rates. This data justifies further investigation into epithelial responses to thermoplasty.

5.4.3 Limitations of this study

This pooled study aimed to overcome the limitations relating to small sample size in previous published studies examining the biopsy remodelling effects of thermoplasty. 119 participants were included in this study, which is approximately five times more than in any individual study to date. However, one consequence of using existing data, collected at several research centres, is that the available data outcomes were not consistent across the whole population. There were large

gaps in the data set; for example, reticular basement membrane and epithelial integrity data was more limited than airway smooth muscle mass data. Different centres had also measured different clinical outcomes; for example, some centres used the asthma control test (ACT), while others used the asthma control questionnaire (ACQ), as a measure of asthma control. Due to this, some of the correlations investigated included fewer than 15 participants. For this reason the data has been presented with 'n' numbers for each variable in the tables; those with small numbers of participants should be interpreted with caution.

The biopsy analysis was largely undertaken at each centre, with a small number of exceptions (Paris performed the biopsy analysis for the Marseille cohort, and Leicester performed the analysis for the cohorts from Basel, Southampton and Glasgow). Therefore there may be inter-centre variations in the way that some of the biopsy outcomes were analysed and recorded. However, our baseline values between centres were generally comparable, suggesting that this was not a major factor. There were also small, but statistically significant, differences in clinical characteristics at baseline between the different study cohorts contributing to the study, with participant populations at some centres seemingly having more severe or less severe disease than the overall study average. This is an expected characteristic of a 'real-world' study, and reflects the fact that no standardised guidelines exist to assist clinicians in deciding whether a patient should undergo bronchial thermoplasty. That said, the response to treatment was uniform between centres, both in terms of clinical outcomes and biopsy remodelling outcomes, and therefore it is unlikely that this individuation of inclusion criteria across cohorts significantly affected the analysis.

There was no control group in the study, chiefly because this is a 'real-world' study, and also due to the difficulty identifying a control population for an invasive treatment such as thermoplasty. All clinical comparisons were necessarily made against pre-treatment data. It is therefore not possible to rule out a degree of responder bias in outcomes such as the asthma questionnaires. However, biopsy analysts were blinded to patient identity and stage of treatment, so the biopsy

results are unlikely to be affected in this same way. It is not possible in this study to comment on whether some improvements might be seen as a result of the three courses of high-dose oral corticosteroids given during treatment, and a possible improved adherence to prescribed asthma medications as a result of close follow-up and clinician contact that occurs during thermoplasty treatment.

It is possible that the method for collecting bronchial biopsies could itself affect the biopsy changes, through damaging the structures of the bronchial wall with the biopsy forceps. However, the results of this study, and others, consistently show improvements across the remodelling features assessed. If the differences in biopsy features were to occur as a result of the biopsy process, then the clear response to treatment would not be seen. Therefore, although this phenomena cannot be ruled out, it does not seem to be important to the overall results of the study.

Finally, although this study presents data regarding microscopic structural remodelling changes after thermoplasty, it is unable to offer any information relating to the effect this may have functional parameters beyond the clinical data presented, such as spirometric values. For example, it is not able to clarify whether airway smooth muscle responds to stimuli differently after thermoplasty.

5.4.4 Future research

Although this is the largest study investigating remodelling changes after thermoplasty it is still limited by relatively smaller sample sizes for reticular basement membrane thickness and epithelial integrity, as compared to that for airway smooth muscle mass. The trends in the data suggested that these features (particularly epithelial integrity) could be important factors in the clinical responses after thermoplasty, and therefore more data for these outcomes is urgently needed. If the suggested relationship between epithelial integrity improvements and clinical improvements is shown to be a real effect, then further work should be undertaken to investigate whether the thermoplasty catheter could be re-designed to optimise contact with the epithelium, perhaps by

including more than four wires on the thermoplasty catheter, in a bid to potentially increase the therapeutic benefit.

Additionally, further investigation into the relationships between remodelling changes and inflammatory markers is needed, in order to help understand the mechanism by which these remodelling changes occur, and identify if the beneficial clinical effects come about directly as a result of structural change, or as a result of the effects structural airway components have on inflammatory pathways and cellular function.

In summary, this study adds significant knowledge regarding the remodelling responses to thermoplasty and how these relate to clinical outcomes. It challenges the understanding that airway smooth muscle mass reduction is the key contributor to clinical benefits, and suggests that improvements in epithelial integrity may play a more important role.

6 Conclusion and discussion

6.1 Introduction

Significant gaps remain in the understanding of the mechanisms by which licensed and investigational treatments for severe asthma exert their beneficial effect. It is also not fully understood why clinical responses to severe asthma treatments are heterogeneous across the asthma population, ranging from complete resolution of symptoms and exacerbations, to no beneficial effect at all. A small number of biomarkers have been identified to predict responses to some asthma treatments, but even in patients that exhibit predictive biomarkers to a certain therapy, the response to treatment may be poor. In this thesis I have attempted to explore the mechanisms of two different treatment approaches for severe asthma, in order to better understand their mechanisms of effect. I have primarily investigated the effects on airway remodelling, but have also investigated inflammatory responses and how this relates, or not, to remodelling. The central hypothesis of this thesis was that airway remodelling changes, and in particular those seen in the epithelium, are keys determinants of the clinical responses seen to novel asthma treatments.

6.2 Summary of findings

I hypothesised that treatment with the anti-interleukin-13 monoclonal antibody tralokinumab would have a beneficial effect on airway eosinophilic inflammation and airway remodelling, with particularly improvements in the airway epithelium. In chapter 3 I report the findings from a phase 2, multicentre, randomised, double-blind, parallel-group, placebo-controlled, 12-week trial of tralokinumab in participants with inadequately controlled moderate-to-severe asthma. The change from baseline to Week 12 was assessed in bronchial, blood, and sputum eosinophil counts, fractional exhaled nitric oxide (FeNO), blood IgE

concentrations, airway physiology, measures of airway inflammation, and remodelling assessed on bronchial biopsies, and quantitative computed tomography (CT).

I hypothesised that inhibition of IL-13 with tralokinumab would reduce eosinophil migration from the vascular space into the lung tissue, and subsequently into the airway. With less inflammatory burden in the airway tissue, I expected to see improvements in measures of epithelial health and remodelling assessed by bronchial biopsies. A range of measures was used to assess the epithelium, including epithelial integrity (as a measure of the epithelial barrier function), reticular basement membrane thickening (a marker of sub-epithelial matrix deposition and remodelling), and MUC5AC expression (a measure of mucus production).

Contrary to my hypotheses, in this patient group tralokinumab showed no significant effect on eosinophilic inflammation in the blood, sputum or bronchial tissue. There was also no consistent observed effect on markers of remodelling and epithelial health measured on bronchial biopsy samples. There was a significant reduction in exhaled nitric oxide and blood IgE in tralokinumab treated subjects compared to placebo, confirming engagement with the target receptor and a biological effect, but this did not translate into inflammation or remodelling improvements.

Previous *in vitro* studies have shown that IL-13 plays a role in epithelial cell proliferation [179], subepithelial fibrosis (i.e. increased reticular basement membrane thickness), goblet cell metaplasia, and mucus hypersecretion, in addition to effects on smooth muscle cell proliferation and numerous pro-inflammatory mediators [180]. However, these effects have not previously been investigated *in vivo*. This study confirms that remodelling effects are not exhibited *in vivo*; the findings also highlighting the potential difficulties that arise when attempting to extrapolate *in vitro* results, where experiments are isolated from the complexities of interacting biological pathways, to real asthma patients. The

lack of inflammatory and remodelling effects in this study explains the limited clinical improvements seen with anti-IL-13 treatment reported elsewhere [67, 125].

The lack of effects seen with anti-IL-13 is almost certainly due to the overlapping biological effects of interleukin-13 with interleukin-4. IL-4 and IL-13 share common receptor targets (chiefly the IL-4 alpha receptor), and it is likely that inhibiting one of IL-4 or IL-13 in isolation is inadequate to effect significant biological changes *in vivo*. Further evidence to support this concept is shown in recent results of late stage clinical trials of dupilumab, a monoclonal antibody which binds directly to the alpha subunit of the IL-4 receptor, and therefore blocks both IL-4 and IL-13 signalling pathways [65]. Phase III clinical trials of dupilumab have shown significant clinical benefits in asthma patients [65], in contrast to lack of biological and clinical effect of blocking IL-13 in isolation [67, 125]. The effect of dupilumab on remodelling is not known.

My hypothesis regarding the benefits of anti-interlekin-13 treatment was disproved. This study, alongside others, does not support the ongoing development of agents acting solely on IL-13 in the treatment of asthma, as the effects on inflammation, remodelling and clinical parameters are inadequate.

In chapters 4 and 5 I report the results of two related studies; firstly, a small trial investigating remodelling effects following bronchial thermoplasty treatment, and their relationship to a broad range of clinical and physiological parameters; and secondly, a much larger pooled analysis of remodelling responses to thermoplasty and the relationships to important clinical outcomes such as asthma control and exacerbation rates.

In response to bronchial thermoplasty my data gives robust evidence of clinical benefit, with significant reductions in asthma exacerbation rates and improvements in asthma control and asthma symptoms. There was minimal or no effect seen in a large range of lung function measures. These clinical findings are consistent with previous studies [95, 135, 136].

In patients treated with bronchial thermoplasty I have measured and observed a reduction in airway smooth muscle mass in line with previous small biopsy studies [90-94]. I have demonstrated that this reduction occurs acutely (in the first 6 weeks), and is maintained at least 12 months after completion of treatment. Reticular basement membrane thickness also decreases following thermoplasty, as previously shown [90, 93, 94], and remains decreased for at least 12 months. Both smooth muscle mass and reticular basement membrane thickness appear to return to 'normal' levels after thermoplasty, as would be observed in a non-asthmatic airway. It is reassuring that these improvements appear to remain stable beyond 12 months, suggesting that the beneficial effect of thermoplasty on remodelling features is long-lasting, and supports its use in clinical practice as a 'one-off' treatment in severe asthma. This lasting remodelling effect at 12 months is consistent with the clinical improvement (in asthma exacerbation rates and asthma symptoms) seen at 12 months in the largest thermoplasty clinical trial [95]. Investigation into the long term clinical improvements following thermoplasty (beyond 10 years) is currently in progress. In the chapter 5 cohort follow-up biopsies were available up to three years after thermoplasty in a very small number of patients (data not shown). These biopsies showed that the reductions in airway smooth muscle mass and reticular basement membrane thickness were maintained up to three years, although the number of biopsies available was too few for statistical analysis. Remodelling effects beyond 1 year have not been investigated in a substantially large patient cohort, and this should be undertaken in future. The cohort of patients included in chapter 5 could potentially be used for this, although a significant proportion of these patients may decline additional bronchoscopic procedures with biopsies.

I also observed a rapid and sustained improvements in epithelial integrity following thermoplasty treatment; the first time this has been reported. During thermoplasty the radiofrequency energy is applied to the airway wall, and therefore the epithelium is highly likely to be directly affected by the treatment. No robust data has been previously published regarding changes in the epithelial

structure or function following thermoplasty. I anticipated that there would be an initial injury to the epithelium due to the direct heat effect, followed by repair and regrowth of healthy epithelium in the medium to long term. Data from chapter 4 of this thesis has contributed to a mechanistic study investigating the effects of thermoplasty *in silico*, *in vitro* and *in vivo* [132]. Data from this study demonstrated that bronchial epithelial cells *in vitro* were damaged immediately after heating to 65°C, before exhibiting repair and regrowth back to baseline levels within 14 days (see chapter 1). The *in vitro* experiments were not continued beyond 14 days. In chapters 4 and 5 of this thesis, the earliest point of follow-up biopsies was after 14 days, and these samples showed that epithelial integrity was improved compared to baseline. This suggests that the regrowth process demonstrated *in vitro* up to 14 days continues beyond this time point. Demonstration of an initial epithelial insult *in vivo* would involve undertaking bronchial biopsies within the first one or two weeks after thermoplasty, and would therefore be difficult to do in practice. Further bronchoscopy and biopsies from the areas of the airway treated at thermoplasty within 14 days would likely carry an unacceptably high level of risk for the patient.

In both chapters 4 and 5, direct correlations between clinical and remodelling improvements were inconsistent and sparse. Despite this, it cannot be ignored that there were large improvements in both remodelling and clinical outcomes, so a causative relationship cannot be ruled out, even if this is not a linear correlation. To further explore this I undertook a responder group analysis in chapter 5. Even with reasonably good sized patient groups (n=10 to 75, depending on outcome), change in airway smooth muscle mass showed no numerical or statistical difference between responders and non-responders for asthma control and quality of life scores, and exacerbation rates. Although the patient groups were smaller for reticular basement membrane thickness and epithelial integrity (n=5 to 21), here we do see some differences between responder and non-responder groups. For reticular basement membrane thickness, asthma control and symptom score ‘responders’ appeared to actually have an increase in basement

membrane thickness, with non-responders having a reduction ($p<0.05$). This is an unexpected finding given that basement membrane thickening is seen as detrimental in severe asthma. One would suppose that reducing membrane thickening would improve asthma outcomes, but this did not appear to be the case. The between-group changes in epithelial integrity did not reach statistical significance, but showed large numerical differences between responders and non-responders for asthma quality of life, asthma control, and asthma exacerbations. Patients with a good clinical response had more than a two-fold greater mean improvement in epithelial integrity than those without. This supports my hypothesis that epithelial integrity is an important contributor to the clinical improvements seen after thermoplasty. Increased epithelial integrity may reduce susceptibility to insults from inhaled allergens and pathogens, but more studies are needed to explore this further.

In this population of severe asthmatics the relationships between baseline biopsy remodelling features and baseline clinical features were limited, and where relationships were seen they were often in opposition to the direction expected, based on the principles of the treatment.

Epithelial integrity was positively correlated with the number of years since asthma diagnosis. One explanation for this may be that those patients who have had an asthma diagnosis for longer have been established on asthma treatment for longer, potentially allowing opportunity for their treatment to augment a repair process in the epithelium. It may also be that patients who progress from diagnosis to thermoplasty treatment over a short time have a more aggressive or severe disease pattern, possibly accompanied by increased damage to the epithelium. A protective or repair-augmenting process related to baseline asthma therapy is also suggested by the positive correlations between inhaled corticosteroid dose and epithelial area, and oral corticosteroid dose and epithelial integrity. This suggests that corticosteroids promote epithelial growth and repair, although it should be noted that patients with the highest epithelial area values demonstrate a degree of epithelial hyperplasia, which is not necessarily beneficial

[57]. The weak relationship seen between epithelial thickness and asthma control questionnaire scores perhaps suggests that very high levels of epithelial thickness (i.e. epithelial hyperplasia) are detrimental to asthma control.

Airway smooth muscle mass at baseline demonstrated correlations with smoking history, exacerbation rate and asthma quality of life scores. However, despite being statistically significant these relationships were weak, with Pearson's correlation coefficients of 0.2-0.3. Therefore, airway smooth muscle mass appears to be a poor predictor of asthma control in this population of severe uncontrolled asthmatics (chapters 4 and 5).

It has been suggested, through computer modelling, that only a small proportion of the airway smooth muscle is heated to the temperatures required to effect a response in smooth muscle cells [132]. Although not proven, it follows that a significant amount of the airway epithelial surface is heated to a high temperature during thermoplasty, due to its proximity to the thermoplasty catheter. The importance of epithelial repair in the treatment of severe asthma has largely been ignored to now, but the present findings suggest that further investigation is justified.

Interestingly, this data also suggests that remodelling responses and exacerbation rate reductions are attenuated in asthmatics with a greater than 10 pack-year history of smoking. Although this group of smokers did still see clinical benefit, it may be worth the clinician taking into consideration the extent of a patient's smoking history when deciding whether to recommend treatment with bronchial thermoplasty.

Overall, the lack of robust and consistent correlations between pre-treatment remodelling and clinical features challenges the notion that remodelling changes in the airways of severe asthmatics directly account for poor asthma clinical outcomes. Remodelling changes in severe asthma are clearly demonstrated in the work presented in this thesis and other work, but it remains unclear whether remodelling is simply a consequence of severe uncontrolled disease, or an

independent driver of poor clinical outcomes. Following treatment with anti-interleukin-13 therapy there is no significant improvement in features of airway remodelling, and only a small clinical effect. However, marked improvements in response to thermoplasty are seen in both remodelling and clinical outcomes, despite direct correlations between the two being limited. Taken together this suggests that remodelling changes are important to clinical outcomes in severe asthma, and that therapy to reverse remodelling directly may lead to clinical benefit. However, it remains a possibility that both the remodelling and clinical improvements seen are a consequence of other unidentified mechanisms, rather than remodelling reversal directly causing clinical improvements.

6.3 Limitations

The anti-interleukin-13 study was generally well designed and executed, although was short in duration at 12 weeks. Adequate biopsy samples were obtained for all participants, but the number of participants able to provide adequate sputum samples was limited, and therefore this data should be interpreted with caution. No specific biomarkers were used to select the patient population, and so it remains a possibility that a subgroup of asthma patients who would respond to this therapy was missed. However, none of the IL-13 associated biomarkers investigated in the phase III STRATOS trial robustly showed such a relationship [125], and the lack of effect has been consistent across several large and well-designed studies. Further specific limitations are discussed in chapter 3.

The thermoplasty studies presented in chapters 4 and 5 have significant limitations. Firstly, these were observational unblinded studies without pre-specified inclusion and exclusion criteria, other than those safety criteria applied generally to thermoplasty treatment. A significant proportion of the patients included were either not eligible for, or had failed, treatment with monoclonal antibodies targeting T2 inflammation. It is therefore possible that many of the

patients included in this study had additional co-morbidities and confounding medical problems contributing to the burden of their asthma symptoms. Although a limitation to investigation of the effects of thermoplasty, this does reflect the patient population that is considered for thermoplasty treatment in clinical practice. Despite a lack of clear selection criteria the effects of thermoplasty were still significant.

Despite attempts to bolster patient numbers by undertaking a pooled analysis involving many thermoplasty centres (chapter 5), there remained substantial data gaps in both clinical and remodelling elements of the work presented here. Some of the outcomes explored included less than 10 subjects, which greatly limits how reliable and robust some of the conclusions are. This was due to the 'real-world' nature of this study, where research protocols were accommodated around the clinical care of the patient. Data presented here for associations with airway smooth muscle mass responses can be taken as robust, with good sized patient groups. However, data for reticular basement membrane and epithelial integrity correlations against clinical outcomes should be seen as exploratory, with larger studies needed to confirm the findings of this thesis.

6.4 Future work

Although there was a limited effect overall in the anti-IL-13 study, it is possible that the treatment is having an effect at the genetic or transcription level. Bronchial brush samples were collected as part of the MESOS study and are presently being processed for transcriptomic data; the results have not contributed to this thesis. Although there was no overall effect in this study, possible changes at a transcriptome level may give insight into variations between those subjects who did exhibit some response to treatment, and those who did not, as well as helping to understand the interaction between IL-13 and IL-4 in more detail.

Investigation should be undertaken into the mechanistic and remodelling effects of other novel treatments for asthma, especially those which have demonstrated significant reductions in asthma exacerbation events. There is very limited data demonstrating remodelling effects in response to anti-IL-5 therapy [127, 138], and no data in the recently licensed anti-IL-4/13 antibody dupilumab, or investigational agents such as anti-TSLP (tezepelumab). Additionally, fevipiprant (DP2 antagonist) has demonstrated effects on airway remodelling [97], and phase III evaluation of exacerbation benefits is in progress. Should this be successful then thorough characterisation of the relationship between remodelling and exacerbation reduction should be undertaken. Demonstration of the detailed mechanisms by which these drugs lead to clinical benefits *in vivo* should help to clarify some of the complexity in the pathogenesis of asthma, and potentially help to identify new ways to approach and optimise treatments.

Chapters 4 and 5 suggest an important role for epithelial repair in the clinical benefits seen following thermoplasty treatment. Detailed analysis of this relationship was unfortunately limited by small participant numbers with data for both epithelial remodelling changes and clinical outcomes. Future work should focus on further characterising the relationship between epithelial repair and clinical response. Larger studies examining epithelial biopsy responses should therefore be undertaken.

More work is needed to further our understanding of the mechanism by which bronchial thermoplasty achieves its effect on remodelling. Although the microscopic remodelling effects have been consistently demonstrated, the mechanisms by which this occurs remain somewhat of a mystery. This mechanistic uncertainty is highlighted by the observed effect in the untreated right middle lobe [92], which suggests either a systemic response to thermoplasty leading to uniform improvements in the airway structure, or heat transmission through the lung tissue. The latter explanation seems unlikely, especially in light of modelling data suggesting that heat transmitted to the airway wall dissipates quickly in the tissue and is unlikely to transfer over larger distances between different regions

of the lung [132]. It seems implausible that sufficient heat could be transferred to adjacent airways, even with repeated activations of the thermoplasty catheter. A systemic cellular response seems more likely, although no robust data for this currently exists.

As discussed in chapter 5, the mechanism by which thermoplasty leads to such a profound reduction in exacerbations needs further investigation. Changes in the lung microbiome are seen in asthma, and different microbiome profiles are associated with different phenotypes of asthma [181, 182]. Exposure to high temperatures during thermoplasty could alter the lung microbiome and affect disease behaviour; hence this could be a contributor to reductions in exacerbation rates. This hypothesis could be explored by characterising the microbiome before and after thermoplasty, and relating this to clinical outcomes.

The airway epithelium acts as a barrier between inhaled pathogens and allergens, and the lung parenchyma [183], and therefore improvements in epithelial integrity may reduce susceptibility to inhaled insults. Epithelial cells also release inflammatory cytokines on exposure to viruses [184], which may be altered following thermoplasty. This could be investigated by undertaking viral or allergen challenge testing before and after thermoplasty, and comparing any changes observed to improvements in epithelial remodelling.

In clinical practice the patients selected for thermoplasty are typically those with severe disease who are not eligible for, or have failed, treatment with biologics targeting the T2 inflammatory pathway. Although evidence is limited, T2 biologics have not shown consistent or significant effects on features of airway remodelling. It could be suggested that using thermoplasty to reverse airway remodelling changes, prior to starting biologic treatment to control inflammation, may lead to an augmented treatment response. This could be testing in a prospective randomised clinical trial.

There were some differences in the study populations between the contributing centres in the pooled thermoplasty study reported in chapter 5. This highlights the

lack of current guidelines informing which severe asthma patients should undergo bronchial thermoplasty, resulting in different treatment decisions depending on where the patient is seen. In future it will be important to build on the work in this thesis to determine predictors of response to thermoplasty, and use these to develop guidelines and patient selection criteria. If thermoplasty mechanisms could be clarified in more detail, it may allow better patient selection and pre-treatment optimisation, as well as the possibility of further improving the way in which thermoplasty is delivered.

6.5 Summary

Airways remodelling is an important contributing factor to clinical manifestations of asthma. I have investigated the mechanism of action of two different treatments for severe asthma: an investigational monoclonal antibody inhibiting the interleukin-13 pathway; and the licensed asthma treatment bronchial thermoplasty. Anti-IL-13 did not effect significant changes in eosinophilic inflammation or remodelling, and its clinical effect has been disappointing. Development of therapy targeting IL-13 in isolation for severe asthma has therefore ceased as a result of this study and others.

Bronchial thermoplasty does lead to significant improvements in clinical and remodelling outcomes. However, in contrast to existing understanding, the reduction in airway smooth muscle mass may not be the most important factor leading to clinical improvement. I have shown for the first time that there is significant improvements in the structure and integrity of the epithelium following thermoplasty treatment. I have also suggested that improvements in the epithelium may be more important to the clinical benefits of thermoplasty, although a small sample size in my data limits firm conclusions in this regard. Future work should aim to characterise changes in the epithelium in greater detail,

which may allow further optimisation of the thermoplasty procedure to target epithelial repair more effectively.

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