Trial Title: Early Video Assisted Thoracoscopic Surgery (VATS) or Intrapleural Enzyme Therapy (IET) in Pleural Infection - a feasibility randomised trial.

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The key investigators have no conflicts of interests to declare

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

Pleural infection is a serious complication of pneumonia where infected fluid collects around the lung in a large abscess. It can affect anyone, and occurs in 40 patients every day in the UK. Treatment requires antibiotics and drainage of fluid using a chest tube inserted with local anaesthetic between the ribs, and admission to hospital for 2 weeks.

When these treatments fail, patients either die (about 20% of cases) or are referred for major surgery (a further 20%). Surgery is important when initial treatment fails, but has several side effects and is not an option for elderly and sick patients, where the death rate is 40%.

A new treatment (called Intrapleural Enzyme Therapy or IET) can be given through the chest tube early in treatment, which improves drainage and reduces the need for surgery and the time spent in hospital. Keyhole surgery is also now available to drain infected fluid (Video Assisted Thoracoscopic Surgery or VATS), and some people believe that this should occur early in treatment to prevent death and long hospital admissions, but this has not been proven. Early treatment with either IET or VATS may therefore improve care but we do not know the long-term effects (e.g. restriction in breathing) or impact on quality of life.

In this study, we will consult with patients to understand what factors are important to them when treating this disease. This will help us to understand what should be measured in a study to best improve care. We will conduct a study where patients are randomised (assigned by computer) to usual treatment (chest tube and antibiotics), early VATS or early IET. We will measure whether it is acceptable to patients to be randomised in this way and whether a larger study in the future is important and possible.

3. SYNOPSIS

Trial Title	Early Video Assisted Thoracoscopic Surgery (VATS) or Intrapleural Enzyme Therapy (IET) in Pleural Infection - a feasibility randomised trial.	
Internal ref. no. (or short title)	MIST 3	
Clinical Phase	Feasibility	
Trial Design	Randomised trial	
Trial Participants	Adults with pleural infection requiring admission to hospital for antibiotics and chest tube drainage. Defined as: 1) A clinical presentation compatible with pleural infection 2) A pleural collection with a chest drain in situ 3) Has pleural fluid requiring drainage which is either: • purulent **or** • gram stain positive **or** • culture positive **or** • acidic with a pH <7.2 **or** • low pleural fluid glucose (<2mmol / L) in the absence of accurate pH measurement 4) Residual collection/ongoing sepsis after 24h standard care 5) Willing and able to give written informed consent	
Planned Sample Size	Total 75 randomised (25 in each arm); however more participants will be required to be screened to fulfil the randomised requirement	
Treatment duration	Whilst as an inpatient for pleural infection (from 48 hours to 7 days post treatment, whilst an inpatient only)	
Follow up duration	2 months (optional follow up at 6 months) . Any participants randomised post 1st June 2021 will only receive the 2 month follow up visit.	
Planned Trial Period	24 months	
	Objectives	Outcome Measures
Primary	To assess the feasibility of randomising 75 participants with pleural infection to standard care, early VATS or early IET.	Recruitment rate, retention rate and the proportion of participants screened, who consented to be randomised and who consented to be interviewed.
Secondary	1. Explore the risks/benefits from a participant perspective of a referral to standard care, VATS or IET treatment strategy 2. Understand the acceptability of randomisation in a surgery versus non-surgery trial.	1. Conduct structured interviews with a proportion of randomised participants and carers (Oxford recruiting site only); 2. Proportion of participants who accepted/did not accept to be randomised. Conduct structured interviews with a proportion participants to collect information about their concerns and reasons for accepting/not accepting randomisation.

	3. Establish feasibility of collecting accurate long-term (6 month,) outcomes in randomised participants including mortality, hospital stay, readmissions, lung function (optional), further surgery, functional ability, participant reported outcomes and quality of life. 4. Assess feasibility of trial interventions 5. Establish treatment costs including standard care, intrapleural drugs, surgery, initial and subsequent hospitalisation, outpatient, A&E and primary care contacts. 6. Assess which outcomes of pleural infection are most important to the participants; 7. Proportionate adverse events for the intervention arms	3. Review completeness of data collected up to 6 months from randomisation, regarding mortality, length of hospital stay (time from starting intervention until discharge), number of hospital readmissions, completion of lung function tests (FEV1/FVC) (optional), proportion of participants requiring further surgery. Assess the number of qualitative assessments completed such as functional assessments, questionnaires and visual analogue scores. Collect data on quality of life. 4. Record type of surgery (VATS, thoracotomy) and time to surgery (from randomisation to surgery point of surgical intervention) in the surgical arm and details of compliance (proportion initiating treatment/completing treatment/requiring dose reductions/missed doses) in each interventional arm along with the reasons for non-completion. 5. Costs of surgery will be assessed using a micro-costing study evaluating staff time, theatre time and consumables. Other healthcare resource use will be obtained from participants' trial records; hospital records; and participant self-report through questionnaires. Resource use will be costed using appropriate unit costs. 6. Perform structured qualitative interviews with a proportion of participants who have had pleural infection to collect information on their priorities of care. 7. Record agreed adverse events
Investigational Medicinal Product(s) and interventions	1. Recombinant human deoxyribonuclease (DNAse)And Recombinant human tissue plasminogen activator (tPA, Alteplase) 2. Video Assisted Thoracoscopic Surgery	

	3. Chest drain insertion, broad spectrum antibiotics and Intrapleural saline flushes (standard care)
Formulation, Dose, Route of Administration	DNAse 5mg BD (diluted in 30mls sterile water) intrapleural Alteplase 10mg BD (diluted in 30mls sterile water) intrapleural

***Some of the study assessments and visits have been made optional in order to streamline the trial pathway, following the slow recruitment due to COVID-19. This will reduce the data collection burden on sites and focus on the essential data required to meet the study outcomes. The maximum follow up time has been shortened to 2 months to facilitate a 4 month recruitment extension (Apr – Jul 2021) due to COVID-19. The 6 month follow up visit is now optional. Those participants randomised after 1st June 2021 will only be required to have a 2 month follow up visit in keeping with the trial timelines.**

4. ABBREVIATIONS

AE	Adverse event
CI	Chief Investigator
CRF	Case Report Form
CRP	C-reactive Protein
CT	Computerised Tomography Scan
CTRG	Clinical Trials and Research Governance
DNase	Deoxyribonuclease
EPS	Extracellular Polymeric Substance
FEV1	Forced Exploratory Volume
FVC	Forced Vital Capacity
GCP	Good Clinical Practice
GP	General Practitioner
HRA	Health Research Authority
IET	Intrapleural Enzyme Therapy
MIST1	Multi Centre Intra-Pleural Sepsis Trial 1
MIST2	Multi Centre Intra-Pleural Sepsis Trial 2
NHS	National Health Service
ORTU	Oxford Respiratory Trials Unit
PI	Principal Investigator
QoL	Quality of Life

RCT	Randomised Controlled Trial
REC	Research Ethics Committee
SAE	Serious Adverse Event
TPA	Tissue Plasminogen Activator
TSC	Trial Steering Committee
SOP	Standard Operating Procedure
VATS	Video Assisted Thoracic Surgery

5. BACKGROUND AND RATIONALE

Introduction

Infection of the pleural space is common and the incidence is increasing in both adult (1, 2) and paediatric (3) populations. There are currently around 80,000 cases per year in the US and UK (estimated 15,000 new cases per year in the UK). These infections carry a significant health burden; over 35% are fatal or require thoracic surgery (4); 26% of such participants require a hospital admission lasting more than a month (4); the associated estimated cost of care is around £5900 per participant (internal audit data).

Current clinical care

Standard treatment for pleural infection, advocated in guidelines from all major respiratory specialist societies (5, 6), is a combination of appropriate antibiotics and drainage of infected pleural fluid/pus with a chest tube. More complex surgical drainage techniques (e.g. video assisted thoracoscopic surgical pleural drainage, open thoracotomy with decortication, or rib resection and open drainage (5, 6)) is advocated in participants with a "poor likely response to medical therapy", or a poor response to initial treatment. Definitive surgical treatment in selected participants with pleural infection is essential. Pleural infection is a progressive disease with pleural fibrosis developing with time (7); this can prevent effective drainage with the least invasive surgical techniques (VATS), and precipitates the need for open thoracotomy which is associated with higher adverse event rates (see below). Early surgery may be appropriate as the infection is debilitating, and there are progressively increasing anaesthetic and perioperative risk. Previous studies demonstrate that around 60% of participants will respond favourably to medical treatment (4), therefore participants are generally treated with a combination of antibiotics and chest tube drainage initially, with referral for surgical intervention in those who have evidence of ongoing sepsis syndrome despite these treatment measures. This pathway is based on expert opinion rather than empirical evidence and an adequately powered, randomised controlled trial is needed to establish the optimal treatment pathway.

Surgery for pleural infection

The timely use of surgical drainage techniques has been a cornerstone of the treatment of pleural infection for many years (8), and is accepted to be sometimes life-saving. Such treatment is not based on large randomised trials, but large cohort studies such as a recent analysis of 4,424 cases of adult pleural infection suggest effective surgical drainage is associated with improved outcome (1). This data is collected from US 'billing' records, and is affected by reporting and selection bias, but it and other small surgical series (9-13), strongly support the importance of surgery that is advocated by standard treatment guidelines (5, 6).

Some authors have advocated surgery as immediate treatment for all participants with pleural infection (13-15), although two moderate sized clinical trials in children showed no clinical benefit and greater cost

from this more radical approach (16, 17). Early surgery in adults has been advocated in pleural infection (18) on the basis of two randomised studies which compared standard care (antibiotics and chest tube, plus fibrinolytics in one study) to early VATS (19, 20). Both demonstrate earlier hospital discharge and lower mortality with VATS, but are underpowered and methodologically flawed (unclear criteria for medical failure, lack of objective decision-making criteria, no blinding).

The disadvantages of surgical drainage are substantial and preclude its use in all participants. Surgical thoracic procedures carry associated anaesthetic/perioperative risks (21) (operative mortality ~2%, major complication rate ~8% in reported VATs series), and thoracotomy also causes substantial post-operative pain. 61% of participants experience some pain at one year after surgery and 3-5% describe this as severe (22). 66% of participants require analgesia at six months and 38% of participants still have pain 3 years after surgery, falling to 30% at 4 years (23). Video assisted thoracoscopic (VATS) drainage significantly improves on this adverse event rate. However, 4% of participants still experience significant pain at 2 years (24), and a proportion (reported variously from 8 to 59% (25-27)) of VATS procedures require conversion to open thoracotomy at the time of surgery, with the attendant increase in morbidity.

Despite the likely benefit of surgery in selected participants, there is evidence to suggest that older participants with more co-morbidity receive less access to this treatment (perhaps because of concerns about anaesthetic / perioperative risk). We have collated surgical empyema case series from the UK (13, 28-31) and US (1, 10, 32-37) (including a very large recent cohort (1)), and demonstrated that the typical age of participants in these series is 49.5 years in the UK and 52.6 years in the USA. This is significantly below the median age of 61 years seen in an unselected and well documented UK sample of 454 participants (unpublished data) (4). Within this sample, those who received surgery were significantly younger with less co-morbidity than the group as a whole (surgery group age 52.5 SD 16.0 years, non-surgical group age 61.6 SD 17.6 years, difference 9 years, 95% CI: 4.8 to 13.2, $p<0.001$, unpaired t-test). This age threshold is associated with a large difference in mortality (no. of deaths in participants aged <60 = 11/212 (5.2%), deaths in those >60 = 87/242 (36%), difference = 30.8%, 95% CI: 24 to 37.5%, $p<0.001$. OR for death by age cut-off = 10.3, 95% CI: 5.3 to 19.9).

Thus, surgical drainage of pleural infection remains a vital therapy in those not responding to medical treatment, but whether it has a role earlier in the treatment pathway is, as yet, unclear. It is possible that early surgery will result in better outcomes in the short and longer term, and that this vital therapy is avoided in those who may need it most (such as the elderly).

Reasons for failed medical therapy

Standard 'medical' therapy for pleural infection (chest tube drainage and antibiotics) often fails; this may be due to a number of reasons:

1. The presence of thick infected pleural fluid which cannot easily drain down the pleural catheter. Infected fluid is thick due to free, uncoiled, DNA liberated from dead leukocytes that forms 'tangles' in the abscess fluid, creating a gel.
2. The presence of locules which partition the fluid into separate and undrainable pockets. Locules are due to the development of fibrinous septations within the infected collection.
3. The presence of resistant collections of infecting organisms in bacterial structures known as "biofilms". A biofilm is a community of micro-organisms attached to a surface, producing extracellular polymeric substance (EPS). The organisms exhibit an altered phenotype compared with their corresponding planktonic cells and the EPS is a complex matrix, made up of both fibrin and free DNA, which serves as a storage facility for nutrients and entraps other microbes and

non-cellular materials. Biofilm bacterial cells withstand host immune responses, and are much less susceptible to antibiotics than their non-attached individual planktonic counterparts.

Potentially, each of these problems is amenable to therapeutic intervention with intrapleural adjunctive therapies. The fibrinous septations can be disrupted by fibrinolytic agents, the thick pleural fluid can be thinned with Deoxyribonuclease (DNase) and there is ex-vivo experimental data in support of DNase as a biofilm disruptor and an agent capable of decreasing biofilm formation in a number of bacterial infections, including several key microbiological organisms in pleural infection (*Strep Pneumonia*, *Enterococcus*, *Staph Aureus*, *Staph Epidermidis* and *Pseudomonas Aeruginosa*) (38-42).

Evidence for intrapleural adjunctive therapies

For many years, intrapleural streptokinase alone was advocated as a treatment with which to improve drainage from infected pleural collections. Case series and small randomised studies suggested improved drainage with streptokinase. However, the largest randomised study to date (MIST1 (4)) including 454 participants, and a meta-analysis of the 5 methodologically sound fibrinolytic studies (43) suggested no benefit from the use of intrapleural fibrinolytic on important clinical outcomes.

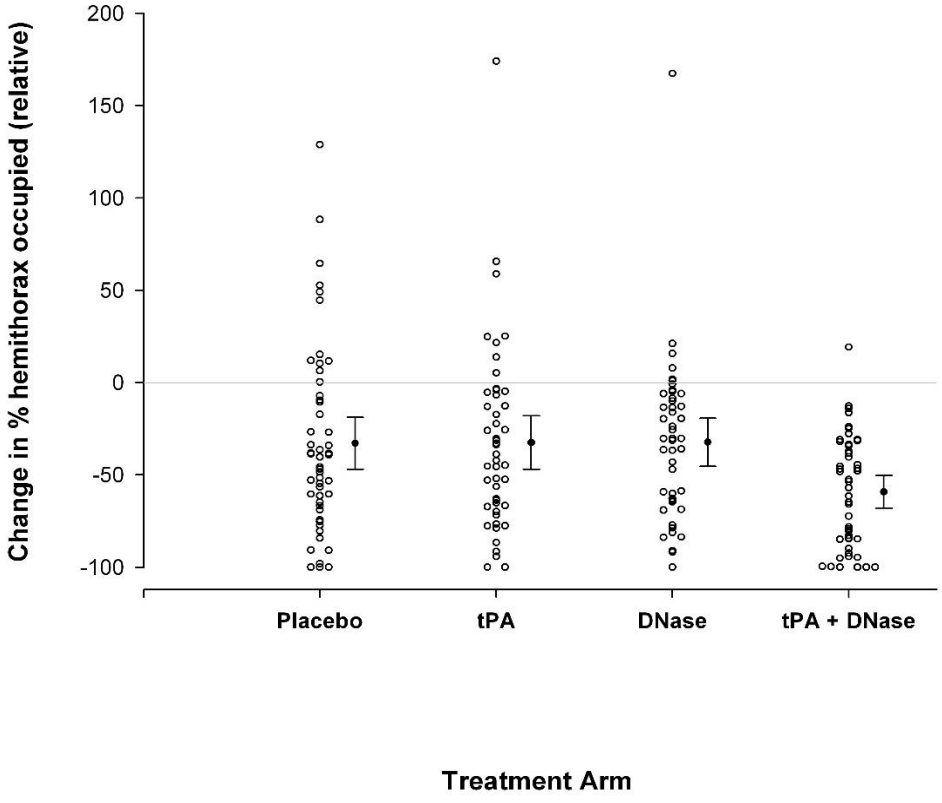

Figure 1. Primary outcome measure (radiographic improvement) from the MIST2 study.

On the basis of this negative result, the MIST2 (44) study was conducted as an initial randomised assessment of the use of combination fibrinolytic (tPA) with intrapleural DNase. 210 participants were randomised in a 2 x 2 factorial double blind placebo controlled study, with radiographic drainage as the

primary outcome measure. The results of this study demonstrated that tPA alone or DNase alone were no better than placebo in improving the chest radiograph. However, combination therapy (tPA + DNase) resulted in significant treatment interaction and was significantly better than placebo in improving the chest radiograph (relative improvement in % hemithorax occupied by pleural fluid versus placebo = 22.8%, 95% CI: 7.1 to 28.9, p=0.002) (Figure 1). This treatment effect appeared to be independent of pleural fluid purulence, which was a minimisation factor for the study, well balanced between the treatment arms and a pre-planned subgroup analysis (p value for interaction between pleural fluid purulence and treatment effect = 0.95).

The improvement in the primary outcome measure was associated with strong signals toward an improvement in some clinically important outcomes which were secondary outcome measures for the purposes of the MIST2 study. There was evidence at 3 months post randomization that combination (tPA + DNase) treatment was associated with a decrease in surgical rate (placebo surgical rate 9/50 (18.0%), tPA + DNase surgical rate 2/47 (4.3%), estimated odds ratio for surgery vs placebo = 0.20, 95% CI: 0.02 to 1.02, p=0.052). This suggests that although MIST2 was underpowered to accurately assess change in surgical outcome, combination tPA + DNase therapy was associated with a potentially large reduction in need for surgery. This reduction, if proven in a larger study, would be highly clinically relevant (80% reduction in surgical rate) and potentially decrease treatment costs and morbidity for this disease.

In addition, combination tPA + DNase therapy was associated with a reduction in hospital stay compared to placebo (duration of hospital stay in days; placebo mean 14.9 days (SD 14.6), combination mean 11.0 (SD 9.4), difference -4.8 days, 95% CI: -10.4 to 0.1, p=0.06). The MIST2 study was not powered to accurately estimate this treatment effect, but if real would represent a substantial decrease in hospital stay (30% absolute reduction) with the attendant savings in cost and morbidity.

The cost of combination tPA + DNase treatment is not trivial, estimated at around £960 per participant. However, should the decrease in surgical rate and decrease in hospital stay prove to be true in larger trials, there are potential cost savings using this treatment.

Thus, intrapleural combination tPA + DNase therapy has been shown to improve the standard clinically used surrogate (chest radiograph) in pleural infection, and may have important beneficial effects on reducing surgery rate and hospital stay.

Rationale for this study

Assessing the early use of VATS or IET requires a phase III randomised controlled trial to directly compare the early introduction of these treatments to conventional care. The recent MIST 2 trial concluded that IET improves drainage and reduces the need for surgery and hospital stay but has not been compared directly with surgery. The study will also take into account the selection bias of previous trials and will randomise all participants enrolled, despite fitness for surgery, to any treatment arm. The analysis will be performed as per intention to treat, despite a proportion of participants in the surgical treatment arm who are likely not to be fit enough to undergo surgical intervention.

Before undertaking a large trial it is important to establish the key outcome measures which are important to participants to allow for relevant outcomes in a subsequent randomised controlled trial (RCT). Information also needs to be collected on feasibility of recruitment, participant acceptability and the ability

to collect outcome data. The trial proposed will address the feasibility of randomising participants to standard care, early VATS or early IET by undertaking qualitative interviews both with a proportion of participants who have participated in the trial but also with those who have refused.

This combination of outcomes and objectives will establish whether a larger RCT can be undertaken with participant focussed outcome measures established through detailed interviews with people who have been directly involved in any process of care for a pleural infection.

PPI Input and Feedback

A PPI group has been convened for the trial. An introductory meeting took place in October 2017 to gather the views and thoughts of patients, partners and carers who have undergone similar treatment to the MIST3 trial. This proved to be an extremely positive day with lots of interesting feedback. Consequently these views were taken into account in the writing of this protocol and the accompanying trial paperwork. Since then, the PPI group met again when the trial paperwork was established.

The trial team explained the rationale of the trial and invited the group to ask questions and provide feedback. The group were given the document pack to take away and comment on. The majority of comments were returned informing the trial team a questionnaire chosen to ask participants during the trial was felt not to be suitable due to limited ability, therefore this has now been replaced. We plan to meet on a frequent basis throughout the course of the trial.

6. OBJECTIVES AND OUTCOME MEASURES

	Objectives	Outcome Measures
Primary	To assess the feasibility of randomising 75 participants with pleural infection to standard care, early VATS or early IET.	Recruitment rate, retention rate and the proportion of participants screened, who consented to be randomised, who consented to be interviewed.
Secondary	1. Explore the risks/benefits from a participant/carer perspective of a referral to standard care, VATS or IET treatment strategy as well as which outcomes of pleural infection are most important to the participants;	1. Perform structured qualitative interviews with a selection of participants who have had pleural infection and their carers (carer interviews at Oxford recruiting site only)
	2. Understand the acceptability of randomisation in a surgery versus non-surgery trial.	2. Proportion of participants who accepted/did not accept to be randomised. Conduct structured interviews with a proportion of of participants to collect information about their concerns and reasons for accepting/not accepting randomisation.
	3. Establish feasibility of collecting accurate long-term (6	3. Review completeness of data collected up to 6 months from

	month) outcomes in randomised participants including mortality, hospital stay, readmissions, lung function (optional), further surgery, functional ability, participant reported outcomes and quality of life.	randomisation, regarding mortality, length of hospital stay (time from starting intervention until discharge), number of hospital readmissions, completion of lung function tests (FEV1/FVC) (optional), proportion of participants requiring further surgery. Assess the number of qualitative assessments completed such as functional assessments, questionnaires and visual analogue scores. Collect data on quality of life.
	4. Assess feasibility of trial interventions	4. Record type of surgery (VATS, thoracotomy) and time surgery (from randomisation to surgery point of surgical intervention) in the surgical arm and details of compliance (proportion initiating treatment/completing treatment/requiring dose reductions/missed doses)in each interventional arm along with the reasons for non-completion.
	5. Establish treatment costs including standard care, intrapleural drugs, surgery, initial and subsequent hospitalisation, outpatient, A&E and primary care contacts.	5. Costs of surgery will be assessed using a micro-costing study evaluating staff time, theatre time and consumables. Other healthcare resource use will be obtained from participants' trial records; hospital records; and participant self-report through questionnaires. Resource use will be costed using appropriate unit costs.
	6. Assess which outcomes of pleural infection are most important to the participants;	6. Perform structured qualitative interviews with a proportion of participants who have had pleural infection to collect information on their priorities of care.
	7. Proportion of adverse events for the intervention arms	7. Record agreed adverse events

7. TRIAL DESIGN

Multi-centre, open-label, randomised three-arm parallel arm, feasibility study to determine whether randomising participants to standard care, intrapleural enzyme therapy or early VATS is possible and

acceptable to participants with pleural infection and a prospective cohort of participants refusing randomisation.

Design and Randomisation

Participant Population
Participants will be approached initially by the clinical team as in-patients, who are suspected of having pleural infection. The aim is to enrol all participants with pleural infection and then assess who would be willing to undergo randomisation. It will be explained to participants that, if pleural infection is confirmed, they will be randomised to receive either referral to standard care, referral for IET or referral for Early VATS (as per the local surgeon's clinical view) via the agreed pathways.

Confirmation of diagnosis and randomisation
Participants may be consented prior to pathological confirmation of pleural infection as the diagnostic procedure is often performed at the same time as a chest drain is inserted, but randomisation will only occur once pathological / radiological confirmation has been obtained, with randomisation occurring within 24 hours of confirmation of diagnosis.

As fluid may drain effectively after initial drain insertion, it is permitted, according to local investigator preference, to wait for an initial drainage period before offering entry to the trial (which includes up to 24 hours as above). All participants will initially receive a small bore chest tube (<15F) and antibiotics once diagnosis is confirmed (standard care as per current national guidelines) to prevent a delay in treatment initiation and those participants in whom drainage occurs successfully will not be randomised (and not counted towards the denominator for this feasibility study), but outcomes kept with their consent.

Follow up
All participants randomised will be carefully followed up as per the follow up schedule and outcomes collected in order to permit assessment of the feasibility of randomisation and trial recruitment, and retention through until final follow up. Factors which affect acceptance of randomisation will be explored by specific structured interviews in a proportion of participants randomised/not randomised during the trial period. However, in the participants who decline randomisation and interview but consent to follow up, this will be restricted to a short telephone call at 2 weeks asking the participant their reasons for declining randomisation, and a further telephone follow up call at 2 months to document death or need for surgery. These telephone calls will be conducted by the sites and recorded on the specific CRF.

Some participants may be considered to require immediate surgery (for example, in the presence of solid pleural material on ultrasound where the physician does not consider a chest tube drainage attempt would be reasonable). Similarly some patients may drain effectively within 24 hours. The frequency of this scenario will be captured on the screening logs but they will not be randomised and will not be followed up as part of the trial.

Surgical and IET Exclusions
Specific consideration was given to the possibility of excluding participants who are considered "unfit for surgical intervention" or who may be "unsuitable for IET" from this randomised trial. However, including these participants is particularly important in this study for a number of reasons. Firstly, the study is assessing the feasibility and acceptability of randomising to surgical versus IET versus standard treatment, rather than the actual performance of surgery or IET. Secondly, IET has the specific advantage over surgery that it is applicable to "all comers" with pleural infection (25), including the frail and elderly in whom clinical outcomes are the poorest, but IET may not be used in certain circumstances where surgery is preferable (for example, in those with major haemorrhage). As we envisage the larger phase III

trial to include all comers with pleural infection, inclusion of all participants in the feasibility study is therefore scientifically required for consistency.

This study therefore randomises participants for a surgical opinion (rather than for surgical intervention), with the receiving surgeon deciding on what intervention (if any) is required or possible. Surgical intervention will be according to the surgical SOP developed by the trial team. Similarly, participants in the IET arm will be randomised to "IET intended treatment" with the local physician considering if it is safe to give this treatment. All analysis will be by intention to treat.

Interventions for randomised participants
Participants will be randomised 1:1:1 to the three treatment arms.

1. Standard Care
As per current treatment guidelines (BTS 2010 (5)), participants will be admitted to hospital and started on broad spectrum antibiotics as per local guidelines and until results of any positive microbiology. A chest tube (minimum 12F in size) will be inserted using image guidance and local anaesthetic, and the participant will be monitored with radiology, blood and clinical parameters to assess for treatment failure. This will be assessed at 3-5 days and be according to objective decision making criteria which will be documented (please see below).

As not all participants with pleural infection are considered fit enough to undergo surgical intervention, objective criteria for "medical treatment failure" will be recorded in all cases using objective criteria. These will be measured at 3-5 days post study inclusion, will be recorded on the CRFs, and are:

- The presence of a residual and clinically significant pleural collection as judged by the local PI, based on current radiology (chest radiograph, ultrasound and/or CT); and at least one of the following:

1) Clinical evidence of ongoing sepsis as manifested by factors such as otherwise unexplained persistent fever, tachycardia and hypotension (on clinical discretion)
2) A serum CRP (C-reactive protein) that fails to fall by more than or equal to 50% compared to the baseline value prior to initiation of medical treatment
3) A lack of significant response in the peripheral blood white-cell count as judged by the local investigator.

Standard care is received by thousands of patients in the UK each year with a mean inpatient hospital stay of 5 days before consideration of additional treatments in the form of IET or surgery. Most patients will require chest tube drainage with regular saline flushes for the duration of this period. All of these patients will have access to additional treatments if medical treatment failure is confirmed, as defined by the criteria above, as is normal care.

*** Crossovers from the standard care arm to the IET arm or VATS arm will be permitted once participants have been deemed to require additional treatment after a further 48 hours of standard care.** Any crossover prior to completing a further 48 hours of standard care post randomisation would constitute a protocol deviation.

2. IET arm
Through the chest tube inserted during usual clinical care, intrapleural tPA (10mg bd) and DNase (5mg bd) will be administered as per our previous randomised trial protocol (44) 12 hourly over 72 hours, to start as soon as possible after randomisation as per recruiting sites' local administration protocols.

Sites will be able to determine doses on a participant by participant basis but must not exceed these doses. Centres will be permitted to use lower doses than this as per their local guidelines, and doses will be recorded on the CRFs. Recent studies have demonstrated the safety and feasibility of administering the two agents in a single session (i.e. DNase and tPA in one intrapleural administration, followed by 1 hour of clamping, then repeating the procedure 12 hourly) and this will be the schedule used in this randomised trial, to ease pragmatic delivery of the protocol.

Some participants may not be considered suitable to undergo IET treatment – the reasons for this will be recorded in the CRFs and the participants will remain in this treatment group. In which case after a further 48 hours of standard care, if still deemed to be required additional therapy, these patients can be offered alternative intervention including large volume saline pleural irrigation therapy or surgical treatment as clinically necessary. Any crossover prior to completing a further 48 hours of standard care post randomisation would constitute a protocol deviation.

3. Early VATS arm
Participants assigned to VATS will be referred immediately post randomisation to local surgical services, and VATS conducted according to standard surgical standards (defined as a trial specific instruction for this trial). As above, the decision on requirement for and safety of conducting VATS will be at the discretion of the receiving surgeon, and according to the surgical SOP. Variation in timing of surgery, surgical bed and operation room availability (from randomisation to surgical event), and the proportion of participants considered "fit" enough for surgery on surgical review (i.e. the number who actually undergo a surgical procedure) will be collected as part of the study, as these variations are key outcomes of this trial.

Not all hospitals have access to surgery in the same hospital, and these participants will need to be transferred to achieve a surgical treatment – hence the rationale of minimising by centre to ensure that balance is achieved across the randomised groups across all centres. All participants in the VATS arm will be referred for prompt surgical review; if the participant is considered not fit for surgery, the surgical team will dictate further management which may include a number of treatments (including for example an increase in the size of the chest tube). If after 48h no treatment on the surgical TSP has been found to be suitable, these patients may continue on the standard care arm with interventions such as large volume saline pleural irrigation IET may be given if no other treatment is deemed clinically appropriate. If required, these patients can be discussed with the trial team.

***In the event of disruption or restriction of surgical services due to COVID-19 pressures, eligible patients can still be randomised. If they are allocated to the surgical arm, and receive a prompt and favourable surgical opinion i.e. early VATS would have been feasible outside of COVID then please indicate this on the CRF.**

Standard Treatment in all arms
In the IET and "control" arms, the size of the chest drain inserted is at the discretion of the local clinicians but at least 12F in size is generally recommended. To ensure high quality care, all participants will be treated with antibiotics according to microbiological sensitivities (where available – estimated positive cultures in 60% of cases according to our previous published data (28)) and with empirical antibiotic therapy according to local prevalence and national guidelines (8). All participants will be treated with thromboprophylaxis and supported nutritionally according to best practice, guided by standard operating procedures which will be written for this study.

The use of imaging (such as thoracic CT or ultrasound) is at the discretion of the local physician/surgeon, but it is recommended that all participants planned for surgery undergo a CT prior to VATS.

In the IET and standard treatment arms, if there is insufficient clinical response on the objective "medical failure" criteria listed above at 72 hours post randomisation, surgical referral as per national guidelines is recommended, and will be recorded on the inpatient CRF, including type of surgery undertaken.

Data collection
Data collection will be performed by the research team on the participant's clinical condition, pathology results and outcomes. The participant's radiology will be anonymised and transferred to Oxford as part of the analysis. Participants will complete questionnaires, supported by members of the research team when necessary. Pleural fluid samples will be collected and sent to Oxford for analysis. All data will be identified by a unique patient identifier.

Follow up (post discharge)
Follow up visits will be undertaken alongside normal clinical care. This is commonly

- Within the first 2 (</+ 2 week) weeks post discharge, (face to face recommended)
- At approximately 2 months (+/- 2 weeks) (face to face optional)
- Optional 6 months (</+ 2 week)

Specific to this trial, a follow up point at 2 weeks is suggested post discharge / intervention to assess response to ongoing antibiotic therapy. The responsible clinician is permitted to stop antibiotic therapy at the two week follow up point if adequate response (regardless of assigned treatment group), with a general recommendation for 4 to 6 weeks of antibiotic treatment. If the participant is deemed to be progressing well and would clinically not require any further face-to-face follow up, in light of the COVID-19 pandemic, it would be reasonable to conduct further follow ups (i.e. at 2 months and 6 months) remotely.

In-depth Participant Interviews
Qualitative interviews will be performed on a proportion of participantsafter the participant has recovered from their acute illness regarding their priorities of care. These interviews will either be performed by trained members of the ORTU team or by Oxford Brookes University. In addition a proportion of those participants, who refused randomisation but consented to be interviewed, will also be approached to take part, and, any themes arising from these two groups will be incorporated into the design of the subsequent randomised controlled trial. A proportion of carers from Oxford participants randomised or refused randomisation but consented to interview will also be approached. All interviews will be performed by a trained member of staff based at the Oxford Respiratory Trials Unit/Oxford Brookes University, the interviews will be performed either face to face, over the phone or via Skype. The interviews will be audio recorded and these recordings will be stored electronically on the ORTU network drive. Interviews performed by Oxford Brookes University will be transferred to ORTU via Oxfile. Audio files will be sent securely to a professional transcription company, with whom the University has a contracts and confidentiality agreements. The transcriptions will be anonymised and the transcriptionist will delete the recording when they have completed their work and returned the transcript.

8. PARTICIPANT IDENTIFICATION

8.1. Trial Participants

All participants with pleural infection fulfilling the inclusion / exclusion criteria are eligible for the trial. Screening logs will be kept, documenting reasons for non-inclusions.

8.2. Inclusion Criteria

1) A clinical presentation compatible with pleural infection AND
2) A pleural collection with a chest drain in situ

3) Has pleural fluid requiring drainage which is either:
    - purulent **or**
    - gram stain positive **or**
    - culture positive **or**
    - acidic with a pH <7.2 **or**
    - low pleural fluid glucose (<2mmol / L) in the absence of accurate pH measurement **or** septated pleural fluid on ultrasound which is likely secondary to pleural infection (on the basis of local investigator view).
4) Residual collection or ongoing sepsis after 24 hours of standard care
5) Willing and able to give written informed consent

8.3. Exclusion Criteria

- Age <18 years
- Pleural collection not amenable to chest tube drainage
- Chest tube already in place for >= 72 hours
- Has previously received intra-pleural fibrinolytics and /or DNase for this empyema
- Has a known sensitivity to DNase or tissue plasminogen activator
- Has had a previous pneumonectomy on the side of infection
- Participants who are pregnant or lactating
- Estimated survival less than three months from a different pathology to this empyema, (e.g. metastatic lung carcinoma)

9. TRIAL PROCEDURES

9.1. Recruitment

Participants with either confirmed or suspected pleural infection will be identified by any member of the clinical team. Due to the nature of the trial, the participants will all be under inpatient care at the time and can be offered participation early in their admission. The clinical team will approach participants and either the clinical or research team will then provide the participant with the participant information sheet and be available to answer any questions. Participants will be identified through respiratory and general wards or from outpatient referrals, clinics and ambulatory care.

9.2. Screening and Eligibility Assessment

There is no maximum duration between screening and randomisation but due to the nature of the disease, treatment must not be delayed, so it is likely that participants will have less than 24 hours to consider enrolment. Day 0 should be considered as being **first contact** with the PI team (</=3 days from first signs of pleural infection), and a decision to randomise needs to be made by the end of Day 1. If the participant remains eligible and the drain stays in, then randomisation is possible. The pleural fluid samples which are necessary to confirm eligibility are taken as part of clinical care and are not trial specific and thus do not require prior consent. If a participant is consented prior to pleural fluid samples being obtained these samples will be transferred to the central site for storage and analysis as per the consent form.

9.3. Informed Consent

Consent can be obtained **prior** to confirmation of pleural infection in participants who are likely to have a pleural aspiration and chest drain insertion in the same procedure. These participants will be randomised

once the eligibility criteria have been confirmed. If pleural infection is not confirmed the participants will not need to participate further in the trial.

The participant must personally sign and date the latest approved version of the Informed Consent form before any trial specific procedures are performed.

Written and verbal versions of the Participant Information and Informed Consent will be presented to the participants detailing no less than: the exact nature of the trial; 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 under no obligation to take part in the study and is free to withdraw from the trial at any time for any reason without prejudice to future care, without affecting their legal rights and with no obligation to give the reason for withdrawal.

Although it is usually a requirement in clinical studies that a participant is offered 24 hours in which to decide whether to take part in a study, the nature of the disease process in question (pleural infection) and the intervention (intrapleural agents which improve drainage of infected material) suggest that delay of more than a few hours in administering the medication may be detrimental to participant care. On this basis, a shortenedd period of reflection will be offered to participants considering participation in the study, although no form of coercion or pressure will be used. This strategy has proved robust in previous clinical studies of pleural infection (MIST1 and MIST2) and will be specifically addressed in the ethics application.

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. The person who obtained the consent must be suitably qualified and experienced, and have been authorised to do so by the Principal Investigator and have been delegated this responsibility. A copy of the signed Informed Consent will be given to the participant. The original signed form will be retained at the trial site with a copy emailed to ORTU to a trial specific inbox.

9.4. Randomisation, blinding and code-breaking

Randomisation will occur via a web-based system with minimisation for centre and a validated score of risk in pleural infection (the RAPID score, scored in 3 categories = low, moderate and high).

Randomisation will occur once pleural infection has been confirmed by the documented inclusion criteria. This may occur after the initial aspiration or once a chest drain has been inserted.

Participants will be randomised 1:1:1 to standard care, IET or early VATS surgery. All participants will require chest tube insertion, therefore randomisation can occur after tube insertion (up to 24 hours post insertion).

The trial will not be blinded so no un-blinding procedures are required.

9.5. Baseline Assessments

***Some of the study assessments and visits have been made optional in order to streamline the trial pathway, following the slow recruitment due to COVID-19. This will reduce the data collection burden on sites and focus on the essential data required to meet the study outcomes. The maximum follow up time has been shortened to 2 months to facilitate a 4 month recruitment extension (Apr – Jul 2021) due to COVID-19. The 6 month follow up visit is now optional. Those participants randomised after 1st June 2021 will only be required to have a 2 month follow up visit in keeping with the trial timelines.**

1. Baseline data collected will include:

1. Participant demographics including co-morbidities (at enrolment)
2. Recent blood test results as part of usual clinical care including RAPID parameters where available (within 1 week) (see trial specific instructions)
3. Recent radiology results (within 1 week)
4. Details of the symptoms the participant has had for the current pleural infection (at enrolment)
5. Details of the treatment the participant has had for the current pleural infection (at enrolment)
6. Previous spirometry if available (within 12 months)(optional)
7. Details of any previous intrapleural treatment or thoracic surgery
8. Ultrasound findings (one image at enrolment)
9. Vital signs (first set of observations recorded in hospital including blood pressure, heart rate, temperature, respiratory rate and oxygen requirement)
10. Patient weight (in kilograms)

2. Initial intervention

All participants will have 20mls of blood and 20mls of pleural fluid taken for standard care, and 20mls of blood and 20mls of pleural fluid to be sent to the coordinating centre for storage future use with the participants consent (i.e. total of 40mls blood and 40mls pleural fluid)*. These samples should be taken on the day of enrolment (+24 hours if needed).

***As a result of the COVID-19 pandemic, laboratory processing of research samples has been suspended. Therefore, no research samples for future storage are being collected currently. Once restrictions are lifted these will be reinstated. The time period of suspension will be documented within the trial master file.**

Data should be collected on:

1. Fluid purulence
2. Biochemical results (including LDH, pH and glucose)
3. Chest drain size (if required)
4. Pleural fluid microbiology results (gram stain and culture) (once only)
5. Blood culture results (if available) (once only)

9.6. Subsequent Visits

1. The initial inpatient period

Data should be collected on:

1. Cumulative volume of pleural fluid drainage
2. Blood results including renal function and inflammatory markers – see below for frequency
3. Antibiotic treatment
4. Duration of drainage
5. Any chest tube displacement or blockage
6. Details of trial procedure – e.g. whether all intrapleural treatment was completed, any missed doses, date and type of surgery, reason surgical intervention was not undertaken, time from randomisation until surgery
7. Details of subsequent pleural interventions
8. Requirement for surgery due to treatment failure on objective criteria
9. Adverse events (for surgery using the modified Clavien-Dando classification, and all others on standard criteria)
10. Pain score (100mm VAS) every day until chest drain removal and at discharge (optional).
11. IPAQ-S7S and EQ-5D-5L questionnaires*
12. Hospital Anxiety and Depression Scale (HADS) score (once only - within 72 hours of admission)*

*These questionnaires can be completed remotely (over the phone) to minimise patient contact in light of the COVID-19 pandemic

Clinical assessments will be conducted by a member of the clinical or research team.

The ultrasound image can either be a baseline image (prior to drain insertion) or randomisation image (showing residual collection following initial period of drainage prior to randomisation). This should be captured and uploaded onto the image CRF.. Chest x-rays may be performed at varying points throughout admission to guide clinical care. For the purposes of the study, as a minimum, 2 chest x-rays are required – the admission chest x-ray (day 0) and the last chest x-ray prior to discharge (appropriately labelled 'day X' when labelled onto image CRF).

Blood tests including inflammatory markers are to be taken aspart of routine clinical care, and therefore are not specifically required for the trial if not clinically indicated. These tests will then be repeated at outpatient follow up appointments as detailed in the trial flow chart (see Appendix A).

VAS booklets will be completed once a day by the participant (optional).

The data collection should last until drain removal or day 7, if chest drain still in situ.

2. Discharge

Length of initial hospital stay from diagnosis to discharge including any social care through patients' Electronic Patient Records (EPR), and information should be also be collected on specialty wards, diagnoses and procedure codes.

At discharge, data will be collected on treatment received and completed, death as well as whether or not any serious adverse events occurred, related to pleural infection. Spirometry and pain score (100mm VAS) should be performed at the time of discharge*. If spirometry is not performed for any reason, this should be recorded on the discharge CRF.

Participants will ideally be provided with the Home VAS booklet questionnaire at discharge*. As an alternative, this can be posted out to the participant following discharge.

*Inpatient VAS, Home VAS and spirometry are optional but encouraged where possible

3. Qualitative Interviews

Participants (all approached during the study who agree to be randomised, or agree to be interviewed) and their carers (Oxford only - with consent) will be approached for participation in qualitative interviews regarding their experiences during the trial or their reasons for refusing randomisation. This will aim to establish priorities of care and therefore important outcomes in the planned multicentre randomised controlled trial. It is anticipated that the interviews will not take place until the participant is discharged and appropriately recovered (i.e. at one of the early out-patient reviews). The interviews will be undertaken by members of the research team trained in qualitative methodology.

4. Follow up Visits post randomisation

Follow up will occur at 2 weeks, 2 months, and then at 6 months. +/- 2 weeks for all visits.
Data collected will be:

1. Height to be measured at 2 week follow up*(optional)
2. Weight (at each visit)*(optional)
3. Spirometry (FEV1 and FVC) at 2 weeks and 6 month follow up*∞(optional)
4. Duration of antibiotic therapy in total since discharge from hospital
5. Further hospital admission(s)
6. Date of death (if applicable)
7. Details if participant suffered side effects possibly attributable to the trial intervention since initial hospital discharge
8. Further interventions needed, including further surgery
9. Evidence of malignancy
10. Exercise ability (via the IPAQ-S7S questionnaire) .
11. Specific questions suggested by the MIST3 participant group, including:
    a. Do you feel back to normal?
    b. Time to return to normal work / function at home
12. Generic health-related quality of life (QoL) as measured using the Euroqol 5 Dimensions 5 Levels (EQ-5D-5L) questionnaire.
13. Information on subsequent hospitalisations (including ward transfers, and diagnoses and procedure codes will be obtained from participants' EPR records. Information on out-patient, A&E and primary care contacts will be obtained from participant questionnaires administered at each follow-up).
14. Chest x-ray will be performed at all visits as part of standard care*

Participants will be asked to complete a Home VAS booklet once a week post discharge until their 2 month follow up appointment (optional).

It is preferable if follow ups to 2 months occur face to face, to allow assessments such as chest x-ray and ultrasound. If this is not possible an attempt will be made to contact the participant to complete the follow up CRF by telephone. A 6 month telephone follow up is optional.

*These will not be expected if the follow up appointment was conducted remotely. For all other data items, these should be obtainable remotely.

∞ Spirometry may not be available due to the COVID-19 pandemic. If it is not possible to be performed this should be recorded in the CRF.

9.7 Sample Handling

Samples for routine clinical care will be conducted as per local hospital practice.

The additional 20mls of blood and pleural fluid will be put in to transport tubes and sent to the coordinating centre (as per a trial specific procedure) and process / stored as per established Oxford Respiratory Trials Unit Standard Operating Procedures, and will be stored for future research separate to this protocol with the consent of the participant.

As a result of the COVID-19 pandemic, laboratory processing of research samples has been suspended. Therefore, no research samples for future storage are being collected currently. Once restrictions are lifted these will be reinstated. The time period of suspension will be documented within the trial master file.

9.8 Discontinuation/Withdrawal of Participants from Trial Treatment

During the course of the study a participant may choose to withdraw early from the study treatment at any time. This may happen for several reasons, including but not limited to:

- The occurrence of what the participant perceives as an intolerable AE.
- Inability to comply with study procedures
- Participant decision

Participants may choose to stop treatment and/or study assessments but may remain on study follow-up.

Participants may also withdraw their consent, meaning that they wish to withdraw from the study completely.

Participants have the right to withdraw from the trial at any time without having to give a reason and this will not affect their future care.

a) Withdrawal of a participant from the trial should be under the guidance of the principal investigator (in liaison with the ORTU team as appropriate). Withdrawal details will be recorded on the relevant CRF.

b) Participants are <u>only</u> withdrawn if they specifically request no further data collection. In the event of participants not wishing to attend visits, or to discontinue treatment, they are not considered withdrawn but this will be recorded as a file note/protocol deviation. Should a participant decide to withdraw, all efforts will be made to complete and report the observations as thoroughly as possible.

c) For participants moving from the area, every effort should be made for the participant to be followed up at another centre, or for follow up via GP.

d) Participants have a right to request the destruction of samples upon request.

9.9 Definition of End of Trial

Trial closure will either be when the last medical note review is performed at 12 months or at the direction of the Trial Steering Committee (TSC)

10 Trial Intervention

10.1 Treatment Description

Recombinant human deoxyribonuclease is a sterile solution already licensed for use in nebulised form for the reduction of sputum viscosity in participants with cystic fibrosis. The standard dose is 2.5 to 5mg once or twice daily. It is well tolerated; rash, voice alteration, chest pain and laryngitis are the main reported side effects when administered as in inhaled solution. In animal studies it appears to be well tolerated in inhalation doses 180-fold higher than routinely used doses. It requires storage at 2-8^{O}C.

Recombinant human tissue plasminogen activator (tPA, Alteplase) is already licensed for use in myocardial infarction. The standard dose is <100mg. With this use, its main side effects are the risk of systemic bleeding associated with systemic fibrinolytics. With intra-pleural use, such adverse events are not reported and another fibrinolytic (Streptokinase), with a similar adverse event profile when used systemically, does not cause an excess of bleeding when used in the pleural space.

Use of combination tPA + DNase in the MIST2 study was not associated with an increased incidence of serious adverse events compared to either placebo or individual DNase or tPA. Bleeding events were captured as serious adverse events for the purposes of the MIST2 study, and no excess of bleeding events was seen compared to placebo in any group.

The solutions will be made up and administered by clinical staff as per local protocols.

10.2 Storage of Trial Treatment

Trial medication for this trial will be from the usual clinical supplies used in hospitals taking part in this trial (the MIST2 regime is used as standard care in selected patients in all the recruiting centres). Each course of trial treatments will be pre-prepared and dispensed to the ward as per local guidelines and the normal use of these medications.

10.3 Compliance with Trial Treatment

All the trial treatments will be administered whilst the participant is in hospital so it will be possible to accurately document participant compliance. If there are compliance issues the reasons for these will be collected as part of the feasibility assessment.

10.4 Accountability of the Trial Treatment

Trial drugs used will be those available via the NHS system (manufactured by Roche UK and Boehringer Ingelheim UK) and thus trial pack preparation is not required. Compliance will be recorded on the CRFs (number of completed doses) with no need for drug vial accountability.

10.5 Concomitant Medication

Participants may not receive any intra-pleural therapy other than the trial drugs and simple saline flushes to maintain chest tube patency (if required – this does not include irrigation with large volumes of saline (>120mls per day) which is not permitted in this study). Specifically, intra-pleural antibiotic therapy, or

fibrinolytic or DNase therapy other than the trial drugs may not be given. Participants may not receive intra-pleural fibrinolytic therapy other than the trial medications without discussion with the chief investigator or deputy. It will be recorded whether the participant was anti-coagulated with therapeutic doses of warfarin or heparin (or its derivatives) or received any systemic fibrinolytic therapy on the report forms.

10.6 Post-trial Treatment

The trial treatment will not be continued outside the trial, with a maximum of 3 days' worth of dosing in all cases.

10.7 Other Interventions

There are no other specific interventions expected in this trial, other than surgical treatments which are specified in the surgical SOP.

11 SAFETY REPORTING

11.1 Definitions

Adverse Event (AE)	Any untoward medical occurrence in a participant to whom a medicinal product has been administered, including occurrences which are not necessarily caused by or related to that product.
Adverse Reaction (AR)	An untoward and unintended response in a participant to an investigational medicinal product which is related to any dose administered to that participant. The phrase "response to an investigational medicinal product" means that a causal relationship between a trial medication and an AE is at least a reasonable possibility, i.e. the relationship cannot be ruled out. All cases judged by either the reporting medically qualified professional or the Sponsor as having a reasonable suspected causal relationship to the trial medication qualify as adverse reactions.
Serious Adverse Event (SAE)	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. *NOTE: Pregnancy is not, in itself an SAE. In the event that a participant or his/her partner becomes pregnant whilst taking part in a clinical trial or during a stage where the foetus could have been exposed to the medicinal product (in the case of the active substance or one of its metabolites having a long half-life) the pregnancy should be followed up by the investigator until delivery for congenital abnormality or birth defect, at which point it would fall within the definition of "serious".
Serious Adverse Reaction (SAR)	An adverse event that is both serious and, in the opinion of the reporting Investigator, believed with reasonable probability to be due to one of the trial treatments, based on the information provided.
Suspected Unexpected Serious Adverse Reaction (SUSAR)	A serious adverse reaction, the nature and severity of which is not consistent with the Reference Safety Information for the medicinal product in question set out: • in the case of a product with a marketing authorisation, in the approved summary of product characteristics (SmPC) for that product • in the case of any other investigational medicinal product, in the approved investigator's brochure (IB) relating to the trial in question.

NB: to avoid confusion or misunderstanding of the difference between the terms "serious" and "severe", the following note of clarification is provided: "Severe" is often used to describe intensity of a specific event, which may be of relatively minor medical significance. "Seriousness" is the regulatory definition supplied above.

Any pregnancy occurring during the clinical trial and the outcome of the pregnancy should be recorded and followed up for congenital abnormality or birth defect, at which point it would fall within the definition of "serious".

11.2 Assessment results outside of normal parameters as AEs and SAEs

As pleural infection patients are generally unwell, no specific blood parameters will be considered to constitute an AE or SAE, with the exception of deranged clotting which in the judgement of the investigator is due to IET therapy and of sufficient abnormality to justify reporting. If any subset of coagulation profile more than doubles after IET treatment, the trial fellow will review.

11.3 Causality

The relationship of each adverse event to the trial medication must be determined by a medically qualified individual according to the following definitions:

Unrelated – Where an event is not considered to be related to the IMP / intervention

Possibly Related – although a relationship to the IMP / intervention cannot be completely ruled out, the nature of the event, the underlying disease, concomitant medication or temporal relationship make other explanations possible.

Probably Related – the temporal relationship and absence of a more likely explanation suggest the event could be related to the IMP / intervention

Definitely Related – the known effects of the IMP, its therapeutic class or based on challenge testing suggests that the IMP / intervention is the most likely cause.

All SAEs labelled possibly, probably or definitely related will be considered as related to the IMP.

11.4 Procedures for Recording Adverse Events

All AEs occurring during the initial trial period (to 7 days post treatment (whilst an in- participant)) will be recorded to ensure all data on adverse outcomes from the IET or surgery or standard care are captured. Known and well recognised complications of pleural infection, surgery or IET therapy will be recorded as part of the CRFs for the study, but (even if serious) are not subject to SAE reporting timelines if a known and documented complication of therapy (see section 11.5.1).

The following information will be recorded: description, date of onset and end date, severity, assessment of relatedness to trial medication, other suspect drug or device and action taken. Follow-up information should be provided as necessary.

The severity of events will be assessed as one of the following: mild, moderate or severe.

AEs considered related to the trial medication as judged by a medically qualified investigator or the Sponsor will be followed either until resolution, or the event is considered stable.

It will be left to the Investigator's clinical judgment to decide whether or not an AE is of sufficient severity to require the participant's removal from treatment. A participant may also voluntarily discontinue from treatment due to what he or she perceives as an intolerable AE. Normal follow up within the trial will continue.

11.5 Reporting Procedures for Serious Adverse Events

The safety reporting period is for 7 days post treatment (whilst an in-participant) (or 7 days post initial intervention for the pleural infection if surgical treatment is delayed). Serious adverse events which are not in the forseeable natural history of complications of pleural infection or treatment for this condition (which includes all of the complications listed in paragraph 11.5.1) are reportable in the first to 7 days post treatment (whilst an in-participant) (or 7 days post initial intervention for the pleural infection if surgical treatment is delayed).

All serious adverse events are recorded on the CRFs as part of the study in the first to 7 days post treatment (whilst an in-participant) (or 7 days post initial intervention for the pleural infection if surgical treatment is delayed).

Thereafter, only those serious adverse events which are considered directly attributable (related) to the treatment for pleural infection (not including any of the mentioned foreseeable complications) according

to local Investigator opinion will be recorded over the further 6 month follow up period. There will be no adverse event reporting beyond this but outcomes collected on CRF's.

11.5.1. Events exempt from immediate reporting as SAEs

Specific SAEs which do not require immediate reporting in this trial are those associated with the natural history of pleural infection or treatment for this condition.

Foreseeable complications of pleural infection are mortality (approximately 20% at 6 months), respiratory failure, admission to intensive care, complications of antibiotic therapy, worsening sepsis, requirement for emergency or other surgery, deep vein thrombosis and death due to progressive infection, as well as readmission with infection within a month. If these known complications occur, and are judged to be due to sepsis or as a direct result of infection, this does not need to be immediately reported but will be recorded on the CRFs.

In addition; each treatment arm has foreseeable complications (as listed here) and do not require expedited reporting:

1. For the standard care arm, these are related to the chest tube insertion procedure and include:

Bleeding, wound site infection, pain, major organ perforation, bronchopleural fistula.

2. For the IET arm:

Intrapleural bleeding, allergic reaction, systemic bleeding and pain.

3. Surgical arm:

There is list of well-established surgical complications which will form part of the surgical SOP. These include complications during the procedure requiring conversion of the 'keyhole' surgery into an open surgical procedure, such as uncontrollable bleeding and failure of the lung to fully re-expand. Post-operative complications include pain, wound infection, prolonged air leak, repeat operation, blood transfusion, respiratory failure and the need for a tracheostomy.

Similarly, further interventions for pleural infection at any stage (including the need for surgery, or further surgical or pleural intervention) will be recorded on the CRFs and not as an immediately reported SAE.

11.5.2. Procedure for immediate reporting of Serious Adverse Events

All SAEs (other than those defined in the protocol as not requiring reporting) must be reported on the ORTU SAE reporting form to ORTU as soon as possible of the Site Study Team becoming aware of the event. ORTU will perform an initial check of the report, request any additional information, and ensure it is reviewed by a nominated Medical Reviewer (including Expectedness Assessment). It will also be reviewed at the next Trial Safety Oversight Group meeting. All SAE information must be recorded on an SAE form and scanned and emailed, to ORTU respiratorytrialsunit@ouh.nhs.uk Additional and further requested information (follow-up or corrections to the original case) will be detailed on a new SAE Report Form and scanned/emailed to ORTU.

11.6 Expectedness

Expectedness for the IET arm is determined according to the Summary of Product Characteristics.

Expectedness for the surgical arm is determined according to the surgical SOP.

Expectedness for the standard care arm is determined according to the following list of expected events:

Bleeding, wound site infection, pain, major organ perforation, bronchopleural fistula.

Related and Unexpected SAE
In the event of an SAE (defined as reportable in this protocol) that is assessed as being 'related' to a trial intervention and 'unexpected' will be reported to the REC that gave a favourable opinion of the study.

Reports of related and unexpected SAEs should be submitted within 15 working days of ORTU becoming aware of the event, using the HRA report of serious adverse event form (see HRA website).

12 STATISTICS

12.1 Statistical Analysis Plan (SAP)

The outline of the statistical analysis is included here. A separate Statistical Analysis Plan will not be drafted for this study. All statistical analysis will be conducted by the Centre for Statistics in Medicine, University of Oxford. All results will be reported according to the CONSORT 2010 statement: extension to randomised pilot and feasibility trials (Eldridge SM et al, BMJ 2016;355:i5239).

12.2 Description of Statistical Methods

The feasibility outcomes (recruitment rate, acceptability of randomisation, retention rate) will be reported as proportions together with 95% confidence intervals. These will be used to assess whether a definitive trial is feasible. Descriptive statistics will be used to describe the demographics between the groups. For categorical variables, the number (and percentage) will be reported for each treatment group and overall. For continuous variables, means and standard deviation (or medians in interquartile range) will be reported for each treatment group and overall. Comparisons between treatment arms for the clinical outcomes will be reported using descriptive statistics only as this feasibility trial is not powered for definitive conclusions to be drawn. No statistical tests will be undertaken. These will be based on multivariable linear (for continuous outcomes) or logistic (for binary outcomes) regression adjusted for stratification factors and important prognostic factors and will be reported as an adjusted difference in means (for continuous outcomes) or in proportions (for binary outcomes). Treatment comparisons will be reported for the intention-to-treat population (all randomised participants will be analysed according to their allocated treatment group irrespective of which treatment they actually receive) as treatment effects together with 95% confidence intervals for the two main comparisons: (1) VATS vs IET; (2) VATS vs Standard Care.

Compliance to the interventions will be reported.

To establish the feasibility of collecting accurate long-term outcomes in randomised participants, we will present the completeness of the outcomes across the duration of the trial. The outcome measures collected in this trial will be used to inform the sample size for the future definitive phase III RCT, if it is feasible to be undertaken.

Adverse events and serious adverse events will be reported by treatment arm on the safety population only (all patients who received the allocated treatment).

It is anticipated that STATA (StataCorp LP) or other appropriate validated statistical software will be used for analysis.

Interviews will be digitally audio-recorded, transcribed verbatim, and anonymised before being uploaded to NVivo data management software. The interview data will be analysed using Thematic Analysis. Audio-recordings will be listened to and transcripts read and re-read for familiarisation, then open-coded to develop an initial code list. Codes will then be grouped into categories, and data explored to identify connections and to develop a descriptive account of the dataset as a whole. The analysis will focus on the acceptability of trial processes to patients, individual and group equipoise, and the patient experience of pleural infection and treatment.

12.3 Sample Size Determination

As a feasibility trial, no formal sample size calculations were performed or possible. However, the primary purpose of this study is to assess if recruitment to a larger, definitive trial is feasible, and the recruitment target of 75 randomised participants in a number of UK centres over 18 months has been chosen based on this aspect, and recent current recruitment to our observational study in pleural infection (PILOT which recruited 20 participants per month in 20 centres). Extrapolating this data to be obtained from this study, if 75 participants can be randomised in 18 months from 5 centres to this surgical trial, a future phase III study will be able to recruit 480 suitable participants from 20 centres over 2 years.

This number of participants is sufficient for a definitive two-arm trial comparing IET and VATS, whose primary outcome is hospital stay, in which our current data suggests a total sample size of 432 participants are required randomised 1:1 (rationale: using information encompassing a clinically meaningful difference in hospital stay of more than 3 days (mean hospital stay in IET arm = 11.8 days,(3), mean hospital stay in VATS arm = 8.5 days,(21, 22), assumed both arms has the same SD of 10 days, 90% power, 5% significance level), randomising 1:1 between IET and VATS requires 194 participants per arm, totaling 388 participants. Allowing a 10% attrition rate, the estimated total sample size for the larger definitive trial is 432 (216 in each arm) participants randomised).

Thus, demonstration of successful randomisation of 75 participants over 18 months of recruitment from a number of centres would demonstrate that a phase III trial of this size, in this population and with similar randomised groups, is feasible. The primary outcome(s) of a future phase III trial will be informed by work conducted in this feasibility trial.

All participants who consent to interviews but not to randomisation into the study will be included in the analyses of the relevant qualitative outcomes.

Crossovers from the standard care arm to the VATS arm or to the IET arm will be permitted once treatment on the standard care arm has been deemed to have failed after a further 48 hours (as is current BTS guideline standard practice). Crossovers will be recorded and a per-protocol analysis will be conducted.

12.4 Analysis Populations

The study will be analysed on intention to treat, with included populations as specified above.

12.5 Decision Points

No interim analysis will be conducted. The Trial Steering Committee will review the recruitment rate regularly throughout the trial.

12.6 Stopping Rules

No formal stopping rules are planned.

12.7 The Level of Statistical Significance

Not applicable

12.8 Procedure for Accounting for Missing, Unused, and Spurious Data.

Missing data will be reported for the key feasibility and clinical outcomes, but no adjustment will be undertaken.

12.9 Procedures for Reporting any Deviation(s) from the Original Statistical Plan

Any changes/deviations from the statistical analysis outlined here will be described and justified in the final statistical report.

12.10 Health Economics Analysis

Initial Health Economic Analysis will be undertaken, to inform a potential larger trial, and will be the subject of a specific Health Economic Analysis plan to be written during trial recruitment, using the parameters collected.

12.11 Criteria for the Termination of the Trial

No specific premature closure / 'stopping rules' are defined for the TSC. However, it is anticipated that the TSC will only advocate trial closure where there is proof beyond reasonable doubt that one treatment arm is clearly superior to the other such that continuation in the trial would result in significant participant disadvantage.

12.12 Cost-effectiveness analysis

This is a feasibility trial, the main aim will be central monitoring to assess whether we can obtain the resource use, cost and main outcome data. As part of the central monitoring procedures by ORTU, where appropriate, queries necessary to perform a cost-effectiveness analysis in a full definitive trial will be recorded.

We will assess, the response rates to the EQ-5D-5L and resource use questionnaires administered to patients, and evaluate patterns of missing data.

Reason for missing data, a pilot of the micro-costing study used to evaluate the costs of trial surgical intervention and assessments of whether we obtain reliable costs for participants undergoing surgery will be recorded.

In addition, we will assess if we can obtain all the relevant information required to generate costs of hospitalisation from participants' EPR records, including: dates of hospitalisation, dates of ward transfers, and diagnoses and procedure codes. In addition, we will assess if we can obtain all the relevant information required to generate costs of hospitalisation from participants' EPR records, including: dates of hospitalisation, dates of ward transfers, and diagnoses and procedure codes.

Crossovers from the standard care arm or the VATS arm to the IET arm will be permitted and recorded.

13 DATA MANAGEMENT

13.1 Source Data

Source documents are where data are first recorded. These include, but are not limited to, hospital records (from which medical history and previous and concurrent medication may be obtained), clinical and office charts, laboratory and pharmacy records, and medical imaging.

Data required for the conduct and analysis of this trial will be collected on Case Report Forms (CRFs). This may be transcribed or summarised from source documents, or may be collected directly in trial CRFs. CRF entries will be considered source data if the CRF is the site of the original recording (e.g. there is no previous written or electronic record of data).

13.2 Access to Data

Direct access will be granted to authorised representatives from the Sponsor, host institution and the regulatory authorities to permit trial-related monitoring, audits and inspections.

13.3 Data Recording and Record Keeping

Data will be entered into a secure, validated, GCP-compliant electronic data management system. All staff performing data entry will be appropriately trained prior to access being granted. Access is controlled by individual user accounts, and a full audit trail is kept of all modifications made to data.

Standard Operating Procedures (SOPs) will be followed to maximise completeness and accuracy of trial data. The processes for quality assurance of study data will be detailed in the study monitoring plan, data management plan, and other associated documents.

Participants will only be identified in all trial documents and datasets (other than the signed consent form) by a unique trial-specific number or code. The name and any other identifying detail will NOT be included in any trial data electronic file.

All trial documents will be stored securely. Both paper and electronic trial data will be retained through an archiving service for a period as described in the Data Management Plan.

14 QUALITY ASSURANCE PROCEDURES

14.1 Risk assessment

The trial will be conducted in accordance with the current approved protocol, GCP, relevant regulations and standard operating procedures. A risk assessment and monitoring plan will be prepared before the study opens and will be reviewed as necessary over the course of the trial to reflect significant changes to the protocol or outcomes of monitoring activities.

14.2 Monitoring

Regular monitoring will be performed according to the trial 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 trial specific Monitoring Plan. Following written standard operating procedures, the monitors will verify that the clinical trial is conducted and data are generated, documented and reported in compliance with the protocol, GCP and the applicable regulatory requirements.

14.3 Trial Committees

14.3.1 Trial Management Group

Trial Management Group will meet regularly throughout the trial to discuss the day to day management of the trial, a TMG charter will be written detailing all of the requirements:

Members:
CI
Research Fellow
Trial Manager
Data Manager
Clinical Trials Assistant

14.3.2 Trial Steering Committee

The Trial Steering Committee will meet on a 6 monthly basis throughout the trial to assess the progress of the trial. A TSC charter will be written detailing the requirements of this committee and its members.

Members:
Independent Chair
CI
Independent Member
Non-Independent Member
Independent Member
Research Fellow
Trial manager
Data Manager
PPI Rep

14.3.3 Safety Monitoring Committee

The Oxford Respiratory Trials Unit (ORTU) will conduct a review of all SAEs for the trial reported during the reporting period and cumulatively. The aims of this committee include:

- To pick up any trends, such as increases in un/expected events, and take appropriate action
- To seek additional advice or information from investigators where required
- To evaluate the risk of the trial continuing and take appropriate action where necessary

15. PROTOCOL DEVIATIONS

A trial related deviation is a departure from the ethically approved trial protocol or other trial document or process (e.g. consent process or IMP administration) 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 trial master file.

The Oxford Respiratory Trials Unit has Standard Operating Procedures for deviations and breaches which will be used throughout.

16. ETHICAL AND REGULATORY CONSIDERATIONS

16.1 Declaration of Helsinki

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

16.3 Approvals

The protocol, informed consent form, participant information sheet and any proposed advertising material will be submitted to an appropriate Research Ethics Committee (REC), HRA (where required), and host institution(s) 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

Eligible participants will be given detailed information and the opportunity to discuss the trial further with a member of the trial team. Participants are generally given 24 hours 'thinking time' thereafter to consider enrolling in a trial. It is recognised that clinical circumstances in this trial are likely to make this impossible. The participants will be asked to consent to trial entry, the collection of information about their care, and collection of subsequent data sheets. All will be appropriately anonymised.

The safety profile of the intra-pleural medications appear reasonable from the previous study, however are not fully defined and this is an outcome of the trial. This risk will be covered by specific consent.

16.5 Reporting

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

16.6 Participant Confidentiality

The trial staff will ensure that the participants' anonymity is maintained. The participants will be identified only by a participant ID number on all trial documents and any electronic database, with the exception of the CRF, where participant initials may be added. All documents will be stored securely and only accessible by trial staff and authorised personnel. The trial will comply with the General Data Protection Regulation (UK GDPR) and Data Protection Act 2018.

For further information on how UK GDPR and associated data protection legislation impacts on research please, University of Oxford researchers see https://researchsupport.admin.ox.ac.uk/policy/data/checklist and https://researchsupport.admin.ox.ac.uk/policy/data/practical and OUH researchers see https://www.ouh.nhs.uk/privacy/default.aspx

Participants consenting to be interviewed, will have their details sent to Oxford from nhs.net email accounts at sites to the trial specific nhs.net email account. Oxford Brookes University staff will also have access to trial specific inbox to obtain these details but research passports will be in place.

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

Funding is provided in full by a NIHR Research for Patient Benefit Grant.

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.

18 PUBLICATION POLICY

The preparation of a manuscript for rapid publication will be the sole responsibility of the trial's Chief Investigator. High priority will be given to this. Any detailed reports of the study prepared by Boehringer or Roche for internal use and for submission to regulatory authorities will be submitted to the Steering Committee for review within an appropriate period of time, prior to their dissemination and will not be submitted without approval from TSC.

The primary report is planned to be with all co-investigators and recruiters named in the author list, but subject to specific journals which limit the number of authors, this may be in the name of the "MIST3 investigators group" with the trial fellow(s) as specified by the CI, and Chief Investigator named, and all other contributors listed with their roles in the acknowledgment section.

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

No specific IP is expected in this trial.

20. ARCHIVING

All trial documentation will be archived at Restore Datacare, ORTU's archiving facility.

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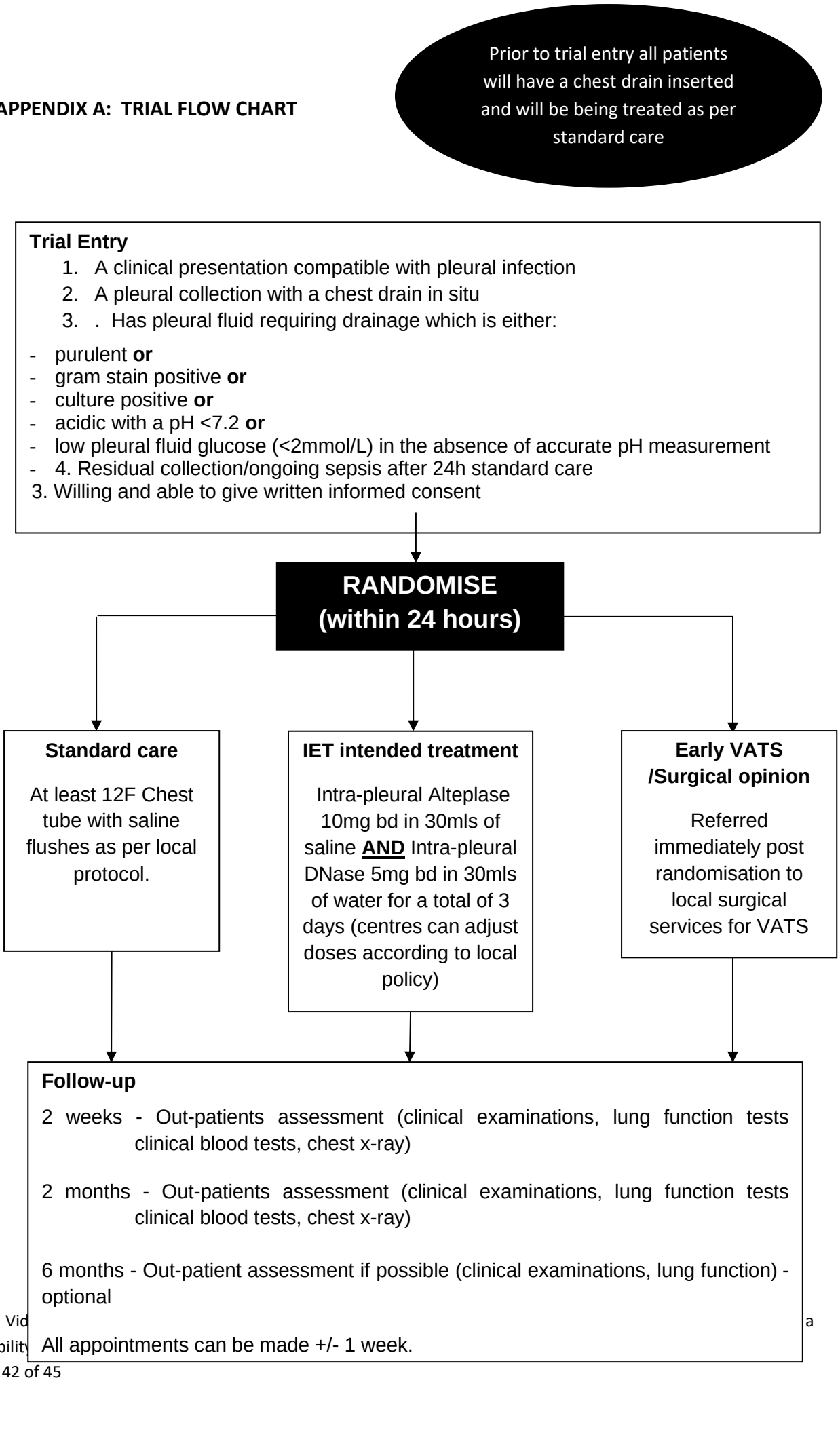
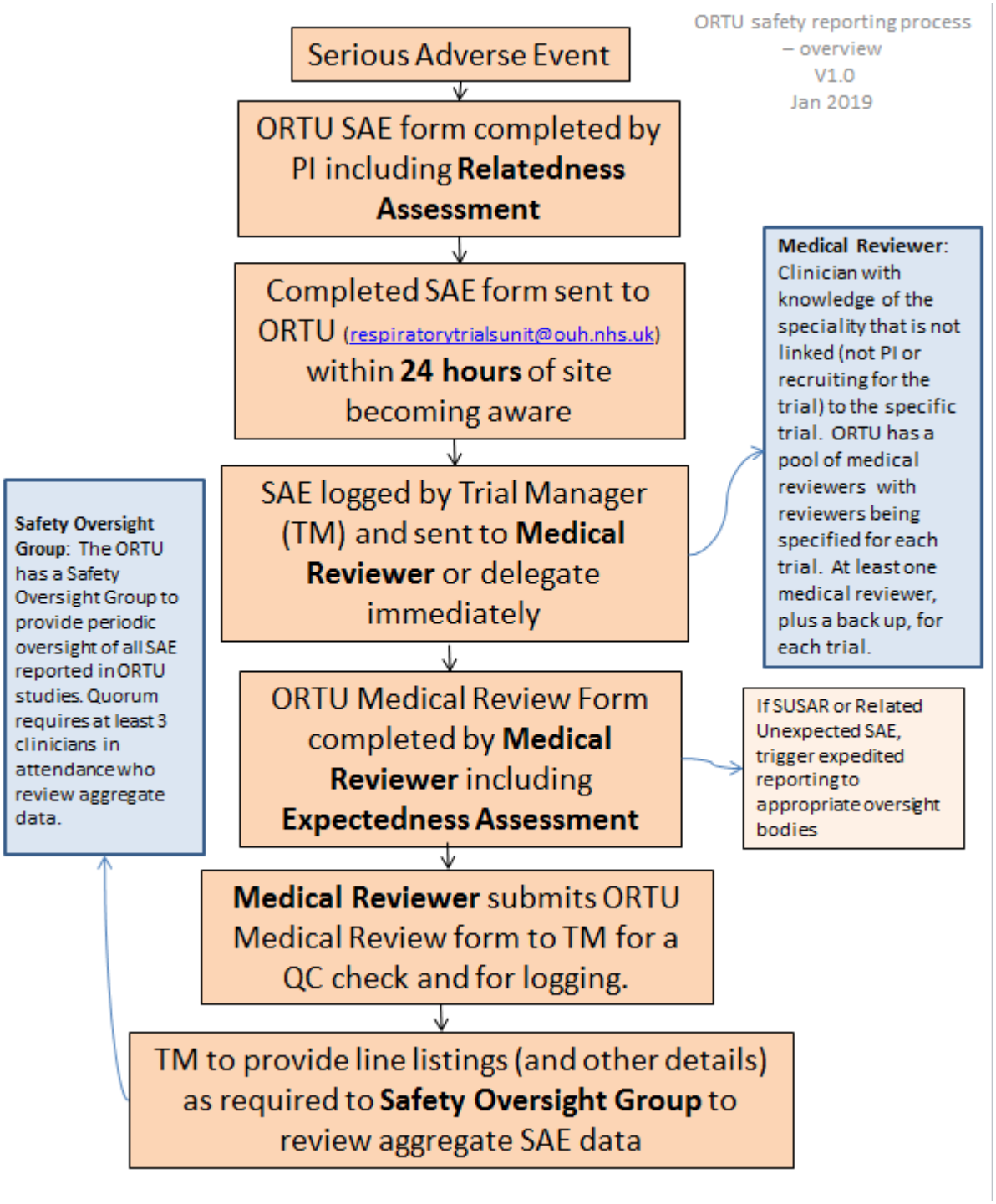
22. APPENDIX A: TRIAL FLOW CHART



23. APPENDIX B: SAE REPORTING FLOW CHART



24. AMENDMENT HISTORY

Amendment No.	Protocol Version No.	Date issued	Author(s) of changes	Details of Changes made
Minor 1	V3.0	31Jul2019	Dr Eihab Bedawi	Minor change to clarify that the trial intervention solution will be made up and administered by clinical staff as per local protocols and not as per TSPs.
Minor 2	V4.0	21Jan2020	Dr Eihab Bedawi	Minor changes made through the protocol bringing it in line with the information being collected during the participant visits on CRF. Clarification on how the participant's interviews will be performed and the addition of pain to the post-operative complications of the surgical arm in the safety section.
Minor 3	V5.0	13Feb2020	Dr Eihab Bedawi	Clarification on the use of a transcription service provider for the qualitative interviews. Clarification on randomisation arm crossovers, standard care arm can cross to VATS or IET if required. Additional inclusion and exclusion criteria added.
Minor 4	V6.0	22Jul2020	Dr Eihab Bedawi	Clarification that research samples have been suspended during COVID pandemic. Change to how questionnaires are completed and how follow up appointments can be undertaken remotely, detailing which assessments can and cannot be undertaken.
Minor 7	V7.0	21Oct2020	Dr Eihab Bedawi	P8 and 8.2 inclusion of another inclusion criteria "A pleural collection with a chest drain in situ". Minor typos picked up by our PPI rep.
Sub 8	V8.0	05Nov2020	Dr Eihab Bedawi and Professor Rahman	Inclusion of using Oxford Brookes University as a collaborator to perform the qualitative interviews.
Minor 9	V9.0	21Jan2021	Professor Rahman and Dr Eihab Bedawi	Amendment to the trial design section, updating the information on cross over of treatment between the 3 treatment arms.
Minor 10	V10.0	14Apr2021	Professor Rahman and Dr Eihab Bedawi	Amendment to make some of the trial visits optional in order to streamline the process, following the slow

				recruitment due to COVID-19, this will enable sites to recruit to target but adding less burden to complete all visits and assessments, focusing on the essential data to meet the trial outcomes. Appendix A flowchart updated inline with previous amendment for inclusion criteria.