ATROSAB / ATROSIMAB

Biochemical and in vitro characterization
ATROSAB – GENERAL DESCRIPTION

Full length humanized IgG1 antibody specifically blocking TNF-R1

- ATROSAB is the **FIRST NEXT-GENERATION TNF-INHIBITOR**, selectively targeting TNF-R1 without antagonizing TNF-R2
- Selective inhibition of TNF-R1 only mediates pathology, while maintaining immunity and neuroprotection through TNF-R2
- ATROSAB does not have any ADCC or CDC activity ("cytotoxicity")
- ATROSAB inhibits:
  - TNF induced cell death
  - TNF mediated release of IL-8 (HT1080 cells; potency assay)
ATROSIMAB – GENERAL DESCRIPTION

New optimized structure of monovalent antibody serves as a platform technology

- Affinity maturation leads to new variable domain 13.7 having 10-fold stronger binding as compared to ATROSAB
- Binding to identical epitope as ATROSAB
- Fully human sequence
- “Chimeric” Ig-domains CH3/CH1 (CH31) and CH3CLk (CH3k), hinge-less
- Potential combination of FcRn binding and natural dimerization with covalent linkage via disulfide bond
- Inherently less prone to form multimers / aggregation
- New patents filed for new type of monovalent structure in 2016 serving as monovalent, Fab-like antibody platform
ATROSIMAB - BIOACTIVITY

Monovalent antibody reveals superior properties for use in autoimmune indications

• Offers superior pharmacology to ATROSAB, optimized for the treatment of inflammatory indications, including NASH, RA and Crohn’s Disease

• Newly formulated ATROSIMAB
  - Monovalent TNF-R1 engagement
  - Comparable binding to full-length antibody ATROSAB
  - Lacks agonistic activity including with anti-human Fab serum-mediated cross-linking
  - 3.6-fold stronger inhibition of TNF-mediated TNF-R1 activation than ATROSAB
  - PK comparable with other Fc fusion proteins
ATROSIMAB does not activate CRP, IL-6 & IL-8 and is stable in serum over 7 days

Blood samples of C57Bl/6J-huTNFR1-/- mice were taken from the tail vein at the indicated time points. Plasma samples were frozen to -80 °C and analyzed by ELISA for CRP levels at the end of the experiment. Concentrations were determined upon interpolation from a mouse CRP standard curve. Shown is Mean ± SD of three mice per group. Statistical analysis was performed using GraphPad Prism, one-way ANOVA (Time only) and Tukey post-test (p < 0.1).

Coating 1 µg/ml hu TNFR1-Fc; Blocking 2% skim milk in PBS; Detection anti huFab-HRP 1:20,000 diluted in 2% skim milk in PBS. n=3

ATROSIMAB does not induced any increase in IL-6, IL-8 or CRP levels
ATROSIMAB - BIOACTIVITY

Inhibition of TNF-α included IL-8 release (potency)

- IL-8 release from HT-1080 cells triggered by 0.1 nM TNF was inhibited by ATROSIMAB
- ATROSAB, Fab13.7, 0.1 nM TNF and unstimulated cells served as controls
- Shown are mean and SD of one experiment
ATROSAB / ATROSIMAB FOR NASH

*In Vivo* proof of concept studies in high-fat mouse model
**TRANSGENIC HTNF-R KI HIGH-FAT MOUSE MODEL**

Increased body weight in high-fat diet compared to control-diet mice
**TRANSGENIC HTNF-R KI HIGH-FAT MOUSE MODEL**

**Increased Apoptosis**

![Control-Diet](image1) ![High-Fat-Diet](image2)

**Activated Caspase-3**

- Control-Diet
- High-Fat-Diet

**Increased Fibrosis**

![Control-Diet](image3) ![High-Fat-Diet](image4)

**Siris-Red Staining**

- Control-Diet
- High-Fat-Diet

**Increased steatosis, triglycerides and inflammation**

- Steatosis
- Triglyceride Concentration (mg/mL Protein)
- NAFLA Activity Score

**Graphs**

- Activated Caspase-3 (% Positive Cells)
  - High-Fat-Diet (n=5)
  - Control-Diet (n=4)

**Other Information**

- Increased fibrosis
- Increased steatosis, triglycerides and inflammation
ATROSAB reduced Apoptosis, Steatosis, Triglycerides & NAFLD activity score significantly

20 weeks HFD including 4 weeks treatment with Control-Ab or anti-TNFR1-Ab
TNFR-1 Inhibition reduces steatosis in liver tissue of high-fat diet mice

B6-huTNFR1-k/i-mice treated with anti-TNFR1 or control (cetuximab) antibody for 8 weeks following 24 weeks HFD feeding
Decrease of the NAFLD Activity score & its components in liver tissues of high-fat diet mice treated with anti-TNFR-1 antibody

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean Chance in score anti-TNFR-1-Ab vs. Co-Ab</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Steatosis</td>
<td>-1.06</td>
<td>0.011</td>
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<tr>
<td>Inflammation</td>
<td>-0.36</td>
<td>0.212</td>
</tr>
<tr>
<td>Ballooning</td>
<td>-0.21</td>
<td>0.691</td>
</tr>
<tr>
<td>Total NAFLD Activity Score</td>
<td>-1.63</td>
<td>0.039</td>
</tr>
</tbody>
</table>

HFD, Control-Ab (n=6)
HFD, anti-TNFR1-Ab (n=7)
ATROSAB IN NASH

TNFR-1 Inhibition reduces insulin resistance & improves glucose tolerance in high-fat diet mice
ATROSAB IN NASH

ATROSAB is effective in treating NASH in transgenic hTNF-R ki high-fat mouse model

Conclusions

- ATROSAB significantly reduces apoptosis, steatosis, inflammation and fibrosis in the liver of high-fat diet mice
- TNF-R1 inhibition significantly reduces aminotransferase levels in sera, insulin-resistance and improves glucose tolerance
- ATROSAB represents a promising novel therapeutic strategy for the treatment of patients with NASH
ATROSIMAB - BIOACTIVITY

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