

## **Intrapleural and Plasma Processing of LTI-01 (Single Chain Urokinase, scuPA) in a Phase 1b Trial of LTI-01 Intrapleural Fibrinolytic Therapy (IPFT) in Patients with Complicated Parapneumonic Effusions (CPE) or Empyema**

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**Rationale:** We are unaware of prior trials integrating the analysis of fibrinolytics processing into the trial design. This has contributed to uncertainty about effective dosing and making the interpretation of previous trials challenging. Unlike currently available IPFT, scuPA (LTI-01) generates durably active complexes that resist inhibition by plasminogen activator inhibitor-1 (PAI-1). We investigated how LTI-01 is processed in human pleural fluids and plasma and correlate it to clinical outcomes.

**Methods:** This was an open-label, dose-escalation safety trial (ANZCT Registry Trial ID: ACTRN12616001442493). Subjects with CPE/empyema, loculation and failure to drain were studied (n=14). There were five dose-escalation cohorts: 50,000 IU; 100,000 IU; 200,000 IU; 400,000 IU (n=3 in all these groups); and 800,000 IU (n=2). LTI-01 was given daily for up to 3 days. Pleural fluids and plasma were collected prior to and 3 and 23 hours after dosing with LTI-01. Biochemical analyses included uPA antigen, PA activity, fibrinolytic activity, PAI-1 antigen and activity, D-dimers and bioactive uPA/ $\alpha_2$ macroglobulin complexes, performed as previously reported.

**Results:** Increments of pleural fluid uPA antigen occurred consistently after LTI-01 IPFT (median increase  $\sim 10^4$ – $10^6$  pg/ml,  $p < 0.01$  by grouped analysis), but were not observed in the plasma of any patient. Pleural fluid PAI-1 antigen was elevated markedly;  $\sim 500$ – $4000$  pg/ml in all pleural fluids. Active PAI-1 fell from a baseline of  $\sim 200$  ng/ml in pleural fluids to undetectable levels at 3 hours after dosing ( $p < 0.01$ ) but reverted to baseline at 23 hours after LTI-01 IPFT. Pleural fluid plasminogen concentrations were unchanged after LTI-01 IPFT, while fibrinolytic and PA activities increased variably. Pleural fluid d-dimers increased at 3 hours post treatment ( $p < 0.01$ ); this suggests that locally increased fibrinolysis supported the reductions in pleural opacification, plasma CRP, and white cell/neutrophil counts that were observed in the 14 enrolled patients. Bioactive, PAI-1-resistant uPA/ $\alpha_2$ macroglobulin complexes were increased in pleural fluid after LTI-01 IPFT for up to 23 hours and were similar in plasma before and after LTI-01 IPFT.

**Conclusions:** Processing of LTI-01 within pleural fluids resembles that reported in our preclinical pleural injury models, including empyema in rabbits. Durable, bioactive, PAI-1-resistant complexes of uPA/ $\alpha_2$ macroglobulin were generated by LTI-01 IPFT. Treatment with LTI-01 activated intrapleural fibrinolysis, which coincided with reductions of pleural opacification. There was no systemic fibrinolysis, which correlated with the absence of any observed bleeding.

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