Hepatic immune cell profiling of the GAN diet-induced obese and biopsy-confirmed mouse model of NASH with advanced fibrosis and HCC

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Background & Aim

Non-alcoholic steatohepatitis (NASH) has become an emerging risk factor for the development of liver fibrosis and hepatocellular carcinoma (HCC). Recent data suggest that NASH-HCC patients are less responsive to HCC-targeted immunotherapies, likely owing to NASH-related aberrant immune cell regulation. To gain further insight into immune cell regulations in NASH-driven HCC, we profiled liver leukocyte populations in the translational Gubra Amylin NASH (GAN) diet-induced obese (DIO) and biopsy-confirmed mouse model of advanced fibrosing NASH and HCC.

Methods

C57BL/6JRj male mice were fed the GAN diet or chow diet for 58 weeks before randomization. Prior to randomization all animals underwent liver biopsy for histological confirmation (fibrosis stage 3, steatosis score 3, inflammation score ≥2) using the non-alcoholic fatty liver disease activity scoring (NAS) and fibrosis staging system. Animals were terminated at 70 weeks on diet. Liver samples were stained with two panels of fluorescently labelled antibodies to detect and quantify leukocyte subsets, including monocytes/macrophages, neutrophils, T-cells, B-cells, Natural killer (NK) and NK T-cells and analysed by flow cytometry.

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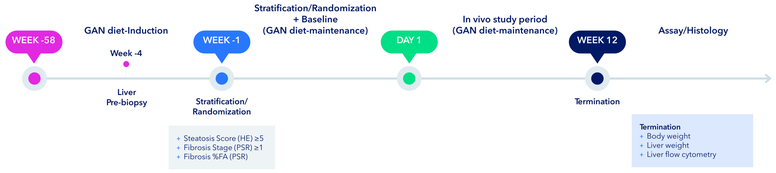
1 Study Outline



Group	Animal	Gender	Number of animals	Treatment	Administration route	Dosing Frequency	Dose
1	LEAN-CHOW	Male	10	**Vehicle**	SC	QD	-
2	DIO-NASH	Male	16	**Vehicle**	SC	QD	-

2 Metabolic Parameters

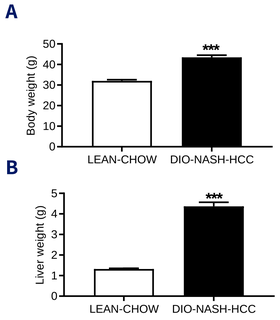


Figure 1. DIO-NASH-HCC mice show increased body weight and hepatomegaly. **(A)** Terminal body weight. **(B)** Terminal liver weight. ***p<0.001 compared to Chow control (Dunnett's test one factor linear model).

3 Hepatic leukocyte markers analyzed by flow cytometry

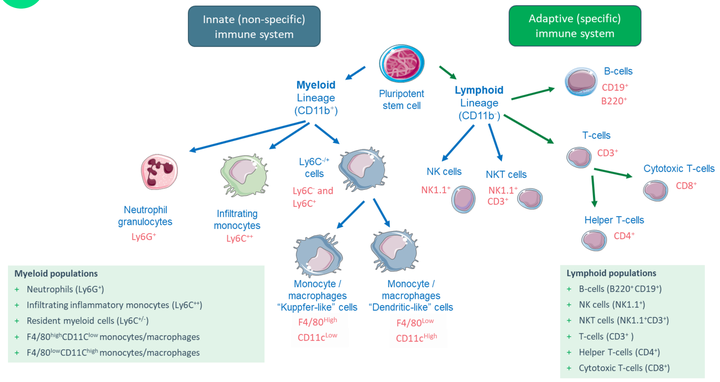


Figure 2. Liver myeloid and lymphoid leukocyte subsets analyzed by flow cytometry.

4 Hepatic leukocyte subsets in GAN DIO-NASH-HCC mice

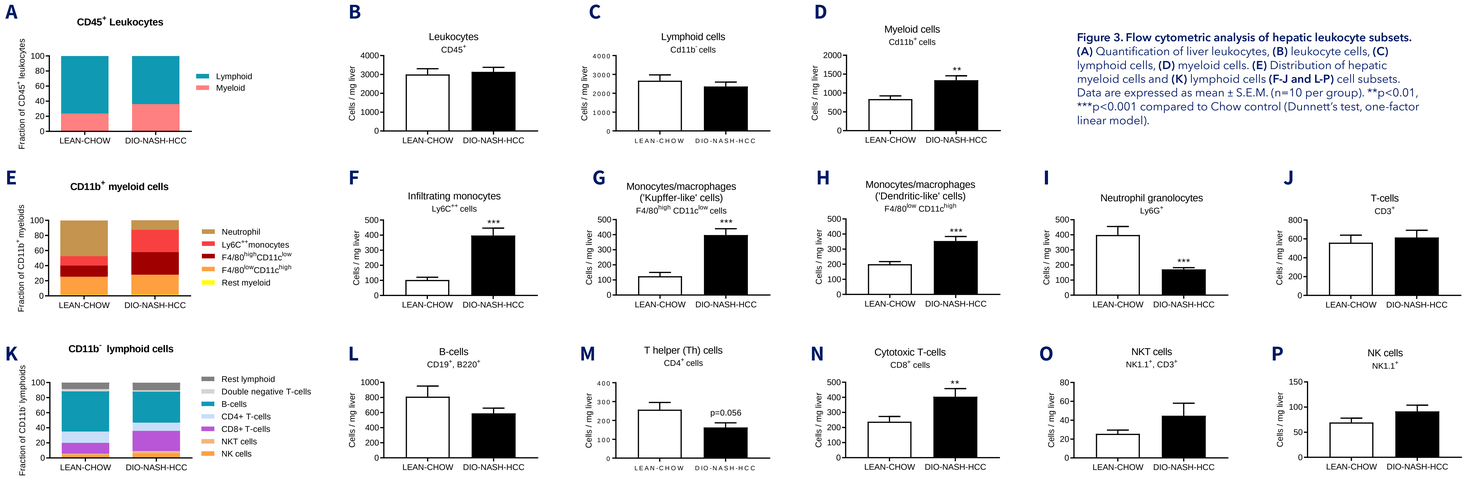


Figure 3. Flow cytometric analysis of hepatic leukocyte subsets. **(A)** Quantification of liver leukocytes, **(B)** leukocyte cells, **(C)** lymphoid cells, **(D)** myeloid cells. **(E)** Distribution of hepatic myeloid cells and **(K)** lymphoid cells **(F-J and L-P)** cell subsets. Data are expressed as mean ± S.E.M. (n=10 per group). **p<0.01, ***p<0.001 compared to Chow control (Dunnett's test, one-factor linear model).

Conclusion

- The GAN DIO-NASH-HCC mouse is a translational model of HCC development on the background of severe NASH and fibrosis
- DIO-NASH-HCC mice demonstrate marked expansion of hepatic resident and infiltrating monocyte/macrophage and specific T-cell populations, notably CD8+ T cells
- CD8+ T cells have been implicated in NASH-driven HCC and reduced responsiveness to HCC-targeted immunotherapies in NASH patients

Liver flow cytometry can provide detailed information of the mode of action of compounds with potential therapeutic effects in NASH-driven HCC.

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