EX-VIVO BLOOD ASSAYS DEMONSTRATE SYSTEMIC ANTI-INFLAMMATORY ACTIVITY OF G1090N, A NOVEL FORMULATION OF NITAZOXANIDE; AND SUPPORT FURTHER CLINICAL EVALUATION IN ACLF

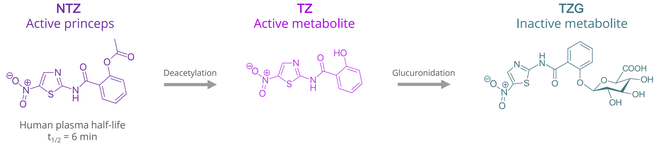
Simon Debaecker[1], Marie Bobowski-Gerard[1], Alexandra Pruvost[1], Manon Clarisse[1], Virginie Galoseau[1], Anthony Rabat[1], Jérémy Magnanensi[1], Camille Vanbésien[1], Jean-Marie Brusq[1], Jonel Trebicka[2], Bart Staels[3], Zouher Majd[1], Sakina Sayah Jeanne[1]

[1]GENFIT SA, Loos France
[2]Hospital of the Goethe University Frankfurt, University Hospital Münster, Germany
[3]Univ.Lille, Inserm, CHU Lille and Institut Pasteur de Lille, U1011-EGID, Lille, France

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BACKGROUND & AIM

- Acute-on-chronic liver failure (ACLF) is a severe syndrome characterized by acute decompensation of chronic liver disease, multiple organ failures, and high short-term mortality[1]. Systemic inflammation driven by bacteria or pathogen-associated molecular patterns (PAMPs) such as lipopolysaccharide (LPS), is a key driver of ACLF[2]
- G1090N, a novel formulation of nitazoxanide (NTZ), is an investigational drug under development for the treatment of ACLF. A phase 1 clinical trial assessed pharmacokinetics, safety and tolerability of single and multiple oral doses of G1090N (NCT07110441)



- NTZ has shown promising effects on hepatic encephalopathy (HE) in clinical studies[1, 2, 3]. In animal models of ACLF, NTZ has been shown to protect against systemic inflammation and organ damage, which could be in part mediated by direct anti-inflammatory activity on immune cells[6-9]

- **This study first aims to evaluate the ability of G1090N to counteract inflammation through ex-vivo stimulation of peripheral blood mononuclear cells (PBMCs) from subjects enrolled in a Phase 1 study**
- **To confirm the activity of TZ in a clinically relevant inflammatory setting, the compound was also assessed in whole blood from cirrhotic subjects**

METHODS & STATISTICS

Ex-vivo stimulation of PBMCs from subjects enrolled in the Phase 1 study

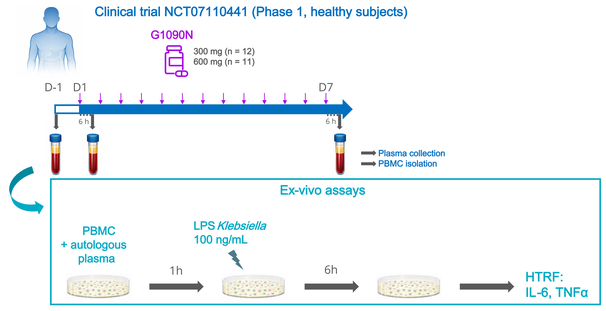
- Healthy volunteers enrolled in the Phase 1 trial NCT07110441 received G1090N (300 or 600 mg BID) for 1 day or 7 days. PBMCs and autologous plasma were isolated by centrifugation from blood collected at the following timepoints: baseline (D-1), 6 h after the first dose (D1), and 6 h after the last dose (D7). Following thawing, PBMCs were cultured for 1 h in the presence of 50% autologous plasma, then stimulated with LPS (100 ng/mL) for 6 h in the presence of 5 mM $MgCl_2$. IL-6 and TNFα levels were quantified in culture supernatants by Homogenous Time-Resolved Fluorescence (HTRF)
- Cytokine secretion ratios are displayed as a box plot representing the median, interquartile range, whiskers and outliers. Non-parametric statistical analyses were performed as cytokine ratios did not follow a normal distribution. Normalized cytokine levels were compared with the reference LPS condition (100%) using a one-sample Wilcoxon signed-rank test (*: p<0.05, **: p<0.01, ***: p<0.001)

Assessment of TZ activity in blood samples from cirrhotic and healthy subjects

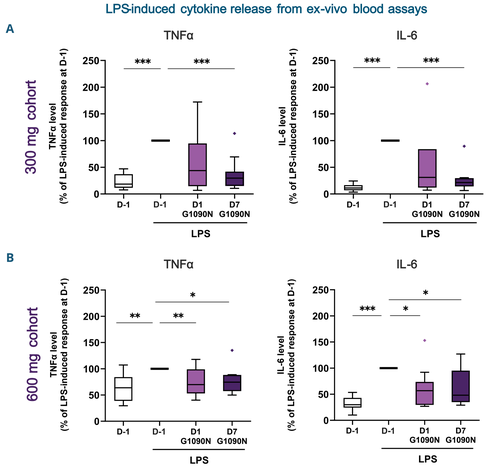
- Fresh whole blood samples from healthy donors (n = 13 pooled samples) were collected at the French Blood Establishment (Etablissement Français du Sang, EFS), while samples from cirrhotic subjects (n = 13) were collected at Cerballiance laboratories and Assistance Publique–Hôpitaux de Paris (APHP). Blood was diluted 1/5 in culture medium and incubated with TZ (60-120 µM for healthy donors and 10-120 µM for cirrhotic subjects) for 1 h at 37°C prior to stimulation with LPS (50 ng/mL) in the presence of 5 mM $MgCl_2$. Following 4 h incubation, supernatants were collected and IL-6 and TNFα levels were quantified by HTRF
- Data are presented as box plots showing the median, interquartile range, whiskers and outliers
- As data did not follow a normal distribution, non-parametric statistical tests were used. Comparisons versus the LPS control condition (Vehicle, Veh.) were performed using one-sample Wilcoxon tests (*: p<0.05, **: p<0.01, ***: p<0.001). Comparisons between healthy donor pools and cirrhotic donor samples were performed using a two-tailed Mann-Whitney test ($: p<0.05, $$: p<0.01, $$$: p<0.001)

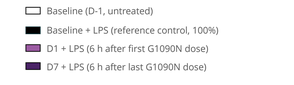
RESULTS

EX-VIVO STIMULATION OF PBMCs AND PLASMA FROM HEALTHY SUBJECTS WHO RECEIVED A SINGLE DOSE OR REPEATED DOSES OF G1090N



LPS-induced cytokine release from ex-vivo blood assays





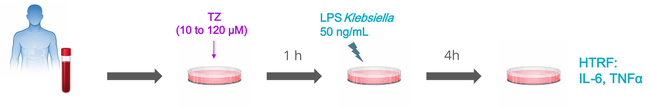
A. Relative TNFα and IL-6 levels following 300 mg G1090N treatment, compared to baseline. n = 6 for D1, n = 12 for D-1 and D7

B. Relative TNFα and IL-6 levels following 600 mg G1090N treatment, compared to baseline. n = 11

→ Treatment with G1090N reduced LPS-induced pro-inflammatory cytokine release by PBMCs, whatever the dose and the treatment setting

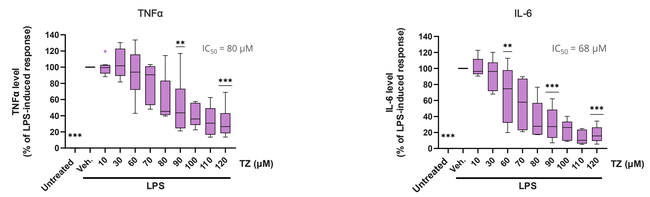
→ These findings indicate that doses used in the first-in-human study were sufficient to achieve ex-vivo anti-inflammatory activity

ASSESSMENT OF TZ ACTIVITY IN BLOOD SAMPLES FROM CIRRHOTIC AND HEALTHY SUBJECTS

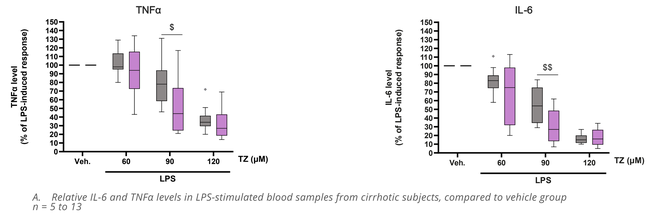


Human whole blood (EDTA)
- Healthy (n = 13 pools)
- Cirrhotic (n = 13)

LPS-induced cytokine release in cirrhotic subjects



LPS-induced cytokine release in cirrhotic vs healthy subjects



A. Relative IL-6 and TNFα levels in LPS-stimulated blood samples from cirrhotic subjects, compared to vehicle group n = 5 to 13

B. Relative IL-6 and TNFα levels in LPS-stimulated blood samples from cirrhotic and healthy subjects, compared to vehicle group n = 13

- TZ dose-dependently inhibited LPS-induced IL-6 and TNFα secretion in cirrhotic blood, reaching maximal inhibition of 86% and 67%, respectively, at concentrations between 110 and 120 µM
- Greater anti-inflammatory activity was observed at intermediate TZ concentration (i.e. 90 µM) compared with blood from healthy subjects

→ TZ demonstrated robust anti-inflammatory activity in both cirrhotic and healthy blood samples, with enhanced apparent potency in cirrhotic samples

CONCLUSION

- **Exploratory analyses using PBMCs and autologous plasma collected after single and repeated G1090N administration in the NCT07110441 clinical trial confirmed the anti-inflammatory activity of G1090N, indicating translatability of previous preclinical findings**
- **Anti-inflammatory activity was also demonstrated in whole blood from cirrhotic subjects, supporting the potential of NTZ in conditions associated with systemic inflammation**
- **Together with previously reported organ-protective effects in preclinical models, these findings support further clinical development of G1090N in ACLF**

REFERENCES

1. Arroyo et al., J Hepatol, 2021 74(3): 670-685
2. Clària et al, 2023, JHEP Rep. 5(9):100807
3. Elrakaybi et al., Int J Pharm Sci Res, 2015 6 (11): 4657-67
4. Glal et al., J Hepatobiliary Pancreat Sci, 2021 28: 812-824
5. Abd-Elsalam et al., J Clin Gastroenterol, 2019 53: 226-230
6. Legry et al., J Hepatol 2022 77: 359-360
7. Bobowski-Gerard et al., Hepatol. 2025 82 (S1): S1918
8. Klein et al., J Hepatol. 2026 84 (S1): S163-S164
9. Shou et al., Inflammation 2019 Vol. 42:1336–1349

DISCLOSURE | JT is a consultant for GENFIT SA and a member of EF-CLIFF, which is sponsored by GENFIT - BS is a consultant for GENFIT SA - G1090N (nitazoxanide formulation) is an investigational drug under development for ACLF. In this study, we have used tizoxanide, the active metabolite of nitazoxanide, for in vitro experiments.
We thank Audrey Pechberty from GENFIT's team for her valuable contribution.