The AXL inhibitor bemcentinib reduces inflammation and fibrosis by inducing a dynamic change in macrophages and CD8+T cells subsets during experimental NASH

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1 Introduction

Tyro3, AXL and MERTK are receptor tyrosine kinases activated by the ligand GAS6 (1). AXL signaling is increased in nonalcoholic steatohepatitis (NASH) patients and promotes fibrosis through activation of hepatic stellate cells and inflammatory macrophages (2). Recent data shows that bemcentinib reduces experimental NASH by inhibiting AXL signaling and increasing hepatocyte survival through increased Gas6 (2). In recent years the role of the innate and adaptive immune system in NASH has gained attention (3,4).

2 Aim

We aim to determine which immune cells express AXL and how AXL inhibition affects the liver immune profile during NASH. This may provide clues for new NASH-directed therapies.

3 Methods

Mice were fed chow or a high-fat methionine-restricted choline-deficient (HFCD) diet for 8 weeks to induce NASH, receiving daily doses of bemcentinib (3, 10, 30 and 100 mg/kg) or vehicle by oral gavage for the last two weeks. Hepatic inflammatory and fibrotic gene expression were measured by qPCR.

Time-of-flight mass cytometry (CyTOF) was utilized to analyze the expression of more than 40 markers on single cells from dissociated livers. Cells were acquired on the Fluidigm Helios and cell populations were studied using machine learning.

5 Conclusions

Bemcentinib reduces inflammation and fibrosis by inducing a dynamic change in liver macrophages and CD8+T cells subsets from pro-inflammatory towards tissue remodeling and antifibrotic phenotypes.

6 Acknowledgements

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7 References

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4 Results

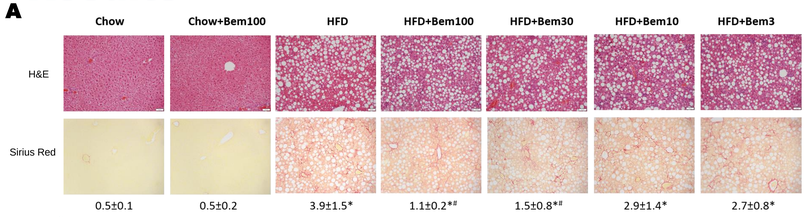


Figure 1. Dose-response effect of bemcentinib (Bem) in liver fibrosis in HFD-fed mice. A) Representative images of liver sections after H&E and Sirius Red staining from mice fed for 4 weeks with chow or HFD diet that received vehicle or Bem at the indicated dose (mg/kg) for the last 2 weeks. Magnification 20X. Sirius Red quantifications (px/µm) are shown under representative pictures. **B)** Fibrogenic and **C)** inflammatory gene expression. Student's t test; *P ≤ .05 vs control mice; #P ≤ .1 vs HFD-fed mice, n=5/group.

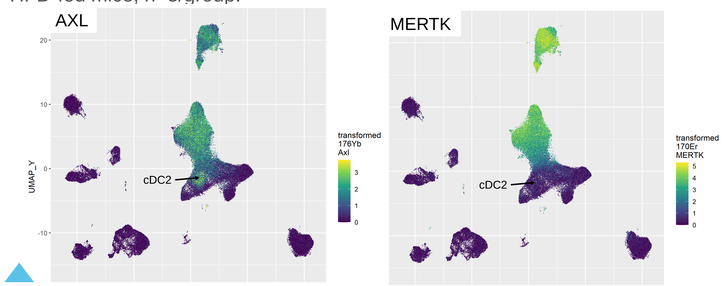
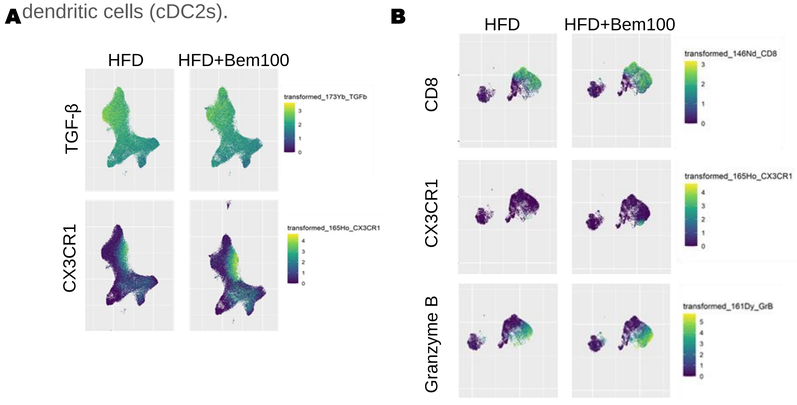


Figure 2. UMAP of immune cells colored by AXL and MERTK expression. CyTOF analysis revealed that AXL was co-expressed with MERTK in infiltrating macrophages and Kupffer cells, but exclusively expressed in conventional type 2 dendritic cells (cDC2s).



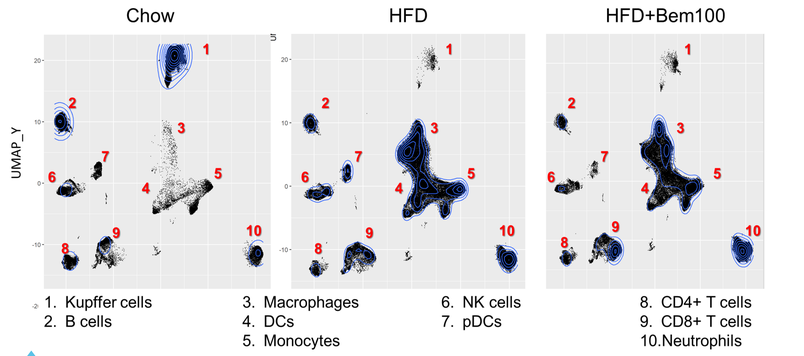


Figure 3. UMAP of immune cell populations with topographical density linens. Compared to chow animals, NASH (HFD) strongly reduced the amount of Kupffer cells and increased infiltration of monocytes/macrophages and CD8+ T-cells. Bemcentinib partially rescued NASH-induced loss of Kupffer cells and reduced the proportion of both plasmacytoid dendritic cells (pDCs) and subsets of recruited macrophages.

Figure 4. Zoomed in UMAP of macrophages and CD8+ T-cells. A) Bemcentinib induced a shift in the composition of the resident and recruited macrophages from TGF-β high to TGF-β med subgroup with an increased influx of CX3CR1high macrophages, associated with tissue remodeling and anti-inflammatory phenotype (5). **B)** Moreover, bemcentinib enhanced the expression of a subtype of CD8+ tissue resident memory T-cells characterized by the expression of CX3CR1 and Granzyme B high known to promote hepatic stellate cell apoptosis (6).

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