Mouse and rat models of advanced NASH and progressive fibrosis based on choline-deficient, amino acid defined high-fat diet (CDAA-HFD) induction

**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.

<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>
</tr>
</thead>
<tbody>
<tr>
<td>Strain</td>
<td>Male C57BL/6J mouse</td>
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</table>

**Key model traits**

- CDAA diet high in fat, fructose and cholesterol for 6-12 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.

**Study outline**

- **Diet**: 45% fat and 28% fructose 1% cholesterol, 0.1% methionine No choline
- **Strain**: Male C57BL/6J mouse
- **Research diets**: A16092201
- **Minimum 6 weeks on diet to develop hallmarks of fibrosing NASH**
- **CDAA-HFD induction**: Choline-deficient, amino acid defined high-fat diet (CDAA-HFD) induction for 6-12 weeks prior to study start.
- **Non-metabolic associated advanced NASH and progressive fibrotic development**
- **Histopathological assessment**: NAFLD Activity Score and Fibrosis Stage.
- **Mouse and rat models of advanced NASH and progressive fibrosis**
- **CCDA-HFD mouse model**: Choline-deficient, 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.

**Study outline**

- **CDAA-HFD induction**: Week -6/12
- **Randomization + Baseline**: Week -1
- **First Dose**: Day 0
- **In vivo study period**: Chronic repeated dosing (PO/SC, QD/BID)
- **Body weight (QD)**
- **Food intake (QD) Week 1-2**
- **Food intake (QW) Week 3-8**
- **Assay/Histology**: Week 8
  - **Liver biopsy histology**: NAFLD Activity Score (HE)
  - Fibrosis Stage (PSR)
  - Morphometric analysis:
    - Steatosis (HE)
    - Inflammation (Gal-3) (IHC)
    - Collagen (Col1a1) (IHC)
    - Fibrosis (PSR)
    - Stellate cell activation (α-SMA) (IHC)
  - **Tissue/Blood samples**:
    - Liver for RNAseq (optional)
    - Terminal plasma for sponsor
    - Terminal liver for sponsor

**Key model traits**

- CDAA diet high in fat, fructose and cholesterol for 6-12 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.
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.

<table>
<thead>
<tr>
<th></th>
<th>CHOW</th>
<th>CDAA-HFD (20w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>30.0 ± 0.4</td>
<td>24.1 ± 0.3</td>
</tr>
<tr>
<td>Liver weight (g)</td>
<td>1.3 ± 0.0</td>
<td>2 ± 0.1</td>
</tr>
<tr>
<td>Plasma ALT (U/L)</td>
<td>22 ± 1.5</td>
<td>301 ± 20</td>
</tr>
<tr>
<td>Liver steatosis (HE) (% FA)</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>
</tr>
<tr>
<td>Liver fibrosis (PSR) (% FA)</td>
<td>1.04 ± 0.1</td>
<td>8.76 ± 0.2</td>
</tr>
</tbody>
</table>

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).

HE and PSR staining

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.