Study Title: Primary Pneumothorax Fluorescein-Enhanced Thoracoscopy (The PREFECT Study)

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There are no potential conflicts of interest.

Confidentiality Statement

This document contains confidential information that must not be disclosed to anyone other than the Sponsor, the Investigator Team, HRA, host organisation, and members of the Research Ethics Committee, unless authorised to do so.

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2. LAY SUMMARY

Pneumothorax is air in the chest which causes the lung to collapse. This can occur spontaneously without any other injury to the chest. Primary spontaneous pneumothorax (PSP) usually refers to young patients without known lung disease, who were traditionally thought to have normal lungs. This view is now being challenged, but the true reason for their lung to leak air (and hence collapse) is not known.

This study will use a new technique to show up abnormal areas in the lung by asking patients to inhale a drug called fluorescein. Fluorescein glows bright green under ultraviolet (UV) light. Therefore, when a patient has surgery to prevent another collapsed lung, we can use UV light to show where the fluorescein is coming abnormally close to the lung surface.

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We think these areas are the source of the air leak and will therefore have a different structure with some chemicals being over- (or under-) produced. The lung structure (histology) will be looked at under the microscope and the chemicals being produced (mRNA) will be analysed. Importantly, we will be comparing areas that look normal and abnormal in the same patient and also with other patients who have not had a pneumothorax (the control group).

This is the first time that this study is being undertaken, so we will be testing our technique on up to 8 patients at the beginning to make sure we are getting good quality samples, before we perform the full histology and mRNA testing on another 20 patients.

3. SYNOPSIS

Study Title	**Primary Pneumothorax Fluorescein-Enhanced Thoracoscopy (The PREFECT Study)**		
Internal ref. no. / short title	**PREFECT**		
Study registration	ISRCTN tbc		
Sponsor	University of Oxford Clinical Trials and Research Governance Joint Research Office 1st floor, Boundary Brook House Churchill Drive Headington Oxford OX3 7GB E-mail: ctrg@admin.ox.ac.uk		
Funder	NIHR Academic Clinical Lectureship (For Dr R Hallifax) Medical Sciences Internal Fund: Pump-Priming grant (University of Oxford, Medical Sciences Division)		
Study Design	Exploratory/hypothesis-generating		
Study Participants	Patients undergoing thoracic surgery for pneumothorax. Comparison group: patients undergoing thoracic surgery for non-pneumothorax (e.g. lobectomy or wedge resection for lung cancer).		
Sample Size	Initial 4-8 patients to optimise pathway Full analysis: no specific sample size calculation: 10 patients in each group (20 total)		
Planned Study Period	Study duration: 24 months Individual participants will only be enrolled until their thoracic surgical procedure and will not require any additional follow-up		
Planned Recruitment period	September 2019 to July 2021		
	Objectives	Outcome Measures	Timepoint(s)
Primary	Assess the feasibility of using nebulised Fluorescein to highlight and identify abnormal	1. Descriptive comparison of histological findings in areas highlighted by fluorescein and those	Histological comparison: 1-2 weeks post-surgery.

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	areas of visceral pleura (at time of surgery).	not-highlighted (control within patient) 2. Comparison with non-pneumothorax control patients	
Secondary	1. Genomic analysis of normal and abnormal areas of pleura and subpleural parenchyma 2. Develop an image analysis protocol to quantify auto-fluorescence 3. Determine association between measures of lung inhomogeneity (using laser gas analyser) and histological and proteomic analysis 4. Determine whether upregulated proteins (identified by genomic analysis) are also found in blood samples	1. Quantitative comparison of genomic analysis between abnormal and normal areas of lung within patients, and compared to control subjects. 2. Correlation of autofluorescence, genomic data, and degree of lung inhomogeneity. 3. Quantitative genomic analysis of peripheral blood to compare with lung genomic analysis findings – objective 1)	1. Interim analysis of pilot samples (8 patients) at 12 months 2. Image analysis will be ongoing throughout study 3. Full analysis of remaining (12) patients at 24 months 4. Blood analysis after completion of lung tissue genomic analysis: 22-24 months.
Intervention(s)	• All patients are undergoing surgery for pneumothorax recurrence prevention (as part of their standard care). • During surgery, they will inhale fluorescein to highlight areas of abnormalities within the lung. These areas will be marked for analysis. • Lung specimens will be examined in two ways: 1. Under the microscope to look at histological abnormalities 2. mRNA extraction and sequencing will look at the gene expression within the lung specimen		
Comparator	The comparator group are non-pneumothorax patients undergoing surgery for another indication. They will undergo the same intervention with fluorescein and lung biopsy analysis.		

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4. ABBREVIATIONS

AE	Adverse event
AR	Adverse reaction
BMI	Body mass index
CI	Chief Investigator
COPD	Chronic Obstructive Pulmonary Disease
CRF	Case Report Form
CT	Computed Tomography
CTRG	Clinical Trials and Research Governance
DMC/DMSC	Data Monitoring Committee / Data Monitoring and Safety Committee
DLCO	Diffusing Capacity for Carbon Monoxide
ELC	Emphysema-like change
FEV1	Forced Expiratory Volume in 1 Second
FRC	Functional Residual Capacity
FVC	Forced Vital Capacity
GP	General Practitioner
IMP	Investigational Medicinal Product
kCO	Diffusing Capacity (corrected for alveolar volume)
LGA	Laser Gas Analyser
MHRA	Medicines and Healthcare products Regulatory Agency
MMP	Matrix Metalloproteinase
mRNA	Messenger Ribonucleic acid
NHS	National Health Service
Nrf2	Nuclear Factor Erythroid2-related Factor 2
ORTU	Oxford Respiratory Trials Unit
PEFR	Peak Expiratory Flow Rate
PFT	Pulmonary Function Testing
PI	Principal Investigator
PIL	Participant/ Patient Information Leaflet
PSP	Primary Spontaneous Pneumothorax
R&D	NHS Trust R&D Department
REC	Research Ethics Committee
SAE	Serious Adverse Event
SAR	Serious Adverse Reaction

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SOP	Standard Operating Procedure
SUSAR	Suspected Unexpected Serious Adverse Reactions
TLC	Total Lung Capacity
TIMP	Tissue Inhibitor of Metalloproteinase
TMF	Trial Master File
TSG	Oxford University Hospitals Trust / University of Oxford Trials Safety Group
VA	Alveolar Volume
VATS	Video-Assisted Thoracoscopic Surgery

5. BACKGROUND AND RATIONALE

i. Importance

Pneumothorax - air in the pleural space - is a common pathology. Primary spontaneous pneumothorax (PSP) conventionally refers to patients developing a pneumothorax, in the absence of trauma, with no underlying established lung pathology. PSP occurs in ~3,000 patients per year in the UK[1, 2].

ii. Pathophysiology

PSP is more common in taller patients with low body-mass index (BMI) and smokers. Although PSP occurs in patients without evidence of underlying established lung pathology, most patients have emphysema-like changes (ELC), i.e. blebs and bullae, in the lungs on Computed Tomography (CT) scans. One study found these changes bilaterally in the upper lung zones in 81% of non-smoking patients with PSP but not in healthy controls[3]. Thoracoscopic investigation of 250 healthy individuals with no prior history of pleural disease (treated with thoracoscopic sympathectomy) demonstrated a 6% incidence of apical blebs. These were more prevalent in slim individuals (BMI <22kg/m^2) who smoked[4].

However, it is unclear how often these lesions are the actual site of air leakage. A landmark paper examined patients with PSP at thoracoscopy using inhaled fluorescein-enhanced autofluorescence[5]. Autofluoresence allows visualisation of gas under ultraviolet light. In these patients it demonstrated areas in the visceral pleura which the inhaled gas comes (abnormally) close to the pleural surface. These areas appear normal on plain white light thoracoscopy, and do not necessarily correspond to areas with blebs and bullae, but are areas of parenchymal abnormality or “pleural porosity”[6]. The true nature of these lesions are unclear, but are thought to be areas of disrupted mesothelial cells at the visceral pleura replaced by an inflammatory elastofibrotic layer with increased porosity, allowing air leakage. A further study using inhaled fluorescein-enhanced autofluorescence in a pig model found greater “inflammation” (subjectively described by histopathologists) in those areas with high fluorescein signal[7].

iii. Abnormal elastolysis

The reason why airways inflammation should result in degradation of elastic fibres, and hence formation of a porous elastofibrotic layer, is not clear. One theory is that there is an imbalance in the protease-antiprotease and oxidant-antioxidant systems. Matrix metalloproteinases (MMP) are a group of zinc- and calcium dependent endopeptidases that can damage the basement membrane (between pulmonary epithelium and alveloli)[8]. MMP-2 and MMP-9, two interstitial collagenases, have been postulated to be pathogenic in other lung diseases[9], including asthma and COPD[10]. Two Taiwanese groups examined

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of surgical resection specimens for patients undergoing surgery for PSP and demonstrated overexpression of MMPs[8, 11]. The first compared lung tissue from 15 PSP patients with that from 20 patients with stage I non-small cell lung cancer who were used as controls[8]. Immunohistochemistry showed overexpression of MMP-2, MMP-7 and MMP-9 in the tissue of PSP patients compared to controls[8]. The second non-comparative study of 91 patients also found that high MMP-2 and MMP-9 expression and that higher levels were more frequent in those patient presenting with a recurrence of their pneumothorax compared to those with first episode[11]. The same group of patients was examined for expression of nuclear factor erythroid 2-related factor 2 (Nrf2) in alveolar type I pneumocytes. NrF2 is a regulator of redox homeostasis and inflammatory response. It is believed to play a cytoprotective role in detoxification and chemoprevention[12]. High Nrf2 expression was seen in those patients without previous recurrence, suggesting a protective effect[12].

A more recent study specifically looked at the gene expression in lung biopsy specimens of adolescents undergoing VATS for PSP, comparing 9 paired samples: from visually abnormal (bullae) and macroscopically “normal” site (>3cm distant) [13]. Blood samples were also taken from another 15 PSP patients, and 23 age-matched controls. Microarray gene expression analysis showed overexpression of MMP-1, MMP-9, IL-8 (Interleukin-8 is known to up-regulate MMP-9 production), and TIMP-1 (Tissue Inhibitor of Metalloproteinase-1) [13]. The authors compared the activity of MMP-9 (as a ratio of the expression of MMP-9 to TIMP-1) in peripheral blood samples taken at the time of the pneumothorax (acute phase) and 3 months later (recovery), confirming that MMP-9/TIMP ratio was significantly higher in the acute phase, but also that MMP-9/TIMP ratios was significantly greater in patients with PSP compared to healthy controls at all time points [13].

There are a number of limitations to this study: firstly, is assumption that macroscopically “normal looking” lung at VATS is sufficiently normal to act as a “control” within that patient. The previous fluoroscopic studies (outlined above) demonstrate that pleural porosity in fact occurs as macroscopically normal lung and not at bullous lung tissue[6]. Secondly, there was no comparison made with lung tissue from control patients who had not suffered with a pneumothorax. And thirdly, although the author specifically chose to recruit adolescents (<20 years old), this is not representative of the typical patient presenting in the UK with PSP[2].

Nevertheless, these studies provide interesting insight in the potential pathogenic overexpression of MMPs and the role of oxidative stress response in PSP.

iv. Need for the study

The above theories of pleural porosity will be assessed via a combination of histological analysis of subpleural architecture and mRNA expression. Autofluorescence highlights areas of abnormality to allow comparison within the same patient and the additional comparison to control patients will provide additional power to assess differences in mRNA expression in patients with apparently “normal” lung (without pneumothorax).

6. OBJECTIVES AND OUTCOME MEASURES

Objectives	**Outcome Measures**	**Timepoint(s) of evaluation of this outcome measure (if**

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		applicable)
Primary Objective 1. Assess the feasibility of using nebulised fluorescein (at time of surgery) to highlight and identify abnormal areas of visceral pleura in patients with recurrent primary pneumothorax.	1. Descriptive comparison of histological findings in areas highlighted by fluorescein and those not-highlighted (control within patient) 2. Comparison with non-pneumothorax control patients	Histological comparison: 1-2 weeks post-surgery.
Secondary Objective 1. Genomic analysis of normal and abnormal areas of pleura and subpleural parenchyma 2. Develop semi-automated image analysis to quantify auto-fluorescence 3. Determine association between measures of lung inhomogeneity (using laser gas analyser) and histological and proteomic analysis 4. Determine whether upregulated proteins (identified by genomic analysis) are also found in blood samples	1. Quantitative comparison of genomic analysis between abnormal and normal areas of lung within patients, and compared to control subjects. 2. Correlation of autofluorescence and genomic data with degree of lung inhomogeneity. 3. Quantitative genomic analysis of peripheral blood to compare with lung genomic analysis findings – objective 1)	1. Interim analysis of pilot samples (8 patients) at 12 months 2. Image analysis will be ongoing throughout study 3. Full analysis of remaining (12) patients at 18-22 months 4. Blood analysis after completion of lung tissue genomic analysis: 22-24 months.

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7. STUDY DESIGN

PREFECT is an exploratory open study of patients in Oxford, who are undergoing surgery for pneumothorax (or other indication in the control group).

Patients will be involved in the study for up to 2-3 weeks until their scheduled Thoracic surgery. See Flowchart in Appendix A. They will not be required to attend any additional study specific follow-up visits.

8. PARTICIPANT IDENTIFICATION

8.1. Study Participants

Participants will be recruited from patients already undergoing video-assisted thoracoscopic surgery (VATS) for pneumothorax recurrence prevention in Oxford. Control participants will be recruited from those undergoing VATS for another condition in which the lung specimens will be surgically removed: including lobectomies or wedge resections for isolated parenchymal conditions (e.g. solitary lung cancers or metastases). The control patients will not have a history of pneumothorax or other pleural disease.

8.2. Inclusion Criteria

1. Patient undergoing Video-assisted Thoracoscopic Surgery (VATS) for pneumothorax recurrence prevention, or ongoing air leak (pneumothorax group)
   OR
   Thoracic surgery for another indication, for example, early stage lung cancer or metastasis resection (control group).
2. Male or female aged between 16 and 55 years (inclusive)
3. Willing and able to consent to participation.

8.3. Exclusion Criteria

1. Inability to consent or comply with the trial requirements.
2. Patients in the control group should not have demonstrable emphysema (on CT scanning) and should not have experienced a spontaneous pneumothorax in the past.
3. Allergy to fluorescein.
4. Any other significant disease or disorder which, in the opinion of the Investigator, may either put the participants at risk because of participation in the study, or may influence the result of the trial, or the participant's ability to participate in the study.

9. PROTOCOL PROCEDURES

Refer to Appendix A for a schedule of study procedures.

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9.1. Recruitment

Pneumothorax patients will be enrolled from Respiratory and Pleural Clinics (when referring for surgery), thoracic surgical clinic, or thoracic pre-admission clinics. Control patients will be identified at Lung MDT and recruited from lung cancer clinic.

9.2. Screening and Eligibility Assessment

All patients referred for thoracic surgery for pneumothorax recurrence prevention in Oxford will be screened and assessed for eligibility by the Chief Investigator or the clinical team.

9.3. Informed Consent

Once an eligible patient is identified and agrees to participate in the study, informed written consent will be obtained by the principal investigator or other suitably qualified delegated personnel. The participant will be asked to sign and date the latest approved version of the Informed Consent form before any study specific procedures are performed.

Written and verbal versions of the Participant Information and Consent form will be presented to the participants detailing no less than: the exact nature of the study; what it will involve for the participant; the implications and constraints of the protocol; the known side effects and any risks involved in taking part. It will be clearly stated that the participant is free to withdraw from the study at any time for any reason without prejudice to future care, and with no obligation to give the reason for withdrawal.

The participant will be allowed up to one week to consider the information, and the opportunity to question the Investigator, their GP or other independent parties to decide whether they will participate in the study. Written Informed Consent will then be obtained by means of participant dated signature and dated signature of the person who presented and obtained the Informed Consent.

A copy of the signed Consent form will be given to the participant, a copy will be filed with patient's medical records and another copy will also be kept at the ORTU.

9.4. Enrolment

Enrolment will be completed in person by signing the consent form. There is no randomisation in this study.

9.5. Blinding and code-breaking

All analyses will be performed blinded to clinical data and all other investigation results to avoid bias.

9.6. Description of study intervention(s), comparators and study procedures (clinical)

Patients enrolled prior to elective surgery will be invited to undergo pulmonary function testing (PFTs) and LGA testing prior to their planned surgical procedure, which is typically a period of 1-2 months. Pneumothorax patients may not routinely undergo PFTs prior to surgery, but will be asked to undertake this as part of the study. Those enrolled as an inpatient with ongoing pneumothorax will not undergo PFTs, as this a contra-indication.

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Control patients will have PFTs as per standard care. They will also be invited to undergo LGA testing if possible, but should not delay their treatment (e.g. their timeline may be limited if the patient is on a cancer treatment pathway). The Fluorescein-enhanced assessment will occur during the planned surgical procedure.

9.6.1. Pulmonary Function Testing (PFTs) and Laser Gas Analyser (LGA)

PFTs and Laser Gas Analyser testing will occur in the Lung Function Laboratory at the Churchill Hospital, Oxford.

PFTs

Standard lung function testing will performed on all patients, as per NHS Trust and national guidelines. They will occur in the Lung Function Laboratory at the Churchill Hospital, Oxford, ideally prior to the LGA testing. This will include:

- Spirometry: FEV1 (Forced Expiratory Volume in 1 second), FVC (Forced Vital Capacity), FEV1/FVC ratio, and PEFR (Peak Expiratory Flow Rate).
- Body plethysmography: TLC (Total Lung Capacity), Vital capacity (VC), Functional residual capacity (FRC) and RV (Residual Volume)
- Diffusion capacity: DLCO (Diffusing Capacity for carbon monoxide), VA (Alveolar Volume), kCO (DLCO/VA diffusing capacity corrected for alveolar volume)

These parameters will allow assessment of any previously undiagnosed obstructive or restrictive defect, and any abnormality of oxygen transfer.

LGA

The LGA assessment will consist of a 30 min period when the patient breathes either through a facemask or through a mouthpiece while wearing a noseclip. The mouthpiece will be connected to the gas analyser. The patient initially breathes air (baseline) and then for the middle ten minutes breathes 100% oxygen (N_2 washout period) and back to air again.

9.6.2. Fluorescein-enhanced autofluorescence

Surgery

The indications for surgery will be: on-going persistent air leak (as an inpatient) or, electively, for ipsilateral recurrence or first contralateral recurrence.

VATS will be undertaken at Oxford University Hospital NHS Foundation Trust by the Thoracic surgery team. Pre-operative preparation and general anaesthetic will be conducted as standard by the anaesthetist. The VATS procedure will be conducted by one of consultant thoracic surgeons or by one of the thoracic registrars under direct supervision. With the patient in the lateral position, a standard 2- or 3-port technique will be used to access the thoracic cavity and deflate the lung to allow inspection. The video equipment (“Stack”) and thoracoscopy must have the capability to switch to Ultraviolet (UV) light.

The optimal time to detect Fluorescein in the lung is 20-30mins after inhalation[7]. Therefore, the fluorescein 10% aqueous solution (5ml vial) will be administered to the patient via a nebuliser just prior to general anaesthestic induction. One or two administrations may be required. Fluorescein glows bright green under UV light. Therefore, during the procedure the typical “white light” view will be switched to

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UV light. Under UV light, abnormal areas will be highlighted by bright green due to the inhaled gas containing fluorescein coming close to the visceral pleural surface.

Typically, VATS will involve wedge resection including an area of visible bullae at the lung apex ("bullectomy"). This will be performed as usual, but prior to resection, two or three areas within the resection margin will be marked (e.g. using surgical sutures) according to the presence or absence of pleural porosity. These areas will provide the lung biopsy specimens to be examined by histology and gene expression.

The surgical procedure will be concluded and immediate post-operative period will continue as standard practice in OUH Trust.

All patients will be asked to have a blood sample taken, which will be spun, the mRNA extracted from the serum and frozen for analysis.

9.6.3. Gene expression analysis (mRNA)

mRNA Extraction

After the surgery, the lung specimen will be immediately taken to the histopathology department. At each marked site, 3 replicate samples will be taken. These samples will be directly transferred to the Nuffield Department of Medicine Research Building (NDMRB) and mRNA will be extracted using a standard kit (e.g. RNeasy) on the same day and frozen for batch analysis at a later date.

mRNA Sequencing

mRNA samples will be provided to Oxford Genomics Centre (Wellcome Centre for Human Genetics) for sequencing. This will consist of library preparation then sequencing on a latest generation platform (e.g. NovaSeq6000), providing up to 20million reads per sample. Raw sequencing data will be transferred to the bioinformaticians at the Weatherall Institute of Molecular Medicine (Oxford) for analysis.

Assessment of gene expression in blood samples

Once upregulated genes have been identified by mRNA sequencing on lung specimens (as above), blood samples will be tested to determine whether these same proteins can be identified on blood serum. This will potentially allow a less invasive way (i.e. blood taking, rather than surgery) of identifying individuals who may be at greater risk of recurrence.

9.6.4. Histological analysis

Histological specimens will be examined by the pathology team at OUH. The histopathology team will be blinded as to the whether the specimens were taken from the "normal" or "abnormal" sites (according to autofluorescence). The lung specimens will be examined using immunostaining for relevant markers (e.g. elastic tissue fibres (Van Geison), EVG, and MMP).

9.6.5. Auto-fluorescence image analysis

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During surgery, video recordings and still images will be taken and stored for further image processing. Fluorescence quantification will be undertaken using image analysis software (such as Image J, NIH, or SigmaScan Pro, Systat). For each lesion, relative fluorescence intensities will be determined by comparing the centre of the lesion and at surrounding areas with no macroscopic evidence of fluorescence. The degree of auto-fluorescence will then be analysed to determine whether it correlates to differential gene expression.

9.7. Baseline assessments

In addition to the assessments described above, baseline demographic (e.g. age, sex, height, weight) and medical history (e.g. previous pneumothorax, other comorbidities) and family history will be collected at enrolment.

9.8. Follow-up

Thoracic surgery marks the end of the patient's involvement in the study. There is no need for any special safety observation period following the Pulmonary Function Testing and Laser Gas Analyser assessment.

9.9. Sample Handling

The samples taken from patients will be a lung specimen and a blood sample.

The lung specimen will be taken from patients undergoing surgical lung resection/biopsy for standard care. For pneumothorax patients this is typically a lung specimen of 50 x 16 x 13mm in size. Samples for control patients (e.g. wedge resection or lobectomies) are larger. As described above, 3 areas within this specimen will be marked during surgery and will then be further analysed.

9.9.1 Sample handling for study purposes

At each site, 3 thin slices (replicates) will be taken and immediately transferred to NDMRB for mRNA extraction (as per 9.6.3). In addition, at each site, lung specimens will be examined histologically using immunostaining (as per 9.6.4) by a co-investigator in the Pathology department.

9.9.2 Sample handling

Once the study has finished, any samples left after the analysis will be transferred into the ORTU collection under HTA licence number 12217 (if participant has given consent for their further use in research).

9.9.3 Blood sample

On the day of their surgery, patients will be asked to have a blood sample taken, which will be spun and the mRNA will be extracted from the serum (as per 9.6.3). These samples will be stored in a freezer based at the Oxford Respiratory Trials unit at the Churchill hospital, until the lung sample mRNA analysis has been completed and target protein identified. Serum mRNA will then be analysed to determine in the same proteins can be identified in the serum.

9.10. Early Discontinuation/Withdrawal of Participants

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In consenting to the study, patients are consenting to treatment according to the study protocol, follow-up and data collection. If a patient wishes to withdraw from the study, the investigator should nevertheless explain the importance of remaining in follow-up, or failing this of allowing routine follow-up data to be used for study purposes. If the patient explicitly states their wish not to contribute further data to the study, the patient should be withdrawn, the investigator should complete the withdrawal form as part of the CRF and the ORTU should be informed in writing. Data collected up to the point of withdrawal can still be included in the study or data collection can continue through medical notes as long as the patient agrees to this. If a patient requests full withdrawal, then samples will be destroyed and data not used.

9.11. Definition of End of Study

The study will close at receipt of last lung tissue samples from last participant.

10. SAFETY REPORTING

Safety reporting will apply from the first study assessment (e.g. either LGA testing or anaesthetic at surgery) and will end when the participant completes the study. SAEs will be followed up until event resolution or stabilisation for all participants.

10.1. Definition of Serious Adverse Events

A serious adverse event is any untoward medical occurrence that:

- results in death
- is life-threatening
- requires inpatient hospitalisation or prolongation of existing hospitalisation
- results in persistent or significant disability/incapacity
- consists of a congenital anomaly or birth defect.

Other ‘important medical events’ may also be considered a serious adverse event when, based upon appropriate medical judgement, the event may jeopardise the participant and may require medical or surgical intervention to prevent one of the outcomes listed above.

NOTE: The term "life-threatening" in the definition of "serious" refers to an event in which the participant was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe.

10.2. Reporting Procedures for Serious Adverse Events

A serious adverse event (SAE) occurring to a participant should be reported to the REC that gave a favourable opinion of the study where in the opinion of the Chief Investigator the event was ‘related’ (resulted from administration of any of the research procedures) and ‘unexpected’ in relation to those procedures. Reports of related and unexpected SAEs should be submitted within 15 working days of the Chief Investigator becoming aware of the event, using the HRA report of serious adverse event form (see HRA website).

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10.3. Expectedness

Complications secondary to the thoracic surgery, classed as "expected" may include: bleeding, infection, venous thromboembolic disease, chronic scar pain and recurrence of pneumothorax. There are no expected adverse reactions related to performing the PFTs or LGA testing.

Inhaled fluorescein has been used in this way before (see Background section) with no reported side effects, except for a slight yellow discolouration of the mouth, which goes away after 1-2days.

11. STATISTICS AND ANALYSIS

PREFECT is an exploratory study. Statistical analysis will be performed by co-investigators.

11.1. Statistical Analysis Plan (SAP)

The plan for the statistical analysis of the study is outlined below. There is not a separate SAP document in use for the trial.

11.2. Description of the Statistical Methods

Briefly, mRNA analysis identifies highly expressed genes by multiple "reads" of genomic data. To increase the statistical power, sample replicates will be taken and multiple data reads. This analysis will be conducted in collaboration with bioinformaticians at the Weatherall Institute of Molecular Medicine, in accordance with good practice[14]. Quantified comparison of highly expressed genes will be made between normal and abnormal samples in the same patient and between patients (pneumothorax vs control patients).

11.3. Sample Size Determination

PREFECT is an exploratory study. Patients will be screened from pleural clinics and thoracic surgical clinics. Initially up to 8 patients will be recruited to determine the ideal delivery of fluorescein, how to visualise and digitally capture the autofluorescence images, how to mark the biopsy sites during surgery, and determine the ideal lung tissue size and method for mRNA extraction. These first patients will not have full PFTs, LGA assessment or mRNA sequencing, but will otherwise be managed as per protocol. If this pathway is optimized at less than 8 patients then this phase can be completed early.

After pathway optimization (up to 8 patients), 20 patients will be recruited for full analysis (i.e. up to 28 in total). As hypothesis generating work, the power calculation of sample size is not possible, but the aim is to recruit: 10 undergoing pneumothorax surgery (pneumothorax patients) and 10 undergoing thoracic surgery for other indication (control patient) requiring wedge resection or lobectomy.

11.4. Analysis populations

PREFECT is an exploratory study. Analysis will be undertaken of all participants who undergo the procedure (and hence have fluorescein at the time of their surgery) and mRNA expression analysis will be performed on all suitable samples (i.e. have a large enough component of useable mRNA extracted).

11.5. Decision points

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Interim assessment will be made after the first 4-8 patients in order to determine that the patient pathway is “optimised”: fluorescein administration, video recording, marking of the areas within the lung specimen, transfer of lung specimen to pathology lab, taking of small sample for mRNA analysis, and mRNA extraction.

11.6. Criteria for the Termination of the Trial

The study will terminate following full recruitment, completion of all study procedures of at least 20 patients, or should a significant safety concern emerge, as determined by Study management Group or Sponsor.

12. DATA MANAGEMENT

The plan for the data management of the study is outlined below. There is not a separate Data Management document in use for the study.

12.1. Source Data

Source documents are hospital records, mRNA analysis data (from WIMM), descriptive histological analysis, quantitative autofluorescence data, and clinical records.

All documents will be stored safely in confidential conditions. On all study-specific documents, other than the signed consent, the participant will be referred to by the trial number, not by name.

12.2. Access to Data

Direct access will be granted to authorised representatives from the Sponsor and host institution for monitoring and/or audit of the study to ensure compliance with regulations.

12.3. Data Recording and Record Keeping

All data will be stored in accordance with the requirements of the General Data Protection Regulation (GDPR) and Data Protection Act 2018.

Each patient will be assigned a trial number (e.g. PRE-01). All samples and data will then be stored under this anonymous trial number. Data generated will be analysed by the Chief Investigator (Dr Rob Hallifax) and Ms Nicki Gray (a collaborator at the Weatherall Institute of Molecular Medicine) on password-protected PCs on the University of Oxford network. Any paper-based identifiable data at each site will be kept in a locked cabinet, in a locked or ID-access controlled area. If the patient agrees for their samples to be used in future research, the consent form will be held be retained for the life of the sample to meet HTA traceability requirements.

De-identified research data will be kept (on a secure PC on the University network) for 5 years after the conclusion of the trial.

Participants' personal data (for example name, telephone number and address) will be available to the local trial team. For the purposes of monitoring, where it is deemed necessary, patients' trial files and

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clinical records will be available to members of the trial team, the R&D office, members of the regulatory authorities or Sponsor representatives.

Responsible members of the University of Oxford or the host NHS Trust may be given access to data for monitoring and/or audit of the study to ensure we are complying with regulations.

This is explained in the patient information sheet and forms part of the consent form.

13. QUALITY ASSURANCE PROCEDURES

The study may be monitored, or audited in accordance with the current approved protocol, GCP, relevant regulations and standard operating procedures.

13.1. Risk assessment

A detailed Risk Assessment will be conducted before the trial starts by the Study Management Group and an appropriate Monitoring Plan will be developed.

13.2. Study monitoring

Regular monitoring will be performed according to the study specific Monitoring Plan. Data will be evaluated for compliance with the protocol and accuracy in relation to source documents as these are defined in the study specific Monitoring Plan. Following written standard operating procedures, the monitors will verify that the clinical study is conducted and data are generated, documented and reported in compliance with the protocol, GCP and the applicable regulatory requirements.

13.3. Study Committees

This small exploratory hypothesis generating study will have oversight from a Study Management Group, which will consist of the CI and co-investigators, and meet at least every 6 months to assess recruitment, any logistical and safety issues. As the study is a small, exploratory, low risk study, a formal Data Monitoring Committee is not required.

14. PROTOCOL DEVIATIONS

A study related deviation is a departure from the ethically approved study protocol or other study document or process (e.g. consent process or administration of study intervention) or from Good Clinical Practice (GCP) or any applicable regulatory requirements. Any deviations from the protocol will be documented in a protocol deviation form and filed in the study master file.

15. SERIOUS BREACHES

A “serious breach” is a breach of the protocol or of the conditions or principles of Good Clinical Practice which is likely to affect to a significant degree –

(a) the safety or physical or mental integrity of the trial subjects; or

(b) the scientific value of the research.

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In the event that a serious breach is suspected the Sponsor must be contacted within 1 working day. In collaboration with the CI, the serious breach will be reviewed by the Sponsor and, if appropriate, the Sponsor will report it to the approving REC committee and the relevant NHS host organisation within seven calendar days.

16. ETHICAL AND REGULATORY CONSIDERATIONS

16.1. Declaration of Helsinki

The Investigator will ensure that this study is conducted in accordance with the principles of the Declaration of Helsinki.

16.2. Guidelines for Good Clinical Practice

The Investigator will ensure that this study is conducted in accordance with relevant regulations and with Good Clinical Practice.

16.3. Approvals

Following Sponsor approval the protocol, informed consent form, participant information sheet will be submitted to an appropriate Research Ethics Committee (REC), HRA and host institutions for written approval.

The Investigator will submit and, where necessary, obtain approval from the above parties for all substantial amendments to the original approved documents.

16.4. Other Ethical Considerations

It is possible that whilst undergoing investigation as part of their participation in this study, a participant may have an incidental abnormality identified. In these cases, the participant will be counselled by the investigator and referred on for further clinical investigation as deemed appropriate and agreed.

16.5. Reporting

The CI shall submit once a year throughout the study, or on request, an Annual Progress report to the REC Committee, HRA (where required) host organisation, Sponsor and funder (where required). In addition, an End of Study notification and final report will be submitted to the same parties.

16.6. Participant Confidentiality

The study will comply with the General Data Protection Regulation (GDPR) and Data Protection Act 2018, which require data to be de-identified as soon as it is practical to do so. The processing of the personal data of participants will be minimised by making use of a unique participant study number only on all study documents and any electronic database(s). All documents will be stored securely and only accessible by study staff and authorised personnel. The study staff will safeguard the privacy of participants' personal data.

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16.7. Expenses and Benefits

Reasonable travel expenses for any visits additional to normal care will be reimbursed on production of receipts, or a mileage allowance provided as appropriate.

17. FINANCE AND INSURANCE

17.1. Funding

This study is funded by a Medical Sciences Internal Fund: Pump-Priming grant from University of Oxford, Medical Sciences Division.The funding for salary of the Chief Investigator (Dr Rob Hallifax) is via a National Institute for Health Research (NIHR) Academic Clinical Lectureship. The Laser Gas Analyser is supported by a grant from the NIHR Biomedical Research Council (BRC) for translational research.

17.2. Insurance

The University has a specialist insurance policy in place which would operate in the event of any participant suffering harm as a result of their involvement in the research (Newline Underwriting Management Ltd, at Lloyd's of London). NHS indemnity operates in respect of the clinical treatment that is provided.

17.3. Contractual arrangements

Appropriate contractual arrangements will be put in place with all third parties.

18. PUBLICATION POLICY

The preparation of a manuscript for rapid publication will be a priority for and responsibility of the Chief Investigator. The Study Management Group will also take responsibility for reviewing drafts of any manuscripts, abstracts, press releases and other publications arising from this study. It is anticipated that an initial report would be completed within six months of the study's closure.

All publications will include a list of investigators, and named authors will include the study's Chief Investigator, and co-investigators as a minimum. Authors will be determined in accordance with ICMJE guidelines and other contributors to the study will be acknowledged. Authors will acknowledge that the study has been sponsored by the University of Oxford, UK.

19. DEVELOPMENT OF A NEW PRODUCT/ PROCESS OR THE GENERATION OF INTELLECTUAL PROPERTY

Ownership of IP generated by employees of the University vests in the University. The protection and exploitation of any new IP is managed by the University's technology transfer office, Oxford University Innovations.

20. ARCHIVING

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De-identified research will be kept (on a secure PC on the University network) for 5 years after the conclusion of the trial. Any paper-based identifiable data at each site will be kept in a locked cabinet, in a locked or ID-access controlled area in ORTU.

21. REFERENCES

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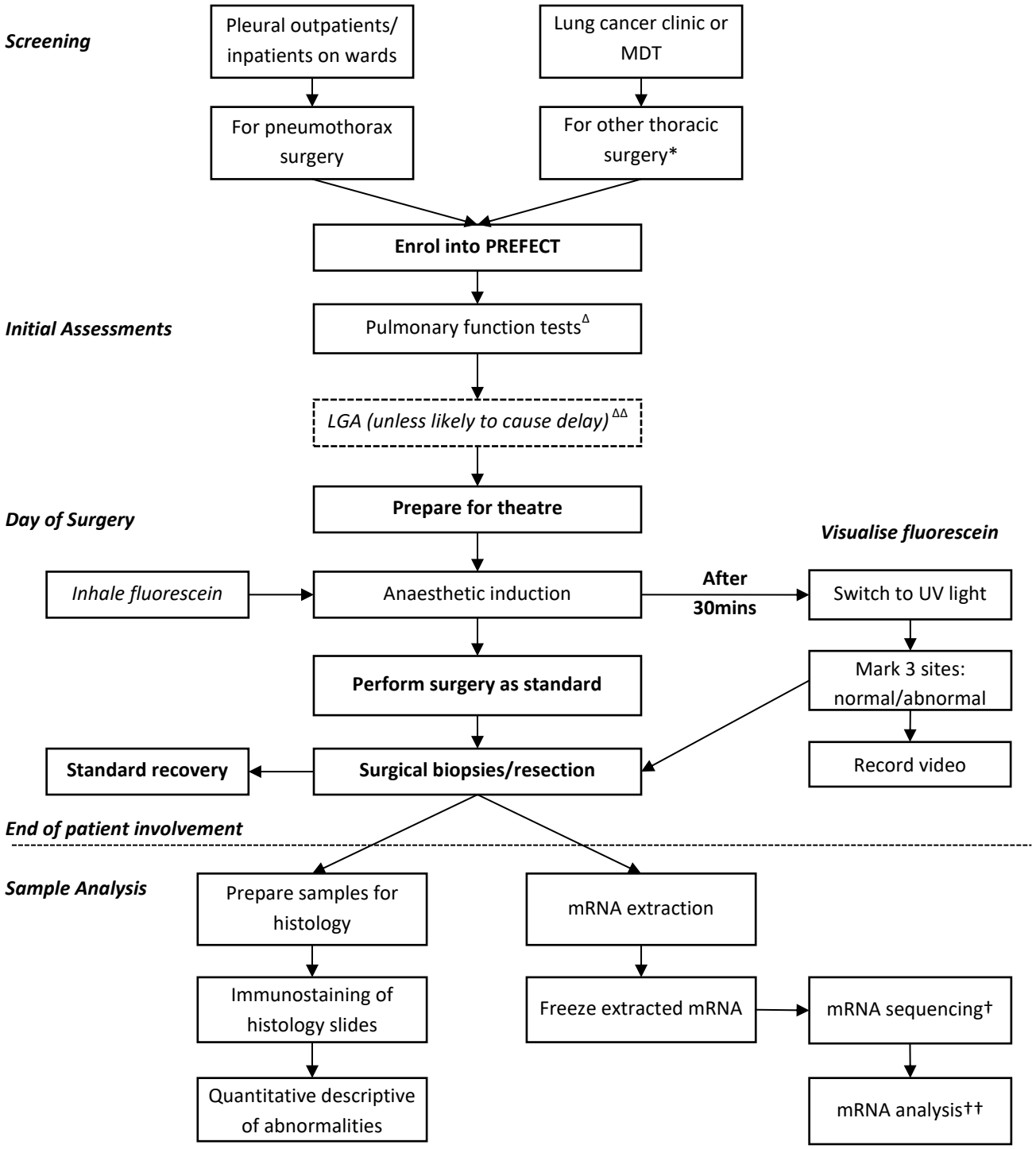
22. APPENDIX A: STUDY FLOW CHART



MDT: Multi-disciplinary team. LGA: laser gas analyser testing. UV: ultraviolet. mRNA: messenger Ribonucleic acid. * Thoracic surgical procedure for another indication, requiring lung resection (e.g. wedge resection or lobectomy). Δ Pulmonary function tests will not be performed on inpatients with ongoing pneumothorax. ΔΔLGA will only be performed in the control group if it does not cause a delay to their treatment pathway. †Performed at Wellcome Trust Centre for Genetics. ††Performed with Weatherall Institute for Molecular Medicine.

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23. APPENDIX B: SCHEDULE OF STUDY PROCEDURES

		Schedule of visits & Follow-up				
Study Procedures	Pleural and Thoracic Surgery Clinics	Consent/ Enrolment	Surgery	24-72 hrs post sample collection	3-6 months post collection	After lung mRNA analysis complete
Screening	**X**					
Lung function testing		**X**				
Laser Gas Analyser assessment		**X**				
Administration of Fluorescein			**X**			
Lung specimen and blood sample collection			**X**			
RNA extraction and freezing			**X**	**X**		
Histological analysis				**X**		
Lung mRNA sequencing & analysis					**X**	
Blood mRNA sequencing & analysis						**X**

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24. APPENDIX C: AMENDMENT HISTORY

Amendment No.	Protocol Version No.	Date issued	Author(s) of changes	Details of Changes made

Protocol amendments must be submitted to the Sponsor for approval prior to submission to the REC committee and HRA (where required).

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