CLM-022*, A DUAL INHIBITOR OF PRIMING AND ACTIVATION STEPS OF NLRP3 INFLAMMASOME, AS A POTENTIAL TREATMENT FOR ACUTE AND CHRONIC INFLAMMATORY LATE-STAGE LIVER DISEASES

Hana El Khatib[1], Alexandra Caron[1], Elodie Delecroix[1], Victor Launay[1], Maryse Malysiak[1], Valérie Daix[1], Simon Debaecker[1], Guillaume Vidal[1], Dean Hum1, Bart Staels[2], Sakina Sayah Jeanne[1]

[1]*GENFIT S.A., Loos, France,*
[2]*Univ. Lille, INSERM, CHU Lille, Institut Pasteur de Lille, U1011-EGID, Lille, France*
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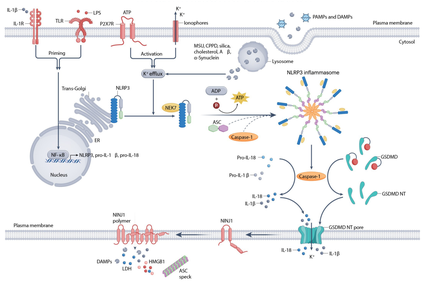
BACKGROUND & AIM

- Inflammation is a common element in the pathogenesis of most chronic liver diseases leading to fibrosis, cirrhosis and liver failure. Due to the close connection with the intestine via the portal circulation, the liver is constantly exposed to gut-derived microbial particles identified as pathogen-associated molecular patterns (PAMPs), which activate resident immune cells. In addition to the intestinal- or virus-derived PAMPs, hepatic innate immune cells are also activated by damage-associated molecular patterns (DAMPs), which are released from injured parenchymal and non-parenchymal cells (Tanwar 2020)

Figure 1: Molecular mechanisms driving NLRP3 inflammasome activation



- Inflammation is characterized by activation of innate immune cells, production of pro-inflammatory cytokines and pyroptosis which is a lytic form of inflammatory regulated cell death. Pyroptosis is regulated by inflammasomes which are intracellular multiprotein complexes expressed in both parenchymal and non-parenchymal cells of the liver. In response to cellular danger signals (PAMPs, DAMPs), inflammasomes activate caspase-1, release IL-1β and IL-18 (Vande Walle 2024; figure 1)

- **The aim of this work was to characterize the activity of CLM-022 on the priming and activation of NLRP3 activity both in vitro and in vivo**
- **To assess the activity of CLM-022 on inflammasome priming, gene expression was evaluated on LPS-induced PBMCs (peripheral blood mononuclear cells)**
- **To assess the activity and specificity of CLM-022 on inflammasome activation, IL-1β release and pyroptotic cell death was evaluated on LPS-primed THP1 activated with nigericin (WT and NLRP3-KO)**
- **For in vivo studies, efficacy of CLM-022 was assessed on different rodent models:**
  - **A liver injury model, induced in mice by administering acetaminophen, resulting in the deterioration of hepatic function and increase of the inflammasome protein in the liver**
  - **A model of LPS endotoxemia in rats, leading to the induction of systemic inflammation and hepatic impairment**

METHODS & STATISTICS

In vitro LPS-induced PBMC genes expression analysis

- CLM-022 (30 nM) or control vehicle were added to human peripheral blood mononuclear cells (Zenbio) 1 hour before LPS stimulation (1 ng/mL Escherichia coli O111:B4, Sigma Aldrich) for 5 hours. mRNA were extracted with Qiagen Rneasy Mini kit and expression levels of TNFα, IL-1β, NLRP3, IL-6 and COX2 were determined by RT-qPCR and assessed using CFX96 Touch™

In vitro Inflammasome activation in THP-1

- THP-1 WT and THP-1 NLRP3 KO (InvivoGen) cells were differentiated into macrophages with 100 ng/mL of PMA during 72 hours. THP-1 macrophages were primed overnight with 500ng/mL of LPS and activated with 10µM of nigericin. CLM-022 (100nM and 300nM), MCC950 (at 50nM, used as pharmacological reference compound for NLRP3 inhibition) or vehicle were added 30 minutes after nigericin. Kinetic of pyroptotic cell death was assessed with CellTiter-Glo and lactate dehydrogenase measurement (LDH, with the LDH-Glo Cytotoxicity Assay from Promega), IL-1β detection was measured with HTRF IL-1 β (Revvity)

Evaluation of CLM-022 on acetaminophen (APAP)-induced acute liver injury

- C57BL/6 male mice (9 weeks old) received intraperitoneal (i.p.) injection of APAP (300 mg/kg). Mice were treated orally with CLM-022 (5 mg/kg) or control vehicle (Ctrl) 1 hour after APAP administration (n=8-16/group). Mice were sacrificed 24 hours after APAP induction. Blood was collected to measure serum ALAT and ASAT levels using the Daytona plus automate. Livers were harvested and snap-frozen to assess NLRP3 protein expression using the Jess system, with total protein used for normalization (Bio-Techne)

Evaluation of CLM-022 on LPS-induced endotoxemia in rats

- Male Sprague-Dawley rats received a single i.p. injection of 1 mg/kg LPS (Escherichia coli O111:B4). CLM-022 (1 mg/kg) or control vehicle (Ctrl) were administered intravenously 30 minutes after the LPS injection. Serum was collected after 3 hours to measure cytokines by Luminex technology and hepatic markers ALAT and ASAT using the Daytona plus automate

RESULTS

CLM-022 INHIBITS THE NLRP3 PRIMING IN LPS-INDUCED PBMCS

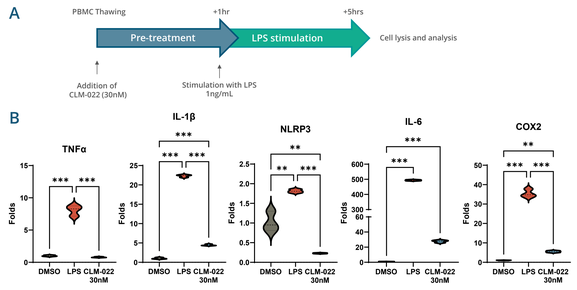


Figure 1: CLM-022 inhibits NLRP3 priming induced by LPS

A. Experimental design.

B. mRNA expression levels of TNFα, IL-1β, NLRP3, IL-6 and COX2 in PBMC were determined by RT-qPCR

CLM-022 INHIBITS PYROPTOSIS INDUCED BY INFLAMMASOME ACTIVATION IN WT BUT NOT IN NLRP3 KO THP-1 CELLS

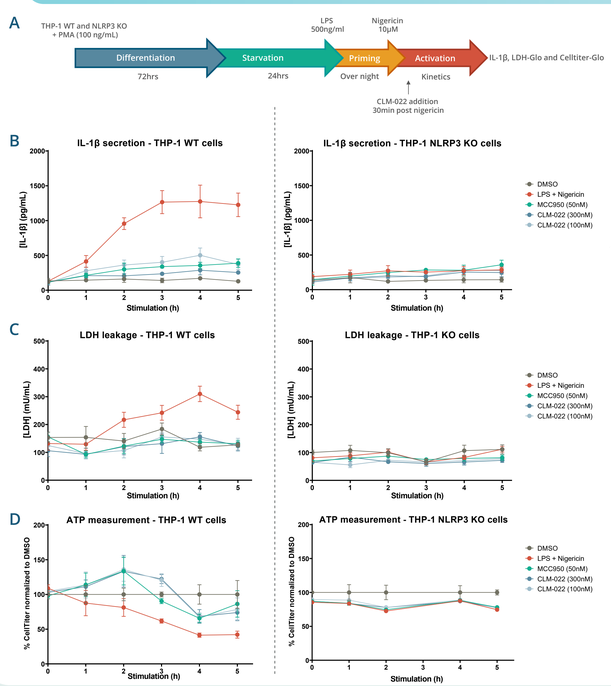


Figure 2: CLM-022 inhibits pyroptosis induced by inflammasome activation in THP-1 WT but not in THP-1 NLRP3 KO cells

Left panels for WT and right panels for NLRP3 KO

A. Experimental design

B. Levels of human IL-1β in cell-free supernatants were quantified using commercially available HTRF (homogeneous time-resolved fluorescence - Revvity)

C. Cytotoxicity was evaluated by LDH release from damaged cells (LDH-Glo Cytotoxicity Assay from Promega)

D. Cell viability was assessed with ATP measurement using CellTiter-Glo luminescent cell viability assay (Promega) directly on treated cells

Results as the mean value ± SD of 6 replicates

CLM-022 IMPROVES HEPATIC FUNCTION IN APAP-INDUCED LIVER INJURY

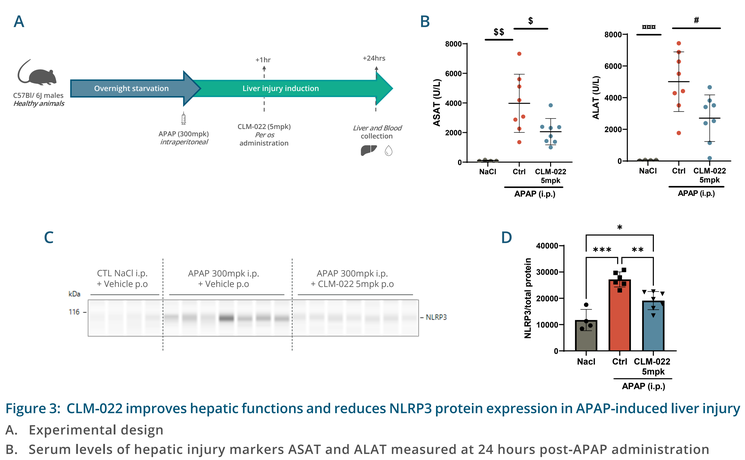


Figure 3: CLM-022 improves hepatic functions and reduces NLRP3 protein expression in APAP-induced liver injury

A. Experimental design

B. Serum levels of hepatic injury markers ASAT and ALAT measured at 24 hours post-APAP administration

C. NLRP3 protein detection 24H post APAP administration. Analysis was performed using a Jess system under reducing conditions and using the 12-230kDa separation system

D. Relative expression of NLRP3 protein

CLM-022 INJECTION ALLEVIATES LPS-INDUCED SYSTEMIC INFLAMMATION AND PROTECTS THE LIVER IN RATS

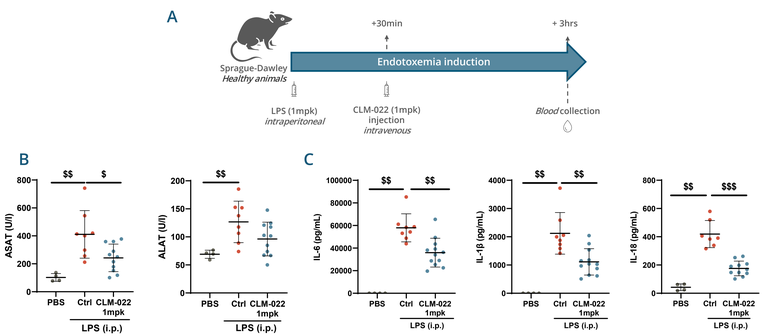


Figure 4: CLM-022 inhibits systemic inflammation and improves hepatic parameters in endotoxemia-induced rats

A. Experimental design

B. Hepatic injury markers ASAT and ALAT assessed by randox system

C. Cytokine measurement assessed using Luminex technology

CONCLUSION

- **In the present study, we demonstrate the ability of CLM-022 to inhibit the NLRP3 inflammasome through the inhibition of 1) NLRP3 priming of LPS-induced human PBMCs, 2) IL-1β production, and 3) pyroptosis in WT human THP-1 macrophages but not in NLRP3 KO THP-1 macrophages**
- **Oral administration of CLM-022 in mice provides hepatic protection in a model of acute liver injury**
- **Intravenous administration of CLM-022 in rat alleviates systemic inflammation and improves hepatic parameters in an endotoxemia model**
- **These findings support the potential of the investigational drug CLM-022 as a treatment of acute and chronic inflammatory late-stage liver diseases, including ACLF**

REFERENCES

1. Tanwar S, Rhodes F, Srivastava A, Trembling PM, Rosenberg WM. 2020. Inflammation and fibrosis in chronic liver diseases including non-alcoholic fatty liver disease and hepatitis C. World J. Gastroenterol. 26:109–33
2. Vande Walle L, Lamkanfi M. 2024. Drugging the NLRP3 inflammasome: from signaling mechanisms to therapeutic targets. Nat Rev Drug Discov. 23(1):43-66

We thank Manuel Monsauret, Florian Depreux, Anthony Rabat, Fériel Berrabah, Nicolas Binienda, Mickaël Karakanat and Morgane Lenne from GENFIT SA for their contribution to this work

Abstract #1841 presented at EASL International Liver Congress™ 2025 | 7 - 10 May, 2025 | Amsterdam, Netherlands