

**Phase 1a/1b Dose-escalation Trial of Intrapleural LTI-01 (Single Chain Urokinase/scuPA)  
in Patients with Complicated Parapneumonic Effusions (CPE) or Empyema**

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Conflict of Interest Statement: Dr. Idell serves as a member of the Board of Directors, Founder and Chief Scientific Officer of Lung Therapeutics, Inc. (LTI) and has an equity position in the company, as does the University of Texas Horizon Fund and The University of Texas Health Science Center at Tyler (UTHSCT). He has a conflict of interest plan acknowledging and managing these declared conflicts of interest through The University of Texas Health Science Center at Tyler (UTHSCT). He is an inventor on a patent USPTO # 7332469 held by the UT Board of Regents and licensed to LTI. Drs. Komissarov and Florova have received funding from LTI and likewise have conflict of interest management plans at UTHSCT. Drs. Rahman and Lee serve as key opinion leaders for LTI and are paid for their input to the company. Dr. Shoemaker is a paid consultant for LTI and Dr. Gillies received payment as Medical Monitor for the study from LTI.

## Abstract

**Background:** Current dosing of intrapleural fibrinolytic therapy (IPFT) in patients with CPE/empyema is empiric, as no dose-escalation trial has previously been done to assess its safety. We hypothesized that LTI-01 (scuPA), which is relatively resistant to PA inhibitor-1 (PAI-1), would be well-tolerated in these subjects.

**Methods:** This was an open-label, dose-escalation trial of LTI-01 IPFT at 50,000-800,000 IU daily for up to 3 days in adult subjects with loculated CPE/empyema and failed pleural drainage. The primary objective was to evaluate safety and tolerability. Secondary objectives included assessments of processing of scuPA in blood and pleural fluid (PF) and of efficacy.

**Results:** LTI-01 was well tolerated with no bleeding, related treatment-emergent adverse events or surgical referrals. uPA antigen increased in pleural fluids at 3 hours after LTI-01 ( $p<0.01$ ) but not in plasma. PF uPA increased at 3h following LTI-01 IPFT, saturated active PAI-1 and generated PAI-1-resistant bioactive complexes, increased PA and fibrinolytic activity and increased D-dimers within PFs. There was no systemic fibrinogenolysis, nor increments in plasma D-dimers. Decreased pleural opacities occurred in all but one subject. Both subjects receiving 800,000 IU required two doses to relieve pleural sepsis, with two other subjects improved at lower doses.

**Conclusion:** LTI-01 IPFT was well-tolerated with no safety signals of concern. No local or systemic bleeding occurred. Processing of LTI-01 IPFT within PFs recapitulates that observed in preclinical modeling. Preliminary efficacy signals, including reduction of pleural opacity were observed.

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## Introduction

Complicated parapneumonic pleural effusions (CPE) or empyema with failed drainage and pleural sepsis are dire consequences of pleural infection and remain important clinical challenges. Serious pleural infections have been reported to occur in about 90,000 patients in the US(1), are increasing in frequency in adults(2) and children(3) and are associated with a mortality rate of up to 20 percent(4). CPE/empyema with loculation, sepsis and failed drainage is often encountered in clinical practice. Patients often have extended hospital stays exceeding hospital Diagnosis Related Group (DRG)-payments to hospitals in the US and can require surgery to relieve pleural sepsis(5). Treatment entails costs of care of more than a third of a \$billion in the US and UK and, by extrapolation, may exceed \$billions world-wide(5, 6).

Intrapleural fibrinolytic therapy (IPFT) has been part of the therapeutic arsenal for CPE/empyema for over sixty years, predicated on the concept that lysis of the intrapleural, extravascular fibrin neomatrix can pharmacologically relieve impaired drainage of purulent collections that support pleural sepsis(7). Because surgery entails morbidity and risks of complications and because some patients with CPE/empyema cannot tolerate surgery, safe and effective pharmacotherapy is desirable. However, the safety profile and efficacy of IPFT in adult patients currently remains unclear at the present time(8-10). A number of agents have been used to achieve effective pleural drainage in adult patients with CPE/empyema, but with variable results in adults(11, 12).

In this phase 1 clinical trial, we used single chain urokinase plasminogen activator; scuPA, as the interventional agent and tested its safety after intrapleural delivery as the primary study objective. LTI-01; scuPA is a new candidate form of IPFT which offers a number of conceptual advantages including relative resistance to inhibition by plasminogen activator inhibitor-1 (PAI-1), the major PA inhibitor within pleural fluids (PFs) (13) and increased bioavailability(13). Extensive analyses including GLP-toxicology studies performed as part of NHLBI SMARTT (Science Moving Towards Translation and Therapy) contract # HHSN268201100014C provide proof of concept that this is a well-tolerated agent. In preclinical testing, scuPA effectively cleared fibrinous collections associated with several forms of pleural injury including empyema(13-18).

A major impediment to interpretation of all trials concerning the efficacy and safety of IPFT to date has been that the dosing of IPFT has been empiric, with dosing of all forms of IPFT subject to arbitrary rather than evidence-based selection(19, 20). We are unaware of any previous dose escalation trials done to establish safety as a prelude to efficacy testing in the IPFT field. In the present report, we sought to address this critical gap in two novel ways. First, we conducted a dose-escalation trial of CHO-cell manufactured recombinant scuPA, otherwise called LTI-01, in patients with CPE/empyema, pleural loculation and failure to drain. Second, this is to our knowledge the first pleural infection trial to integrate comprehensive biochemical analyses of the intrapleural and systemic processing of an IPFT agent into the protocol design. These analyses enabled us to confirm the bioactivity of dose-escalated LTI-01 IPFT in individual human subjects and understand its' contribution to aggregate or individual safety profiles and treatment outcomes.

## Results

**Demographics.** The Intent To Treat (ITT) population comprised all subjects (n=14) who enrolled in the study and who supplied post-screening data. The ITT population was used for summaries of all disposition, demographic and other baseline data. In addition, all other laboratory listings were produced using the ITT population. The demographics of the ITT population are illustrated in Table 1. About three quarters were male with a mean age of 64.9 years (42-89 years). 78.6 percent were Caucasian, with the 3 remainder being of Maori, Tongan or Philippine descent. All subjects had ongoing pneumonia with CPE or empyema with a majority of subjects also recording ongoing chest, abdominal, pleuritic or chest drain site pain (12/14). Comorbidities are listed in section 16.2.4.3 of the CSR (Supplement) and included esophageal reflux disease in 4 patients, COPD in 3, coronary disease in 2, poor dentition, ethanol abuse and head and neck cancer history in 1 patient in these categories. All patients received concomitant medications for their underlying conditions and analgesics, which included morphine in 11/14 patients. Only 1 subject had a BMI exceeding 30 kg/m<sup>2</sup>. All 14 subjects received antibiotics prior to and at screening. After study entry, they were continued on the regimens listed in Table 2 at the discretion of the treating physicians. Individual antibiotic therapy is summarized in CSR Listing 16.2.6.7, Supplement. Kaplan-Meier plots of total days on antibiotic are provided for combined dose levels are provided in the CSR, Figure 14.2.1, Supplement. PF cultures were positive for 4/14 patients at study entry, with one PF clotted and not cultures and 9 yielding no growth. Three of four PF cultures were positive for *Streptococcal* species. Blood cultures were negative in all 14 patients. All subjects met inclusion criteria at screening and had PF that was either purulent, Gram stain positive, culture positive or pH ≤7.2, and had a loculated pleural effusion with failure to drain over 3h of initial observation.

**Safety Results.** No acute intrapleural or systemic bleeding events were observed in any of the LTI-01-treated subjects. There were no consistent effects on heart rate at all dose levels. There were also no consistent effects of treatment on systolic or diastolic blood pressure, respiratory rate or temperature as described in the CSR Listing 16.4.1 and 16.4.2, Supplement. Fever (>37.4°C) was present in only 1 patient. None of the patients required surgical referral following LTI-01 treatment.

A total of 22 treatment-emergent adverse events (TEAEs) were recorded in 7 (50.0%) of the 14 subjects (Table 3). No TEAE was considered to be related to study treatment. All 22 TEAEs were recorded in 7 (50.0%) subjects. TEAEs did not increase with dose level, as 10 TEAEs were recorded in the low dose group (50,000 IU), compared to no such events in the high dose group (800,000 IU). The most frequently occurring TEAEs were hypotension and post-procedural persistent drain fluid (3 events each), and pleuritic pain (2 subjects each). Three TEAEs occurring during active study participation were classified as severe including metastatic squamous cell carcinoma, sinus tachycardia, lung abscess, and 11 TEAEs were classified as of moderate severity. All other TEAEs were mild. There were two further TEAEs of note, neither attributed to LTI-01. One subject (200,000 IU) had a mild post-procedural hematoma on Day 3 pre-dose. The event likely occurred at the time of drain insertion on hospital admission and was considered unlikely to be related to study drug based on the physical appearance of the hematoma. Given improvement in clinical status, reduced opacification on CXR and as a safety precaution, this subject did not receive the scheduled third dose of LTI-01. Two further subjects had study drug withdrawn as a result of a TEAE, both in the 100,000 IU dose group. The first subject did not receive the third scheduled dose of LTI-01 as a safety precaution following migration of the chest drain, which led to chest pain. The second

had moderate prolonged prothrombin time (PT) between Days 3 and 4 which met stopping criteria but was considered unrelated to study drug. There were no life-threatening TEAEs and no TEAEs led to discontinuation from the study. The overall summary of AEs is shown in Table 14.3.1.1, CSR, Supplement.

AEs were further stratified as described in the CSR, Supplemental Data (p46, Section 9.7.1.3). Serious Adverse Events (SAEs) were collected from the time of first study dose to Day 28, hospital discharge or death, whichever came first. Two subjects experienced SAEs within these boundaries. Both were events that led to death. Neither death was attributed to administration of LTI-01. One subject (50,000 IU) with clinical empyema and failure to drain at enrollment died from metastatic squamous cell carcinoma on Day 20. This subject had the condition in addition to clinical CPE meeting Inclusion criteria at the start of the study, given that malignant cells were found in his PF after entry. A second subject (400,000 IU) died of a ruptured lung abscess on Day 86. This subject had a cavitating lung lesion of the right lower lobe, pneumonia, empyema and obstruction of his bronchus intermedius as well as bile duct obstruction at the start of the study. No additional SAEs occurred after Day 28 or after discharge as of database lock (10 May, 2018).

A total of 116 protocol deviations in all 14 subjects occurred during the study. No deviation led to exclusion of subject data from an analysis population. Deviations requiring a protocol waiver are shown in Table 7 of the CSR, Supplement. Protocol deviation descriptions are provided in CSR Appendix 16.1.1. No deviation was considered to affect the study objectives or the integrity of the data collected.

Regarding treatment compliance, six of the 14 subjects did not receive dosing on day 3. In accord with the protocol, second or third LTI-01 doses could be withheld if complete resolution of the pleural process occurred after the prior dose. In one patient in the 50,000 IU group, treatment was stopped after two doses of LTI-01 after which tPA, or tPA/DNase was administered on days 3-5 at the discretion of the treatment team. In a subject in the 100,000 IU group, LTI-01 was not given on day 3 because of an elevated PT; 16 sec. Another patient in the 100,000 IU group did not receive the third dose of LTI-01 because of chest tube migration and required with no further therapy. A subject in the 200,000 IU group and had a chest wall hematoma and did not receive the third dose as the subject was clinically improved following LTI-01 IPFT. In the 800,000 IU group, one patient it was felt that a third dose was not warranted because of radiographic/clinical improvement. In the other patient in the 800,000 IU group, the full third dose was not given because of chest tube blockage and the patient required no further IPFT.

Clinical laboratory testing demonstrated a number of trends. Mean hemoglobin decreased at all time points for the pooled subject population, which was not significantly changed during the treatment phase. Maximum decrease from baseline was -1.19 g/dL by the Day 7 to Day 28 time point. All dose levels >50,000 IU had reductions in mean hemoglobin level from 3 hours post-dose on all dosing days and to the end of the study, except for the Day 3, 3 hours post-dose time point in the 800,000 IU group. Mean leukocyte and neutrophil count decreased from Day 2 (pre-dose) for the pooled subject population. Maximum decreases from baseline were  $-5.3 \times 10^9/L$  (leukocytes) and  $-5.2 \times 10^9/L$  (neutrophils) by the Day 7 to Day 28 time point. Mean platelets increased at all time points for the pooled subject population. The maximum increase from baseline was  $+80.2 \times 10^9/L$  by Day 3, 3 hours post-dose. There was no consistent effect or effect over time of LTI-01 treatment on mean PT, mean activated partial thromboplastin time (aPTT) or mean international normalized ratio (INR) values. One subject had a PT prolongation TEAE. Plasma fibrinogen never fell below the top normal lab range levels in any subject, indicating no evidence

of systemic fibrinogenolysis. All individual clinical laboratory results are presented in data listings (CSR Listing 16.2.8.1, Listing 16.2.8.2, Listing 16.2.8.3, Listing 16.2.8.4). Fibrinogen levels are provided in the CSR (Table 14.2.4.2) and CRP in Table 14.2.3 of the CSR, Supplement including change from baseline values. Total volume of PF drainage after LTI-01 treatment increased and changes from Day 1 pre-dose to 23 hours after final dose are presented in data listings in the and summarized by dose level (CSR (Listing 16.2.6.1 and Table 14.2.1). There were no changes in blood chemistries attributable to LTI-01 IPFT.

**Preliminary Assessments of Efficacy.** As only one subject entered the study with a fever, defervescence was not assessed. No subject was referred to surgery. No subject experienced a life-threatening event prior to hospital discharge. All subjects except Subject 202-1002 had a relative decrease in pleural density area and volume during the study, as confirmed by imaging. Subject 202-1002 had a 4% increase in pleural density area and an 83% increase in pleural density volume. This subject's pleural effusion was found to have malignant squamous cell epithelial cells due to pleural metastases from a head and neck tumor discovered by PF cytology after enrollment. Mean number of days of hospitalization was 13.8 days. Mean number of days on antibiotics was 32.2 days. There was no clear effect of dose level on either parameter, attributable to the low sample size per dose level and lack of a placebo group. Total PF drainage volumes were measured daily from the chest tubes and exhibited increased volumes that tended to increase with dose level (up to 1585.0 mL for the LTI-01 800,000 IU dose level). Total PF drainage volumes from day 1 to 23 hours after final dose; day 4, were 248.7, 525.3, 651.7, 1299.8 and 1585.0 mL for the LTI-01 50,000, 100,000, 200,000, 400,000 and 800,000 IU dose levels, respectively.

Mean plasma CRP decreased at all time points for the pooled subject A maximum decrease from baseline concentrations was found by the Days 7 to 28 time point at -125.4 mg/L. population, with rate of decrease trending lower during the treatment phase (CSR, Fig 14.3.6, p67, Supplement). The changes in this biomarker of systemic inflammation are consistent with improvement of the pleural infection/inflammation. In the LTI-01 400,000 IU group (Day 5), a maximum decrease in mean CRP of -189.0 mg/L was noted. Consistent with these data and including all subjects, a decrease from baseline in mean leukocyte count;  $-5.3 \times 10^9/L$  was noted at all time points from Day 2 (pre-dose) to Days 7 to 28 time. These changes are consistent with improvement in the underlying pneumonia and empyema. The greatest change from baseline in mean leukocyte count was in the LTI-01 200,000 IU group at Day 3 pre-dose to Day 4 to Day 7 at  $-11.6 \times 10^9/L$ . Changes of mean leukocyte counts did not consistently occur at post-dosing intervals, but were consistently seen from Days 4 to 28. Individual changes in leukocyte count were subject to high inter-subject variability (CSR Figure 6, p83, Supplement). Decreases from baseline in mean neutrophil count were observed in all subjects (up to  $-5.2 \times 10^9/L$  comparing baseline to Days 7-28, again consistent with improvement in the underlying pneumonia and empyema (Figure 7 CSR, Supplement).

**Follow up at 3 and 12 Months after LTI-01 IPFT.** Data was available at 3 mo. for all 12 patients known to survive beyond 86 days. At 3 mo., no additional deaths occurred, nor were there any further hospitalizations for pleural disease including surgeries. Information was available for 10/12 subjects at 12 months, with no referrals to surgery or hospitalizations.

**Pharmacokinetic (PK) and pharmacodynamic (PD) analyses.** uPA-related antigen (labeled as urokinase) data are provided in Appendix 16.1.10.1 of the CSR, Supplement. Plasma uPA-related antigen levels (PK) did not significantly increase after dosing on Day 1 at 3-hour post-dose compared with pre-dose (Figure 1) or at other dosing intervals. The largest increase was seen in one of the subjects dosed at 800,000 IU

with an increase of approximately 700 pg/mL post-dose (1446 pg/mL) compared to the pre-dose level (743 pg/mL). PF uPA antigen levels (PD) were much higher than plasma levels ranging from a mean Day 1 postdose (3 hour) level of 376,830 pg/mL in the 50,000 IU dose group compared to 2,129,776 pg/mL in the 800,000 IU dose group. PF levels; PD analyses, increased as dose levels increased at 3h after dosing (Figure 2).

**Intrapleural processing following LTI-01 IPFT and changes in plasma components of the fibrinolytic system.** PF PAI-1 total antigen was significantly increased in PFs of all subjects with CPE/empyema versus levels found in the plasma (Figure 3 A and B). Levels of total PAI-1 at baseline and 23 h (2.1-2.5  $\mu$ g/mL) were higher than those at 3h (1.4-1.8  $\mu$ g/mL;  $p=0.01$ ) and within the same range as PAI-1 in the rabbit model of *Streptococcal* empyema(18). Active PAI-1 was below the detection limit at 3h and elevated at baseline as well as 23h post IPFT (160-200 ng/mL;  $p<0.01$ ). Undetectable levels of PAI-1 activity seen at 3h post dosing are consistent with saturation of the active PAI-1 by t<sub>cu</sub>PA form by conversion of s<sub>cu</sub>PA to its two chain form. Levels of active PAI-1 represented about a third of the total PAI-1 levels in plasma. While active PAI-1 was about a tenth of the total concentration in PFs, levels of PAI-1 activity still exceeded those in plasma by more than 10-fold (Figure 3, panels B and D).

PF PA was undetectable undetectable before LTI-01 IPFT, but was about 1-10  $\mu$ g/mL at 3h after treatment and then fell by about 10-fold to 10-199 ng/ml at 23h. PF fibrinolytic activity (FA) was likewise suppressed at baseline and at 23h post-dosing, but increased to 0.04-0.05 AU at 3h. No PA or fibrinolytic activities were detectable in plasma nor were there any increments in either detected at the 3h post-therapy collection interval. PF plasminogen concentrations were about 10 times < than those in plasma but were unchanged at each interval (Fig 4 c and D). We have previously shown that the form of plasminogen in human CPE/Empyema fluids is mainly glu-plasminogen and amenable to activation by PAs(21), so that the levels of substrate are available within PFs to support its activation to plasmin and the local generation of fibrinolytic activity. Consistent with these observations, supplementation of the predose baseline PFs and at 23h after dosing with two-chain t<sub>cu</sub>PA generated robust levels of PA activity (median 0.7-1.3 AU;  $p<0.01$  versus baseline levels).

PF D-dimers were low ( $\sim$ 1mg/mL) at the baseline and 23h, and significantly higher (4-5mg/mL) at 3h after IPFT ( $P<0.05$ ). The increment of D-dimer levels in PFs at 3h after LTI-01 IPFT (Figure 5A), with unchanged levels in plasma (Figure 5B), is indicative of local but not systemic fibrinolysis. The findings in plasma are consistent with the persistently elevated levels (all >600 mg/dL) observed in plasma of all subjects at all collection intervals and indicate the absence of systemic generation of fibrin(ogen)olytic activity attributable to the administration of LTI-01 IPFT. Bioactive complexes of  $\alpha_2$ macroglobulin-uPA activity that we have shown to be durably active over 24h and PAI-1 resistant were detectable in PFs(13), with a trend towards increased concentrations occurring at 3h after dosing. Median levels of  $\alpha_2$ -macroglobulin/uPA complexes at baseline, 3 and 23h post IPFT were 4, 8-18 and 2-9 nM, respectively. There were no significant variations in the levels of uPA, plasminogen, PAI-1 antigens and PAI-1 activity in plasma during IPFT (not shown).

#### **Individualized assessments of PF derangements of components of the fibrinolytic system and outcomes.**

We next sought to determine whether derangements of the fibrinolytic system in PFs informed about treatment delivery, bioavailability and outcomes in each individual subject treated with LTI-01 IPFT. First, we analyzed the findings in subjects with clear evidence of clinical improvement and pleural drainage.

These patients had relief of pleural sepsis without any switch to another form of IPFT or referral to surgery and had improvement in pleural opacification by either CXR or chest CT imaging. One patient in the 400,000IU group; 102-1005, received all three doses and temporally responded with clinical resolution of pleural sepsis; termed by the clinical team as “The Lazarus effect” and a relative reduction of pleural density of 45%. Interestingly, this patient demonstrated the highest levels of uPA antigen; >100 µg/ml, in post-treatment pleural fluids (Figure 7A) as well as the lowest levels of PAI-1 PF concentrations (Figure 7B). In another patient in the 800,000IU group; 102-1007, clinical resolution of pleural sepsis was achieved with a relative reduction in the pleural density of 36%, so that a third dose of LTI-01 was not administered. This patient had elevated levels of PF uPA >100 µg/ml with low levels of PF PAI-1 compared to other subjects (Figure 7 A, B). In the other patient receiving 800,000 IU, only a portion of the third dose was delivered attributable to chest tube obstruction, but PF was not available for assessment at 3h after that dose. Pleural sepsis likewise resolved in this patient without further IPFT or a referral to surgery and PF PAI-1 levels remained low at days 1 and 2 post-dosing. One additional patient; 102-1001, treated with 50,000 IU/ml drained effectively with a relative reduction of pleural density of 85% by chest X-ray, had relatively low levels of PF PAI-1 with concurrent increments of uPA and resolution of clinical pleural sepsis upon completion of LTI-01 dosing (Figure 7A andB).

Next, we examined the findings in the two patients who died remote to their LTI-01 IPFT. Both had relatively elevated PF levels of PAI-1 during the LTI-01 treatment period. One; 102-1006, who received 3 doses of 400,000 IU LTI-01 IPFT, developed a right lower lobe cavity associated with ipsilateral pneumonia and CPE. This patient also had right hilar and subcarinal adenopathy causing narrowing of the bronchus intermedius and died at d86, 5 days after completion of treatment and had persistently increased PF PAI-1 in the 4 µg/ml range in post-dose PFs at days 2 and 3 despite a relative reduction of pleural density of 31 % at d4. The patient with empyema and concomitant metastatic head and neck cancer; 202-1001, received 3 doses of 50,000 IU LTI-01 IPFT and had a two order of magnitude increment of PF uPA to about 15 µg/ml 3h post-treatment on day 1, but PAI-1 levels increased to almost 4 µg/ml in PF by day 2 pre-dose concurrent with a loculated effusion failed to resolve by CXR at d4.

Of the four patients treated with follow-up tPA or tPA/DNase after LTI-01 IPFT, subject 202-1003 did not receive the third dose of 100,000 IU LTI-01 IPFT because an elevated PT. This patient developed increased white cell counts; ( $+12.6 \times 10^9/L$ ) and neutrophil counts ( $+13.5 \times 10^9/L$ ) at day6, and was the treated with tPA/DNase between days 5 and 8. CRP levels fell between days 2 and 5 during LTI-01 IPFT and at the beginning of tPA/DNase and were unavailable at days 5-8. Interestingly, this patient’s PF uPA increased with decreased PF PAI-1 during the LTI-01 treatment phase (Figure 7 A and B). Subject 202-1002 had 2 doses of 50,000 IU of LTI-01 IPFT and had after which chest ultrasound showing no additional fluid to drain so that the third dose was not administered. The CRP was falling but remained elevated at 230 mg/DL at d3, so that tPA, then tPA/DNase IPFT was given at days 4 and 5 via a newly placed chest tube into a pleural collection that was identified at that time. In this patient, uPA antigen post-dosing of LTI-01 IPFT at day 2 did not increase versus predose levels (Fig 7A) and levels of PF PAI-1 remained elevated, suggesting that delivery of the second dose of LTI-01 could have been inadequate or incomplete. In two other patients; 202-1004 and 202-1005, treated with tPA/DNase after receiving 3 doses of either 200,000 or 400,000 IU respectively, levels of PF uPA were among the highest noted 3h post LTI-01 dosing at which point PAI-1 levels were markedly decreased. In the patient receiving 200,000 IU, CRP levels were lower than in any other patient, falling during LTI-01 IPFT and white/PMN counts were falling. The treating



physician chose to add tPA/DNase because of residual empyema. In the patient receiving 400,000 IU LTI-01, CRP initially fell during LTI-01 IPFT versus baseline, but rose at day 4 despite normal white and neutrophil counts and the patient received tPA/DNase to provide additional assurance that this patient would be ready to travel shortly after hospitalization.

Of the four remaining subjects, two were in the 100,000 IU group, two in the 200,000 IU group. These patients all had relative reductions in pleural density of 15, 83, 71 and 37 percent, respectively with no switch to alternative IPFT or surgical intervention. Both patients in the 100,000 IU group had clear increments of PF uPA post-dosing (Figure 7A) and relatively low; <2 µg/ml; subject 102-1001 or relatively high initial; about 4 mg/ml, levels of PF PAI-1, that fell to about 2 µg/ml after LTI-01 IPFT (subject 103-1001, Figure 7B). Subject 103-1001 received only two doses of PAI-1 as the chest tube migrated and recovered without further intervention beyond customary care. In the remaining two subjects treated with 200,000 IU/ml, post-treatment (3h) PF uPA was about 100 mg/ml and PF PAI-1 fell from about 3.75 µg/ml; subject 102-1003 to about 2 µg/ml or remained within a range of about 2 µg/ml throughout the treatment period (subject 102-1004, Figure 7B). Subject 102-1004 required only two treatments of LTI-01 IPFT as the patient had a chest wall hematoma felt to be due to chest tube insertion. This patient was clinically improving and the PF was felt to be adequately drained.

## Discussion

IPFT has been used for over seventy years after Tillett and colleagues originally used crude preparations of agents designed to clear fibrinous intrapleural collections to expedite pleural drainage(7, 22). Since that time, a number of so-called fibrinolysins, which activate plasminogen to form plasmin and lyse fibrin have been used to treat CPE/empyema(19, 23). A number of plasminogen activators, including streptokinase, urokinase and tissue plasminogen activator (tPA), have subsequently been used over the years, with general success in pediatric patients and checkered results in adults with CEP/empyema, as recently reviewed(11). In previous metaanalyses, it was concluded that there was equivocal evidence supporting the use of IPFT for these patients(8, 9), with a more recent metanalysis unable to assess the impact of IPFT(10). While it is certainly possible that children to IPFT could inherently be more responsive to IPGFT than adults or brought to medical attention earlier, other factors may be equally if not more important in adults with CPE/empyema.

First and foremost, the dosing of IPFT in adults with CPE/empyema has been empiric rather than evidence based, nor is there clear understanding of the bioavailability of any previously studied forms of IPFT in PFs of patients with CPE/empyema(19, 20, 24). Currently used IPFT agents have been used off-the shelf and to our knowledge, no form of IPFT has undergone the rigors needed to attain approval by any regulatory agency. A major initial requirement for such approval is the performance of phase 1 testing in humans to establish the safety of a new drug candidate. After formal GLP toxicologic studies were performed with support of the NIH SMARTT program, a drug product consisting of highly purified active pharmaceutical scuPA conforming to general requirements for regulatory approval was created. This drug product; called LTI-01, was used in this trial, which we believe to be the first dose-escalation safety trial in patients with CPE/empyema to be performed in this field.

The premise for testing LTI-01 is based upon its favorable biochemical properties and unique form of processing in the pleural space. Over the past twenty years, our group has performed a range of preclinical studies and the reported findings strongly suggested that scuPA was effective and well-tolerated in organizing pleural injury. The potential advantages of scuPA IPFT derived from these preclinical studies have been reviewed recently(20) and are briefly summarized here. First, scuPA was found to be well-tolerated in tetracycline-induced pleural injury and empyema in rabbits and was not found to cause intrapleural or systemic bleeding(13-16). Secondly, scuPA IPFT is relatively PAI-1 resistant and thereby resists the immediate and irreversible inactivation by PAI-1 that occurs when serine proteases such as two chain uPA or tPA interact with this inhibitor(13). This issue is of particular importance to the field, given the generally high levels of PAI-1 that occur within pleural fluids, which have previously been reported(15, 25, 26) and confirmed in this study. In addition, levels of total and active PAI-1 are variably increased; by two-three orders of magnitude, in patients with CPE/empyema as demonstrated in pleural fluid samples from patients in the MIST2 trial and in patients from UTHSCT(18). This finding implies that empiric selection of IPFT could predispose to bleeding in patients with relatively low levels of PAI-1 or be insufficient in patients with high PF PAI-1. In rabbits with empyema, scuPA was found to be effective, well-tolerated and durably bioactive(18). These preclinical findings provided a strong premise that enabled acquisition of NHLBI support of the manufacturing of scuPA via the SMARTT program in anticipation of this trial. The scuPA drug substance was well-tolerated in SMARTT-supported GLP toxicology studies supporting advancement to this phase 1a dose escalation trial in patients with CPE/empyema.

The demographic profile of the enrolled patients reflects the disparate causes of pleural infection that often occurs in patients with loculation and failed pleural drainage. The paucity of positive pleural fluid cultures was likely attributable to the prior administration of antibiotics, which were administered to all subjects prior to screening. The range of underlying co-morbidities reflects the commonly seen and relatively advanced age of the patients. Subjects had a mean of about 65 years, comparable to the cohorts previously enrolled in the MIST1 and 2 trials(4, 5). The diversity of the population reflects that often encountered in clinical practice, but a limitation of the study is that only 14 of the 21 subjects that could have been enrolled actually participated and there was no expansion of any of the cohorts as allowed by the 3x3 protocol design.

The present findings provide further data to continue clinical trial testing of LTI-01 IPFT in this population. While 2 patients who received the top dose of 800,000 IU tolerated the drug well and required only two doses to relieve pleural sepsis, recruitment of the third patient to this group was not accomplished. Whether the 800,000 IU dose is the optimal dose that can be carried forward to phase 2 efficacy testing requires further expansion of this cohort. While there was also no identification of a maximally tolerated dose (MTD) of LTI-01 IPFT, the absence of safety signals observed in this trial offers a strong rationale for future dose escalations to identify the optimal candidate dose to be tested in phase 2 testing. Higher dose escalations were not included in the design of this study because of limited drug supply.

LTI-01 did not present safety signals of concern, with the severity of underlying medical conditions in the subject population reflecting all TEAEs. Patients commonly received analgesics in this study, but administration of LTI-01 IPFT was not found to be routinely associated with exacerbations of chest discomfort. Two (2) subjects died during the follow-up period after active study participation. Neither

death was attributed to LTI-01. These observations support the concept that LTI-01 was well-tolerated and safe in the doses that were administered in this trial.

In particular, there were no bleeding events, either local or systemic in the subjects that we enrolled. This observation is of particular importance given bleeding rates of about 5-28 percent that have been reported to be associated with different forms of IPFT in the literature(27, 28), with other studies reporting disparate, albeit lesser incidences(12, 29). The absence of clinical bleeding was anticipated given that bleeding events were not observed in the preclinical studies we reported over the past several years(11, 13, 14, 16, 18, 30-32). Bleeding was likewise not observed in GLP toxicology studies and was limited even though a chest tube was placed in the normal pleural space in the animals receiving doses of scuPA intrapleurally that were at up to about 10-fold in excess of the initial dose used in this study. The initial dose of 50,000 IU was felt to likely be subtherapeutic based on the literature(11, 33), but was intentionally used to determine how well the patients in that group tolerated the study drug and vehicle before advancing the dose. Interestingly, one patient in this group responded to LTI-01 IPFT and had low levels of PF PAI-1 and relatively robust increments of uPA in PFs after dosing. The exclusion criteria we used also had rigorous provisions designed to decrease bleeding risk associated with this trial. These included abnormalities of the screening coagulation profile, known platelet dysfunction or use of anti-platelet agents. Patients could receive anticoagulants in doses commonly used for prophylaxis against deep vein thrombosis but not full anticoagulation, given reports of increased bleeding risk(34, 35). Patients with a glomerular filtration rate of < 30 were also excluded on this basis. In addition, plasma fibrinogen levels were screened, as levels below 100-150 mg/dL have been linked to bleeding risk as extrapolated from that identified in trauma patients(36-38).

All patients maintained supraphysiologic levels of plasma fibrinogen, which is an acute phase reactant and plasma levels remained elevated throughout the treatment period. The data indicate that administration of LTI-01 IPFT was not associated with fibrinogenolysis and that future screening for fibrinogen levels does not need to be routine in the CPE/empyema patient population. There was no consistent impact of LTI-01 IPFT on the coagulation profiles of treated subjects. One patient in the 100,000IU group developed an elevation of the protime (PT) after the second dose of study drug felt to be attributable to the underlying medical condition rather than administration of LTI-01. This patient did not receive the third dose of LTI-01 IPFT, having met the stopping criteria; PT16 seconds, 1 second beyond the administration threshold, had no clinical evidence of bleeding, received oral vitamin K but did not receive a third dose of LTI-01 IPFT.

There was a general resolution of pleural opacification by determination of the resolution observed by either chest X-ray or chest CT imaging, as calculated by the methodology originally reported in the MIST2 trial(5). Relative changes in the percent pleural opacity area (from chest radiography) and/or volume (from chest CT scanning) in this open label study were comparable to findings in the MIST-2 study that showed IPFT therapy with tPA/DNase was more effective than placebo(5). However, this was a safety trial and therefore was not powered to allow precise delineation of efficacy responses. Therefore, this readout needs to be considered as promising but preliminary. Given the limited numbers of patients, the observed resolution of pleural loculations could also reflect ongoing resolution attributable to alternative factors, including effective antibiotic therapy and supportive care. Pleural fluid drainage was increased by administration of LTI-01 IPFT, but a similar has been observed with intrapleural administration of other plasminogen activators such as tPA/ DNase IPFT(5). All baseline assessment approximated the screening

or day 1 predose period and post-treatment assessments approximated the day 4 post treatment interval but some were obtained at later intervals, representing another confounder. Nonetheless, LTI-01 IPFT improved pleural opacification and concurrently alleviated pleural sepsis in four patients, including the two patients treated at the 800,000 IU level, findings that provide a hint of efficacy and a justification to proceed to phase 2 testing. The clinical and radiologic improvements in these patients were temporally associated with the administration of LTI-01 IPFT.

We assessed additional secondary efficacy endpoints, which likewise provided hints of efficacy subject to the same limitations applicable to the radiographic improvements in pleural opacification. Decreases in plasma CRP levels and in white cell and neutrophil counts that generally occurred in the subjects are consistent with improvement of the underlying pleural infections. The CRP changes are most likely, at least in part, related to resolution of the pleural loculations and expedited drainage. As an uncontrolled, open label safety trial, the effects of antibiotics and supportive care could have substantively contributed to these changes. The increments in chest tube drainage are likely attributable to the effects of the LTI-01 on increased pleural microvascular permeability, as has been observed with other IPFT agents. Interestingly, in preclinical studies in rabbits with empyema, increased size of the pleural effusions have been greater with tPA versus scuPA IPFT when used at comparable doses (unpublished data, not shown). Lastly, the 4 subjects who received follow-up IPFT with tPA+/-DNase were all treated at one site and at the discretion of the treating team, which was permitted under the protocol design.

We next sought to assess the bioavailability of the interventional agent in PFs and we interrogated systemic responses of the fibrinolytic system to LTI-01 IPFT. To our knowledge, this is the first study to include such comprehensive biochemical analyses with the administration of any form of IPFT. Interestingly, the levels of PAI-1 antigen were markedly increased versus those that occurred in plasma. The levels of PAI-1 antigen achieved in the PFs of patients in this study were comparable to those previously reported.(21, 25, 39). Consistent with our more recent report, PAI-1 activity was also markedly increased in these PFs (18). The excess of PAI-1 antigen versus active PAI-1 likely relates to cleavage of the serpin or a reversion of a proportion of the total PAI-1 to latency or both processes. The PD studies show that uPA concentrations were markedly increased in pleural fluid, as expected and that the levels of two chain uPA that were generated were able to fully suppress active PAI-1 at all dose escalations we tested. The PK analyses demonstrate that increments of uPA did not occur in the plasma. At all pre-dosing intervals, sufficient plasminogen was present in the PFs to support plasmin generation by LTI-01 IPFT. Increased levels of bioactive uPA- $\alpha_2$ macroglobulin complexes were generally identified within PFs after treatment, suggesting that the mechanism of processing and bioavailability of LTI-01 IPFT used in these subjects was comparable to that of scuPA IPFT (Abbott Laboratories, glycosylated scuPA derived from S2 cells) used in our models of pleural injury and empyema(13, 18). At each dosing level, increments of PA and fibrinolytic activities and D-dimer concentrations were observed, although levels likely varied as a result of the variable levels of PAI-1 and perhaps antiplasmins within the PFs of individual patients. We are unaware of any prior clinical trial in which these assessments have been made or in which the bioavailability/PF activity of any alternative form of IPFT was confirmed.

A potential advantage of the biochemical analyses we conducted was to be able to assess how individual variations in the pleural environment and dosing of IPFT impacted outcomes in individual patients. We found that there were significant variations in the levels of PAI-1 antigen and activity that

could have impacted outcomes. These analyses enabled us to understand why lower dosing of LTI-01 was variably effective in some patients. At lower dosing levels, LTI-01 IPFT successfully expedited a reduction of pleural opacification and drainage with clinical improvement. Such changes were unexpectedly observed, for example, in a patient in the 50,000 IU group, which we had expected to be ineffective. This assumption was based on the literature and our own preclinical analyses based on prorated dosing of clinically common doses of tPA IPFT; 100,000-250,000 IU(11, 33, 40). Our biochemical findings suggest that a patient with relatively low PF PAI-1 can respond to even the lowest dose of appropriately delivered LTI-01 IPFT. Our objective in this trial was to identify a dose to be carried forward to phase 2 testing and the favorable results in the 800,000 IU-treated group are especially encouraging. Notably, the doses of scuPA used in the LTI trial (0.4-6.0 mg) were within the range used in TCN-induced pleural injury (0.2-7.0 mg) and EMP (0.8-7.0 mg) rabbit models and are lower than that used for tPA IPFT (10 mg-100mg)(41) given the broadly comparable specific activities of activated scuPA and tPA (data not shown). We anticipate performing additional testing using this dose and further dose escalations as a part of the phase 2 testing for which we are preparing now with remanufacturing of a new batch of LTI-01. Ultimately, we hope to identify a dose of LTI-01 that is well-tolerated by patients with CPE/empyema and that can be effective in essentially all patients, including those with high levels of PF PAI-1 activity.

In summary, this study offers several contributions that are novel to the field. To our knowledge, we performed the first phase 1 dose-ranging trial of any form of IPFT. The trial was done in order to begin the process of achieving regulatory approval for the indication of failed drainage in loculated CPE/empyema. We found that LTI-01 IPFT was safe at the doses we tested. We integrated the clinical interventions with a comprehensive assessment of the processing of the interventional agent, which is likewise novel to the field. Our findings shed light on the mechanism by which LTI-01 is processed in human PFs and demonstrate that this form of IPFT is bioactive within the setting of CPE/empyema. Bleeding or predisposing systemic derangements of hemostasis did not occur as a result of LTI-01 IPFT. While not powered to address efficacy, hints of that desired outcome were observed and, with demonstration of patient safety, provide a strong premise to advance to further clinical trial testing.

## Methods

**Involved centers and Ethics Approvals.** The trial included a total of 7 sites, of which 3 sites were in New Zealand, including Auckland City Hospital, Auckland; The University of Otago-Christchurch Hospital, Christchurch and Dunedin Hospital, Dunedin. There were 4 sites in Australia, including Westmead Hospital, Westmead; Cairns Hospital, Cairns; Footscray Hospital/Western Health, Footscray and Sir Charles Gairdner Hospital, Nedlands, Perth.

**Trial objectives.** The primary objective was to evaluate the safety and tolerability of escalating doses of intrapleural LTI-01. The secondary objectives were to evaluate pharmacokinetic (PK) and pharmacodynamic (PD) effects of escalating doses of intrapleural LTI-01 and to assess efficacy parameters in subjects treated with escalating doses of intrapleural LTI-01. The intrapleural and systemic processing of LTI-01 was also evaluated by assessments done in pleural fluid and plasma obtained from each subject.

**Diagnosis and main criteria for inclusion.** Male or female subjects  $\geq 21$  years of age with a clinical presentation compatible with pneumonia and a parapneumonic effusion, who had PF requiring drainage

and whose pleural space failed to drain within 3 hours of tube thoracostomy. Subjects were to meet all inclusion criteria and none of the exclusion criteria for eligibility to participate in the study.

**Subjects.** Subjects were  $\geq 21$  years of age, of both genders and all races, were considered for inclusion. Safety precautions included exclusion of cognitively impaired and institutionalized persons, exclusion of subjects with other disease processes that may have interfered with the interpretation of results or situations that may have been harmful to the subjects, exclusion of pregnant women (given the known risks of radiographic assessments to unborn children), and exclusion of children (given insufficient data in adults regarding dosing or AEs). Because of the severity of illness in CPE/empyema patients, and the imperative to rapidly initiate treatment, special considerations to consent procedures were used (Section 5). Furthermore, no control groups or reference therapy groups were deemed appropriate. All subjects underwent tube thoracostomy and attempted drainage of their infected PF prior to study enrollment, and were to be recruited only if their pleural space could not be drained within 3 hours after tube thoracostomy. Subjects were to receive all other customary therapy for CPE/empyema, including antibiotic therapy, medications for other medical conditions or supportive care, apart from those that would have excluded the subject from the trial. The date first subject enrollment was March 7, 2017 and the date on which the last subject was completed was March 26, 2018.

**Identity of Investigational Product.** LTI-01 was supplied as a sterile, single-use vial containing approximately 60,000 IU (0.5 mg) of scuPA in a formulation of 5 mM citrate, 25 mM sodium chloride and 15 mg/mL mannitol, pH 4.5. LTI-01 is a lyophilized drug product designed for reconstitution with 1.0 mL of water for injection. The drug substance was manufactured through NHLBI SMARTT, with lyophilization and vialing completed with support provided by Lung Therapeutics, Inc. Reconstitution was performed by a qualified pharmacist within the clinical site pharmacy. The reconstituted material was administered via chest tube followed by a 'chase' of sterile normal saline solution to bring the total injected volume (LTI-01 plus saline chase) to 30 mL.

**Method of Assigning Subjects to Dose Levels.** Subjects were assigned sequentially to dose level. Blinding was not done, as this was an open label study.

**Selection of Doses in the Study.** The study rationale was based on studies in models of intrapleural injury that demonstrated therapeutic benefit and sufficient duration of effect of LTI-01. Data obtained from the rabbit tetracycline-induced pleural injury, *S. pneumoniae* and *P. multocida* empyema models, and from GLP safety studies(13, 15, 16, 40). It was desirable to initiate dosing at a level that was likely to be at or near a potentially therapeutic dose, since the study subjects had imminently life-threatening conditions. To balance this intention with a safe starting dose, a maximum recommended starting dose (MRSD) was calculated based on an assumption of an intrapleural no observed adverse effect level (NOAEL) of 0.25 mg/kg, converting that dose to the human equivalent dose by allometrically scaling the dose based on body surface area<sup>4</sup> and then adding a 10-fold safety factor to account for interspecies variation. This calculation resulted in an acceptable maximal safe starting dose of ~130,000 IU of LTI-01 for a 60 kg subject<sup>5</sup>. To be conservative, Lung Therapeutics, Inc. initiated dosing at a level approximately 2.5 fold below the above dose. The first 3 subjects enrolled were dosed at 50,000 IU daily for up to 3 days. Dose level was to be doubled in each consecutive cohort of 3 subjects subject to safety review. The lowest dose levels (50,000-100,000 IU) were not anticipated to be optimally active but were similar to the dose level of urokinase (up to 100,000 IU) used in children and adults when delivered by the intrapleural route(11).

**Trial Methodology:** This was a prospective, open-label, dose escalation safety trial. Subjects presenting with symptoms consistent with pneumonia along with CPE/empyema were initially treated by standard of care, including placement of a chest tube and initiation of antibiotics, and were evaluated for participation in the study. Eligible subjects began treatment with intrapleural LTI-01 within approximately 24 hours of initial consent and enrollment. Study treatment was administered as a bolus dose through the chest tube into the pleural space and allowed to stay in the space for 3 hours. Treatment occurred once per day for up to 3 consecutive days. A dose escalation design was used, with up to 5 dose escalation cohorts as shown in Table 1. The first 3 subjects enrolled were given an initial dose of 50,000 IU LTI-01 daily for up to 3 days. Following review of safety data from Cohort A (50,000 IU LTI-01) subjects by a Safety Review Committee (SRC), the dose level was escalated (doubled) in each consecutive group of 3 subjects with dosing for up to 3 days at all dose levels. The format was open label and a 3 by 3 design to conform to regulatory advice and availability of study drug, part of which was reserved for stability studies. A total of 21 patients could have been enrolled. The SRC comprised the Medical Monitor (MM), lead country Study Investigators and the Sponsor Medical representative.

The study protocol permitted completion of dosing after fewer than 3 daily doses in the event of complete resolution of the pleural process after 1 or 2 doses of LTI-01 in any subject. Clinical activity was defined as pleural density improvement of pleural density at Day 4 versus baseline radiographic analysis, as confirmed by chest X-ray and via estimation of the volume of the pleural collection by chest computed tomography (CT) scanning. Dose escalation was not to occur in the event of a dose-limiting toxicity (DLT). A DLT was defined as occurrence of a bleeding event or the same common terminology criteria for adverse events. Common Terminology for Adverse Events (CTCAE) version 4.03 Grade 3 (or higher) nonhematological adverse event (AE) in  $\geq 2$  subjects. For this purpose, bleeding events were defined as an acute bleeding event with  $\geq 2$  g/dL drop in blood hemoglobin; development of melena (indicative of gastrointestinal [GI] bleeding), or hemothorax or pleural fluid (PF)/blood hematocrit  $>50\%$  from the chest tube. In the event of a DLT, the previous dose was to be considered the maximum tolerated dose (MTD) and additional subjects (up to a study total of 21 subjects) could be added to any dose level below or at the MTD for the purpose of obtaining additional efficacy information.

All subjects at all dose levels were observed for 4 days after last intrapleural LTI-01 treatment (or until hospital discharge if sooner). Acceptable clinical stability was to be documented for any individual subject before treatment of a subsequent subject. Blood and PF assessments including CBC, coagulation profile, metabolic profile, fibrinogen and CRP levels done before and at 3 and 123 h after dosing. Chest X-ray, CT scans of the chest and other safety assessments were also performed before and at about 4 days after administration of LTI-01 IPFT.

Blood and PF cultures were obtained, as well as other diagnostic tests deemed appropriate, including atypical pneumonia serology and pneumococcal antigen at Screening. Procedures included chest X-ray and CT imaging and measurement of chest tube drainage, chest tube flushes and drain suction. Use of alternative forms of IPFT including tPA (alteplase)/DNase (dornase alfa), tPA alone or referral for surgery and thoracic surgery including video-assisted thoracostomy or other procedures was to occur as needed after completion of administration of LTI-01 unless exceptional circumstances prevailed in the view of the medical management team. Subjects who required alternative forms of IPFT or were referred for surgery were to be discontinued from the study. The team could, in exceptional circumstances, choose to refer a

subject to surgery prior to completion of the course of LTI-01 IPFT; in such a scenario, the reasons for the decision were to be entered on CRFs.

After identification of an SAE during administration of study drug, LTI-01 administration could be interrupted for up to 24 hours at the discretion of the local PI and restarted if clinically appropriate to complete the delayed trial treatment course. A displaced thoracostomy tube was only to be replaced if clinically indicated for PF drainage and not solely for trial drug administration. If the chest tube displacement occurred over a period of less than 48 hours, the trial treatment course could be restarted and completed, after chest tube replacement, if clinically indicated to complete the planned LTI-01 IPFT. Study drug administration was to be delayed if PT/aPTT was  $>1.7 \times$  control or PT  $>15$  seconds, or fibrinogen was  $<150$  mg/dL, at the last available time point prior to dose administration (typically the labs drawn within 3 hours prior to dose administration). To mitigate against these changes, pharmacologic prophylaxis for deep vein thrombosis (DVT) was delivered as far removed as possible (e.g. 9-12 hours) from the next intrapleural delivery. Use of intermittent compression stockings for DVT prophylaxis could also be considered by the clinical management team. Continued dosing was only allowed if the PT/aPTT was  $\leq 1.7$  and PT  $<15$  seconds and fibrinogen was  $>150$  mg/dL prior to administration of the next dose of LTI-01. If unachievable within 12 hours of scheduled administration, the subject was to be withdrawn from treatment and closely monitored. Subjects with abnormal laboratory parameter values at any scheduled assessment were carefully monitored with management as determined by the clinical team. Similarly, in otherwise stable subjects, transfusion for downward drift of blood hemoglobin that may have reflected marrow suppression as a result of acute illness, hemodilution or IPFT was not considered as a stopping criterion and subjects could continue to be treated with close monitoring at the discretion of the clinical team. Development of bloody PF, confirmed to be hemothorax with a hematocrit of  $>50\%$  that of plasma, or an acute decline of peripheral blood hemoglobin  $>2$  g/dL of hemoglobin with or without hemodynamic instability, or presence of melena indicative of GI bleeding was taken as evidence of significant bleeding. In this scenario, dosing was to be stopped and appropriate care initiated, including fluids and transfusion resuscitation as needed. Surgical control of bleeding complications was provided post-resuscitation as needed. Third LTI-01 doses were not given if there was complete resolution of pleural process after the prior dose, because of chest tube blockage or requirement for alternative therapy.

**Safety Assessments including Adverse Events.** Subjects were monitored for all Treatment Emergent Adverse Events (TEAEs) starting prior to the first study dose (Day 1 at the time of initiation of administration of Dose 1) until 23 hours after last dose of LTI-01. At each contact with the subject, information regarding AEs was elicited by appropriate questioning and examinations. Grade 3 and Grade 4 AEs were collected from the time of first study dose through to Day 7 or hospital discharge, whichever came first. SAEs were collected from the time of first study dose through to Day 28, hospital discharge or death, whichever occurred first. AEs overall are listed in the CSR, Supplemental Data, Table 14.3.1.2). AEs related to study drug (Table 14.3.1.5); AEs by maximum severity (Table 14.3.1.3); AEs by maximum severity and maximum relationship to study drug (Table 14.3.1.6); AEs leading to study drug withdrawal or discontinuation from study (Table 14.3.1.7); and serious AEs (Table 14.3.1.4)

**Efficacy endpoints of the study.** Vital signs were monitored and time to defervescence could not be ascertained as 13/14 patients were afebrile at study entry. Referrals for surgery and mortality during hospitalization, radiographic improvement based on comparison of pre-treatment chest X-ray or CT scan to those done  $23 \pm 3$  hours after the last received daily dose of LTI-01 IPFT, as previously reported(5). First



radiographic study was an upright 2-view chest X-ray. The presence of pleural effusion was confirmed radiographically. If pleural loculation was suggested on the plain chest X-ray, loculation was confirmed using CT scanning of the chest and chest ultrasonography. Completion of CT scanning (high resolution if available) was done unless precluded by the clinical status of the subject. At  $23 \pm 3$  hours after the third (or final) dose of LTI-01, PF related density was compared by follow-up chest X-ray, with absolute change in density to the ipsilateral hemithorax and relative change in pleural opacification versus baseline imaging, as previously described(5). Days of hospitalization for this episode of CPE/empyema, total PF drainage from an initial dose of LTI-01 IPFT until 23 hours after the last LTI-01 IPFT dose versus baseline, stability or decline of white and neutrophil cell counts in blood and C-reactive protein (CRP) levels in plasma were also assessed. Total volume of drainage from the chest tube was documented daily for up to 23 hours after last dose (Day 3 of study drug administration) and totaled for the full course (up to 3 days) of treatment for each subject.

**Central Laboratory Analyses.** These studies were done at UTHSCT and included D-dimer and PA as well as fibrinolytic activity levels in plasma and pleural fluids collected at baseline and at 3 and 23 hours after each dose of LTI-01. Levels of PAI-1 antigen and activity levels were also assessed(18) as were levels of bioactive uPA- $\alpha_2$  macroglobulin complexes(13).

**Pharmacokinetics (PK) and pharmacodynamics (PD) analyses.** These included total uPA antigen PK and PD in plasma and pleural fluids. A uPA ELISA; Quantikine ELISA (Urokinase, R&D Systems, DUpA00, Lot # 171962), was used to perform these studies as LTI-01 is a recombinant form of scuPA that is converted to its active form, two chain uPA. In biological fluids, such as plasma or pleural fluid. The protein plasminogen activator inhibitor 1 (PAI-1) binds to and inhibits the activity of uPA. The assay that was used in this study measures uPA-related antigen and detects single-chain uPA, two-chain uPA, uPA-PAI-1 complexes, and uPA bound to its receptor (uPA-uPAR complexes).

**Prior and Concomitant Therapy.** Subjects were expected to receive customary care including radiologic chest tube placement and initiation of antibiotics prior to enrollment. Subjects could be enrolled after initial therapy if: 1. A chest tube was placed after initial therapy and drainage was found to be impaired, or 2. A chest tube was initially placed along with standard care and impaired drainage developed later. Radiologic tube placement, while not required, was considered justified as large bore tubes had not been proven to be more effective, were associated with greater morbidity, and radiologically placed tubes had the advantage of being located within the PF collection. Subjects could receive all other customary therapy for CPE/empyema. Medications included antibiotic therapy, and medications needed for other medical conditions or supportive care, apart from those that would have excluded the subject from the trial. Adequate analgesia was provided to subjects with CPE/empyema for pain control, to mitigate pain resulting from pleuritis and placement of thoracostomy tube. Pain control was achieved using opioids or acetaminophen; non-steroidal anti-inflammatory drug (NSAID) therapy was not permitted. Prior medication and concomitant medications are listed and summarized, separately, by treatment (CSR Listing 16.2.4.8.1 and Listing 16.2.4.8.2), Supplemental Data. All study drug administration data are listed (CSR Listing 16.2.5.1). In addition, protocol adherence at study drug administration was also recorded and is provided in subject listings. Treatment compliance is summarized by dose level (CSR Table 14.1.3).

Therapeutic doses of anticoagulants, heparin, warfarin, factor Xa or thrombin inhibitor anticoagulants could not be given. Subjects could receive DVT prophylaxis in the form of heparin or derivatives, factor Xa

or thrombin inhibitors. Intrapleural drugs other than LTI-01, or other agents of any kind, were not permitted with the exception of saline flushes used to maintain patency of chest drains. All subjects were to receive antibiotics, based on blood and PF bacterial culture and sensitivity results where positive. Antibiotic regimens used could be at the discretion of the managing physician.

**Anti-drug antibodies (ADA).** Blood samples for testing of ADAs were obtained prior to the first dose and at Day 28 or hospital discharge, whichever occurred first.

**Pharmacokinetics and pharmacodynamics.** Samples of PF and plasma were collected at the intervals stipulated, using methods appropriate for these assessments (Please see Clinical Study Report (CSR), 16.1.10.1., p 1269, Supplemental Data).

**Processing of LTI-01 within pleural fluids and plasma.** Measurement of pleural fluid PAI-1 antigen and activity levels, PA and fibrinolytic activities, D-dimer concentrations, and bioactive, uPA- $\alpha_2$ macroglobulin complexes were measured as previously described(18, 32). Plasma fibrinogen levels were measured in the clinical laboratories at the participating sites.

**Subject Follow-up.** Attempts were made at 3 and 12 months after treatment to document incidence of surgical referral, further hospitalization for CPE/empyema and mortality in treated subjects for whom follow-up is available through the out-patient clinic.

**Statistics.** All statistical analyses were exploratory in nature with descriptive statistics (i.e. mean, standard deviation, median, maximum and minimum) provided for continuous variables. Categorical variables are summarized by frequency distributions (number and percentage of subjects). The intent-to-treat (ITT) population comprised all subjects who enrolled in the study and who provided any post-Screening data. The safety population comprised all subjects who received LTI-01 treatment. All summaries of laboratory data and vital signs data are by actual treatment LTI-01 dose group (from low to high) and combined LTI-01 group (all subjects). Adverse events were coded using MedDRA® Version 18.0. Adverse events and serious adverse events (SAEs) are grouped by system organ class (SOC) and preferred term and are summarized by actual treatment at time of onset of the AE. Adverse event summaries provided are listed in Section 9.7.1.3 of the CSR. Details about the application of the Statistical Analysis Plan are provided in the dedicated section of the CSR Appendix 16.1.9. and on pp 1222-1328 of the CSR, Supplemental Data.

**Study Approval and Registration.** Written informed consent was received from all participants prior to inclusion in the study. Ethics approvals were obtained from all required organizations, including: 1) The Western Sydney Local Health District Human Research Ethics Committee, Westmead Hospital, Westmead NSW 2145, Australia 2) Sir Charles Gairdner and Osborne Park Health Care Group Human Research Ethics Committee Nedlands WA 6009, Australia and 3) Northern B Health and Disability Ethics Committee Health and Disability Ethics Committees, Ministry of Health Wellington 6011, New Zealand. This trial was designed and monitored in accordance with Sponsor procedures, which comply with the ethical principles of Good Clinical Practice (GCP) and International Council on Harmonization (ICH) guidelines, and in accordance with the Declaration of Helsinki. Subjects had the right to refuse participation without explanation and could withdraw from the study at any time at their discretion, without affecting their follow-up care and management. In addition, clinicians managing the subject could choose to embark on alternative treatment at any time if they judged the approach to be in the best interest of the given subject. This consideration took precedence over completion of the protocol elements in a given subject.

Medical confidentiality was preserved under the Declaration of Helsinki and current local, regional and national mandates. All Investigators and the trial team were expected to observe these standards, so as to protect the confidentiality of the enrolled and screened subjects. Personal identifying information was not revealed.

**Author Contributions.** LB, BB, GS, AMS, YCGL served as site Principal Investigators and recruited/managed patients enrolled in this study. SI, YGCL, RWL and NR helped to design and review the protocol. JG and SI served of the Safety Review Committee along with representatives of the Australian and New Zealand sites. JG served as Medical Monitor. SS, SI reviewed and organized the data, including that from the Clinical Study Report (CSR). KS and GF performed statistical analyses. AAK, GF coordinated and performed the sample biochemical analyses, which they performed with KS. TO and WB performed imaging analyses. SI, SS, GF and NR prepared the manuscript, which was reviewed by all authors.

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**Table 1: Baseline Demographics for all Subjects (ITT Population)**

Parameter	Statistics	LTI-01 50,000 IU (N=3)	LTI-01 100,000 IU (N=3)	LTI-01 200,000 IU (N=3)	LTI-01 400,000 IU (N=3)	LTI-01 800,000 IU (N=2)	All LTI-01 (N=14)
Sex							
Female	n (%)	1 (33.3%)	-	-	1 (33.3%)	1 (50.0%)	3 (21.4%)
Male	n (%)	2 (66.7%)	3 (100.0%)	3 (100.0%)	2 (66.7%)	1 (50.0%)	11 (78.6%)
Age (years)	Mean	59.3	52.3	69.0	75.7	70.0	64.9
	Min	52	42	57	72	51	42
	Max	71	61	78	78	89	89
BMI (kg/m <sup>2</sup> )	Mean	24.95	25.27	19.07	26.50	24.15	23.68
	Min	22.4	17.4	15.9	25.1	24.0	15.9
	Max	27.5	33.1	24.7	27.9	24.3	33.1
Race							
White	n (%)	3 (100.0%)	2 (66.7%)	2 (66.7%)	3 (100.0%)	1 (50.0%)	11 (78.6%)
Other	n (%)	-	1 (33.3%)	1 (33.3%)	-	1 (50.0%)	3 (21.4%)

**Legend.** Source: CSR 14.1.2 ITT: Intention to treat. BMI: Body mass index. Data include numbers/category and percentages.

Table 2. Pleural Fluid Characteristics, Cultures and Antibiotic Coverage at Study Entry.

Group	LTI-01 50,000 IU n=3	LTI-01 100,000 IU n=3	LTI-01 200,000 n=3	LTI-01 400,000 IU n=3	LTI-01 800,000 IU n=2
Color/ Purulence	Cloudy	Brown, Purulent	Cloudy	Turbid	No comment
	Blood Stained	Yellow	Purulent	Blood Stained	Turbid
	Turbid	Straw colored	Pus	Blood Stained	-
pH	7.35*	-	6.84	6.91	7.00
	7.2	7.16	6.68	6.76	7.07
	7.2	7.2	-	6.5	-
Culture	N.G.	<i>Strep. constellatus</i>	N.G.	<i>E.coli, Klebsiella pneumoniae</i>	N.G.
	N.G.	N.G.	<i>Strep intermedius</i>	<i>Provitella melaninogenic us, Strep. Oralis, Moraxella catatthalis, Eikenella coorodens</i>	N.G.
	N.G.	N.G.	- (Clotted)	N.G.	-
Antibiotic Coverage	Azithromycin, Amoxicillin	Amoxicillin	Azithromycin, Amoxicillin	Ciprofloxacin	Ceftriaxone
	Pipercillin/tazobactam	Augmentin	Augmentin, Amoxicillin, Azithromycin	Ceftriaxone	Augmentin
	Pipercillin/tazobactam	Clindamycin	Clindamycin	Pipercillin/ tazobactam	-

**Legend:** Source: CSR Listing 16.2.6.7. and Listing 16.2.4.6. and text pp 52-54. N.G.: No growth on aerobic or anaerobic PF cultures. \* The subject had a PF pH of exactly 7.2 at Screening. The subject was enrolled into the study as all other entry criteria were met and no additional safety risk of enrollment was foreseen. The patient entered the study under a protocol exception waiver.

**Table 3: Study Schedule**

STUDY DAY►	SCREEN Day -1	Day 1-2			Day 2-3			Day 3-4			Days 4-7	Days 7-28 or Hospital Discharge
		Within 3 h pre- dose	Hours post- dose		Within 3 h pre- dose	Hours post- dose		Within 3 h pre- dose	Hours post- dose			
EVENT▼			3	23		3	23		3	23		
Informed Consent	X											
Medical/medication History	X											
Inclusion/Exclusion Criteria	X											
Confirmation of Eligibility	X	X <sup>2</sup>										
Demographics (incl. height and weight)	X											
Physical Examination	X									X		
Vital Signs (pre-dose and 3, 6, 12, 18 hours post-dose)	X	X	X	X <sup>6</sup>		X	X <sup>6</sup>		X	X	X*	
Serum Pregnancy Test (WOCBP)	X											
Chest X-ray	X									X		
Chest CT (preferably high resolution)	X									X		
Chest Ultrasonography	X			X			X			X		
Tube thoracostomy under CT guidance and initiation of antibiotics	X											
Review of Blood and PF Screening	X <sup>5</sup>	X										
Dosing		X			X <sup>3</sup>				X <sup>3</sup>			
Clamp Chest Tube <sup>i</sup>		X			X				X			
Blood sample for hematology (local) <sup>1</sup>	X	X	X	X <sup>6</sup>		X	X <sup>6</sup>		X	X	X*	
Blood sample for central lab <sup>1</sup>		X	X	X <sup>6</sup>		X	X <sup>6</sup>		X	X		
Blood sample for ADA <sup>11</sup>		X										X
Blood chemistries	X	X		X <sup>6</sup>			X <sup>6</sup>			X	X*	
CRP (blood)		X		X <sup>6</sup>			X <sup>6</sup>			X	X*	X*
PF collected <sup>4</sup> (half of sample for central lab)		X	X	X		X	X		X	X		
Assessment of PF drainage	X	X <sup>3</sup>		X <sup>3</sup>			X <sup>3</sup>			X		
Adverse Events (post- enrollment)		X	X	X	X	X	X	X	X	X	X <sup>8</sup>	
SAE (post-enrollment)	X	X	X	X	X	X	X	X	X	X	X	X <sup>9</sup>
Concomitant Medications	X	X	X	X	X	X	X	X	X	X	X <sup>10</sup>	X <sup>10</sup>

## Legend: Study Design and Schedule of Assessments

\*As available in medical record.

<sup>1</sup> Complete blood count with differential and platelets, coagulation tests (i.e. PT/PTT, fibrinogen) done locally; half of sample processed for PK/PD at UTHSCT on days when required

<sup>2</sup> Reassessment of PT/PTT, fibrinogen and platelets

<sup>3</sup> PF drainage volume recorded daily once the subject was enrolled. PF volume from the chest tube also recorded during the period of time in between enrollment and the first dose. The total amount of pleural drainage from study entry to completion was totaled from these assessments. Second and third LTI-01 doses were not given if there was complete resolution of pleural process after the prior dose.

<sup>4</sup> PF cell count, hematocrit and protein performed at local lab; half of sample processed for PK/PD at UTHSCT

<sup>5</sup> PF assessment of purulency, Gram stain, culture, pH and volume; blood culture results

<sup>6</sup> 23 hours post-dose sample served as pre-dose sample for next dose and was performed within 3 hours pre-dose

<sup>8</sup> Grade 3 and 4 AEs, and fever

<sup>9</sup> Through Day 28 or hospital discharge, whichever occurred first.

<sup>10</sup> Total days of antibiotics

<sup>11</sup> Blood sample for ADA screening at Day 28 or hospital discharge, whichever occurred first.

**Table 4: Treatment-Emergent Adverse Events by Treatment Group**

	LTI-01 50,000 IU (N=3)	LTI-01 100,000 IU (N=3)	LTI-01 200,000 IU (N=3)	LTI-01 400,000 IU (N=3)	LTI-01 800,000 IU (N=2)	All LTI-01 (N=14)
	Number of subjects (%) Number of TEAEs					
<b>Vascular disorders</b>	<b>1 (33.3%) 1</b>	<b>1 (33.3%) 1</b>	<b>2 (66.7%) 2</b>	<b>0</b>	<b>0</b>	<b>4 (28.6%) 4</b>
Hypotension	1 (33.3%) 1	0	2 (66.7%) 2	0	0	3 (21.4%) 3
Phlebitis	0	1 (33.3%) 1	0	0	0	1 (7.1%) 1
<b>Investigations</b>	<b>1 (33.3%) 1</b>	<b>1 (33.3%) 3</b>	<b>1 (33.3%) 1</b>	<b>0</b>	<b>0</b>	<b>3 (21.4%) 5</b>
Body temperature increased	1 (33.3%) 1	1 (33.3%) 1	0	0	0	2 (14.3%) 2
Hemoglobin decreased	0	0	1 (33.3%) 1	0	0	1 (7.1%) 1
PT prolonged	0	1 (33.3%) 1	0	0	0	1 (7.1%) 1
WBC count increased	0	1 (33.3%) 1	0	0	0	1 (7.1%) 1
<b>Respiratory, thoracic and mediastinal disorders</b>	<b>2 (66.7%) 2</b>	<b>1 (33.3%) 1</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>3 (21.4%) 3</b>
Pleuritic pain	1 (33.3%) 1	1 (33.3%) 1	0	0	0	2 (14.3%) 2
Pleural effusion	1 (33.3%) 1	0	0	0	0	1 (7.1%) 1
<b>Infections and infestations</b>	<b>0</b>	<b>1 (33.3%) 1</b>	<b>0</b>	<b>1 (33.3%) 1</b>	<b>0</b>	<b>2 (14.3%) 2</b>
Lung abscess	0	0	0	1 (33.3%) 1	0	1 (7.1%) 1
Respiratory syncytial virus infection	0	1 (33.3%) 1	0	0	0	1 (7.1%) 1
<b>Injury, poisoning and procedural complications</b>	<b>1 (33.3%) 3</b>	<b>0</b>	<b>1 (33.3%) 1</b>	<b>0</b>	<b>0</b>	<b>2 (14.3%) 4</b>
Post procedural hematoma	0	0	1 (33.3%) 1	0	0	1 (7.1%) 1
Post procedural persistent drain fluid	1 (33.3%) 3	0	0	0	0	1 (7.1%) 3

**Legend:** Table restricted to System Organ Class (SOC)s for which >1 subject reported a TEAE. For all TEAEs, see source: CSR; Table 14.3.1.2, Supplemental Data.

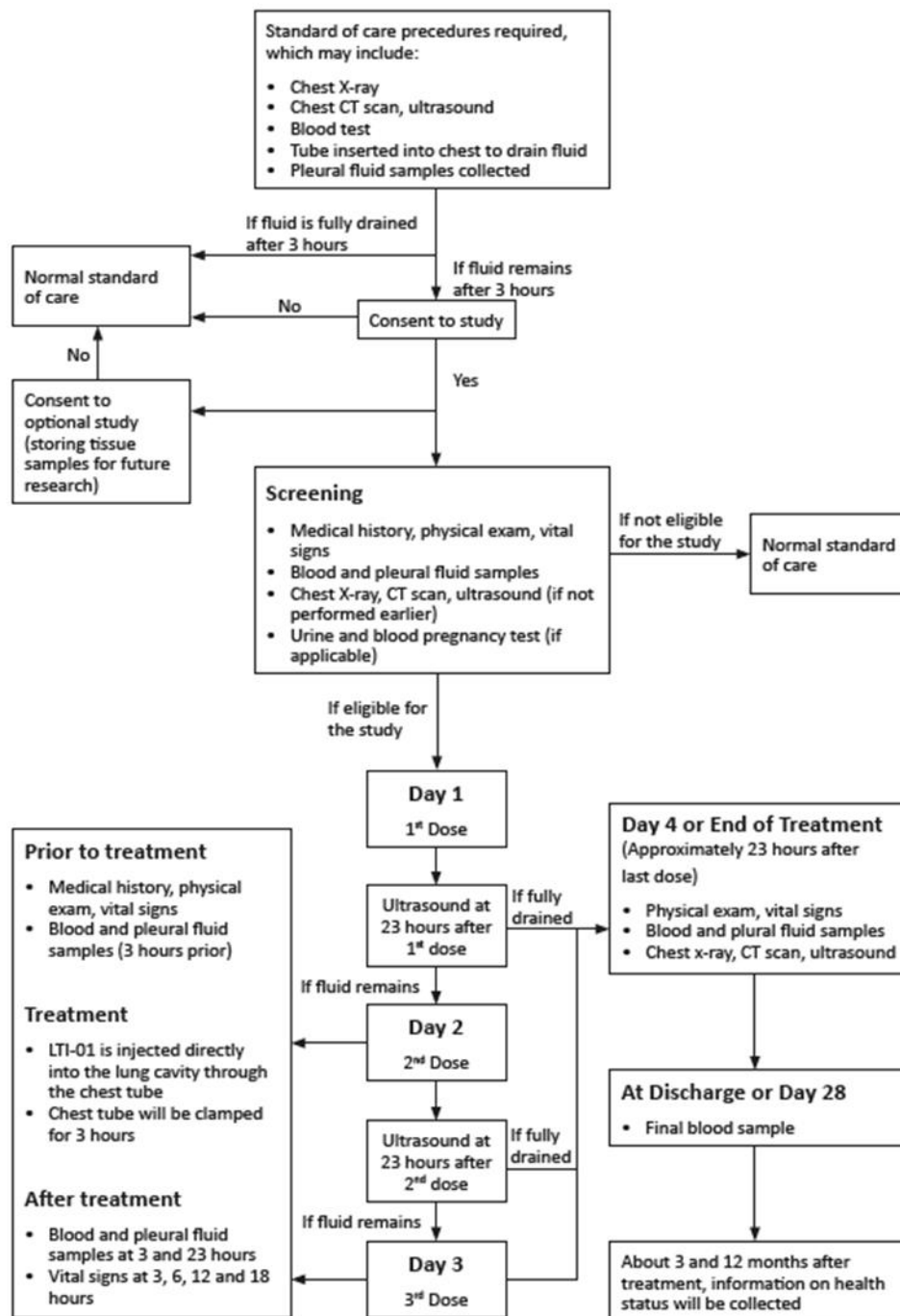
**Table 5: Pleural Density Area and Volume Changes with LTI-01**

Subject ID	Dose of LTI-01 (IU)	Absolute Change in % Pleural Density Area by CXR	Relative Change in % Pleural Density Area (%) By CXR	Absolute Change in Pleural Density Volume (mL) by CT	Relative Change in Pleural Density volume (%) by CT
102-1001	50,000	-42%	-85%	-1041	-69%
202-1001	50,000	1%	4%	279	83%
202-1002	50,000	-5%	-42%	NA	NA
102-1002	100,000	-2%	-15%	-277	-36%
103-1001	100,000	-16%	-83%	NA	NA
202-1003	100,000	-12%	-55%	NA	NA
102-1003	200,000	-14%	-71%	-705	-72%
102-1004	200,000	-6%	-37%	-552	-46%
202-1004	200,000	NA	NA	-334	-62%
102-1005	400,000	NA	NA	-545	-45%
202-1005	400,000	-8%	-38%	-221	-38%
102-1006	400,000	NA	NA	-480	-31%
102-1007	800,000	-6%	-36%	NA	NA
103-1002	800,000	-53%	-85%	NA	NA

Legend. NA = not available. Absolute change is the change of pleural opacification compared as a percentage of pleural density after IPFT versus that before therapy. Relative change in pleural opacification: Change in pleural opacification recorded as the basal opacity-the post-treatment opacification divided by the basal opacification. Only 1 subject (202-1001) had a relative increase in pleural density area (4%) and pleural density volume (83%). This subject's pleural effusion was found to have malignant squamous epithelial cells due to pleural metastases from a head and neck tumor that was discovered by PF cytology after enrollment.

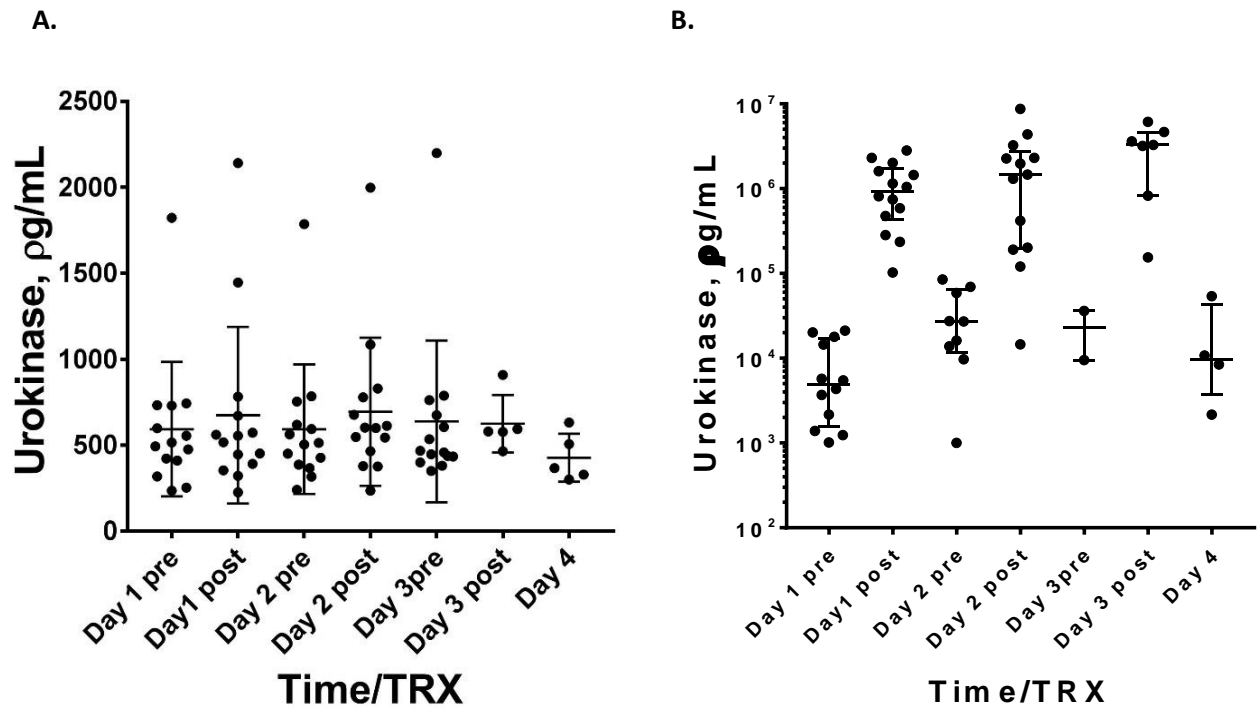


Figure 1. Protocol Flow Chart



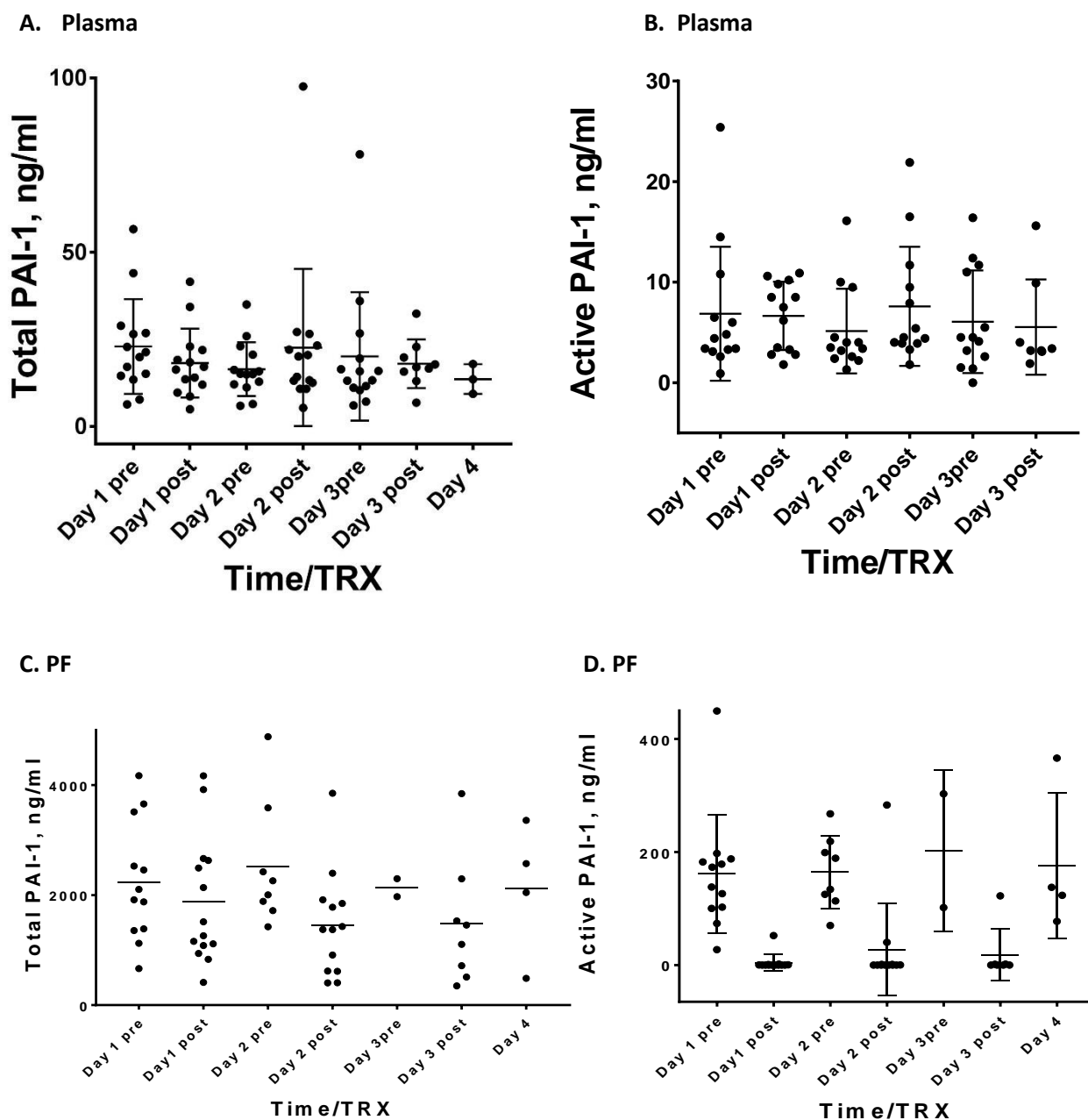
**Legend.** Source: CSR Section 2, Synopsis and Clinical Protocol, Supplemental Data.

Figure 2. Plasma and PF uPA Concentrations before and after LTI-01 IPFT.



**Legend.** Urokinase antigen levels determined by ELISA. All samples collected at each interval and from each dose escalation group are illustrated in the box-dot plots in which the horizontal lines indicate the medians and whiskers indicate the range excluding outlying dots. TRX: LTI-01 treatment. A. Plasma uPA antigen determinations. B: PF uPA antigen determinations (picogram/ml).

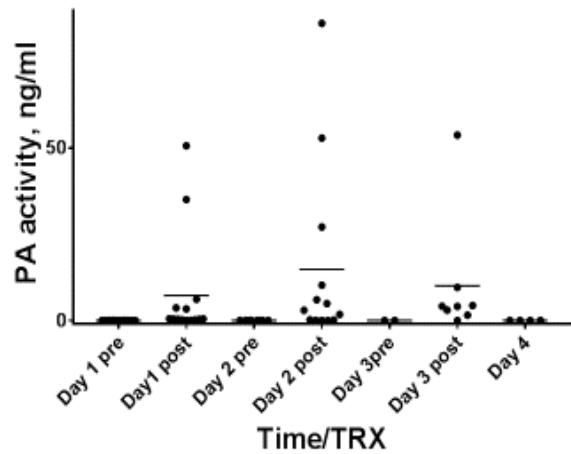
**Figure 3. PF total and active PAI-1 are markedly increased versus levels in plasma in the CPE/empyema Subjects.**



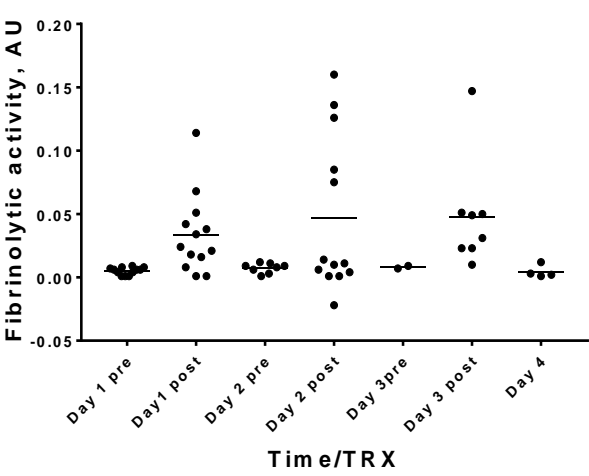
**Legend.** Total PAI-1 was determined by ELISA and PAI-1 activity was determined as we previously reported(18). A and B illustrate plasma PAI-1 antigen levels and activities in the cohort samples all plotted at the same intervals relating to LTII-01 IPFT, respectively. C and D represent the same determinations illustrated in a dot-box plot format with whisker indicative of the rant and outlying values indicated by separate points.

Figure 4.

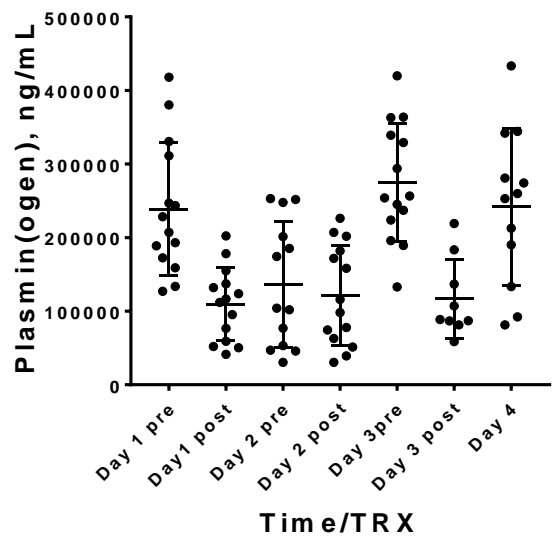
A.



B.



C. Plasma



D. PF

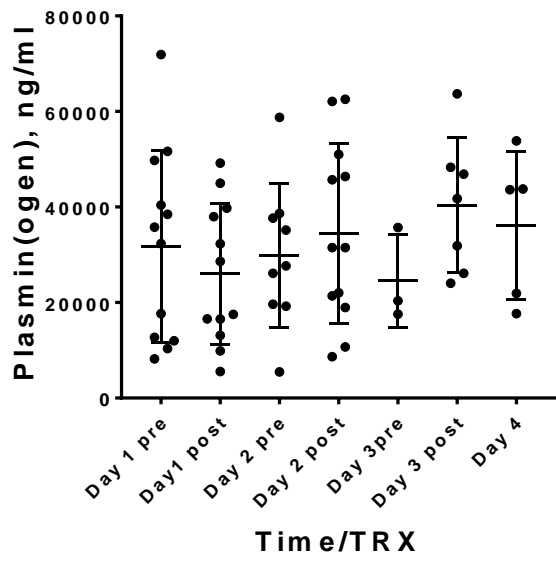
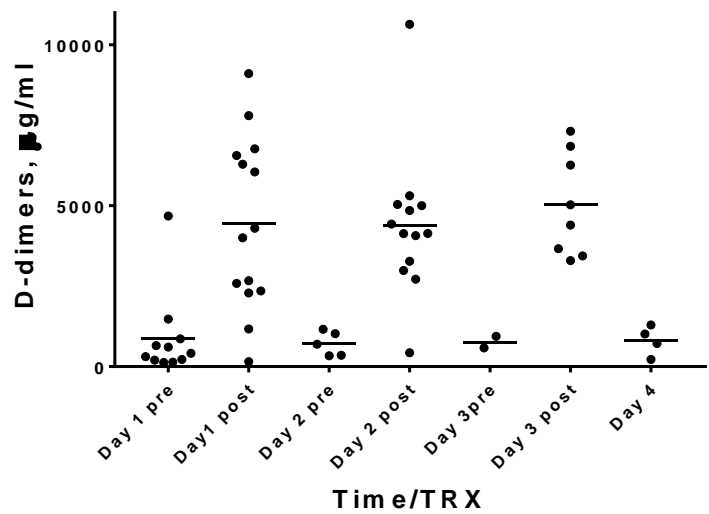
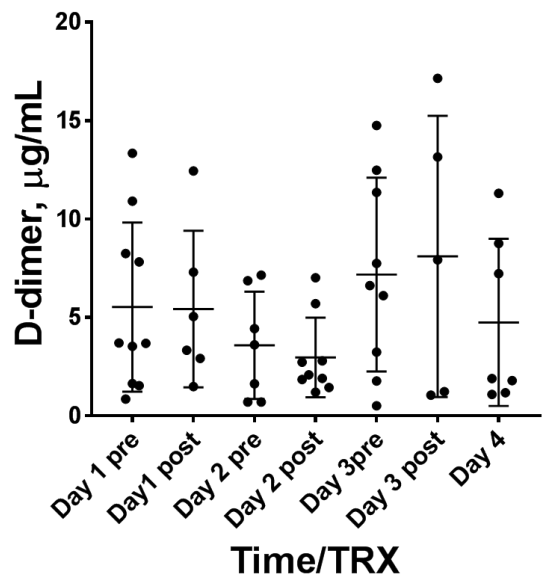


Figure 5. PF D-Dimer levels increased at 3h after LTI-01 IPFT but not in plasma.

A. PFs

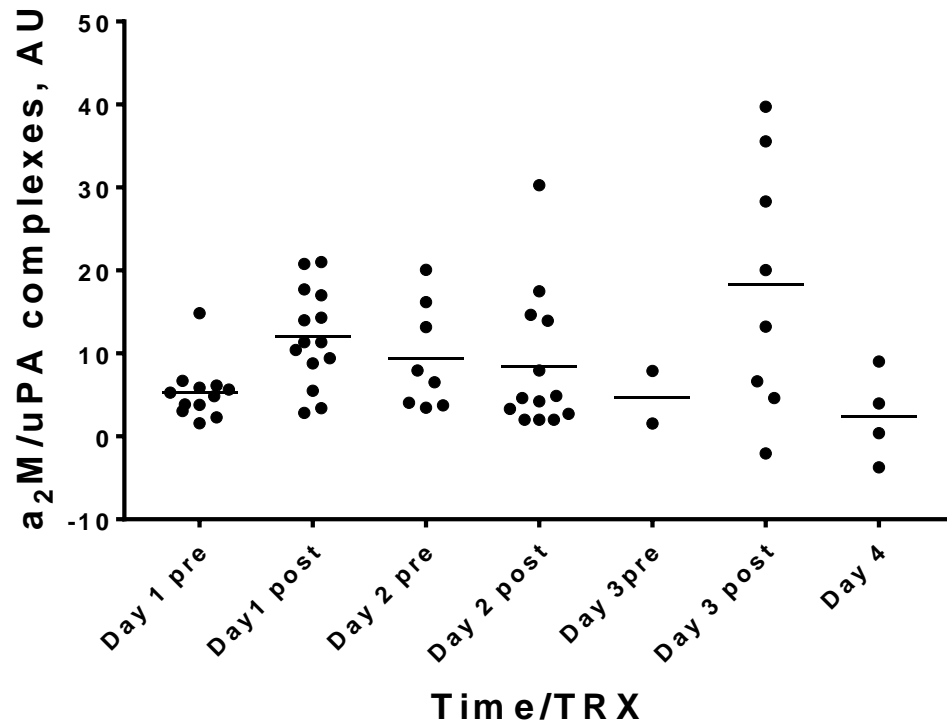


B. Plasma



**Legend.** D-Dimer concentrations in all available PFs (A) and plasma (B) from the trial subjects are illustrated in a dot-box plot format showing the medians, ranges and outlying values. TRX: LTI-01 treatment.

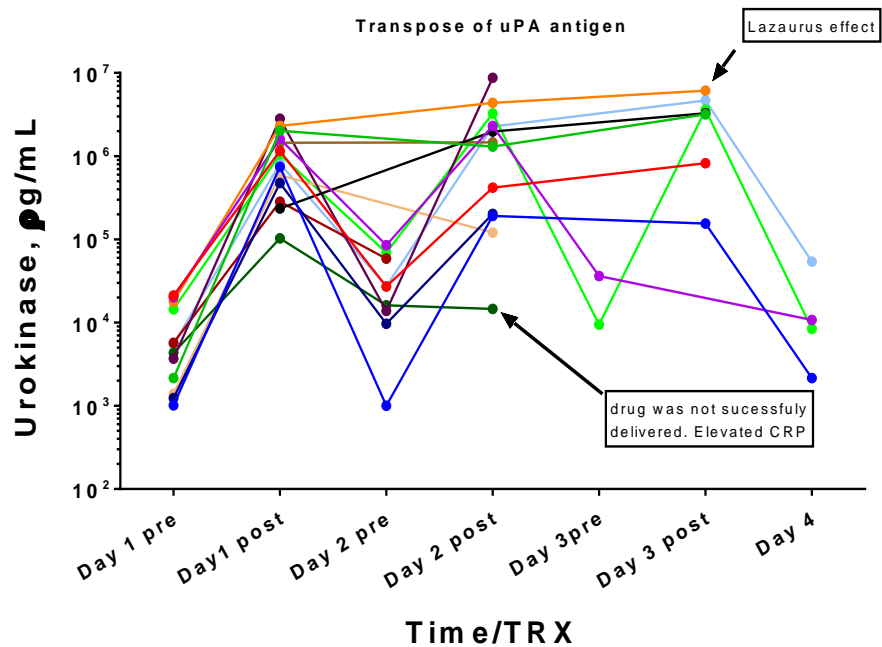
Figure 6. Concentrations of  $\alpha_2$ macroglobulin-uPA complexes in PFs of subjects treated with LTI-01 IPFT.



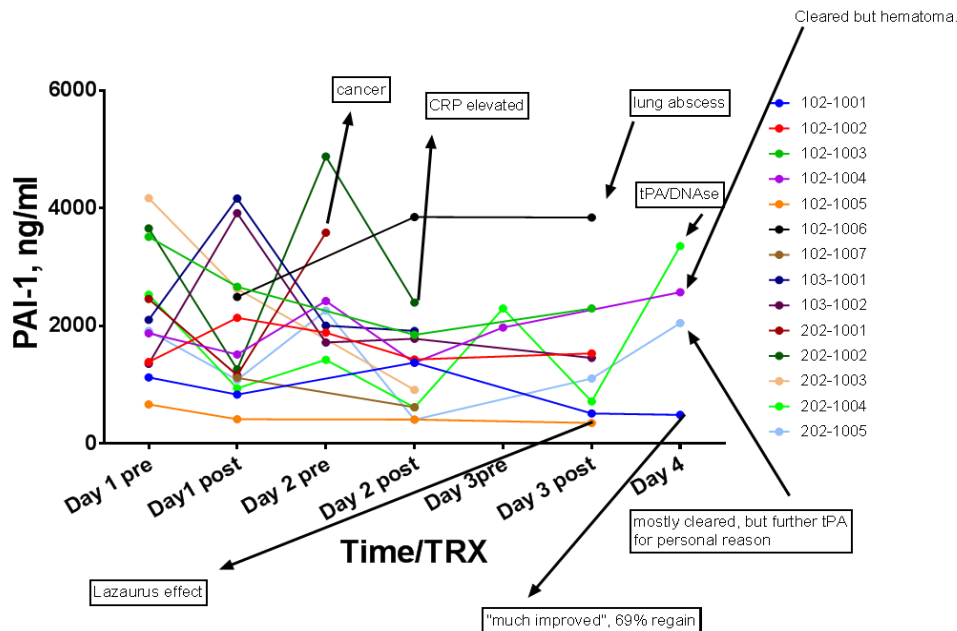
**Legend.** The bioactive , PAI-1 resistant complexes were detected as we previously described(13). The data are illustrated in a box-dot plot format in which the medians are indicated by horizontal lines and the range of the data is otherwise shown here.

Figure 7

A.



B.



**Legend.** A. PF uPA antigen concentrations plotted as line plots for all subjects (n=14). Pre-: within 3h predosing of LTI-01 IPFT. Pos-t: within 3h of administration of the dose of LTI-01 IPFT. B. PAI-1 antigen concentrations in PF at the same intervals pre- and post-dosing of LTI-01 IPFT.

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