PROMETHERA®
BIOSCIENCES

ATROSAB - ATROSIMAB

CLINICAL DATA

January, 28th 2020

promethera.com

ATROSAB / ATROSIMAB

Biochemical and in vitro characterization

ATROSAB – GENERAL DESCRIPTION

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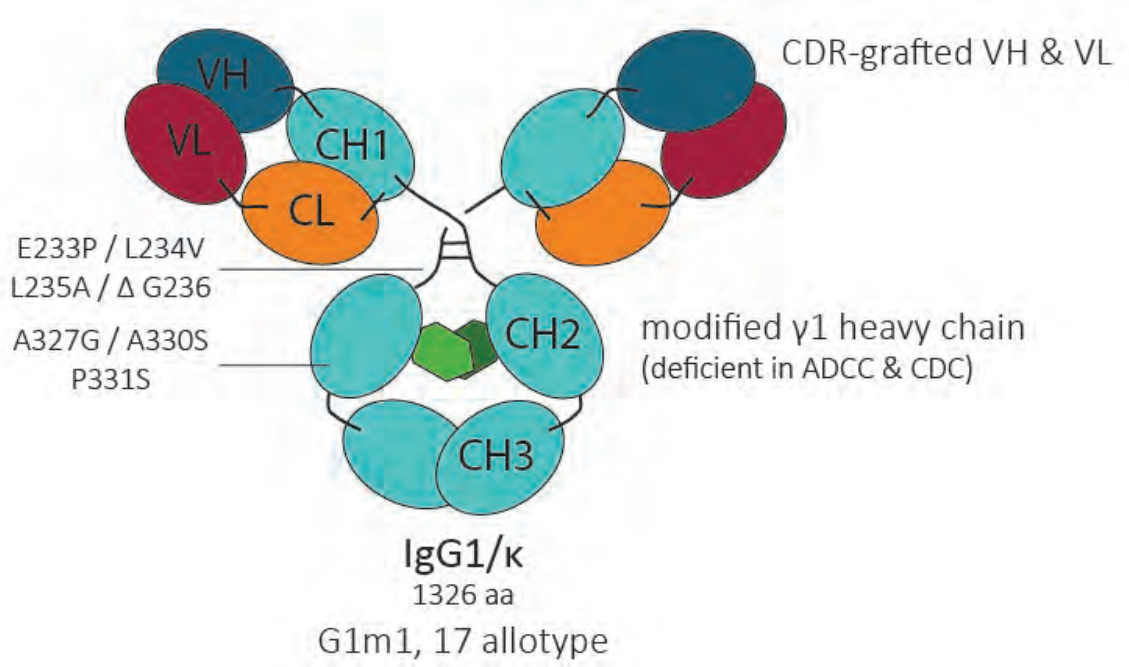
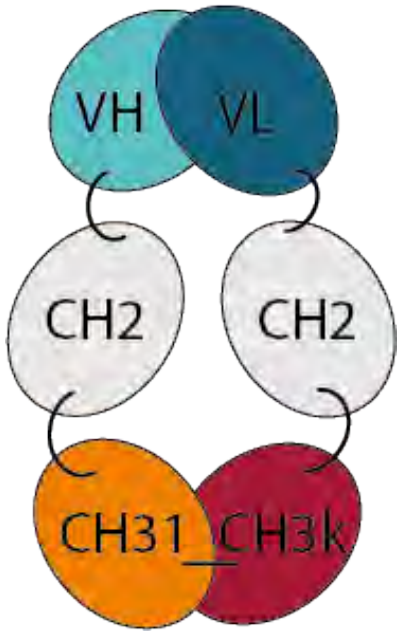
Full legth humanized IgG1 antibody specifically blocking TNF-R1

- 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 (“cytoxicity”)
- 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

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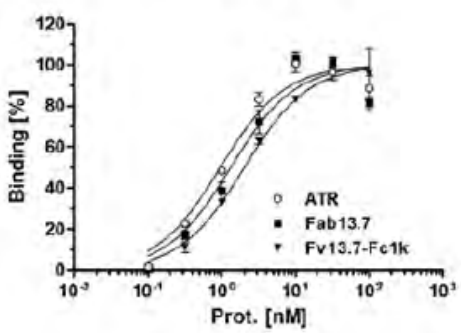
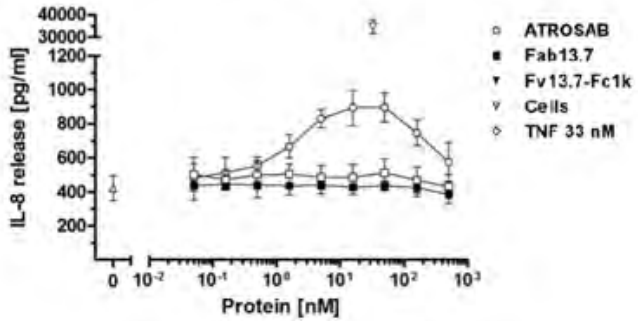
Monovalent antibody reveals superior properties for use in autoimmune indications

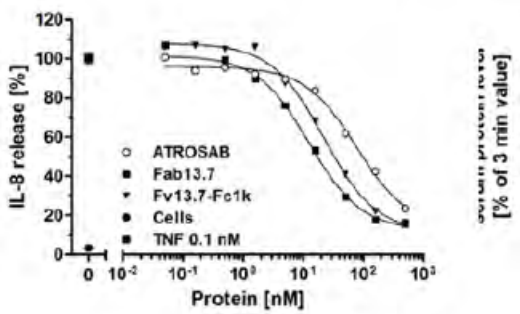
Binding ELISA



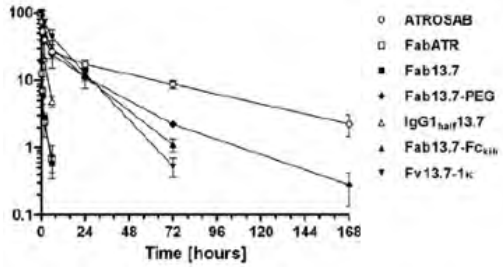
TNFR1 activation



Inhibition of TNFR1 activation



***In vivo* pharmacokinetic**

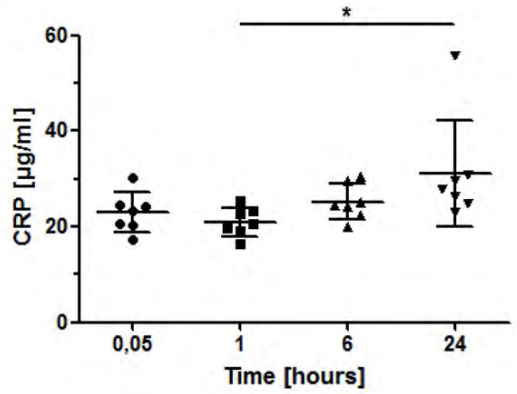


Protein	ATROSAB	Fab13.7	Fv13.7-Fc1k
$T_{1/2,\ terminal}$ [h]	48.99 ± 7.80	1.56 ± 0.07	10.30 ± 0.24
AUC [%*h]	1791.49 ± 189.94	45.42 ± 2.69	1236.89 ± 225.81

- 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

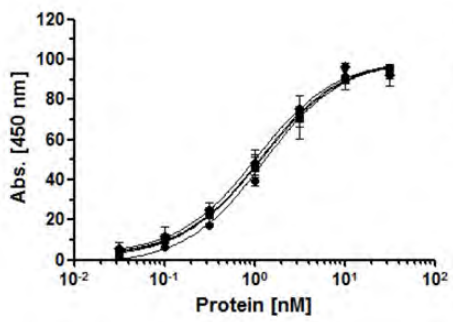
ATROSIMAB - DESCRIPTION

ATROSIMAB does not activate CRP, IL-6 & IL-8 and is stable in serum over 7 days

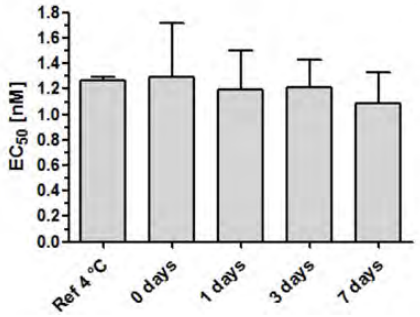


Blood samples of C57BL/6J-huTNFR1$_{ecd}$k/I mice were taken from the tail vein at the indicated time points. Plasma samples were frozen to -80 °C and analyzed by ELISA for CRP levels at the end of the experiment. Concentrations were determined upon interpolation from a mouse CRP standard curve. Shown is Mean ± SD of three mice per group. Statistical analysis was performed using GraphPad Prism, one-way ANOVA (Time only) and Tuckey post-test ($p < 0.1$).

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



Protein	EC50 [nM]
Ref 4 °C	1,3
0 days	1,2
1 days	1,2
3 days	1,2
7 days	1,1

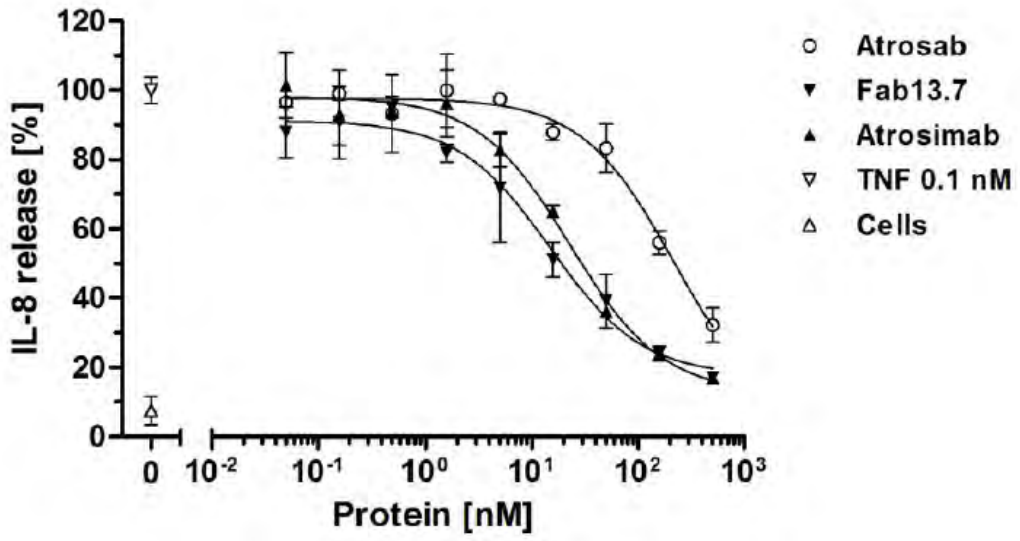


Coating 1 µg/ml hu TNFR1-Fc;
Blocking 2% skim milk in PBS;
Detection anti huFab-HRP 1:20,000 diluted in 2% skim milk in PBS. n=3

ATROSIMAB - 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



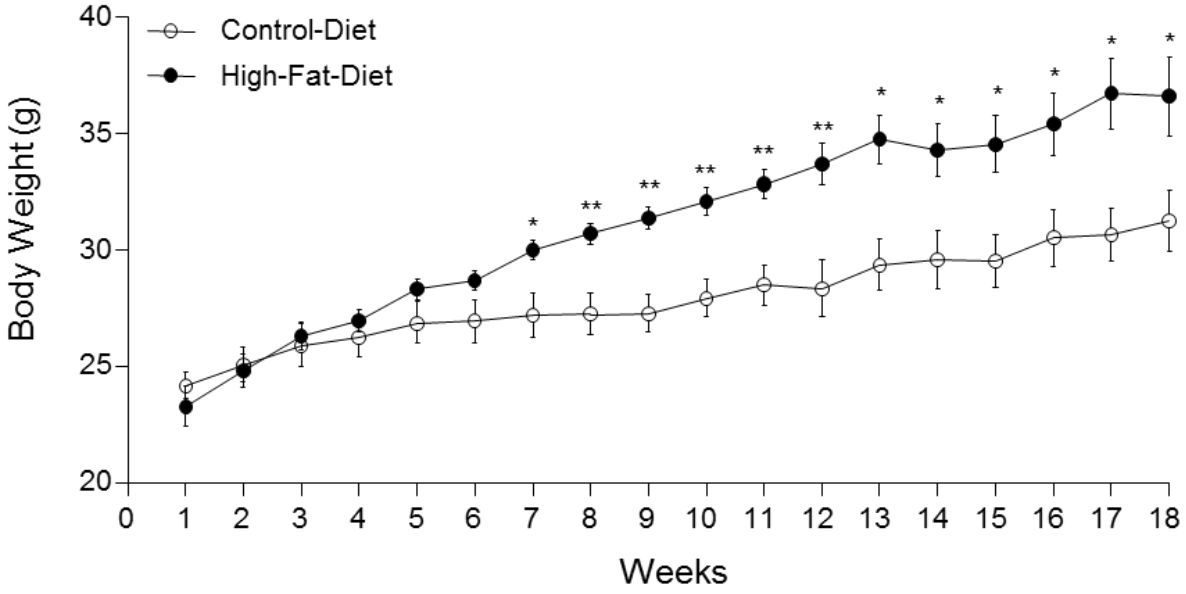
Protein	Atrosab	Fab13.7	Atrosimab
Molecular weight [kDa]	146 (24, 49)	47 (24, 23)	72 (36, 36)
DSC, T_{onset} [°C]	58	76	46
ELISA, EC_{50} [nM]	0.17	0.16	0.43
IL-8 Inhibition, IC_{50} [nM]	216	15	23

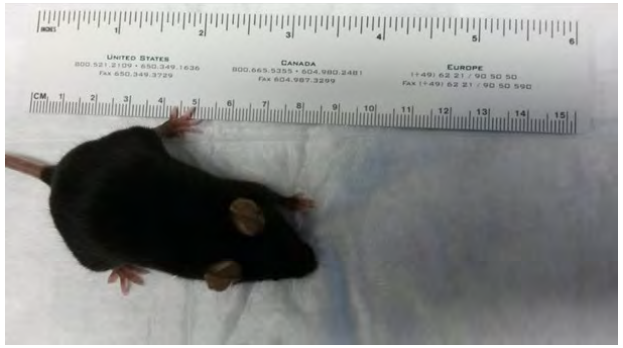
ATROSAB / ATROSIMAB FOR NASH

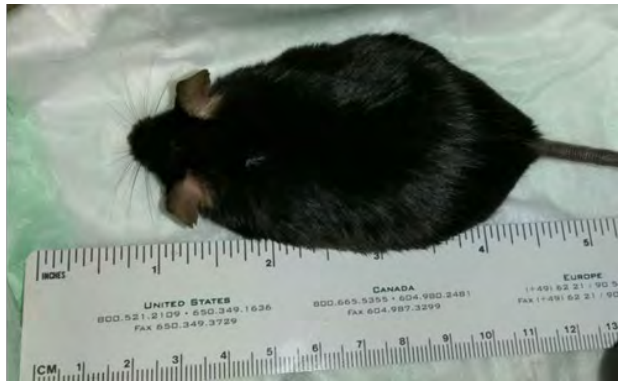
In Vivo proof of concept studies in high-fat mouse model

TRANSGENIC HTNF-R KI HIGH-FAT MOUSE MODEL

Increased body weight in high-fat diet compared to control-diet mice

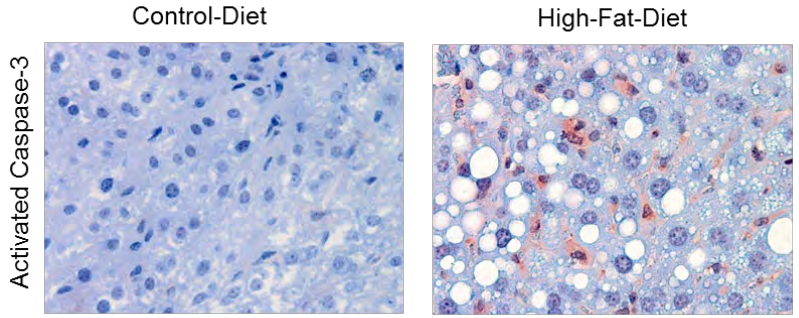


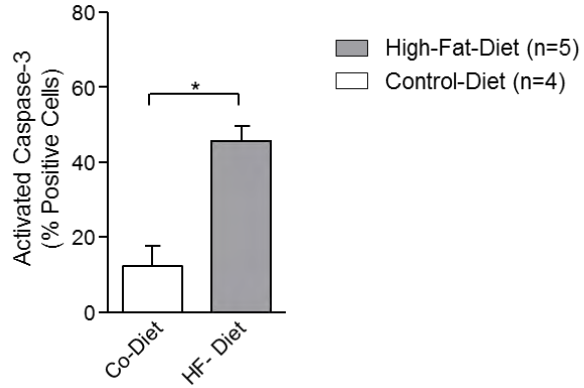




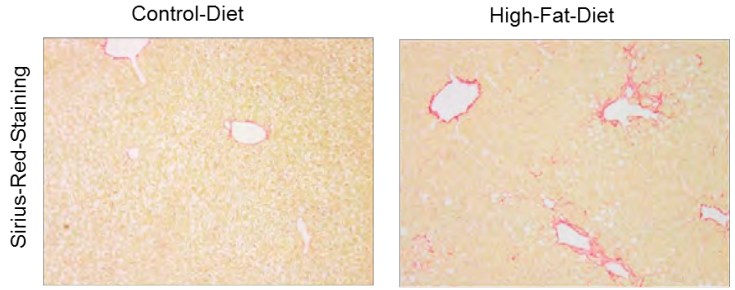
TRANSGENIC HTNF-R KI HIGH-FAT MOUSE MODEL

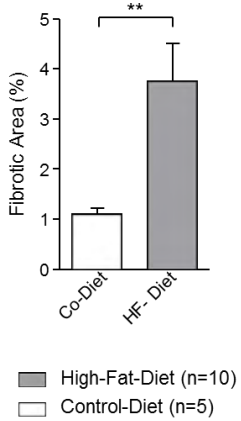
Increased Apoptosis



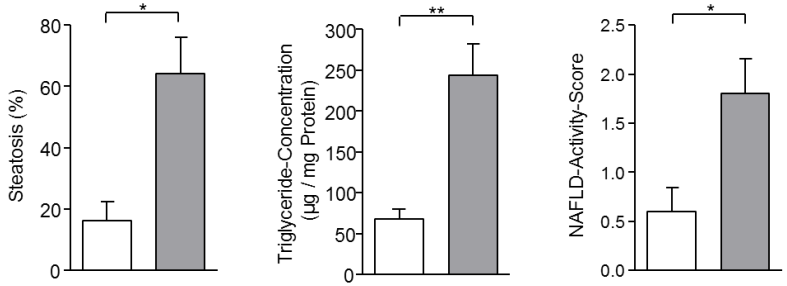


Increased Fibrosis





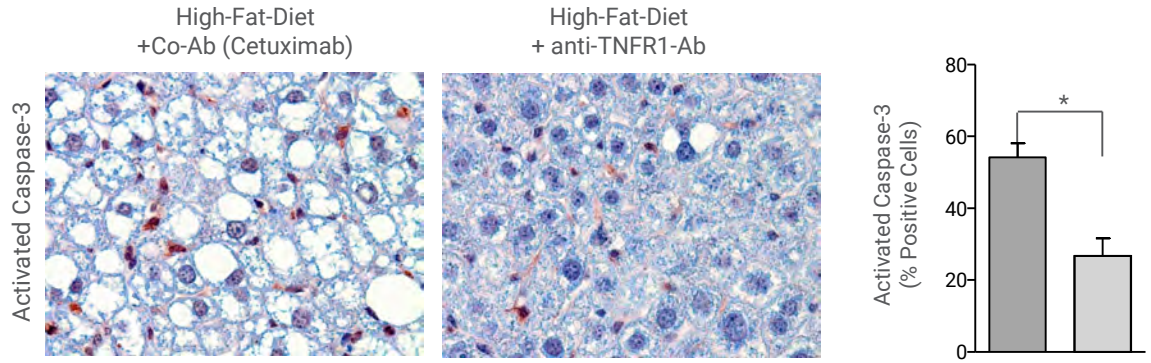
Increased steatosis, triglycerides and inflammation



ATROSAB IN NASH

ATROSAB reduced Apoptosis, Steatosis, Triglyerides & NAFLD activity score significantly

A



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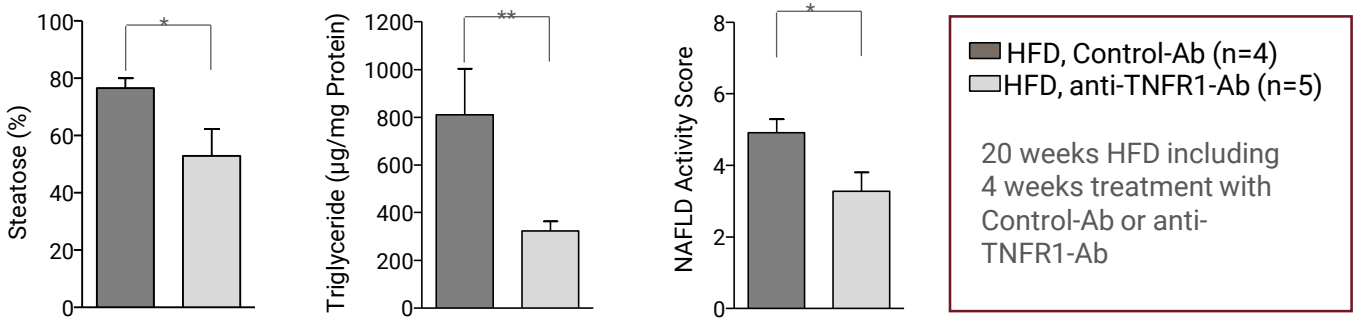
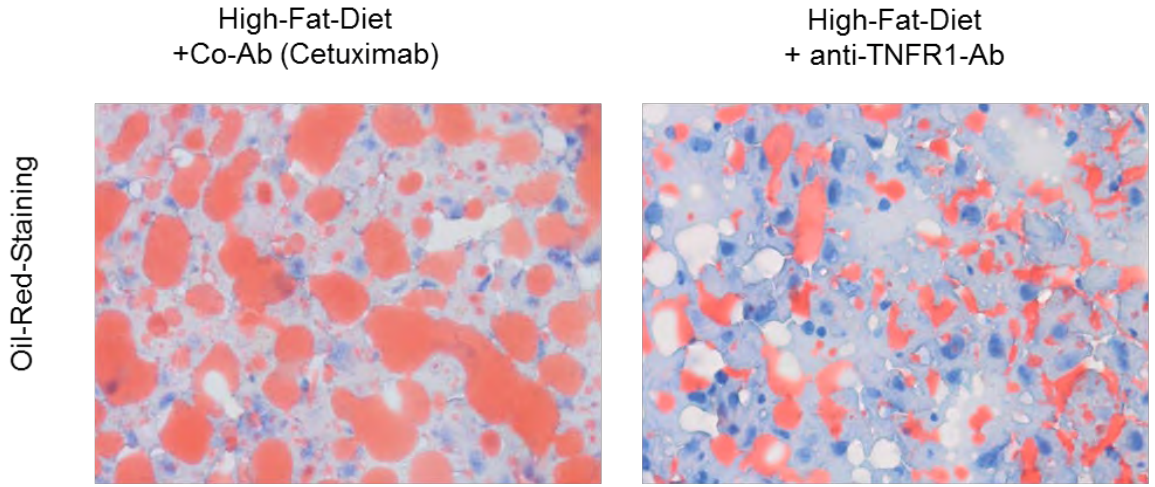


ATROSAB IN NASH

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

ATROSAB IN NASH

Decrease of the NAFLD Activity score & its components in liver tissues of high-fat diet mice treated with anti-TNFR-1 antibody

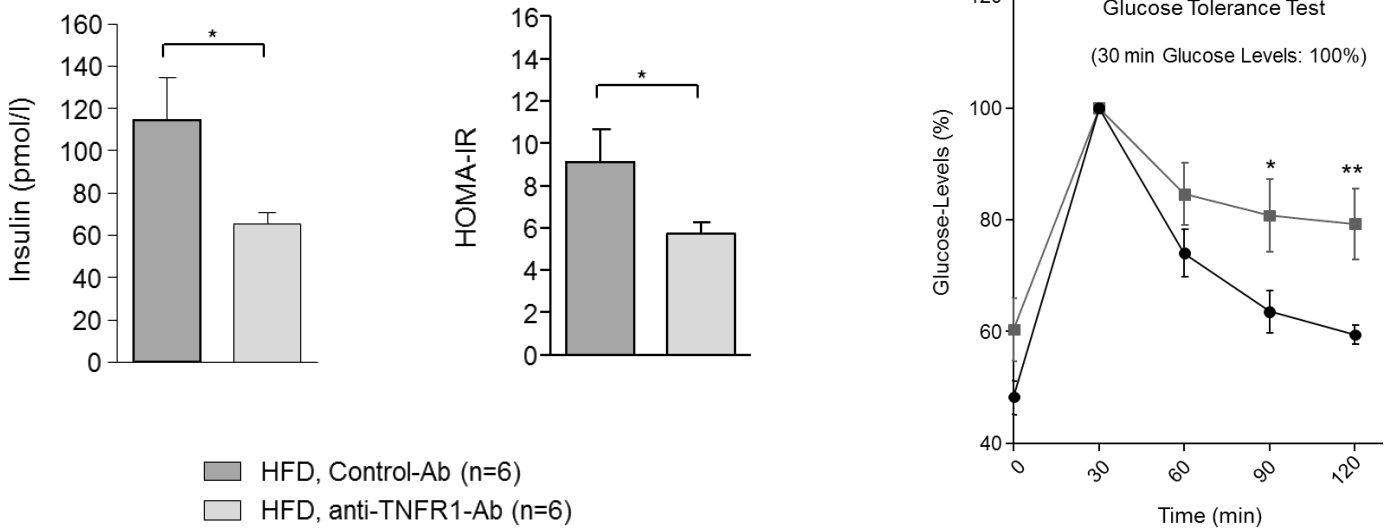
Variable	Mean Chance in score anti-TNFR-1-Ab vs. Co-Ab	P-Value
Steatosis	-1.06	0.011
Inflammation	-0.36	0.212
Ballooning	-0.21	0.691
Total NAFLD Activity Score	-1.63	0.039

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

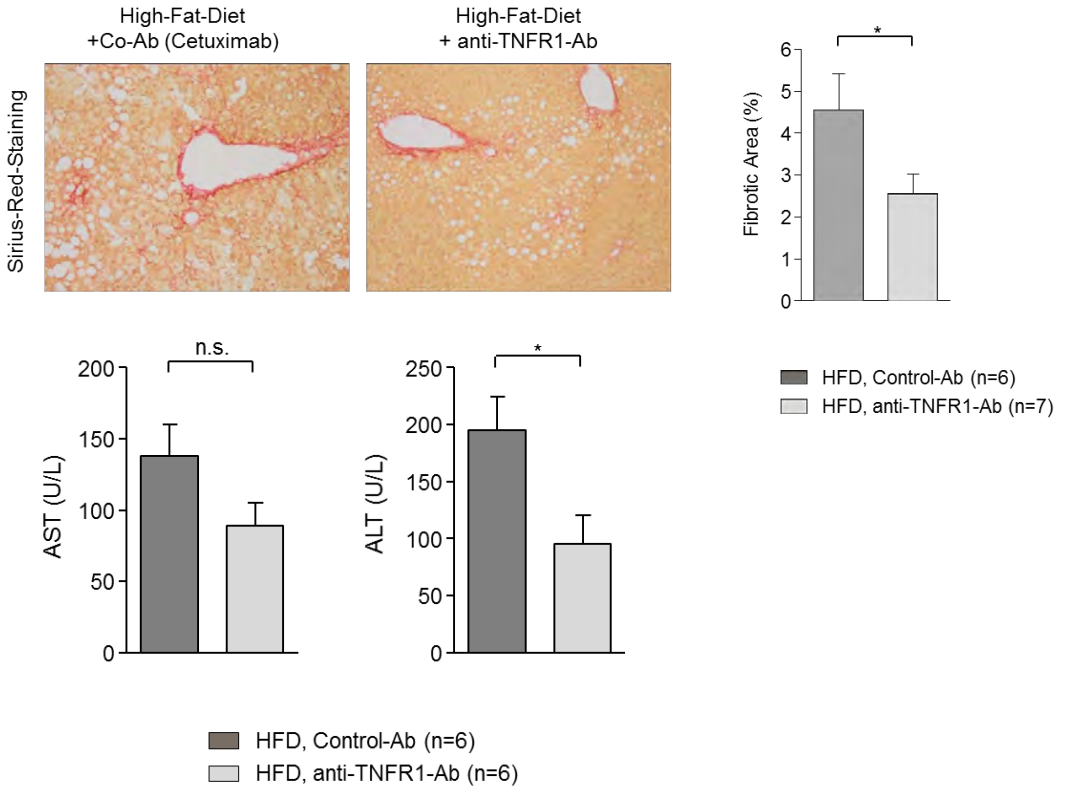


ATROSAB IN NASH

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



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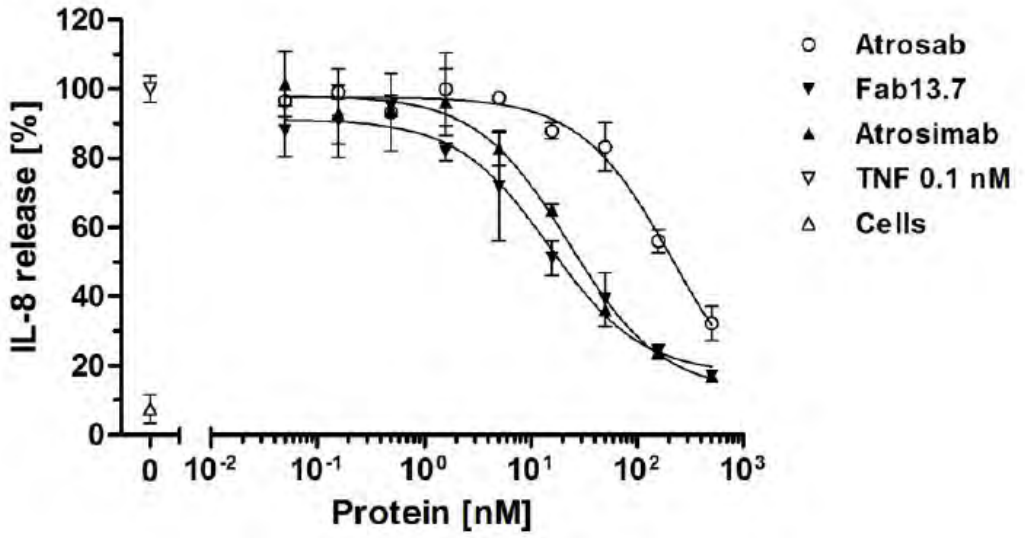

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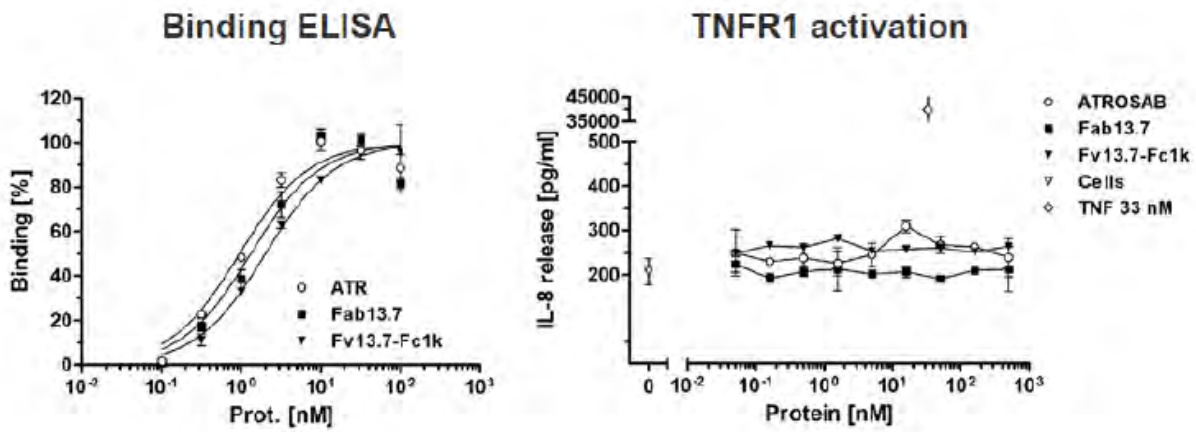
ATROSIMAB CHARACTERIZATION

Monovalent antibody reveals superior properties for use in autoimmune indications



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- Lacks any agonistic activity
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