# Ultrastructural assessment of liver sinusoidal endothelial cell capillarisation in metabolic dysfunction-associated steatotic liver disease and its modulation by lanifibranor

S. Chotkoe [1], G. Wettstein [2], J. De Man [1], S. Thys [3], I. Pintelon [3], Y. Lu [1], N. Beyene [1], D. van der Graaff [4], B. De Winter [1], W. De Vos [3], L. Vonghia [4], W. Kwanten [4], S. Francque [4]

[1] University of Antwerp, Laboratory of Experimental Medicine and Pediatrics, Wilrijk, Belgium; [2] Inventiva Pharma, Daix, France;
[3] University of Antwerp, Laboratory of Cell Biology and Histology, Antwerp Centre for Advanced Microscopy, Wilrijk, Belgium;
[4] Unversity Hospital Antwerp, Edegem, Belgium

**Contact information**
Shivani.Chotkoe@uantwerpen.be

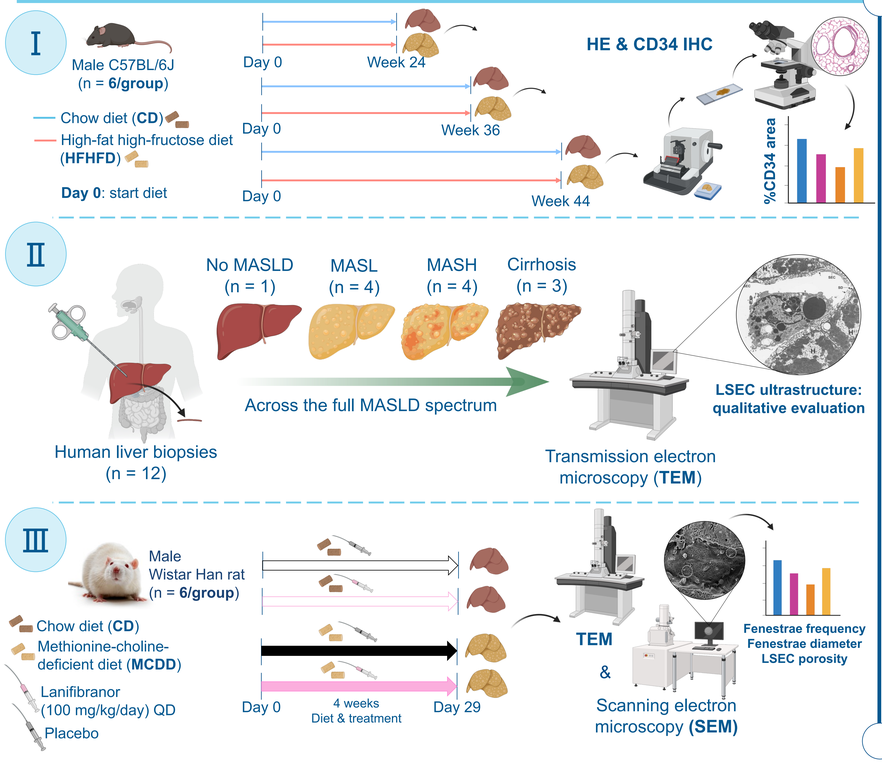
## Introduction & Aim

**MASLD** is associated with an increased **intrahepatic vascular resistance** driven by liver sinusoidal endothelial cell (**LSEC**) dysfunction and loss of fenestrae (**capillarisation**), contributing to disease progression.

Using CD34 immunohistochemistry (IHC), we previously demonstrated that capillarisation is already present in isolated steatosis in both patients and rat models, with further increases observed in MASH patients. Treatment with **lanifibranor**, a pan-PPAR (**peroxisome proliferator-activated receptor**) agonist, reduced CD34 staining. However, **ultrastructural** characterisation of LSEC fenestrae in MASLD remain incomplete.

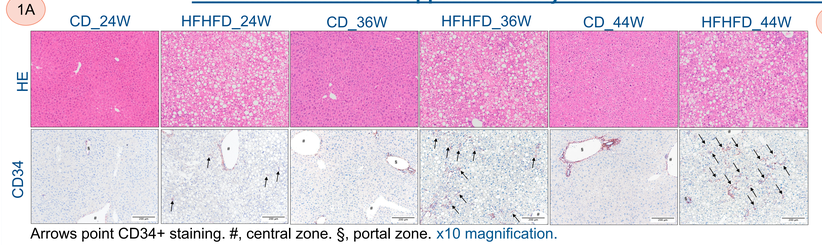
In this study: (i) we investigated the progression of capillarisation in a mouse model of MASLD at histology, and at the **ultrastructural** level we **(ii)** explored sinusoidal remodelling across the MASLD spectrum in human liver tissue, **(iii)** evaluated the effects of lanifibranor on capillarisation in a rat model of isolated steatosis.

## Methods




## Results

### Vascular modification appears as early as in steatosis liver and increases as the disease progresses to MASH and fibrosis




Arrows point CD34+ staining. #, central zone. §, portal zone. x10 magnification.

- Mice under HFHFD progress from isolated steatosis (week 24 and 36) to MASH with minimal fibrosis (not shown) at week 44.
- No CD34 staining is visible in CD mice but there is an increase in CD34 staining in HFHFD-fed mice from week 24 to week 44.

| % CD34 staining | week 24 | week 36 | week 44 |
|---|---|---|---|
| CD | 0.1 ± 0.01 | 0.2 ± 0.03 | 0.1 ± 0.03 |
| HFHFD | 0.6 ± 0.2 | 2.0 ± 0.5 | 2.6 ± 0.8 |
| p | NS | <0.01 | <0.0001 |

### 2 TEM pictures from patients demonstrated a modification of sinusoidal architecture as the disease progresses toward cirrhosis

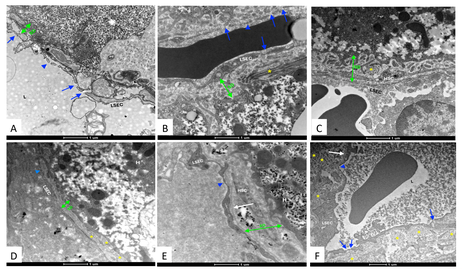



**Figure 2. A) no MASLD** sample with normal architecture of thin LSECs with abundant fenestrae organised in sieve plates. **B) MASL** with **F1** fibrosis and **C) F2** fibrosis had small collagen bundles and HSC processes. Importantly, sieve plates and fenestrae remained present.
**D)** In **MASH** with **F2** fibrosis and **E) F3** fibrosis, perisinusoidal fibrosis, elongated HSCs, LSEC thickening, narrowed fenestrae, and the presence of a prominent basement membrane in one of the F3 samples were observed, indicating a clear pattern of LSEC capillarisation, however with some heterogeneity between samples.
**F)** In **MASH cirrhosis** (**F4**) the ultrastructural features represent an advanced stage of sinusoidal remodelling with pronounced perisinusoidal fibrosis, a prominent basement membrane and a few remaining narrowed fenestrae, wherein the sinusoid becomes almost indistinguishable from a capillary-like vessel.

▲: Fenestra ▲: Sieve plate ⇕SD: Space of Disse ●: Collagen fibrils ▲: Basal membrane H: Hepatocytes HSC: Hepatic stellate cell LSEC: Liver sinusoidal endothelial cell

### 3A Lanifibranor maintains LSEC phenotype and prevents vascular architecture modification

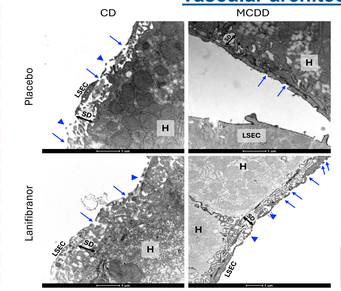



**Figure 3. A)** TEM micrographs.
CD-fed placebo-treated livers showed clear fine structures of the sinusoids, the LSECs and their fenestrae organised in sieve plates.

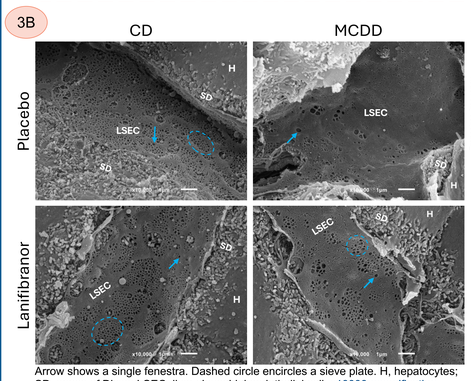
In contrast, in the MCDD livers, LSECs showed loss of fenestrae and the formation of capillaries, however with some heterogeneity. no indication of ECM deposition yet.

Treatment with lanifibranor recovered LSEC fenestrae in steatosis, while in CD-fed rats lanifibranor did not have an effect.

▲: Fenestra ⇕SD: Space of Disse H: Hepatocytes ▲: Sieve plate LSEC: Liver sinusoidal endothelial cell

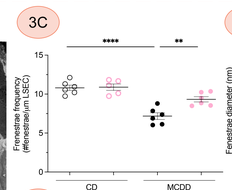
scale bar: 1 µm.

### 3B

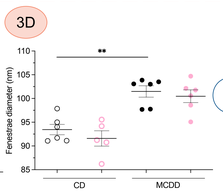



Arrow shows a single fenestra. Dashed circle encircles a sieve plate. H, hepatocytes; SD, space of Disse; LSEC, liver sinusoidal endothelial cell. x10000 magnification.

### 3C




### 3D




### 3E

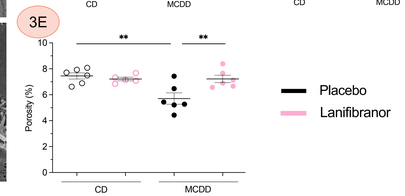



**Figure 3. B)** SEM micrographs. Quantitative assessment of the fenestrae and LSEC areas revealed that in steatosis compared to the healthy counterparts, **C)** fenestrae frequency is significantly decreased, **D)** fenestrae diameter is significantly increased and **E)** LSEC porosity is significantly decreased.
Treatment with lanifibranor in MCDD-fed rats did not affect fenestrae diameter but it significantly improved fenestrae frequency and ultimately recovered fenestrae porosity. In CD-fed rats, lanifibranor did not have an effect on the fenestrae.

| | fenestrae frequency (fenestrae/µm²) | fenestrae diameter (nm) | LSEC porosity (%) |
|---|---|---|---|
| CD + placebo | 10.8 ± 0.3 | 93.5 ± 1.1 | 7.5 ± 0.2 |
| MCDD + placebo | 7.2 ± 0.4 | 101.5 ± 1.2 | 5.7 ± 0.4 |
| p (CD vs MCDD) | <0.0001 | <0.01 | <0.01 |
| MCDD + lanifibranor | 9.3 ± 0.3 | 100.5 ± 1.4 | 7.2 ± 0.3 |
| p (MCDD placebo vs lanifibranor) | <0.01 | NS | <0.01 |

## Conclusions

This study demonstrated, using CD34 staining, that in an HFHFD mouse model of MASLD capillarisation increases with disease progression from isolated steatosis to MASH.

Furthermore, using TEM, at the ultrastructural level in liver biopsies of MASLD patients, loss of fenestrae is observed more frequent as disease activity and severity increases.

Finally, using TEM and SEM, in a rat model of isolated steatosis, loss of fenestrae was observed, mainly by a decreased fenestrae frequency, resulting in decreased LSEC porosity, and treatment with lanifibranor significantly inhibited loss of fenestrae in steatosis, without impact on healthy livers.

**These findings support that LSEC capillarisation:**

- Develops early in MASLD
- Is heterogeneous
- Progresses with disease activity and severity
- Is reversible after treatment with lanifibranor

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Shivani Chotkoe
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