## GS002

### Anti-fibrotic effect of rifaximin in early alcohol-related liver disease: a double-blind, randomised, placebo-controlled trial

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**Background and aims:** Alcohol is the leading cause of liver cirrhosis and liver-related mortality. Alcohol abstinence remains the only therapeutic option. The gut-liver axis with gut dysbiosis and "leaky gut" is considered a key driver of inflammation and fibrogenesis in alcohol-related liver disease (ALD). Rifaximin-α is known to reduce recurrent hepatic encephalopathy and has been linked to improved gut barrier function and reduced systemic inflammation in cirrhosis. We hypothesized that long term rifaximin-αwould reduce inflammation and fibrosis in patients with ALD.
**Method:** We conducted an 18-months randomised double-blind trial in patients with biopsy-verified ALD and no previous decompensation. We stratified patients according to fibrosis stage and alcohol abstinence within the previous six months. Patients were randomly allocated 1:1 to 550 mg rifaximin-α twice daily or placebo. The primary end point was an improvement of at least one fibrosis stage. Secondary outcomes included progression in fibrosis stage, lobular inflammation, ballooning, steatosis, and transient elastography (TE). We assessed liver biopsies and performed the statistical analyses blinded.
**Results:** We randomised 136 patients, 108 (79%) completed the trial with paired liver biopsies of adequate quality and 14 dropped out in each group. At baseline, the distribution of fibrosis stages (F0/F1/F2/F3/F4) were 5/31/49/17/6, 84% were male, median TE 8.7 kPa (IQR = 6.5–11.8) and mean age was 59 ± 6 years. The proportion of patients who improved according to fibrosis stage was 26% in the rifaximin-α group and 28% in the placebo group (OR = 1.10, p = 0.821). The proportion who remained stable or progressed in fibrosis stage was 48% and 26% in the rifaximin-α group, versus 30% and 43% in the placebo group and consequently, rifaximin-α reduced the risk of progression compared to placebo (OR = 0.37, p = 0.035) (Figure). Rifaximin-α reduced lobular inflammation compared to placebo (OR = 0.48, p = 0.067), but did not affect hepatic steatosis or ballooning. TE decreased from a median of 8.8 kPa to 8.3 kPa in the rifaximin-α group and 8.7 kPa to 8.3 kPa in the placebo group (p = 0.757). In total, 13 SAEs were reported, and the most frequent reported adverse event (n = 20) was diarrhoea with no difference between the groups.

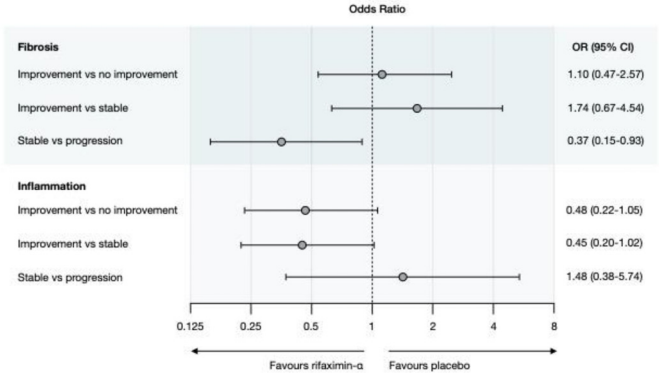


**Figure**: Rifaximin-α protects against fibrosis progression and reduces lobular inflammation compared to placebo.

**Conclusion:** Our results show that 18 months treatment with rifaximin-α does not improve fibrosis in early ALD. However, rifaximin-α seems to prevent histological progression of liver fibrosis and tends to decrease hepatic inflammation.

## GS003

### The spatial distribution and detailed composition of infiltrating immune cells define autoimmune- and checkpoint-therapy associated hepatitis

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**Background and aims:** Immune-checkpoint blockade (ICB) therapy has revolutionized clinical oncology, but frequently results in immune-related adverse events (irAE), such as ICB-Hepatitis. To date, there is limited information regarding key involved immune subsets and their spatial interaction in ICB-Hepatitis-moreover, differences to spontaneous autoimmune hepatitis (AIH) remain unclear. We therefore set out to perform a detailed analysis of the immune cell composition and their spatial interaction in the liver in in both disease entities.
**Method:** We performed high dimensional, spatially resolved analysis of immune cell populations in liver biopsies of patients with ICB-Hepatitis (n = 15), AIH (n = 22) or control tissue (n = 10), using a 40-marker Imaging Mass Cytometry (IMC) panel with single-cell resolution (1 $\mu m^2$). Single cells were segmented and normalized utilizing a machine learning pipeline and subsequently classified high dimensionally. In addition, PBMCs from ICB-Hepatitis- and AIH-patients were analyzed by mass cytometry.
**Results:** Analysis of the composition of the immune infiltrate revealed major differences between ICB-Hepatitis and AIH. An unbiased clustering identified disease-specific, phenotypically well defined, immune cell cluster, which show distinct spatial interaction patterns. Specifically, $CD8^+$ T cell clustering resulted in enrichment of several Ki-$67^+$ Granzyme $B^+$ T-$bet^+$ Cluster in ICB-Hepatitis. In contrast, AIH was defined by a higher expression of exhaustion markers (PD-$1^+$ $TOX^+$ $Eomes^+$ cluster) as well as a tissue resident memory (Trm) phenotype. Remarkably, one Granzyme $B^+$ $CD8^+$ T cell cluster and the Trm cluster correlated with ALT levels in ICB-Hepatitis and AIH, respectively.
Clustering of tissue phenotypes according to spatial liver zonation revealed a biased accumulation of $CD8^+$ T cells near the central vein in ICB-Hepatitis, while in AIH a closer interaction with the periportal triad was observed. Neighborhood analysis identified closely engaged immune cell clusters in both diseases. In ICB-Hepatitis, we observed significant interactions between myeloid clusters and $CD8^+$ T cells, especially between a Ki-$67^+$ CD8+ T cell cluster and metabolically distinct macrophage populations. In AIH, a stronger B-T cell interaction was noted.
**Conclusion:** The composition of the immune infiltrate, as well as a distinct spatial distribution and interaction between immune