The CDAA-HFD rodent models of advanced NASH and progressive fibrosis.

### CCDA-HFD mouse model

The CDAA-HFD model is based on choline-deficient L-amino acid defined high fat diet (CDAA-HFD) induction for 6-12 weeks prior to study start. The CDAA-HFD mouse exhibits non-metabolic associated advanced NASH and progressive fibrotic development being objectively evaluated by histopathological assessment including NAFLD Activity Score and Fibrosis Stage.

### Key model traits

- CDAA diet high in fat, fructose and cholesterol for up to 20 weeks.
- Non-obesity without metabolic disease.
- Early onset of steatosis and fibrosis.
- Fast disease progression to bridging fibrosis (stage F3).
- Clinical histopathological endpoints.
- Prophylactic and therapeutic drug efficacy.

<table>
<thead>
<tr>
<th>Diet</th>
<th>45% fat and 28% fructose 1% cholesterol, 0.1% methionine No choline</th>
<th>A16092201 Research diets Minimum 6 weeks on diet to develop hallmarks of fibrosing NASH</th>
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<tbody>
<tr>
<td>Strain</td>
<td>Male C57BL/6J mice</td>
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### Study outline

#### Assay/Histology

- Chronic repeated dosing (PO/SC, QD/BID)
- Body weight (Q3)
- Food intake (QD) Week 1-2
- Food intake (QW) Week 3-8

#### In vivo study period (CDAA-HFD maintenance)

- Day 0 First Dose
- Day -3 Randomization
- Randomization + Baseline (CDAA-HFD maintenance) Week 8

#### Terminal liver biochemistry:

- Liver TG/TC
- Liver HP

#### Terminal liver histology:

- NAFLD Activity Score (HE)
- Fibrosis Stage (PSR)
- Morphometric analysis:
  - Steatosis (HE)
  - Inflammation (Gal-3) (IHC)
  - Collagen (Col1a1) (IHC)
  - Fibrosis (PSR)
  - Stellate cell activation (a-SMA) (IHC)

#### Tissue/Blood samples:

- Liver for RNAseq (optional)
- Terminal plasma for sponsor
- Terminal liver for sponsor
Metabolic, biochemical and histopathological profile

CDAA-HFD mice are lean and show non-metabolic driven increases in hepatomegaly, plasma liver enzymes, steatosis, inflammation and fibrosis.

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<tr>
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<th>CHOW</th>
<th>CDAA-HFD (20w)</th>
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<tbody>
<tr>
<td>Body weight (g)</td>
<td>30.0 ± 0.4</td>
<td>24.1 ± 0.3</td>
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<tr>
<td>Liver weight (g)</td>
<td>1.3 ± 0.0</td>
<td>2 ± 0.1</td>
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<tr>
<td>Plasma ALT (U/L)</td>
<td>22 ± 1.5</td>
<td>301 ± 20</td>
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<tr>
<td>Liver steatosis (HE)</td>
<td>1.02 ± 0.1</td>
<td>24.9 ± 0.8</td>
</tr>
<tr>
<td>Liver inflammation (Gal-3) (FA)</td>
<td>1.44 ± 0.1</td>
<td>12.9 ± 0.3</td>
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<tr>
<td>Liver fibrosis (PSR)</td>
<td>1.04 ± 0.1</td>
<td>8.76 ± 0.2</td>
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Clinical histopathological scores

CDAA-HFD mice show early disease onset and fast progression of liver fibrosis, as determined using the clinical-derived NAFLD Activity Scoring and Fibrosis staging system (Kleiner, 2005).

Histopathological NAFLD Activity Score and Fibrosis Stage

Assessment of NAFLD Activity Score and Fibrosis stage 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-α/d agonist Elafibranor.

CDAA-HFD rat model

The CDAA-HFD rat model is based on choline-deficient L-amino acid defined high fat diet (CDAA-HFD) induction for 4-6 weeks prior to study start. CDAA-HFD rats are lean and show non-metabolic driven early disease onset and fast progression to advanced NASH with fibrosis and cirrhosis, as evaluated by clinical-derived NAFLD Activity Score and Fibrosis Stage.

**HE and PSR staining**
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.