

# Staging by Thoracoscopy in potentially radically treatable Lung Cancer associated with Minimal Pleural Effusion (STRATIFY): protocol of a prospective, multicentre, observational study

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## ABSTRACT

**Introduction** Recurrence rate following radical therapy for lung cancer remains high, potentially reflecting occult metastatic disease, and better staging tools are required. Minimal pleural effusion (mini-PE) is associated with particularly high recurrence risk and is defined as an ipsilateral pleural collection (<1/3 hemithorax on chest radiograph), which is either too small to safely aspirate fluid for cytology using a needle, or from which fluid cytology is negative. Thoracoscopy (local anaesthetic thoracoscopy (LAT) or video-assisted thoracoscopic surgery (VATS)) is the gold-standard diagnostic test for pleural malignancy in patients with larger symptomatic effusions. Staging by Thoracoscopy in potentially radically treatable Lung Cancer associated with Minimal Pleural Effusion (STRATIFY) will prospectively evaluate thoracoscopic staging in lung cancer associated-mini-PE for the first time.

**Methods and analysis** STRATIFY is a prospective multicentre observational study. Recruitment opened in January 2020. The primary objective is to determine the prevalence of detectable occult pleural metastases (OPM). Secondary objectives include assessment of technical feasibility and safety, and the impact of thoracoscopy results on treatment plans, overall survival and recurrence free survival. Inclusion criteria are (1) suspected/confirmed stages I–III lung cancer, (2) mini-PE, (3) Performance Status 0–2 (4), radical treatment feasible if OPM excluded, (5) ≥16 years old and (6) informed consent. Exclusion criteria are any metastatic disease or contraindication to the chosen thoracoscopy method (LAT/VATS). All patients have LAT or VATS within 7 (±5) days of registration, with results returned to lung cancer teams for treatment planning. Following an interim analysis, the sample size was reduced from 96 to 50, based on a lower-than-expected OPM rate. An MRI substudy was removed in November 2022 due to pandemic-related

## WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Minimal pleural effusion (mini-PE), defined as small ipsilateral effusion in patients with otherwise curable lung cancer, is associated with significantly inferior survival. Prior retrospective series estimate mini-PE may be due to Occult Pleural Metastases (OPM) in up to 80% of cases. The true prevalence of OPM in this setting is uncertain and the utility, safety and impact of thoracoscopic staging of mini-PE has not been evaluated.

## WHAT THIS STUDY ADDS

⇒ Staging by Thoracoscopy in potentially radically treatable Lung Cancer associated with Minimal Pleural Effusion will determine the true prevalence of OPM in mini-PE in a prospective multicentre study. The safety and impact of thoracoscopic staging will be reported for the first time, ideally using local anaesthetic thoracoscopy, although surgical thoracoscopy is permitted.

## HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ This study will better define the oncological significance of mini-PE in otherwise radically treatable lung cancer, and a potential new role for thoracoscopic staging of such patients for future guidelines.

site setup/recruitment delays. These also necessitated a no-cost recruitment extension until October 2023.

**Ethics and dissemination** Protocol approved by the West of Scotland Research Ethics Committee (Ref: 19/WS/0093). Results will be published in peer-reviewed journals and presented at international meetings.

**Trial registration number** ISRCTN13584097.

## INTRODUCTION

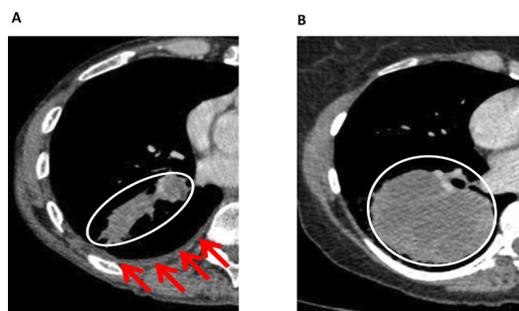
Lung cancer is the most common cause of cancer-related death. Despite major advances in staging and potentially curative treatments (surgery and radiotherapy (RT)), recurrence rates remain unacceptably high. In patients with stages I, II and IIIA, non-small cell lung cancer (NSCLC) 2-year mortality is currently 15%, 30%<sup>1</sup> and 50%,<sup>2</sup> respectively. A likely reason for this is radiologically occult metastatic disease and novel staging tools are urgently required. Recent studies have highlighted minimal pleural effusion (mini-PE) as a marker of particularly high recurrence risk, and excess mortality following radical treatment.<sup>3,4</sup>

Mini-PE has been defined as a small pleural collection ipsilateral to the primary tumour, which is either too small to safely aspirate for a cytology sample, or one that has been aspirated and initial fluid cytology is negative (see figure 1). Mini-PE affects up to 25% of patients presenting with NSCLC,<sup>3</sup> although half of these occur in patients with metastatic disease.<sup>3,4</sup> Since 2014, two large retrospective series have described clear association between excess mortality following radical treatment for stages I–IIIA NSCLC and mini-PE on diagnostic (pretreatment) CT imaging. These series conclude that mini-PE reflects occult pleural metastases (OPM) in up to 80% of patients.<sup>3,4</sup> However, this is based on indirect evidence and supportive follow-up imaging. In both series, it is acknowledged that other factors may have contributed to the adverse survival observed, including benign pleuritis, systemic comorbidities<sup>5,6</sup> and undertreatment due to the suspicion of OPM.

In 2015, 441 consecutive patients, who presented with NSCLC to Glasgow centres over 6 months in 2009 were reviewed retrospectively. Overall, 167/441 had radically treatable NSCLC (stages I–IIIA) and of these 26/167 (16%) had Mini-PE (20/127).<sup>7</sup> In this study, a marked survival disadvantage was found in patients with mini-PE,

as shown in previous series.<sup>3,4</sup> In the Glasgow cohort, more conservative treatment was delivered (more supportive care/palliative RT, less surgery, no radical RT, less chemotherapy) in patients with mini-PE, even though they had apparently radically treatable disease.<sup>7</sup> Mini-PE, therefore, appears to confer excess mortality risk, but there is a notable tail on the survival curves reported in all three prior retrospective series with 10%–20% of patients surviving for several years.<sup>3,4,7</sup> Some mini-PE cases may, therefore, be receiving overly cautious therapy because of inaccurate staging. Precise pleural staging would, therefore, protect patients with OPM from toxicities associated with radical treatments that cannot cure them and encourage radical treatment in patients who can benefit.

Lung cancer staging guidelines that were current at the time of the current study's design either do not specifically address pleural staging (National Institute for Health and Care Excellence CG 121, 2011), or suggest 'a pleural biopsy should be considered' in patients with an effusion, without specifying a modality or biopsy technique (American College of Chest Physicians (2013) and Scottish Intercollegiate Guidelines Network (SIGN) guidelines (SIGN 137, 2014)). Lung cancer teams are, therefore, reliant on CT, Positron Emission Tomography (PET)-CT and pleural aspiration cytology (if this can be performed), all of which are negative by definition in patients with mini-PE. Even in patients with larger, symptomatic PE, CT is limited by a sensitivity of 68% (95% CI 62% to 75%), with a low negative predictive value (65% (95% CI 58% to 72%)).<sup>8,9</sup> With regard to semiquantitative PET-CT, a recent meta-analysis concluded this should not be used for pleural staging, based on a pooled sensitivity of 81% (specificity of 74%), and recommended further studies, particularly in mini-PE.<sup>10</sup> Using current methods, pleural staging is, therefore, an overly subjective process, with treatment decisions based on incomplete data. Instinctively, clinicians have tended to give patients 'the benefit of the doubt', preferring to risk missed metastatic disease than deny a patient 'potentially' radical treatment. However, the adverse prognosis recently associated with mini-PE demands a more objective strategy, particularly considering the toxicities of radical treatment. Additional data are particularly needed regarding the utility and safety of staging thoracoscopy, since it is plausible that most patients could be staged by this technique, ideally local anaesthetic thoracoscopy (LAT), without recourse to video-assisted thoracoscopic surgical (VATS) thoracoscopy, which requires general anaesthesia (GA).



**Figure 1** Minimal pleural effusion (mini-PE) examples. Both panels show axial plane CT images in patients with non-small cell lung cancer (NSCLC). (A) A T2b N1 M0 (stage 2A) NSCLC with associated Mini-PE (red arrows). Based on retrospective data, the HR for death in this case is 2.24 relative to T2b N1 without Mini-PE.<sup>3</sup> (B) A T3 N1 M0 (stage 2B) NSCLC without Mini-PE. Both patients have potentially radically treatable disease (circled).

## Pleural staging by thoracoscopy

VATS thoracoscopy under GA is likely to be a highly sensitive staging tool for mini-PE.<sup>11</sup> In previous studies it has also been combined with pleural lavage cytology (PLC, which involves saline irrigation during surgery in patients without an effusion).<sup>12,13</sup> However, VATS is not a practical option for all patients, in whom non-surgical treatments are frequently required due to comorbidities or patient

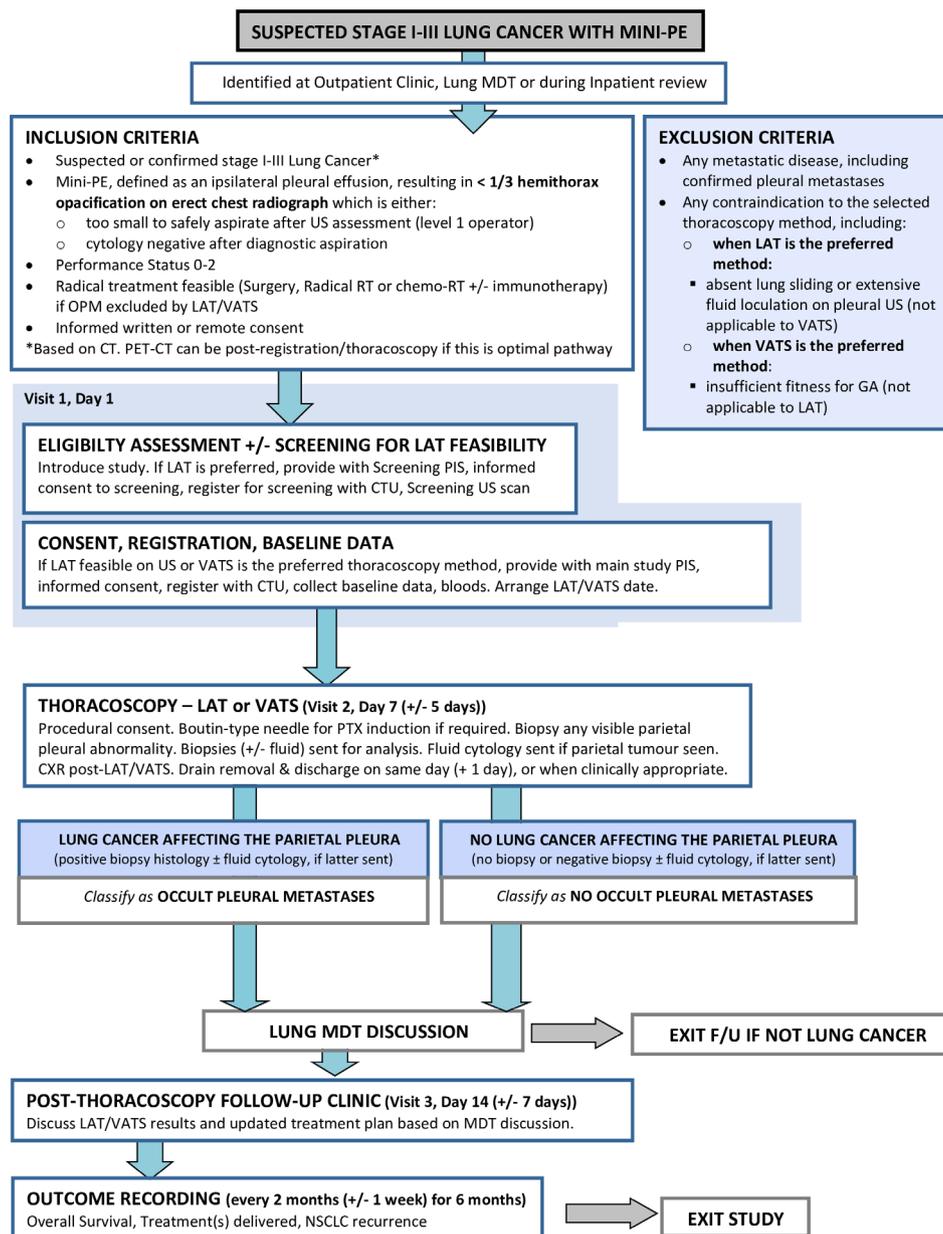
choice. In addition, the significance of PLC results is not clear, since positive results might not necessarily preclude surgical resection.<sup>12</sup> By contrast, LAT is the gold-standard diagnostic test for patients with larger, symptomatic effusions and offers diagnostic performance to equivalent to VATS (sensitivity 93% (95% CI 91% to 94%)) and a low major complication rate (2.3% (95% CI 1.9% to 2.8%).<sup>14</sup> LAT can be performed as a day-case in patients with mini-PE/no PE, but its performance and safety profile may differ in mini-PE, and this has never been prospectively evaluated. Staging by Thoracoscopy in potentially radically treatable Lung Cancer associated with Minimal Pleural Effusion (STRATIFY) will determine the true prevalence of OPM using either LAT or VATS, with sites

encouraged to offer LAT when it is technically feasible. This will be assessed at a dedicated screening visit when LAT is the method preferred by the local team.

## METHODS AND ANALYSIS

### Study design and setting

STRATIFY is a multicentre observational trial, which will be performed according to the UK Policy Framework for Health and Social Care Research. The overall study design is summarised in figure 2. Sample size and associated assumptions are reported under ‘sample size and statistical analysis plan’ section. Eight UK sites will recruit participants. Site selection was based on the availability of



**Figure 2** Study flow chart summarising the design and major study interventions. CT, Computed Tomography; LAT, local anaesthetic thoracoscopy; MDT, multidisciplinary team; mini-PE, minimal pleural effusion; NSCLC, non-small cell lung cancer; OPM, occult pleural metastases; PET-CT, Positron Emission Tomography-Computed Tomography; RT, radiotherapy; VATS, Video Assisted Thoracoscopic Surgery.

**Table 1** Study objectives and associated endpoints

	Objective	Associated endpoint(s)
Primary	To determine the prevalence of detectable OPM in patients with suspected or confirmed stages I–III lung cancer and mini-PE	The prevalence of detectable OPM, as defined by the by the proportion of patients with lung cancer affecting the parietal pleura, based on thoracoscopic sampling (LAT or VATS).
Secondary	To determine the impact of thoracoscopy results on recurrence free and overall survival (RFS and OS) in patients with stages I–III lung cancer and mini-PE	<ul style="list-style-type: none"> <li>▶ Thoracoscopy results, recorded as: OPM demonstrated/not demonstrated</li> <li>▶ RFS, defined as the time from completion of lung cancer treatment to recurrence or death from any cause</li> <li>▶ OS, calculated from thoracoscopy to death from any cause</li> </ul>
	To determine whether staging thoracoscopy is feasible and safe in patients with stages I–III lung cancer and mini-PE	<ul style="list-style-type: none"> <li>▶ LAT feasibility will be recorded as LAT complete/incomplete/not feasible</li> <li>▶ VATS feasibility will be recorded as complete /incomplete/not performed</li> </ul> Safety will be defined by adverse event (AE) and serious AE rates
	To determine the impact of thoracoscopy results on oncological treatment plans in patients with stages I–III lung cancer and mini-PE	<ul style="list-style-type: none"> <li>▶ Thoracoscopy results, recorded as: OPM demonstrated/not demonstrated</li> <li>▶ The treatment plan prior to registration</li> <li>The treatment plan following LAT/VATS</li> </ul>
Exploratory	To determine the diagnostic performance of blood/pleural fluid biomarkers for OPM and/or adverse outcomes in subsequent studies	Venous blood and pleural fluid samples will be collected but not analysed in this study

LAT, local anaesthetic thoracoscopy; Mini-PE, minimal pleural effusion; OPM, occult pleural metastases; VATS, video-assisted thoracoscopic surgery.

a dedicated pleural disease service offering LAT, or ready access to VATS thoracoscopy as an alternative. All sites required integration with their local lung cancer team.

### Study objectives and endpoints

Objectives and their associated endpoints are summarised in [table 1](#).

### Eligibility assessment

All patients will be subject to the following eligibility criteria. There will be no exception to the eligibility requirements at the time of registration. Patients are eligible for the trial if all the inclusion criteria are met and none of the exclusion criteria apply.

### Inclusion criteria

- ▶ Suspected or confirmed stages I–III lung cancer, as defined by at least contrast-enhanced CT\*.
- ▶ Mini-PE, defined as an ipsilateral PE, resulting in <1/3 hemithorax opacification on erect chest radiograph which is either.
  - (1) Too small to safely aspirate after US assessment (level 1 ultrasound operator judgement).
  - (2) Cytology-negative after diagnostic aspiration.
- ▶ Performance Status 0–2.

- ▶ Radical treatment feasible (surgery, radical RT or chemo-RT±immunotherapy) if OPM excluded by thoracoscopy (local principal investigator (PI) judgement).
- ▶ ≥16 years of age.
- ▶ Informed written or remote consent.
  - \* All participants will have contrast-enhanced CT prior to registration, and it is expected that PET-CT will also occur preregistration and prethoracoscopy. However, PET-CT can be completed after registration and after thoracoscopy if this is considered the optimal pathway for the patient. There are no previous data regarding potential false positive PET-CT pleural findings following thoracoscopy (excluding previous reports related to pleurodesis, which will not be performed here). Nevertheless, this is considered sufficiently unlikely to allow the sequencing of these tests to be decided on a per participant basis.

### Exclusion criteria

- ▶ Any metastatic disease, including confirmed pleural metastases.
- ▶ Any contraindication to the selected thoracoscopy method, including:
  - (1) When LAT is the preferred method: absent lung sliding or extensive fluid loculation on pleural ultrasound

(not applicable to VATS); this will be assessed at a dedicated screening visit only applicable when LAT is the preferred approach.

(2) When VATS is the preferred method: insufficient fitness for GA (not applicable to LAT).

- ▶ Uncorrectable bleeding disorder (applicable to both LAT and VATS).

Note that patients with bilateral PE are not excluded but there should be sufficient suspicion of OPM to justify thoracoscopy (in the opinion of the PI), for example, a larger effusion ipsilateral to the primary disease.

### Identification of participants and consent

Potentially eligible patients will be identified and assessed by the respiratory physician/site PI coordinating their care or delegated members of the research team. The study can be introduced at earlier clinic visits if eligibility is likely, and this discussion is clinically appropriate. Potential participants will be given sufficient time (in their own judgement) to consider the commitment required to fulfil trial requirements, and to decide whether to participate. Due to the nature of the trial, and since some patients will be attending 'one-stop' clinics, same-day consent is permissible. Patients may choose to defer consent if they require additional time and will be offered a follow-up telephone call with a member of the study team for this purpose. This call will occur no later than 48 hours after visit 1. In addition, all patients will be made aware that participation is voluntary, and they may withdraw at any time without their standard care being affected. No screening activities related to the trial will be undertaken until informed consent has been obtained. Consent can be obtained face to face or remotely. For remote consent, the patient information sheet (PIS) can be posted or emailed to the patient and then remote consent sought, via telephone or videoconference. The study must have been adequately explained to the patient and the patient must have had the opportunity to ask questions. This must be fully documented in the patient notes. When the subject attends for the first on site clinical visit, consent must be reaffirmed, and signatures of the subject and PI/designee obtained on the consent form. Eligibility will be confirmed by a medical practitioner.

### Screening and registration

If the site PI selects LAT as the optimal thoracoscopy method, formal screening by thoracic ultrasound (TUS) is required as part of visit 1. This is essential to confirm the absence of sonographic exclusion criteria, including absent lung sliding (a surrogate marker of a fixed pleural space not amenable to pneumothorax induction)<sup>15</sup> or extensive fluid loculation, with specific guidance provided in a dedicated Ultrasound Manual (see online supplemental appendix 1). Either of these features will preclude LAT, but are not relevant for VATS thoracoscopy, where they can be overcome by the surgeon. Therefore, if LAT

is the preferred method, an initial screening PIS will be provided, followed by written consent to screening by TUS and allocation of a screening number. The protocol also allows patients to defer formal screening and subsequent consent and registration until the day of LAT (visit 2) if this provides the optimal pathway for the patient or is more practical for the study team. This may be particularly useful if the first study contact is via a remote (eg, video) consultation and/or the patient wishes additional time to consider involvement. Once screening has been completed, eligible patients will be provided with the main study PIS. Those who wish to participate will be registered and a trial number will be allocated. If VATS is selected as the preferred thoracoscopy method, participants are provided with the main PIS immediately, with subsequent consent and registration and without prior TUS screening.

### Study procedures

#### Baseline data collection

At visit 1, baseline data collected will include lung cancer diagnosis status (lung cancer suspected or confirmed), histological subtype, radiological stage and current (prethoracoscopy) treatment plan. Mini-PE laterality, results of any pleural fluid aspiration (if attempted), comorbidities, performance status and staging investigations will also be captured. Baseline organ function and demographics will be recorded.

#### Local anaesthetic thoracoscopy

LAT is performed under conscious sedation, with the patient in the lateral decubitus position. It allows complete visualisation of the parietal and visceral pleural surfaces and directed biopsies. In larger, symptomatic PEs, LAT is the established gold standard diagnostic test, being well-tolerated and feasible as a day-case. In that setting, LAT offers high diagnostic accuracy (sensitivity 92.6%, specificity 100% n=1369 cases) with a low complication rate.<sup>14</sup> The technical feasibility, safety profile and diagnostic performance of LAT in mini-PE will be recorded in the current study since certain procedural modifications are necessary in this setting. The primary adaptation needed is use of a Boutin-type pneumothorax induction needle<sup>14 16</sup> following TUS marking of a suitable entry point, sterile field creation and local anaesthetic infiltration at the chosen access site. This introduces a small volume of air into the pleural space, allowing the lung to drop away from the chest wall under conditions of atmospheric (rather than physiologically negative) pleural pressure and subject to gravity. This ensures the lung is not immediately adjacent to the chest wall during the next stage of the procedure which involves blunt dissection and placement of a 7mm port to act as conduit for the thoracoscope. A dedicated thoracoscopy manual is provided for sites, outlining this and other study specific procedures (see online supplemental appendix 2). These include directions to biopsy



only visible abnormalities on the parietal pleura surface; visceral pleura is not sampled during LAT due to the risk of air-leak. LAT operators are required to complete the thoracoscopy worksheet, included in the thoracoscopy manual and an LAT report form for review by the lung multidisciplinary team (MDT). This latter item (see online supplemental appendix 3) is uploaded onto the electronic health record system (EHR), ideally immediately after the procedure. During LAT, pleural fluid is only sent for routine cytological assessment if pleural biopsies have been taken. This is to maximise diagnostic yield in patients with visible parietal pleural lesions, while avoiding unhelpful uncertainty in patients with macroscopically normal pleura. This uncertainty arises from previous studies of PLC, which although not directly equivalent to LAT fluid cytology, suggested that positive PLC did not dramatically reduce survival in patients who had surgical resection despite this observation.<sup>12</sup> In this setting, therefore, pleural fluid will be banked for later analysis only.

### VATS thoracoscopy

VATS thoracoscopy offers similar high diagnostic sensitivity to LAT and is also safe with a low complication rate.<sup>17 18</sup> However, the procedure requires GA, intubation and single lung ventilation and is therefore not suitable for all patients, including those with major comorbidities. Study-specific guidance for VATS is provided in the thoracoscopy manual (see online supplemental appendix 2), including instructions to only send fluid for cytology if parietal pleural lesions are sampled, as per LAT, for the same reason described above. During VATS, the operator can sample visceral pleural lesions if clinically indicated, since this is standard practice, with routine options available to manage any resulting air-leak. Participants with positive visceral pleural biopsies will not be classified as OPM positive as per the prespecified definition of the primary endpoint.

### Translational research samples

A single blood draw for later translational research will be collected at either visit 1 (eligibility assessment±screening, consent and registration) or visit 2 (LAT/VATS). Pleural fluid samples will be collected during LAT or VATS (visit 2). All samples will be processed and stored in a -80°C freezer within 2 hours of collection. Serum, plasma and pleural fluid samples will all be centrifuged at 2200 g for 15 min at room temperature prior to freezing while whole blood will be frozen immediately without prior processing. Detailed guidance is provided in the sample handling manual (online supplemental appendix 4).

### Post-thoracoscopy results and MDT feedback

Site teams will upload the LAT report form to the EHR and ensure the patient is listed for the next Lung MDT meeting. This records whether parietal pleural

biopsies±pleural fluid samples were sent for routine pathology assessment. The primary endpoint (OPM positive or OPM negative) will be recorded in the study case report form. The final staging and post-thoracoscopy management plan will also be recorded using EHR, but the study team will have no direct input to this decision-making process. A single post-thoracoscopy visit (visit 3) will occur 7 (±7 days) days after thoracoscopy (visit 2). This visit can be virtual or face to face depending on local arrangements. Subsequent study follow-up will involve 2-monthly remote recording of adverse events (AEs), survival, treatment(s)±recurrence.

### Survival

Overall survival (OS) will be recorded from date of registration until death from any cause. Participants alive at 6 months will be censored. Recurrence-free survival (RFS) will be recorded from treatment completion date to disease recurrence or death from any cause.

### Statistical considerations

#### Sample size

The target sample size of 50 patients will allow estimation of the prevalence of OPM, AE rate and the impact on treatment plans with 95% CI bounds not exceeding 10% if the OPM prevalence is ≤15%. This represents a change in estimated prevalence, which was initially set at 70% (requiring a minimum sample size of 96). The initial OPM estimate of 70% reflected solely the retrospective data previously reported.<sup>3 4</sup> The updated estimate of OPM prevalence and sample size calculation acknowledges data from the first 12 recruits to STRATIFY, of whom only one case of OPM has been observed (8.3% OPM rate).<sup>19</sup> The reduction in the sample size from 96 to 50 cases means the trial will no longer have adequate power (80%) to detect an OS HR of 0.5 as planned in previous iterations of this protocol. This original HR corresponded to data from previous retrospective studies, which reported a median OS in OPM positive 6.32 months vs 12.65 months in OPM negative cases.<sup>4</sup> OS differences between OPM positive and OPM negative groups will nevertheless be reported. Post hoc power calculations taking account of the observed prevalence will be performed.

#### Statistical analysis plan

##### Primary efficacy analysis

The estimate of the proportion of cases demonstrating OPM (OPM positive) and the associated 95% CI will use standard statistical methods. The CI will be based on the Clopper-Pearson exact approach.

##### Secondary efficacy analyses

The estimate of the proportions of OPM demonstrated/not demonstrated and LAT complete/LAT incomplete and the associated 95% CIs will use standard statistical methods. The CI will be based on the Clopper-Pearson

exact approach. The comparison of the RFS and OS between OPM positive and OPM negative patients will be illustrated with Kaplan-Meier curves; the HR will be estimated using Cox regression. AE data and the impact on oncological treatment plans will be summarised in tables and listings.

### Exploratory analyses

The number of recruits with banked samples suitable for later analysis will be reported but no other analysis will be performed under this protocol.

### Safety analysis

AE data will be summarised in tables and listings.

### Patient and public involvement

The study has benefited from patient and public involvement (PPI) input throughout the design stage, including input to the original funding application, the study protocol and the content and language used in all patient facing materials, for example, PIS/consent forms. EB (our PPI representative) was a coapplicant on the study funding application in 2018 and has remained involved since. This has included attendance at monthly study management group (SMG) meetings and input to all protocol amendments and any updated patient facing materials.

### Changes to protocol

The protocol described here reflects the current version 5.0, dated 16 November 2022. The following changes were made in previous versions:

#### V.2.0, dated 26 June 2020

- ▶ The definition of the primary endpoint (OPM) was clarified to make it clearer that pleural fluid samples could be sent for routine cytology assessment, alongside parietal pleural biopsies if these were taken

#### V.3.0, dated 25 February 2021

- ▶ The maximum size used to define mini-PE in the inclusion criteria was changed from <40 mm maximum depth on axial CT images to an effusion occupying <1/3 of the hemithorax on erect chest radiograph. The original definition (drawn from previous retrospective mini-PE studies)<sup>3,4</sup> proved difficult to deploy reliably in practice due to variation in where the user could make this measurement.
- ▶ Schedule of assessments updated to include a COVID swab prior to thoracoscopy (Visit 2), in line with COVID-19 guidance at that time.

At this point, a major protocol amendment was undertaken to address significant recruitment challenges, including (1) a change in the diagnostic pathway for lung cancer prompted by COVID-19, with a move to

virtual consultations in many centres, (2) low lung cancer referral rates, which dropped by 60% in some networks and (3) significant delays in opening sites due to UK-wide prioritisation of Urgent Public Health-badged studies. One-to-one sessions with our current sites revealed a series of changes to patient flow and visit scheduling that would make the current protocol, which assumed as series of sequential face to face visits, undeliverable. These discussions also identified other recruitment barriers including the handling of tiny contralateral effusions (currently an exclusion criterion) and use of surgical thoracoscopy (under general anaesthetic) which has become more available in some centres since the original protocol design. Based on this feedback and following PPI review, v 4.0, dated 19 November 2022 was deployed implementing the following changes:

#### V.4.0, dated 16 November 2022

- ▶ Introduction of remote verbal consent as an option, with subsequent written consent at next contact.
- ▶ Allowing completion of screening, consent, registration and baseline data collection on the day of thoracoscopy if this aligns better with local pathways and patient preference.
- ▶ Allowing recruitment earlier in the diagnostic pathway so that STRATIFY pleural staging can be completed without prior histological confirmation of NSCLC. This reduces the burden of invasive tests and necessarily broadens the eligibility criteria to 'suspected or confirmed stages I–III lung cancer'.
- ▶ Allowing STRATIFY pleural staging to be performed by surgical thoracoscopy (ie, under general anaesthetic); this opens the study to centres without access to LAT.
- ▶ Allowing inclusion of cases with bilateral PEs, assuming the collection ipsilateral to the primary is judged to be suspicious, for example, asymmetrically large, with a small contralateral effusion.

A further, final protocol amendment was then made to ensure the study could report on the primary endpoint within its original funding envelope by extending the original recruitment period to 31 October 2023 via a no cost extension from the funder (CSO). This involved the following additional changes:

#### V.5.0, dated 16 November 2022

- ▶ Removal of the MRI substudy, which involved perfusion MRI after registration and prior to thoracoscopy. Overall, 3/42 recruits had completed MRI by this time and these data will be reported in the results publication.
- ▶ Reduction of the sample size from 96 to 50. This was based on review of the OPM prevalence in the first 12 participants (see the Sample size section) and will allow the primary endpoint to be reported with the same precision as originally intended, but with a more realistic recruitment target.

### Definition of end of study

The end of study definition will be the date of last data capture, which will be met when all outstanding data has been returned from all sites, all required data queries have been resolved and the database is finalised for analysis.

### Monitoring, data management and quality assurance

No routine site or telephone monitoring will be performed. If issues arise, an on-site visit or telephone monitoring call will be arranged. The Cancer Research UK (CRUK) Clinical Trials Unit (CTU) will regularly chase outstanding data and queries. Routine requests for missing or queried data will occur quarterly.

### Safety considerations

All AEs and serious AE (SAEs) thought to be related to study procedures will be recorded. This includes AEs resulting from ultrasound, chest radiographs, venous blood sampling, LAT or VATS. Although the MRI substudy has been removed from the current protocol, any AEs or SAEs related to the MRI in the three patients recruited to the substudy prior this point will be reported. This will include any events related to image acquisition, including administration of gadolinium contrast, or the X-ray of orbits (if required) which were the only additional AEs recorded. Safety reporting is overseen by the Pharmacovigilance Department of the CRUK CTU Glasgow as delegated by the trial sponsor.

### Dissemination

Study results, including those related to the MRI substudy removed from the current protocol version, will be presented at national and international scientific meetings and published in full in a peer-reviewed journal.

### Study management

STRATIFY will be coordinated from the CRUK Glasgow CTU. The SMG, comprising the chief investigator, selected coinvestigators, project manager, statistician, trial coordinator, PV coordinator, PPI representative and IT programmer meet monthly to oversee the study.

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**Staging by Thoracoscopy in potentially radically treatable Lung Cancer associated with Minimal Pleural Effusion (STRATIFY): Protocol of a prospective, multicentre, observational study**

**APPENDIX 1: Online Supplement**

**NB: Supplement page numbers in red. Page numbers in black refer to original manuals**

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## Ultrasound Manual

### STRATIFY (L190)

# Staging by Thoracoscopy in potentially Radically Treatable Lung Cancer associated with Minimal Pleural Effusion

Protocol No: STRATIFY2018

Sponsor Ref: GN16ON040

Version 1.1, 22 March 2023



STRATIFY Pleural US Manual

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## STRATIFY Pleural US Manual

## 1 Overview

Patients entered to the Screening stage of the STRATIFY study will undergo a thoracic ultrasound to determine their eligibility for the main STRATIFY study. The aim of this manual is to provide instruction for performing the thoracic ultrasound. This guidance should be followed for all study patients.

## 2 Contact Information

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- Trial name (STRATIFY)
- Your name, email address and telephone number
- Your centre details
- Patient trial number (if applicable)

STRATIFY Pleural US Manual

### 3 Pre-LAT Thoracic Ultrasound Assessment

1. Position patient comfortably in the lateral decubitus position lying on the opposite side to their effusion.
2. Position yourself in front of the patient.
3. Set ultrasound machine to B mode (2D)
4. Identify effusion and ipsilateral hemi-diaphragm.
5. Optimise image with appropriate depth and gain settings
4. Assess effusion:
  - Maximum depth
  - Height in number of rib spaces
  - Extent of loculation
5. Assess for the presence of lung sliding:
  - It is recommended this be assessed at multiple points not just within the safe triangle at the proposed access point
  - Do this at three points
6. Identify a suitable point of entry within the safe triangle.
7. The final decision regarding feasibility of LAT should be made by the local Principal Investigator (PI) or a suitably trained assessor delegated by the PI.



## Thoracoscopy Manual

### STRATIFY (L190)

#### Staging by Thoracoscopy in potentially Radically Treatable Lung Cancer associated with Minimal Pleural Effusion

Protocol No: STRATIFY2018

Sponsor Ref: GN16ON040

Version 1.2, 22<sup>nd</sup> Mar 2023



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## 1 Overview

The aim of this manual is to provide instruction for the technical procedures related to Local Anaesthetic Thoracoscopy (LAT) and Video Assisted Thoracoscopy Surgery (VATS) thoracoscopy in STRATIFY. This guidance is not meant to replace existing protocols and it is acknowledged that practices vary considerably. Nevertheless, the following are minimum requirements that should apply to all study participants. Areas expected to vary between sites are *italicised*.

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- Trial name (STRATIFY)
- Your name, email address and telephone number
- Your centre details
- Patient trial number (if applicable)

### 3 Local Anaesthetic Thoracoscopy (LAT)

#### 3.1 Immediate Pre-LAT Assessment and Safety Procedures

A detailed assessment regarding the safety and feasibility of LAT will have been performed at the formal screening visit. At that visit, an ultrasound scan will have been performed and appropriate blood tests sent in preparation. The following guide is not meant to replace existing pre-LAT checklists that exist in participating units, but should serve as a minimum standard for the immediate pre-LAT assessment, performed on the day of the procedure:

- Review blood results, including full blood count, coagulation screen, renal function and Group and Save (G&S) results. Ensure the G&S is valid and in date. *Note that a second valid result may be required in some centres before provision of blood, if required.*
- Update ECG as appropriate
- Review the patient's medication list. Note:
  - Warfarin should have been stopped at least 3 days pre-procedure and a normalised INR must be confirmed before LAT
  - Clopidogrel must be stopped at least 7 days pre-procedure
  - All DOACs must be stopped at least 2 days pre-procedure
  - Aspirin can be continued
- Ensure the patient has been appropriately fasted (at least 6 hrs prior to procedure time)
- Review the patient's understanding of procedure, answer any questions and complete procedural consent form (*this may be done pre-admission if this is local policy*)
- Secure IV access
  - minimum calibre 20G (Pink); ideally 18G (Green)
  - ideally distal to the elbow on the same side as the effusion
  - flush the venflon once sited to ensure patency
- Prescribe post-procedure analgesia, thromboprophylaxis +/- sedation
- Nursing staff should record routine observations pre-procedure

#### 3.2 Location and Staffing

Local anaesthetic thoracoscopy should be performed in a suitable location *as per local arrangements* (ideally an endoscopy suite or theatre) to ensure sterile conditions can be maintained throughout the procedure. Minimum staffing should include:

- **Primary operator:** Suitably trained, independent operator should be present at all times. This operator should have received formal training and be experienced in Level II Thoracoscopy, including use of a Boutin needle for pneumothorax induction in cases with minimal or no pleural fluid
- **Scrub nurse:** One suitably trained nurse to assist the first operator during the procedure
- A third **adequately trained member** of the team to administer IV sedation and analgesia where necessary is also required. This member of staff may be medical or nursing and

may need to deliver the duties with the second nurse, see below

- A *second nurse* acting as a 'runner' is also recommended. This team member should be available to assist with any non-sterile duties, e.g. performing regular observations, changing fluids, opening equipment packs

### 3.3 Instruments and Equipment

All LAT procedures should be carried out using existing *instruments and equipment at each site*.

Minimum requirements include:

- Sterile gowns and gloves
- Sterile needles and syringe for local anaesthetic administration
- Surgical cut down kit including, scalpel and blunt forceps
- Rigid or semi-rigid thoracoscope
- Routine-type needle
- Port with conical tip trocar and cannula
- Cold light source
- Optical biopsy forceps (double spoon) for use with rigid thoracoscope or appropriate disposable biopsy forceps for use with semi-rigid kit
- De-mister. Either via thoracoscope warmer or suitable sterile de-misting solution
- Chest drain (20F), tubing and bottle
- Sutures
- Chest drain dressing

### 3.4 Positioning, Ultrasound & Site Preparation

#### Positioning

The patient should be positioned in the lateral decubitus position with the affected side lying superiorly. The patient should be made as comfortable as possible. It is recommended that at least one pillow is placed under the head and a further pillow placed underneath the dependant ribcage to avoid unhelpful rib crowding on the affected side. The patient's arms should be flexed and rested in front of their face or extended straight using an arm support.

#### Ultrasound

In addition to the ultrasound performed as part of the eligibility assessment for entry into STRATIFY, on-table ultrasound must be performed after positioning and before access. This is to ensure the anatomy, including the extent of loculation has not changed since screening and to facilitate surface marking, including marking of the optimal site of safe access. On-table US should be focused in the safe triangle. If no fluid is visible, lung sliding in at least one position should be confirmed by a suitably trained operator before proceeding. The diaphragm should

then be identified and its position marked. On the left side, the cardio-pleural angle should also be identified by a surface marking. Finally, a suitable entry site within the safe triangle should be marked in such a way that it remains visible after site clearing and preparation.

### Site preparation

Once the patient has been suitably positioned and a safe site of access marked, a sterile field must be created. The operator must wash their hands using a standard surgical scrub technique before donning sterile gloves and gown. The patient's skin at the access site should be thoroughly cleaned using an *iodine-based solution or equivalent* as per local protocol. The site should be dressed using sterile drapes.

## 3.5 Access

### Local anaesthetic

*1-2% lidocaine (+/- adrenaline)* should be used to anaesthetise the skin and subcutaneous tissues down to the parietal pleura. The maximum dose of 3mg/kg should not be exceeded.

### Incision

Once the skin and underlying tissue have been adequately anaesthetised, an incision in the same plane as the underlying rib should be made. This should be just deep enough to expose underlying subcutaneous fat and just long enough to allow blunt dissection and subsequent entry of the thoracoscopy port.

### Induction of pneumothorax

Given the nature of the STRATIFY study, it is likely that pneumothorax induction will frequently be required and should not dissuade proceeding with LAT. This can be done *with or without direct ultrasound guidance (as per Corcoran et al, Thorax 2015)*, at the discretion of the operator, and based on their current practice. A Boutin-type needle should carefully be inserted into the pleural cavity. A detailed description of the method is not required but this should include initial shallow penetration using the sharp obturator, which should not be inserted into the intercostal space. Prior to this depth, the blunt obturator should be swapped in, allowing safe access to the pleural cavity. Once the parietal pleura has been punctured, the blunt obturator should be removed to allow entrainment of air into the pleural cavity. Ten breaths should be counted to allow a sufficient volume of air to enter the space before replacing the blunt obturator, screwing it in place and removing the entire needle. Blunt dissection should then be performed to create a tract suitable for placement of the thoracoscopy port. This should be done with blunt forceps as per standard practice. Following blunt dissection, it should be possible to insert the port with no (or minimal) resistance.

### 3.6 Inspection & Pleural Fluid Sampling

Insertion of the thoracoscope for visual inspection should be preceded by removal of any pleural effusion using a flexible suction catheter. Note that samples of this pleural fluid should be collected and processed for storage and use in future research as per the [STRATIFY Sample Handling Manual](#). **However, pleural fluid samples should not be sent for cytology analysis unless biopsies are also sent given the uncertain significance of positive results in this clinical context.** Systematic visual inspection of the whole hemi thoracic cavity (apex, costal surface, diaphragm, lung surface) should then be performed. Any abnormalities should be documented on the corresponding [STRATIFY Thoracoscopy Worksheet \(Appendix 1\)](#). Where the lung fails to deflate sufficiently to allow full inspection, operators are advised to:

- a) ensure any fluid sitting on the mediastinal surface of the lung has been completely removed by reinserted a flexible suction catheter around the posterior and anterior borders of the lung
- b) Remove the thoracoscope and allow a larger volume of air to enter the pleural cavity during free breathing: to facilitate this the tip of the port should be directed upwards, so that it lies within the volume of air already within the space

### 3.7 Biopsy Sampling

Once inspection is complete, up to 5 biopsies should be taken from sites of **visible parietal pleural abnormality**. IV analgesia should be administered prior to biopsy. Biopsy sites should be chosen by the primary operator but **should not include areas of visceral pleura**. The sampling of diaphragmatic sites is permitted since this a parietal surface, but given the increased pain and risk associated with this technique, caution is advised unless easily sampleable disease is identified. All biopsies should be collected and processed as per local policy for pleural pathology samples.

### 3.8 Drain Placement and Use of Pleurodesis

Once the operator is satisfied that all necessary biopsies have been taken, a final visual inspection of the pleural cavity should be performed. As per standard practice, this is to ensure haemostasis has been achieved at each biopsy site and to plan drain placement. Care should be taken to minimise the time between removal of the thoracoscope and associated port and insertion of the chest drain, given the potential for the lung to re-expand during this interval. An intercostal drain (*20F Argyle-style is recommended but can be as per local policy*) should be inserted using the stiffened trochar provided at the original access site and directed to the lung apex, if possible. Operators may also choose to direct the drain using a guidewire inserted via the port prior to its removal, e.g. in cases where blunt dissection was technically challenging. Once the drain is in place, it should be connected to an underwater seal or electronic drainage system (e.g. Thopaz®) depending on local policies. The drain should be secured, ideally by 2 sutures, which should also close the wound around the tube. The site should be cleaned of any blood before the application of a suitable dressing.

## 4 Post-LAT Procedures

### 4.1 LAT Report

All LATs should be documented as per local policy in patient notes. In addition, the [STRATIFY Thoracoscopy Worksheet \(Appendix 1\)](#) should be completed, including recording of any biopsies taken marked on the map provided. **This worksheet should be uploaded onto local electronic health records to act as source data for the procedure that will be common to all sites. The completed worksheet data must also be inputted to the STRATIFY MACRO<sup>®</sup> database.**

### 4.2 LAT Pleural Fluid Sample Processing and Storage

Pleural tissue biopsies should be processed and analysed as per normal local policy. Pleural fluid samples should be handled and stored as described in the [STRATIFY Sample Handling Manual](#).

**Note: Pleural fluid samples should not be sent for cytology analysis unless biopsies are also sent given the uncertain significance of positive results in this clinical context.**

### 4.3 Electronic transfer system

Linked anonymised LAT reports should be transferred as .pdf files, annotated by the participant's unique study ID and the date of LAT using the University of Glasgow Transfer Service (<https://transfer.gla.ac.uk/>). This is a secure system with all files transferred in an encrypted format and access strictly controlled and logged. Data files will be uploaded to the service in a password-protected encrypted archive format. When the recipient collects the transferred file a notification is emailed to the sender who will then provide the recipient with the password to unlock the file. See [Appendix 2](#) for full instructions on the transfer process.

### 4.4 Drain Removal, Discharge from Hospital and Follow-up

The intercostal drain should be removed once maximum lung re-expansion has been achieved on a pre-removal chest radiograph. This radiograph should occur at least 1 hour after completion of LAT, and no later than 12 hours after LAT completion. Ideally, drain removal should occur on the same day as the procedure. A further chest radiograph following drain removal is not required, unless clinically indicated. 1-2 stitches should be placed after drain removal and an occlusive dressing applied. The patient should be discharged as soon as clinically appropriate, ideally on the same day as LAT. *Where clinically indicated, or for logistical reasons, patients may be admitted to hospital overnight after LAT.*

Patients should be provided with written details of their follow-up appointment (venue, date, time) to discuss LAT results (Study Visit 4) prior to discharge home. Patients should be discharged with adequate analgesia, a supply of replacement dressings, appropriate clinical worsening advice and contact details for the clinical team.

## 5 Video-Assisted Thoracoscopy Surgery (VATS)

### 5.1 Location and Staffing

VATS should be performed in an operating theatre to ensure sterile conditions can be maintained throughout the procedure. Minimum staffing should include:

- **Primary operator:** Suitably trained, independent operator should be present at all times.
- **Anaesthetist:** Responsible for induction and maintenance of general anaesthesia.
- **Scrub nurse:** One suitably trained nurse to assist the first operator during the procedure
- **A second nurse** acting as a 'runner'; available to assist with any non-sterile duties, e.g., performing regular observations, changing fluids, opening equipment packs

### 5.2 Instruments and Equipment

All VATS procedures in STRATIFY should be carried using existing *instruments and equipment at each site*. Minimum Instrument and Equipment requirements should include:

- Sterile gowns and gloves
- Sterile needles and syringe for local anaesthetic administration
- Surgical cut down kit including, scalpel and blunt forceps
- Rigid or semi-rigid thoracoscope and appropriate biopsy forceps
- Port with Conical tip trocar and cannula
- Cold light source
- Chest drain (20F), tubing and bottle, dressings
- Sutures

### 5.3 Positioning and Site Preparation

The patient should be positioned in the lateral decubitus position with the affected side lying superiorly. A sterile field must be created, including use of an *iodine-based solution or equivalent* as per local protocol. The site should be dressed using sterile drapes applied.

### 5.4 Access

Access should use standard VATS methodology. One or two ports may be inserted.

### 5.5 Inspection & Pleural Fluid Sampling

Insertion of the thoracoscope for visual inspection should be preceded by removal of any pleural effusion using a flexible suction catheter. Note that samples of this pleural fluid should be collected and processed for storage and use in future research as per the [STRATIFY Sample Handling Manual](#).

**Note: Pleural fluid samples should not be sent for cytology analysis unless biopsies are also sent given the uncertain significance of positive results in this clinical context.**

Systematic visual inspection of the entire pleural space (including costal surface, diaphragm, apex, lung surface) should then be performed. Any abnormalities should be documented on the [STRATIFY Thoracoscopy Worksheet \(Appendix 1\)](#).

## 5.6 Biopsy Sampling

Once inspection is complete, up to five biopsies should be taken from different sites of visible pleural abnormality, in addition to clinical biopsies. The number of clinical biopsies taken should be at the discretion of the primary operator in line with their usual clinical practice. Biopsy sites should be chosen by the primary operator and may include areas of visceral pleura. Biopsies for clinical diagnostic use should be collected and processed as per existing local policies.

## 5.7 Drain Placement and Use of Pleurodesis

Once the operator is satisfied that all necessary biopsies have been taken, a final visual inspection of the pleural cavity should be performed. As per standard practice, this is to ensure haemostasis has been achieved at each biopsy site and to plan drain placement. An intercostal drain (at least *20F Argyle-style is recommended but should be as per local policy*) should be inserted using the stiffened trochar provided at the original access site and directed to the lung apex, if possible. Once the drain is in place, it should be connected to an underwater seal or electronic drainage system (e.g., Thopaz®) depending on local policies. The drain should be secured, ideally by 2 sutures, which should also tighten the wound around the tube. The site should be cleaned of any blood before the application of a suitable dressing.

## 6 Post-VATS Thoracoscopy Procedures

### 6.1 Thoracoscopy Report

All thoracoscopies should be *documented as per local policy* in patient notes. In addition, the [STRATIFY Thoracoscopy Worksheet \(Appendix 1\)](#) should be completed, including recording of any biopsies taken on the map provided. **This worksheet should be uploaded onto local electronic records to act as source data for the procedure that will be common to all sites. The completed worksheet data must also be inputted to the STRATIFY MACRO® database.**

### 6.2 Pleural Fluid Sample Processing and Storage

Pleural tissue biopsies should be processed and analysed as per normal local policy. Pleural fluid samples should be handled and stored as described in the [STRATIFY Sample Handling Manual](#). Note: **Pleural fluid samples should not be sent for cytology analysis unless biopsies are also sent given the uncertain significance of positive results in this clinical context.**

### 6.3 Electronic transfer system

Linked anonymised thoracoscopy reports should be transferred as .pdf files, annotated by the participant's unique study ID and the date of thoracoscopy using the University of Glasgow Transfer Service (<https://transfer.gla.ac.uk/>). This is a secure system with all files transferred in an encrypted format and access strictly controlled and logged. Data files will be uploaded to the service in a password-protected encrypted archive format. When the recipient collects the

transferred file a notification is emailed to the sender who will then provide the recipient with the password to unlock the file. See [Appendix 2](#) for full instructions on the transfer process.

#### **6.4 Drain Removal, Discharge from Hospital and Follow-up**

The intercostal drain should be removed once maximum lung re-expansion has been achieved, confirmed by a chest radiograph 1-12 post-VATS. Ideally drain removal should occur on the same day as the procedure unless talc poudrage performed. The patient should be discharged as soon as clinically appropriate, ideally on the same day if possible. *Where clinically indicated, or for logistical reasons, patients may be admitted to hospital overnight after VATS Thoracoscopy.*

Patients should be provided with written details of their follow-up appointment (venue, date, time) to discuss VATS results (Study Visit 4) prior to discharge home. Patients should be discharged with adequate analgesia, a supply of replacement dressings, appropriate clinical worsening advice and contact details for the clinical team

## 7 Appendix 1: Thoracoscopy Worksheet

		<b>STRATIFY THORACOSCOPY WORKSHEET</b>	
		L190 ISRCTN13584097	
<u>Staging by Thoracoscopy in potentially Radically Treatable Lung Cancer associated with Minimal Pleural Effusion</u>			
Patient Initials: (F) ____ (S) ____		Date of Birth: <u>DD / MON / YYYY</u>	Study Number:
GENERAL			
Side:	<input type="checkbox"/> Right	<input type="checkbox"/> Left	Procedure Type <input type="checkbox"/> LAT <input type="checkbox"/> VATS
Septations:	<input type="checkbox"/> Yes	<input type="checkbox"/> No	Volume drained: _____ ml
PROCEDURE DETAILS			
DRUG	OPTION (please ✓)	DOSE	
Pre-medication	<input type="checkbox"/> Oramorph	_____ mg	
	<input type="checkbox"/> Atropine	_____ mg	
	<input type="checkbox"/> Sevredol	_____ mg	
	<input type="checkbox"/> Other, specify (incl unit): _____	_____	
Sedation	<input type="checkbox"/> Midazolam	_____ mg	
	<input type="checkbox"/> Propofol	_____ mg	
	<input type="checkbox"/> Other, specify (incl unit): _____	_____	
	<input type="checkbox"/> General Anaesthesia		
Local anaesthetic	<input type="checkbox"/> Lidocaine <input type="checkbox"/> 1% <input type="checkbox"/> 2% _____ ml	<input type="checkbox"/> Adrenaline inclusion	
	<input type="checkbox"/> Other, specify (incl unit): _____	_____	
Analgesia	<input type="checkbox"/> Alfentanyl	_____ micrograms	
	<input type="checkbox"/> Fentanyl	_____ micrograms	
	<input type="checkbox"/> Morphine	_____ mg	
	<input type="checkbox"/> Other, specify (incl unit): _____	_____	
US on table:	<input type="checkbox"/> Yes <input type="checkbox"/> No	Boutin with US:	<input type="checkbox"/> Yes <input type="checkbox"/> No
Fluid on US:	<input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A	Talc:	<input type="checkbox"/> Yes <input type="checkbox"/> No If yes, dose given: _____ g
Boutin induction:	<input type="checkbox"/> Yes <input type="checkbox"/> No	Drain size:	_____ F
IMMEDIATE COMPLICATIONS: IF NONE TICK HERE <input type="checkbox"/>			
Haemorrhage requiring transfusion:	<input type="checkbox"/> Yes <input type="checkbox"/> No	Failure of procedure:	<input type="checkbox"/> Yes <input type="checkbox"/> No
Hypotension requiring intervention:	<input type="checkbox"/> Yes <input type="checkbox"/> No	Other <input type="checkbox"/> Yes specify:-	_____

BIOPSY DETAILS		
SITE	ABNORMALITY	NO. OF BIOPSIES
1		
2		
3		
4		
5		
6		
7		
8		
9		
10		
11		

**RECORD ABNORMALITY:**

- MACRO - macronodularity
- MICRO - micronodularity
- THICK - pleural thickening
- NORMAL – where site appears normal, but a biopsy is taken\*

*\*If no abnormality is present in a numbered site and no biopsy is taken, leave site row blank*

**RECORD BIOPSIES:**

- Number taken – 1-5
- Unsuccessful - attempted but unsuccessful
- N/A - Not attempted

**FOR REFERENCE:**

RIGHT

LUNG

LEFT

LUNG

INVESTIGATOR SIGNATURE: \_\_\_\_\_

DATE: DD / MON / YYYY

## 8 Appendix 2 - Glasgow University Transfer Service User Instructions

### 8.1 Access the service at <https://transfer.gla.ac.uk/>

### 8.2 Types of User

There are two kinds of users that can access the transfer system:

- Internal: University of Glasgow staff who are allowed to create a drop-off that can be delivered to one or more individuals (whether they are internal or external to the University)
- External: anyone else, anywhere on the Internet, who are only allowed to create a drop-off that is to be delivered to University of Glasgow staff members

### 8.3 Drop off and pick up

- A drop-off is one or more files uploaded to Transfer as a single entity for delivery to a specified person
- A pick-up allows a person to collect the dropped-off files

### 8.4 Creating a drop-off

- When creating a drop-off you:
  - enter identifying information about yourself by logging in or providing you name, organisation and email address
  - enter identifying information about the recipient (name and email address)choose which files should be uploaded to the drop-off
- If the files are successfully uploaded, an email is sent to the recipient explaining that a drop-off has been made with a link to access the drop-off.
- Other information (the internet address and/or hostname from which the drop-off was created, for example) is retained, so that the recipient can verify the identity of the sender
- The recipient has **14 days** to pick-up the files. Each night, drop-offs that are older than 14 days are deleted from the system. If your recipient(s) have not picked them up in that time, you will have to repeat the drop-off

STRATIFY Sample Handling Manual



## Sample Handling Manual

### STRATIFY (L190)

Staging by Thoracoscopy in potentially Radically Treatable Lung Cancer associated with Minimal Pleural Effusion

Version 2.1: 22 March 2023

Protocol No: STRATIFY2018

Sponsor Ref: GN16ON040



## STRATIFY Sample Handling Manual

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STRATIFY Sample Handling Manual

## 1. Contact Information

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**Glasgow Biorepository  
Contacts:** **Alexandra Bell or Clare Orange**  
NHS GG&C Biorepository  
Level 3 Laboratory Medicine /FM Building  
Queen Elizabeth University Hospital  
1345 Govan Road  
Glasgow G51 4TF  
☎ 0141 354 9490  
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✉ [Clare.Orange@ggc.scot.nhs.uk](mailto:Clare.Orange@ggc.scot.nhs.uk)

When contacting, please include the following information:

- Trial name (STRATIFY)
- Your name, email address and telephone number
- Your centre details
- Patient trial number (if applicable)

STRATIFY Sample Handling Manual

## 2. Introduction

The purpose of this manual is to describe the collection, processing, storage and transportation of blood samples for patients who have consented to take part in the translational research aspect of STRATIFY.

## 3. Scope

This manual covers handling of blood and pleural fluid samples at clinical centres.

## 4. Responsibilities

The clinical staff at participating centres are responsible for ensuring that samples are collected, handled, processed and stored at their clinical centre in accordance with these instructions.

Please read this manual carefully and contact the Clinical Research Fellow or Project Manager with any questions. Please ensure that you complete and return the declaration at the end of this document stating that you have received, read and understood this manual.

## 5. Related Documents

- Clinical Trial Protocol: STRATIFY Staging by Thoracoscopy in potentially Radically Treatable Non-Small Cell Lung Cancer associated with Minimal Pleural Effusion
- STRATIFY Local Anaesthetic Thoracoscopy Manual

STRATIFY Sample Handling Manual

## 6. Consumables and Equipment

### 6.1. Equipment

To be provided by the Clinical Site

- Centrifuge (refrigerated)

### 6.2. Consumables

The CRUK CTU will provide the following items:

Item
EDTA tube: VACUETTE® TUBE 6 ml K2EDTA, lavender capped
EDTA K3 tube, 9 ml Lavender capped
SST Clot activator 5ml tube, yellow capped
1.5ml cryovials
Yellow cryovial caps
Red cryovial caps
30ml universal containers for pleural fluid
5.0ml cryovials
Cryolabels
Pipettes
Sample bags (mini grip) 15x20cm
Needles for research blood draw
Syringes for research blood draw
Cryoboxes for cryovials
Cryoboxes for whole blood tubes
Padded envelopes

The clinical site will provide the following items:

Item
Bubble wrap
Indelible marker pen

## STRATIFY Sample Handling Manual

## 7. Sample Collection Schedule

Whole blood, plasma, serum, and pleural fluid samples will be collected from patients according to the schedule of assessments outlined below.

Study Procedure	Visit 1 or Visit 2 or Visit 3*	Visit 3 (LAT)
Plasma sample	X	
Serum sample	X	
Whole blood sample	X	
Pleural fluid sample		X

*\*per protocol, if plasma and serum sample not taken at Visit 1, should be taken at Visit 2 for MRI sub-study patients, or at Visit 3 for patients not participating in MRI sub-study*

## 8. Research Blood Sample Processing, Storage and Shipment

As outlined above, research bloods will be collected and processed at Visit 1 or 2 or 3\* to generate the following samples:

Sample Type	Blood Collection Volume /Tube Type	Manual
Whole blood	10ml of blood collected into 2 x 6ml EDTA tubes (5ml/tube)	Section 8.1
Serum	4ml blood collected into a 5ml Serum Vacuette tube, processed into 4-5 microfuge tubes	Section 8.2
Plasma	10ml blood collected into 2 x 9ml EDTA tubes (8ml/tube), processed into 10-15 microfuge tubes	Section 8.3

*\*per protocol, if plasma and serum sample not taken at Visit 1, should be taken at Visit 2 for MRI sub-study patients, or at Visit 3 for patients not participating in MRI sub-study*

### 8.1. Whole Blood Sample Processing Method

- Samples to be collected at either Visit 1, 2 or 3 as described
- Check expiration date on 6ml EDTA tube; if expired replace with new one
- Collect approximately 5mls of venous blood into a 6ml EDTA tube
- Gently invert the tube 8-10 times
- Complete the STRATIFY Whole Blood label with an indelible pen and place label firmly onto tube, ensuring that the bottom of the label is twisted at the base of the tube.

Please ensure the label is placed on the tube before it is frozen otherwise it will not adhere

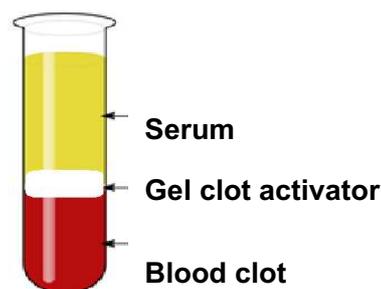
- Immediately place the tube into a small sample storage bag labelled using an indelible pen with the following information:
  - Trial name (STRATIFY)
  - Recruiting Centre
  - Patient Trial Number

## STRATIFY Sample Handling Manual

- Patient initials
- Whole blood
- Place bag into  $-80^{\circ}\text{C}$  ( $\pm 10^{\circ}\text{C}$ ) freezer until ready to ship (see section 9 for shipping instructions)
- Complete the whole blood worksheet with the time sample was frozen and the details of the operator.

## 8.2. Blood collection for isolation of serum

Centrifugation of clotted blood causes separation of blood cells from the serum. Serum moves to the top of the tube and forms the supernatant. The gel layer in the vacutainer serves to separate the blood clot from the serum after centrifugation (see diagram). This top layer of serum can then be carefully removed with a pipette and stored at  $-80^{\circ}\text{C}$ .



Centrifugation should occur as soon as possible after blood has clotted and all specimens should be processed and frozen within **2 hours** of venepuncture.

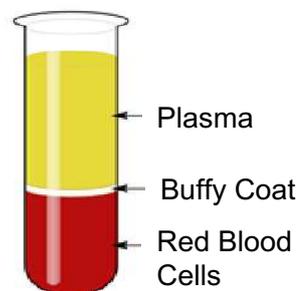
### Method

- Samples to be collected at either Visit 1 or 2 or 3 as described
- Check expiration date on yellow vacutainer; if expired replace with new one
- Collect approximately 4ml of venous blood into 1 yellow vacutainer tube containing SST clot activator
- Gently invert sample 5-6 times
- Record sample collection date and time on serum worksheet
- Allow the sample to clot for 30 minutes at room temperature before centrifugation
- Centrifuge at 2200g for 15 minutes at room temperature
- Record centrifugation time on serum worksheet
- Carefully withdraw the top layer using a pipette and dispense 500 $\mu\text{l}$  aliquots into the DNase/RNase free microfuge tubes. There should be enough serum for 4-5 microfuge tubes. Do not overfill these tubes
- Place a red cap on each tube
- Complete the STRATIFY Serum labels with an indelible marker and stick them securely onto tubes, ensuring that the bottom of the label is twisted around the base of the tube. Please ensure the label is placed on the tube before it is frozen otherwise it will not adhere
- Label the top of the tubes using an indelible marker with the patient trial number, S (serum) and time-point (baseline).
- Place into cryobox labelled using indelible marker with the following information:
  - Trial Name (STRATIFY)
  - Recruiting Centre
  - Patient trial number
  - Patient initials
- Place cryobox into  $-80^{\circ}\text{C}$  ( $\pm 10^{\circ}\text{C}$ ) freezer until ready to ship (see section 5 for shipping instructions)
- Complete the serum worksheet with the time serum samples were frozen, the number of microfuge tubes and the details of the operator.

### 8.3. Plasma Sample Processing

**IMPORTANT: Blood samples must be centrifuged within 1hr of collection to avoid fragmentation, degradation and leukocyte lysis.**

- Centrifugation of un-clotted blood causes separation of blood cells from plasma. A clear layer of plasma will form the supernatant and can then be carefully removed using a pipette.
- The white cells and platelets will form a layer underneath the plasma - this is known as the buffy coat layer. The red blood cells form a layer underneath the buffy coat (see diagram).



#### Method

- Samples to be collected at either Visit 1, 2 or 3 as described
- Check expiration date on 9ml EDTA tubes; if expired replace with new ones
- Collect 8mls of venous blood into 2 x EDTA tubes (approximately 16mls in total)
- Gently invert samples 8-10 times and leave upright prior to centrifugation
- Record the sample collection time on the plasma laboratory worksheet
- Centrifugation should be done **immediately** with these samples as they do not need to clot
- Centrifuge at 2200g for 15 minutes at room temperature
- Record the time of centrifugation on the plasma laboratory worksheet
- Carefully withdraw upper plasma layer using a pipette Transfer 500µl aliquots of plasma into 1.5ml DNase/RNase free microfuge tubes and discard the pellet and any remaining plasma. There should be sufficient plasma for 8-10 microfuge tubes. Do not overfill the tubes
- Place a yellow cap on each of these tubes (these can be re-used from previous step)
- Complete the STRATIFY Plasma labels using an indelible pen and stick them onto the tubes, ensuring that they are secure and the bottom of the label is twisted around the end of the microfuge tube. Please ensure the label is placed on the tube before it is frozen otherwise it will not adhere
- Label the top of the tubes using an indelible marker with the patient trial number, P (plasma) and time-point:
- Place tubes into a cryobox labelled using indelible marker with the following information:
  - Trial name (STRATIFY)
  - Recruiting Centre
  - Patient trial number
  - Patient initials
- Place cryobox into -80°C (+/- 10°C) freezer until ready to ship (see section 5 for shipping instructions)
- Complete the plasma worksheet with the time plasma samples were frozen, the number of microfuge tubes and the details of the operator.

## STRATIFY Sample Handling Manual

**8.4. Pleural Fluid Sampling and isolation of supernatant**

At Visit 3 pleural fluid will be collected during the LAT and processed to generate the following samples:

Sample Type	Blood Collection Volume /Tube Type
Pleural fluid	1pleural fluid collected in a 30ml universal containers and processed into 7-8 x 5ml cryotubes.

- Further information regarding the LAT procedure itself can be found in the accompanying handbook. Once the fluid samples are obtained they should be processed and frozen within 2 hours as follows:
- Centrifuge at 2200 x g for 15 minutes at room temperature
- Record centrifugation time on pleural fluid worksheet
- Carefully withdraw the supernatant using a pipette and dispense 4ml aliquots into the 5ml tubes. There should be enough serum for 7-8 x 5ml tubes. Do not overfill these tubes
- Securely fasten the cap on each tube
- Complete the STRATIFY pleural fluid labels with an indelible marker and stick them securely onto tubes, ensuring that the bottom of the label is twisted around the base of the tube.

Please ensure the label is placed on the tube before it is frozen otherwise it will not adhere

- Label the top of the tubes using an indelible marker with the patient trial number
- Place into cryobox labelled using indelible marker with the following information:
  - Trial Name (STRATIFY)
  - Recruiting Centre
  - Patient trial number
  - Patient initials
- Place cryobox into -80°C (+/- 10°C) freezer until ready to ship (see section 5 for shipping instructions)
- Complete the pleural fluid worksheet with the time serum samples were frozen, the number of microfuge tubes and the details of the operator.

## STRATIFY Sample Handling Manual

## 9. Handling and Transport of Processed Samples

- At the end of the trial, each patient should have the following cryo-boxed samples:
  - 2 x 6 ml tubes with whole blood
  - Up to 5 Serum samples with red lids in 1.5ml tubes
  - Up to 15 Plasma samples with yellow lids in 1.5ml tubes
  - Up to 8 pleural fluid samples in 5.0ml tubes
- Sample tubes must be stored in the cryo-boxes provided by the CTU. Box number and tube position within the cryo-box will require to be completed on the provided STRATIFY sample submission form, prior to shipping.

Samples should be kept at local sites in  $-80^{\circ}\text{C}$  ( $\pm 10^{\circ}\text{C}$ ) storage conditions and will be transferred to the Glasgow Biorepository on dry ice when study recruitment is completed at all sites. The Cancer Research UK Clinical Trials Unit will contact each site to advise when samples are to be shipped and will provide courier instructions

- Samples must be packed securely to avoid breakage during transit and with sufficient dry ice to prevent thawing for at least 2 days to allow for any delays in transport or delivery (2.3 – 4.5 kg per 24 hours). Dry ice and transportation box will be provided by the courier at the time of sample collection. Completed worksheets, and sample submission forms should be packaged with the samples. A receipt will be included in the paperwork for Glasgow Biorepository to record receipt of the samples (see worksheets).
- For queries relating to the transfer of samples to the Glasgow Biorepository, please contact Laura Alexander at Cancer Research UK Clinical Trials Unit, Glasgow (page 3). Please include the trial ID (STRATIFY) in all communications.

STRATIFY Sample Handling Manual

## 10. Worksheets

### 10.1. STRATIFY Whole Blood Worksheet

Patient Study Number: \_\_\_\_\_ Patient Initials: \_\_\_\_\_

Centre Name: \_\_\_\_\_

Time Point	Date and Collection Time	Time Frozen	Operator (Print Name and Sign)
Baseline			

Record whether blood was drawn using peripheral venous access device (e.g. butterfly) or central venous access device (CVAD) here: \_\_\_\_\_

Please describe any deviations from the laboratory manual or issues below:

\_\_\_\_\_

\_\_\_\_\_

#### **Dispatch Details for Whole Blood**

Number of tubes sent: \_\_\_\_\_ Date: \_\_\_\_\_

Staff Responsible: \_\_\_\_\_ (print name) \_\_\_\_\_ (signature)

#### **Whole Blood Sample Receipt (for Glasgow Biorepository use)**

Date/time received: \_\_\_\_\_ Number of samples received: \_\_\_\_\_

Condition of samples on arrival: \_\_\_\_\_

\_\_\_\_\_

Staff responsible: \_\_\_\_\_ (print name) \_\_\_\_\_ (signature)



STRATIFY Sample Handling Manual

**10.3. STRATIFY Plasma Worksheet**

Patient Study Number: \_\_\_\_\_ Patient Initials: \_\_\_\_\_

Centre Name: \_\_\_\_\_

Time Point	Date and Collection Time	Centrifugation start time	Time Frozen	No of Tubes	Operator (Print Name and Sign)
Baseline					

Record whether blood was drawn using peripheral venous access device (e.g. butterfly) or central venous access device (CVAD) here: \_\_\_\_\_

Please describe any deviations from the laboratory manual or issues below:

\_\_\_\_\_

\_\_\_\_\_

**Dispatch Details for Plasma**

Number of tubes sent: \_\_\_\_\_ Date: \_\_\_\_\_

Staff Responsible: \_\_\_\_\_ (print name) \_\_\_\_\_ (signature)

**Plasma Sample Receipt (for Glasgow Biorepository use)**

Date/time received: \_\_\_\_\_ Number of samples received: \_\_\_\_\_

Condition of samples on arrival: \_\_\_\_\_

\_\_\_\_\_

Staff responsible: \_\_\_\_\_ (print name) \_\_\_\_\_ (signature)

STRATIFY Sample Handling Manual

**10.4. STRATIFY Pleural Fluid Worksheet**

Patient Study Number: \_\_\_\_\_ Patient Initials: \_\_\_\_\_

Centre Name: \_\_\_\_\_

Time Point	Date and Collection Time	Time Frozen	Operator (Print Name and Sign)
Visit 3			

Please describe any deviations from the laboratory manual or issues below:

---



---

**Dispatch Details for Pleural Fluid**

Number of tubes sent: \_\_\_\_\_ Date: \_\_\_\_\_

Staff Responsible: \_\_\_\_\_  
(print name) (signature)**Pleural Fluid Sample Receipt (for Glasgow Biorepository use)**

Date/time received: \_\_\_\_\_ Number of samples received: \_\_\_\_\_

Condition of samples on arrival: \_\_\_\_\_

---

Staff responsible: \_\_\_\_\_  
(print name) (signature)

STRATIFY Sample Handling Manual

## 11. Labels

### 11.1. Labels for EDTA whole blood collection tubes

#### STRATIFY Baseline Whole blood (Genomic DNA)

Pt No: \_\_\_\_\_ Initials \_\_\_\_\_

Centre: \_\_\_\_\_

Date: \_\_\_\_\_ Time: \_\_\_\_\_

### 11.2. Labels for 1.5ml microtubes

#### STRATIFY Baseline Serum

Pt No: \_\_\_\_\_ Initials \_\_\_\_\_

Centre: \_\_\_\_\_

Date: \_\_\_\_\_ Time: \_\_\_\_\_

#### STRATIFY Baseline Plasma

Pt No: \_\_\_\_\_ Initials \_\_\_\_\_

Centre: \_\_\_\_\_

Date: \_\_\_\_\_ Time: \_\_\_\_\_

### 11.3. Labels for 5ml cryovials

#### STRATIFY Pleural Fluid

Pt No: \_\_\_\_\_ Initials \_\_\_\_\_

Centre: \_\_\_\_\_

Date: \_\_\_\_\_ Time: \_\_\_\_\_

Timepoint: Visit 3

STRATIFY Sample Handling Manual

## 12. Declaration

I confirm that I have received, read and understood this manual

Name: \_\_\_\_\_

Signature: \_\_\_\_\_

Date: \_\_\_\_\_

Please return this declaration to the Project Manager, CTU Glasgow (see section 2).

**STRATIFY LAT REPORT FORM – MDT SUMMARY**

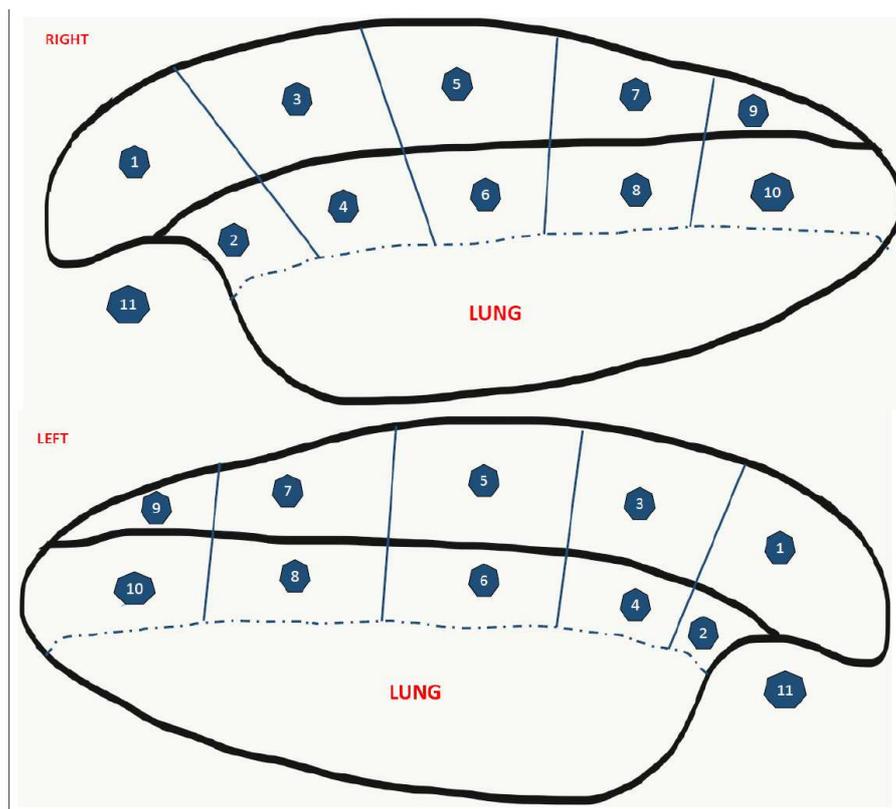
Patient Trial number:

Resp Consultant:

Patient Initials:

LAT Date:

Trial Site:

LAT 1<sup>st</sup> Operator:LAT 2<sup>nd</sup> Operator**Samples Taken:** (NB all sent for urgent processing)

Biopsies taken? Y/N If Y, specify all sites sampled:

Were biopsies taken from visible parietal pleural tumour? Y/N

Fluid sent for cytology: Y/N Volume:

**NOTE:** Fluid is only sent for cytological analysis in patients in whom parietal pleural tumour is visualised. This is to maximise the diagnostic yield of sampling in that context.

The prognostic significance of positive fluid cytology results in patients without parietal pleural tumour is uncertain and may not exceed that of pleural lavage cytology, which would not preclude radical treatment<sup>1,2</sup>.

1. **Lim et al:** Impact of positive pleural lavage cytology on survival in patients having lung resection for non-small cell lung cancer: An international individual patient data meta-analysis (JTCVS, June 2010, Vol 139, Issue 6, Pages 1441-1446)
2. **Lim et al:** Intraoperative pleural lavage cytology is an independent prognostic indicator for staging non-small cell lung cancer (JTCVS, April 2004, Vol 127, Issue 4, pages 1113-1118)