The GAN-CCL4 rodent models of advanced NASH with progressive fibrosis.

**GAN-CCL4 mouse model**

The GAN-CCL4 model is based on GAN diet-induction in combination with CCL4 for 4 weeks prior to study start. The GAN-CCL4 mouse exhibits non-metabolic associated advanced NASH and progressive fibrotic development, objectively evaluated by histopathological assessment including clinically-derived NAFLD Activity Score and Fibrosis Grade.

**Diet**

- 40% fat (palm oil)
- 40% carbohydrates (20% fructose)
- 2% cholesterol

**Strain**

Male C57BL/6J mice

**Study outline**

- **GAN-CCL4 induction**
- **Randomization** + Baseline (GAN-CCL4 maintenance)
- **In vivo study period** (GAN-CCL4 maintenance)
- **Assay/Histology**

**Week -4**

- GAN-CCL4 induction

**Day -3**

- Randomization

**Day 0**

- First Dose

**Week 8**

- Termination (treatment)
  - Plasma ALT/AST
  - Liver weight

**Terminial liver biochemistry:**

- Liver TG/TC
- Liver HP

**Terminal liver histology:**

- NAFLD Activity Score (HE)
- Fibrosis Grade (PSR)
- Morphometric analysis:
  - Steatosis (HE)
  - Inflammation (Gal-3) (HC)
  - Collagen (Col1α1) (HC)
  - Fibrosis (PSR)
  - Stellate cell activation (α-SMA) (HC)

**Tissue/Blood samples:**

- Liver for RNAseq (optional)
- Terminal plasma for sponsor
- Terminal liver for sponsor

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**Key model traits**

- GAN diet high in fat, fructose and cholesterol in combination with CCL4 for up to 12 weeks.
- Non-obesity without metabolic disease.
- Early onset of steatosis and fibrosis.
- Fast disease progression to advanced fibrosis and cirrhosis.
- Clinical histopathological endpoints.
- Prophylactic evaluation of drug efficacy.
Metabolic, biochemical and histopathological profile

GAN-CCL4 mice are lean and show non-metabolic driven increases in hepatomegaly, steatosis, inflammation and fibrosis.

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<thead>
<tr>
<th></th>
<th>CHOW</th>
<th>GAN-CCL4 W12</th>
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<tbody>
<tr>
<td>Body weight (g)</td>
<td>27.8 ± 0.85</td>
<td>25.4 ± 0.56</td>
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<tr>
<td>Liver weight (g)</td>
<td>1.12 ± 0.04</td>
<td>1.41 ± 0.04</td>
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<tr>
<td>Plasma ALT</td>
<td>19.8 ± 1.01</td>
<td>385 ± 32.2</td>
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<tr>
<td>Liver steatosis (HE) (%) FA</td>
<td>1.37 ± 0.04</td>
<td>11.2 ± 0.75</td>
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<tr>
<td>Liver inflammation (Gal-3) (%) FA</td>
<td>0.79 ± 0.04</td>
<td>10.4 ± 0.98</td>
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<tr>
<td>Liver fibrosis (PSR) (%) FA</td>
<td>0.41 ± 0.06</td>
<td>3 ± 0.21</td>
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Clinical histopathological scores

GAN-CCL4 mice show early disease onset and rapid progression of liver fibrosis, as determined using the clinically-derived NAFLD Activity Scoring and Fibrosis Grade (Kleiner, 2005; Ishak, 1995).

Histopathological NAFLD Activity Score and Fibrosis Grade

Assessment of NAFLD Activity Score and Fibrosis Grade allows for evaluation of individual treatment effects on liver histopathology. Effect of 8 weeks of treatment with the PPAR-a/d agonist Elafibranor.
GAN-CCL4 rat model

The GAN-CCL4 rat model is based on GAN diet-induction in combination with CCL4 for 4 weeks prior to study start. GAN-CCL4 rats exhibits non-metabolic associated moderate NASH and progressive fibrotic development, objectively evaluated by histopathological assessment including clinically-derived NAFLD Activity Score and Fibrosis Grade.

HE and PSR staining
Histopathological NAFLD Activity Score and Fibrosis Grade

Assessment of NAFLD Activity Score and Fibrosis Grade allows for evaluation of individual treatment effects on liver histopathology. Effect of 8 weeks of treatment with the PPAR-\(\alpha/d\) agonist Elafibranor.

Histomorphometric evaluation of steatohepatitis and fibrosis

Quantitative assessment of liver steatosis, inflammation and fibrosis by histomorphometric image analysis. Effect of 8 weeks of treatment with the PPAR-\(\alpha/d\) agonist Elafibranor.