EFFICACY OF CLM-022*, AN INHIBITOR OF THE NLRP3 INFLAMMASOME, IN IN VIVO AND IN VITRO PATHOGEN-ASSOCIATED MOLECULAR PATTERNS (PAMPs)-INDUCED DISEASE MODELS

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BACKGROUND & AIM

- Patients with liver cirrhosis are characterized by impaired liver function, severe immune dysfunction, and increased gut permeability. These alterations drive the progression of chronic liver disease through sustained immune activation by gut-derived pathogen associated molecular patterns (PAMPs) and injury-related danger associated molecular patterns (DAMPs), which stimulate innate immune responses via receptors (TLRs and PRRs)[1,2]
- Immune dysfunction and gut barrier disruption lead to a dysregulated inflammatory response and elevated cytokines (TNF-α, IL-6, IL-1β). These events play a key role in the transition to acute decompensation and Acute-on-chronic liver failure (ACLF). Subsequently, ACLF patients are also highly susceptible to sepsis[3]
- CLM-022, a small-molecule inhibitor of inflammasome priming and activation, has demonstrated hepatoprotective and anti-inflammatory effects in preclinical models of acute liver injury and endotoxemia, as presented at the 2025 EASL International Liver Congress[4]

- **The aim of this study was to investigate the immunomodulatory effects of investigational drug CLM-022, on human whole blood exposed to Klebsiella pneumoniae lipopolysaccharide (LPS), a Gram-negative bacteria. The efficacy of CLM-022 was further evaluated in a rodent model of sepsis induced by cecal ligation and puncture (CLP), to explore its potential in improving survival induced by polymicrobial sepsis**

METHODS

• Whole blood assays

Blood from healthy volunteers was collected in lithium heparin tubes at the Etablissement Français du Sang (EFS) and stored at room temperature until use (<20 hours). Blood was diluted by 1/5 in media to increase volume. Whole blood was incubated 4 hours with LPS Klebsiella pneumoniae in presence of a dose range of CLM-022 (0 – 0.03 – 0.1 – 0.3 – 0.5 – 0.7 – and 3 µM) or Dexamethasone (used as pharmacological reference for inflammatory cytokine inhibition). Compounds and LPS were added either concomitantly or 30 min post LPS. Secretion of inflammatory cytokines TNFα, IL-6 and IL-1β was determined by measuring their concentration in the whole blood using an Luminex immunoassay (Biotechne)

• Evaluation of CLM-022 activity on cecal ligation and puncture (CLP)-induced sepsis

Sepsis was induced through cecal ligation and puncture (CLP) surgery in C57BL/6J male mice (ArtImmune, France). Briefly, under anesthesia, an abdominal incision was performed, and the caecum was tightly ligated at half the distance between distal pole and the base of the caecum. The caecum was punctured once through-and-through and replaced in its original position within the abdomen, which was closed with sutures and wound clips. CLM-022 (1 mg/kg) or vehicle was orally administered 3 hours after surgery and then once a day for 6 days (n=20/group). Survival was monitored over 7 days

RESULTS

CLM-022 IMPROVES SURVIVAL IN A MOUSE MODEL OF CLP-INDUCED SEPSIS

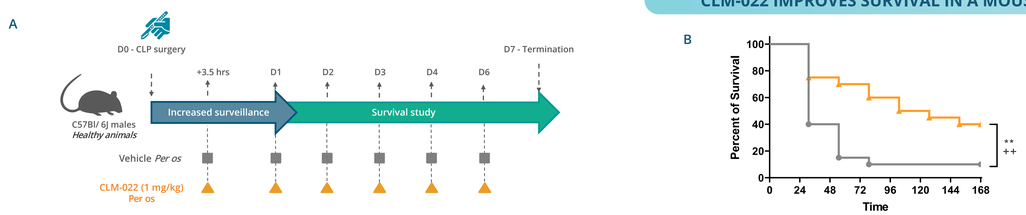


FIGURE 1:
A. Experimental design
B. Survival curves
Statistical analysis: Comparison of survival curves followed by Gehan-Breslow-Wilcoxon test (1) and Log-Rank (2)
***p<0.01, CLP + Vehicle compared to CLP + CLM-022 (1 mg/kg; p.o) treatment*
++p<0.01, CLP + Vehicle 3.5h after CLP at D0, then daily from D1 to D6 compared to CLP (21G needle) + CLM-022 (1 mpk; p.o) treatment

→ In a mouse model of CLP-induced sepsis, CLM-022 administration significantly improved survival with 40% of survival rate compared to 15% for vehicle group

CLM-022 REDUCES PRO-INFLAMMATORY CYTOKINE RELEASE IN HUMAN WHOLE BLOOD ASSAY

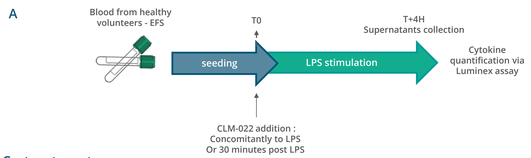
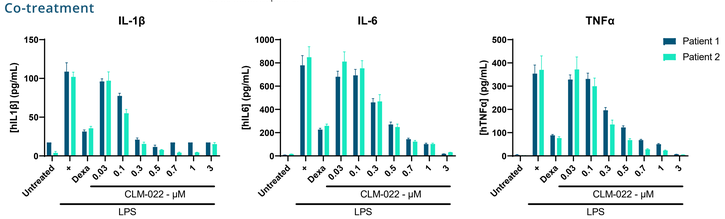
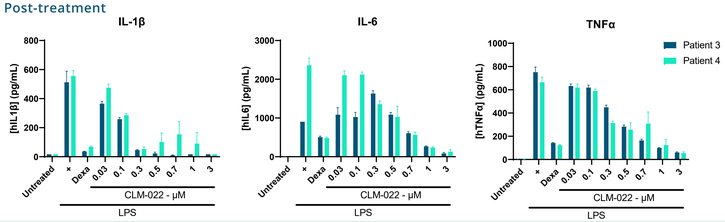


FIGURE 2:
A. Experimental design
B. Effect of a dose range of CLM-022 applied concomitantly with LPS on cytokine secretion measured by Luminex assay 4 hours after stimulation
C. Effect of a dose range of CLM-022 applied 30 min post LPS on cytokine secretion measured by Luminex assay 4 hours after stimulation
D. IC_{50} values were calculated from normalized cytokine release following concomitant application of CLM-022 based on 8 replicates per donor and compiled across two donors (n = 16 total replicates)
E. IC_{50} values were calculated from normalized cytokine release following post-LPS application of CLM-022, based on 8 replicates per donor and compiled across two donors (n = 16 total replicates)
F. The table summarizes the IC_{50} values obtained for each cytokine under both treatment conditions

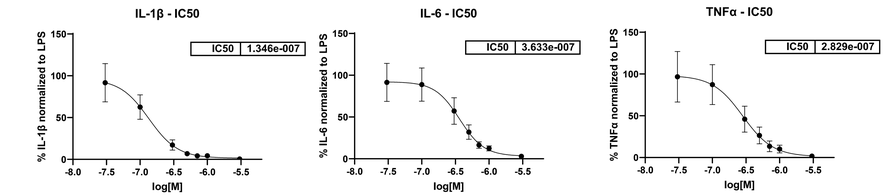
B. Co-treatment



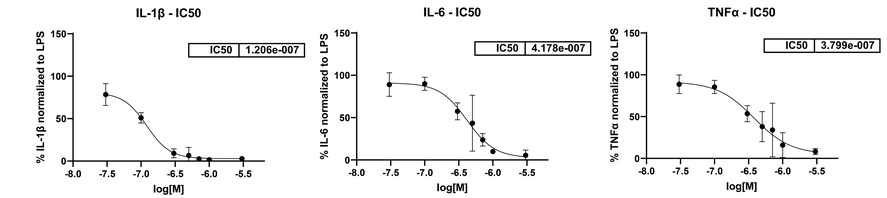
C. Post-treatment



D. Co-treatment



E. Post-treatment



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	CLM-022 application	IC50
IL-1β	Concomitant	134.6 nM
	30 min post LPS	120.6 nM
IL-6	Concomitant	363.3 nM
	30 min post LPS	417.8 nM
TNFα	Concomitant	282.9 nM
	30 min post LPS	379.9 nM

→ CLM-022 dose-dependently inhibits the secretion of IL-1β, IL-6 and TNFα, either when applied concomitantly or following LPS stimulation. IC50 values remain within the same nanomolar range, regardless of whether CLM-022 is administered concomitantly with or following LPS stimulation

CONCLUSION

- **In the present study we demonstrate the ability of investigational drug CLM-022 to improve survival in a mouse model of CLP-induced sepsis (40% vs 15% at day 7)**
- **The investigational drug CLM-022 reduces the secretion of key pro-inflammatory cytokines (IL-1β, IL-6, TNFα) in human whole blood assays at similar nanomolar IC50 values when administered either concomitantly with or following LPS stimulation**
- **These findings support further development of the investigational drug CLM-022 for advanced liver diseases, including acute de compensation of liver cirrhosis and ACLF**

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