Summary of 12wk CDAHFD w/ sIL13Ra2, anti-p40, and anti-IL4 or anti-TGFbeta

Pfizer WORLDWIDE RESEARCH & DEVELOPMENT

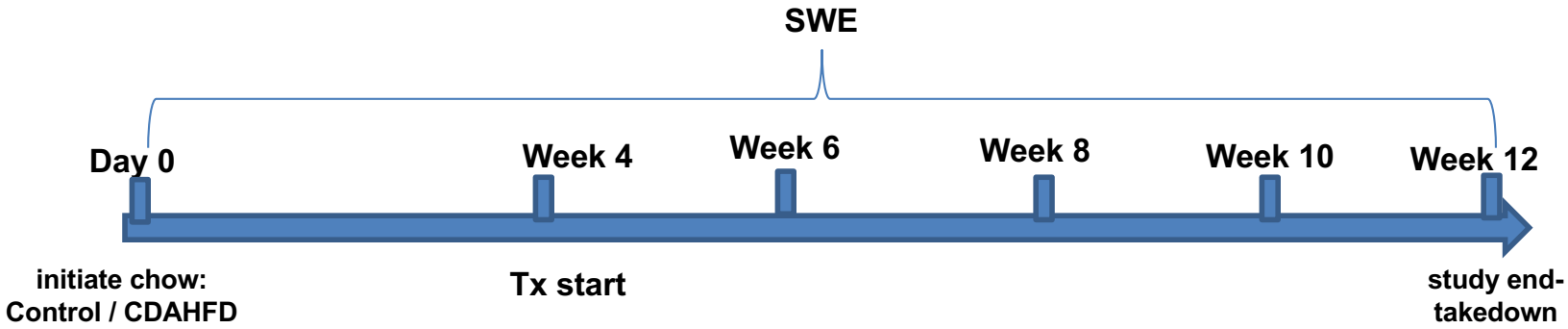
SCIENCE FOR LIFE-CHANGING IMPACT

Experimental Design

Objective: Evaluate effects by various theraputic combinations on fibrosis endpoints in CDAHFD NASH model in mice

Duration – 12wk, treatments start after 4 weeks

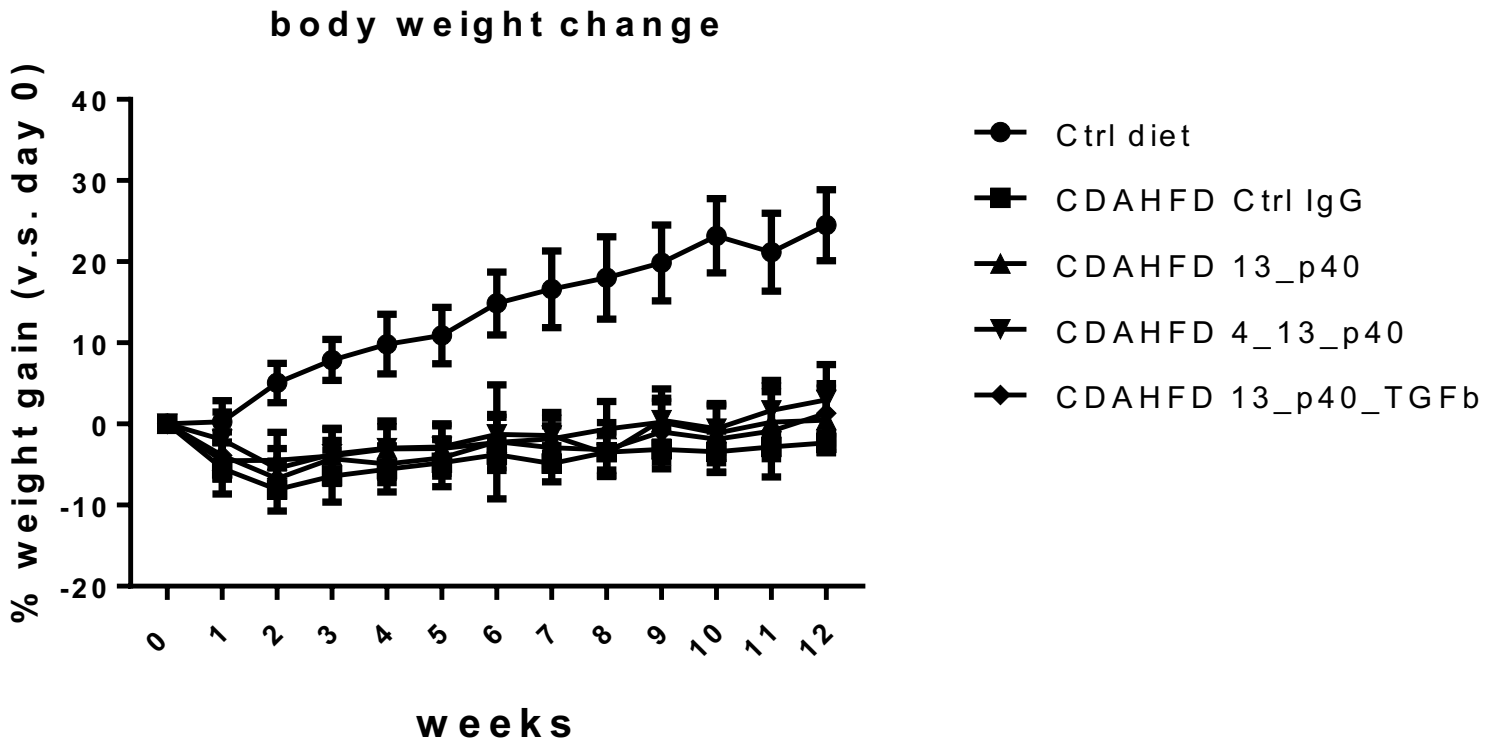
Data collection: weekly body weight, study end liver weight, study end serum liver enzyme, Real-time shear wave (SWE) elastography before and at week 4, 6, 8, 10 and 12 after on CDAHFD diet.



Liver gene expression: a-SMA, Col1a1, Col3a1, Col4a1, Col6a1, MCP-1, IL4, IL5, Siglec5, IL12, IL23

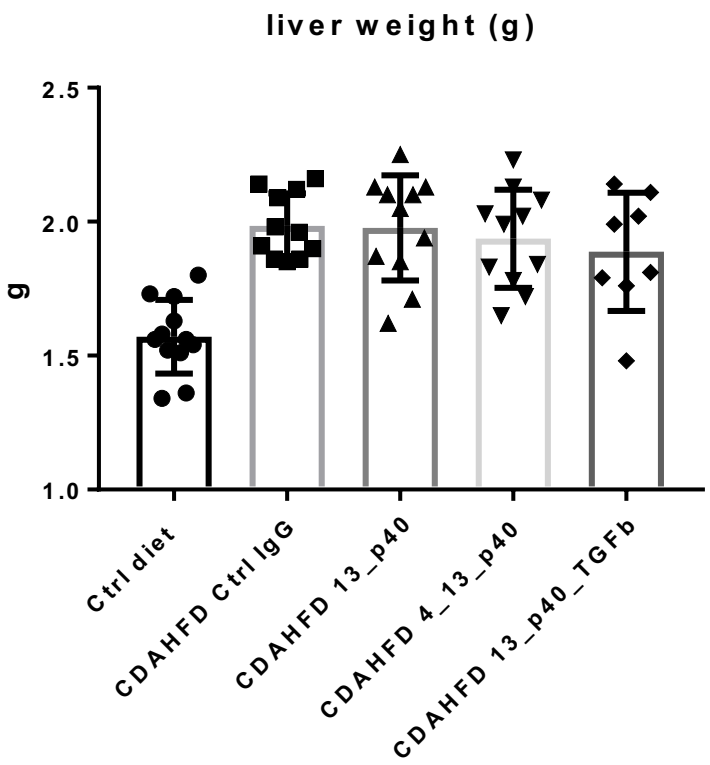
Histopathology: Liver - HE, aSMA IHC, Iba1 IHC, Picrosirius red (PSR)

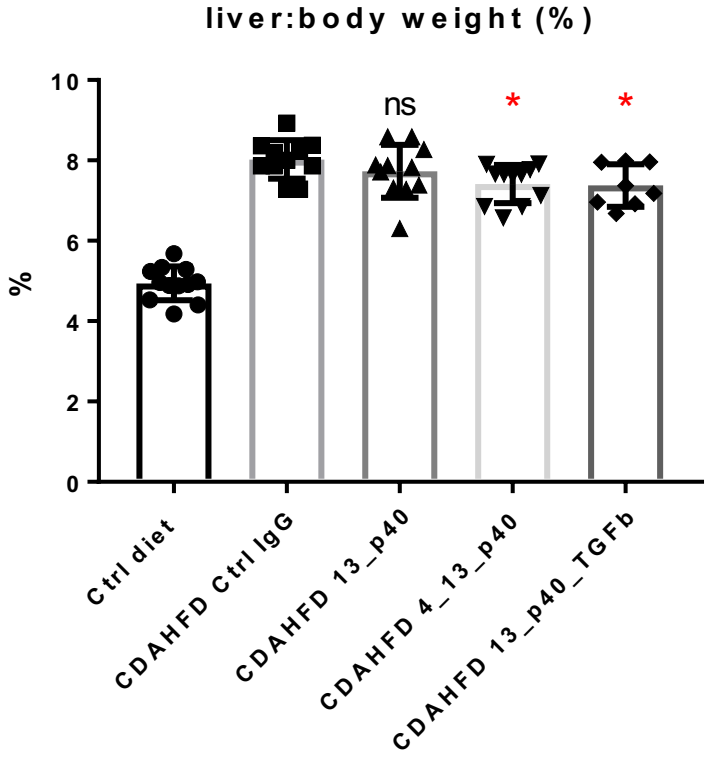
Group ID	Animal ID	Diet	Compound	route	Duration
1	1_12	chow	none		12 wk
2	14_24	CDAHFD	mIgGs		12 wk
3	25_36	CDAHFD	sIL13Ra2, anti-p40		12 wk
4	37_44, 49_52	CDAHFD	anti-IL4, sIL13Ra2, anti-p40		12 wk
5	46_48, 53_60	CDAHFD	sIL13Ra2, anti-p40, anti-TGF-b	subQ, 2x/wk	12 wk
6	61_67	CDAHFD	none		4 wk


body weight change
% weight gain (v.s. day 0)
40
30
20
10
0
-10
-20
0
1
2
3
4
5
6
7
8
9
10
11
12
weeks
Ctrl diet
CDAHFD Ctrl IgG
CDAHFD 13_p40
CDAHFD 4_13_p40
CDAHFD 13_p40_TGFb

Note: exclusion of animals

- In CDAHFD studies, some mice, in both control diet group and CDAHFD diet group, will develop cirrhotic livers. The mechanism for this cirrhosis development is unknown, and we suspect it is related to the purified diets. All mice (4 out of 60 in this study - # 27,42,48,58) that developed cirrhosis were excluded from data analysis.



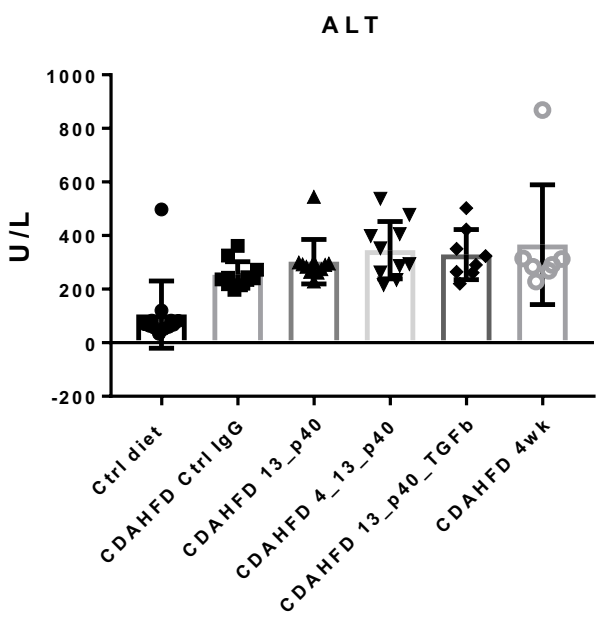


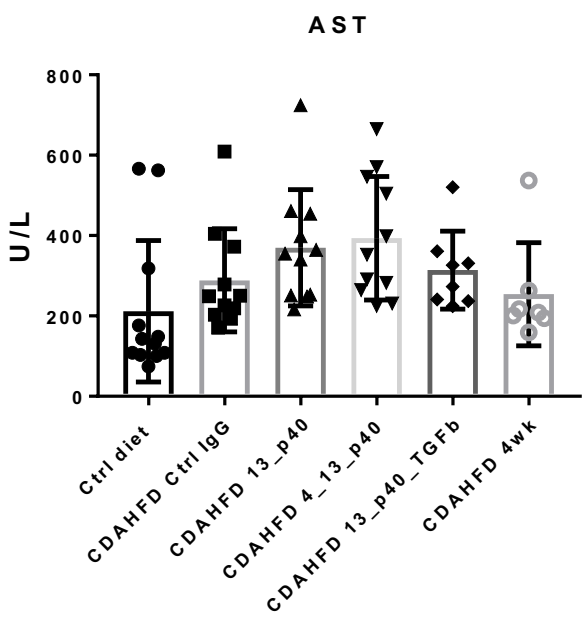
Reduced liver index (liver:body weight ratio) by both triple combinations

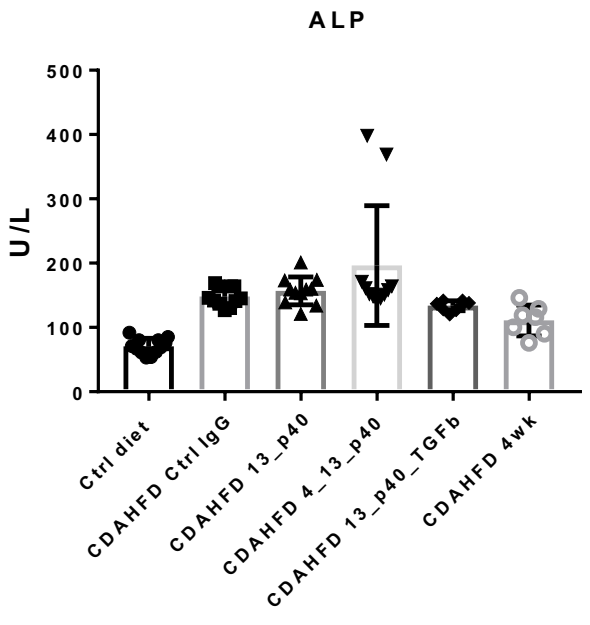
Statistics: treatments v.s. Ctrl IgG
GraphPad one way ANOVA multiple comparisons

Cirrhotic liver excluded

Liver enzymes

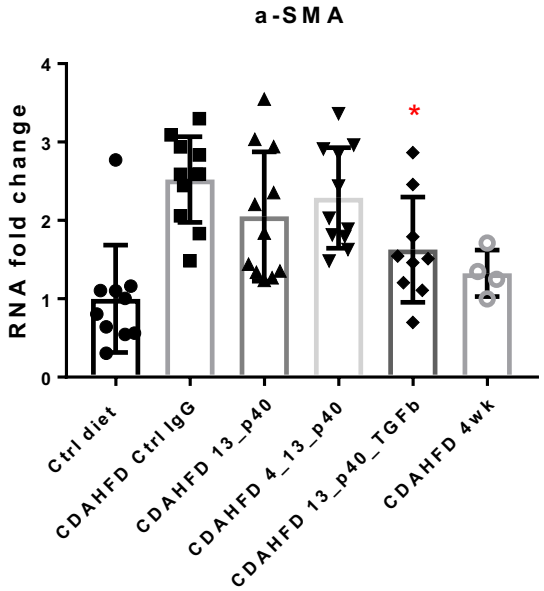


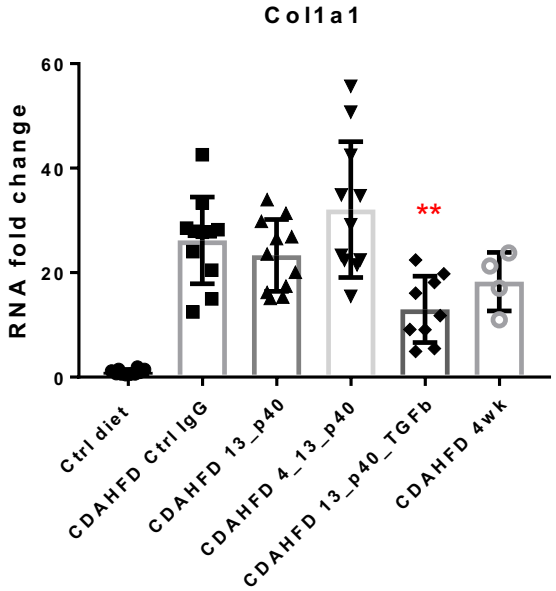




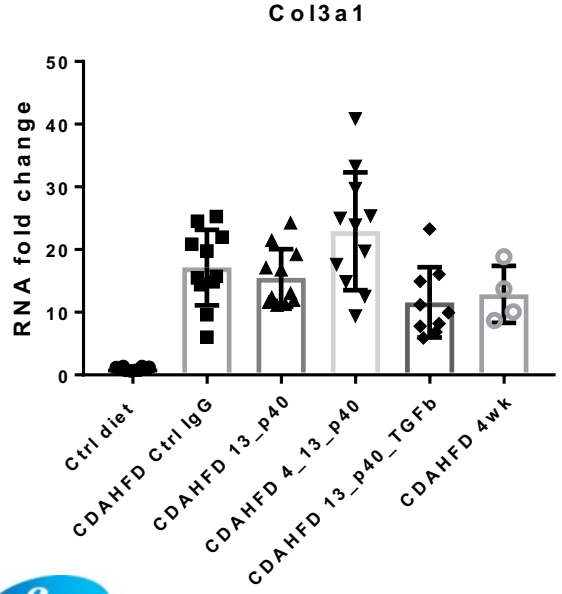
Cirrhotic liver excluded

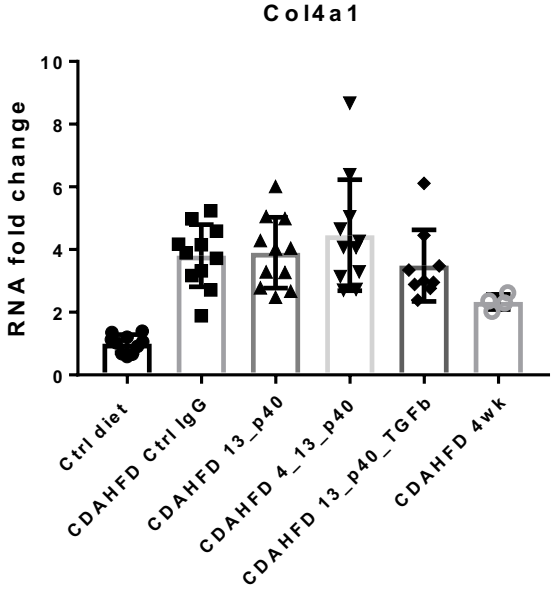
Liver gene expression

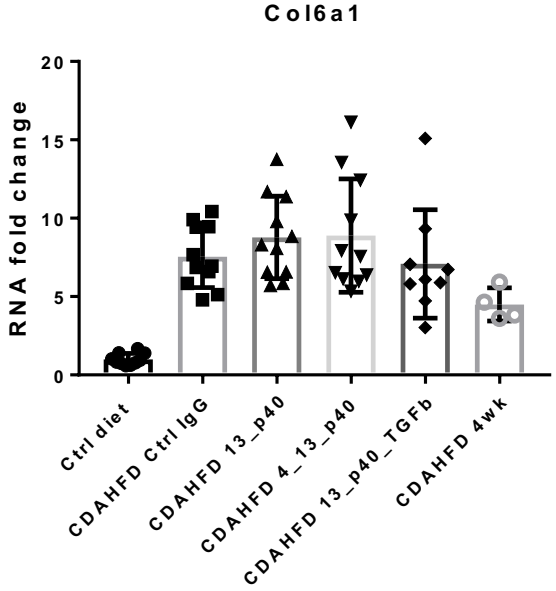




Reduced a-SMA and Col1a1 by TGFb triple combination







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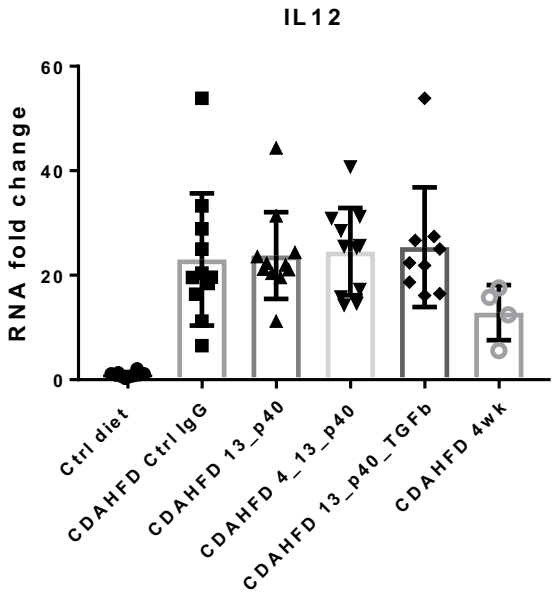
Drug Safety R&D

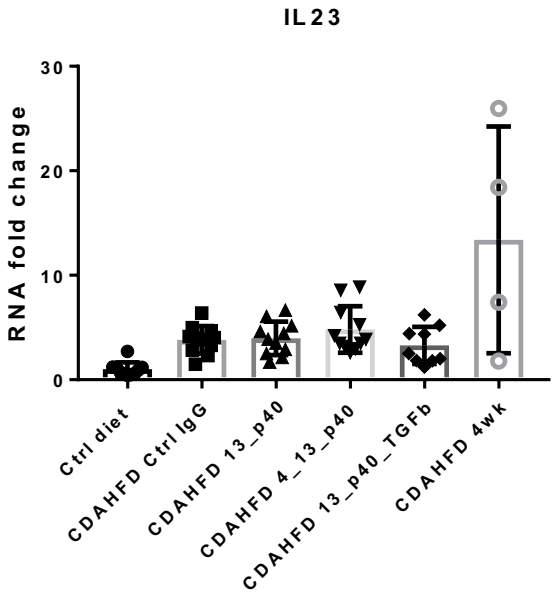
Statistics: treatments v.s. Ctrl IgG
GraphPad one way ANOVA multiple comparisons

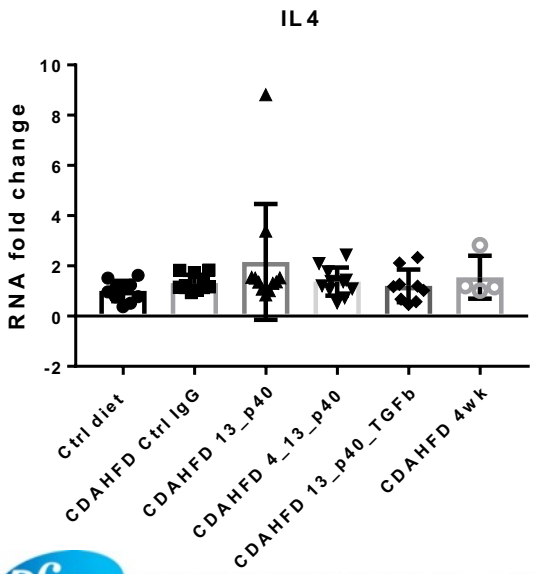
SCIENCE FOR LIFE-CHANGING IMPACT

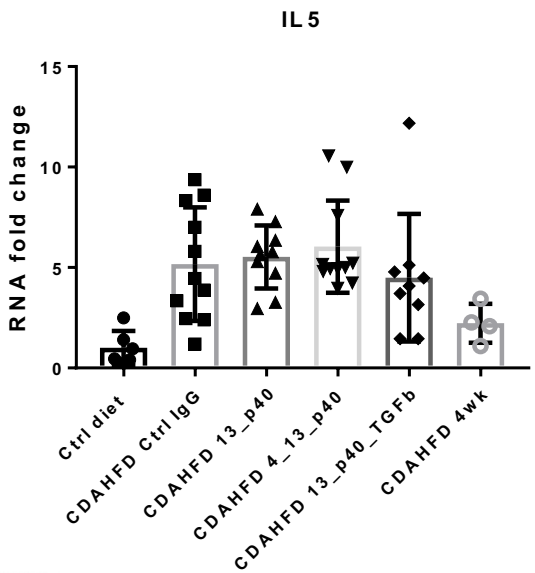
Cirrhotic liver excluded

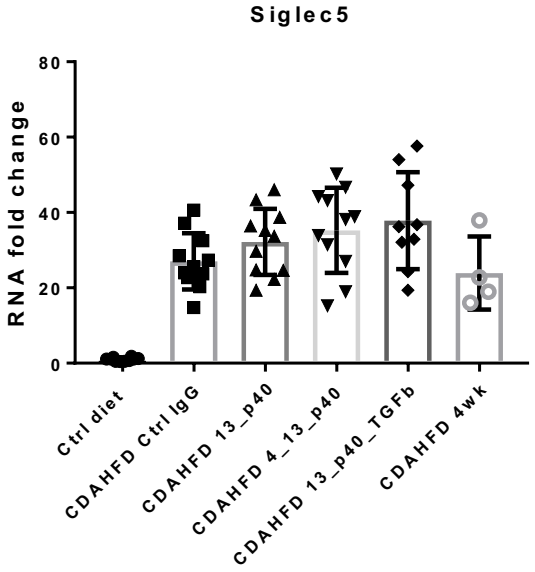
Liver gene expression





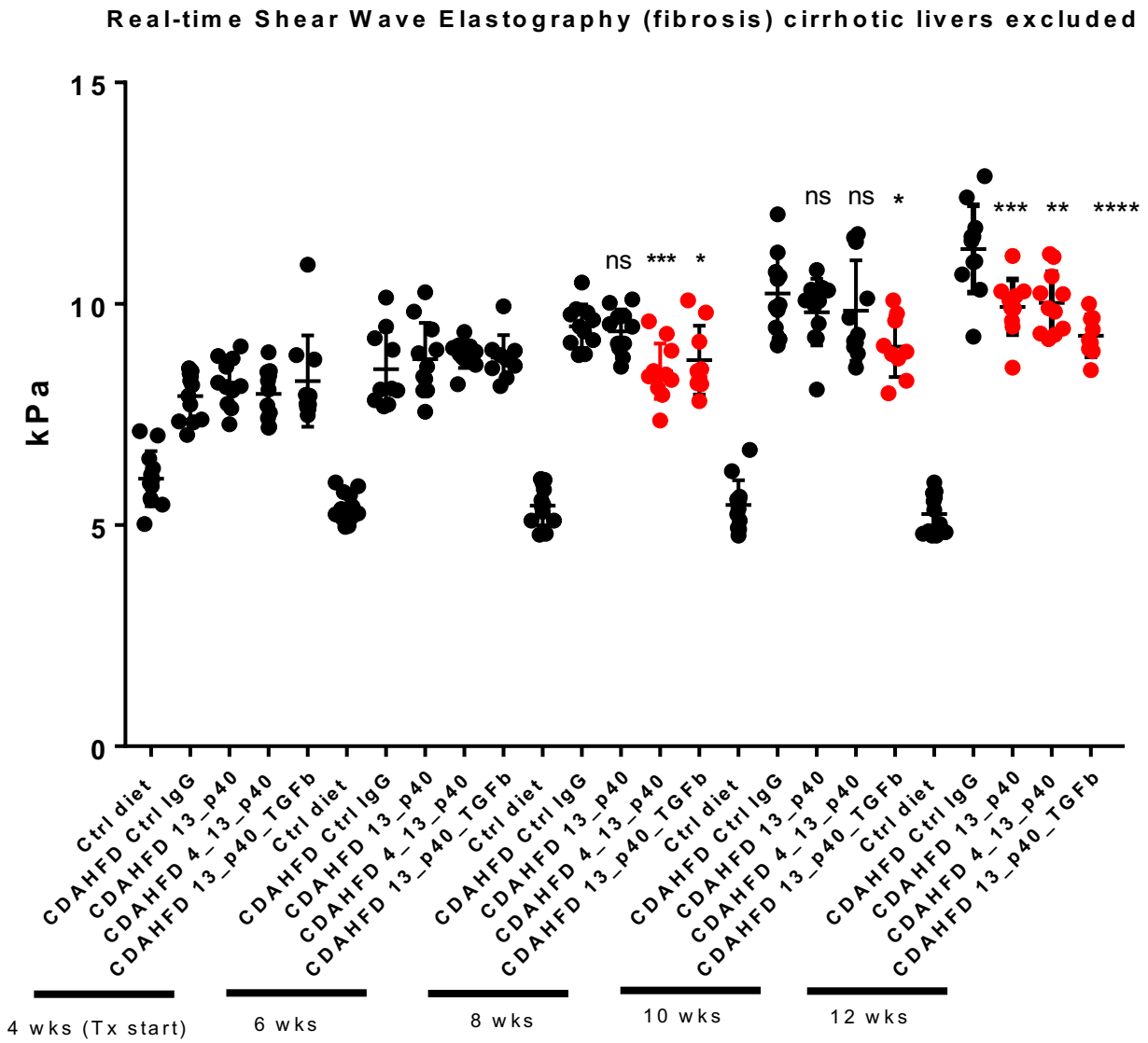






Cirrhotic liver excluded

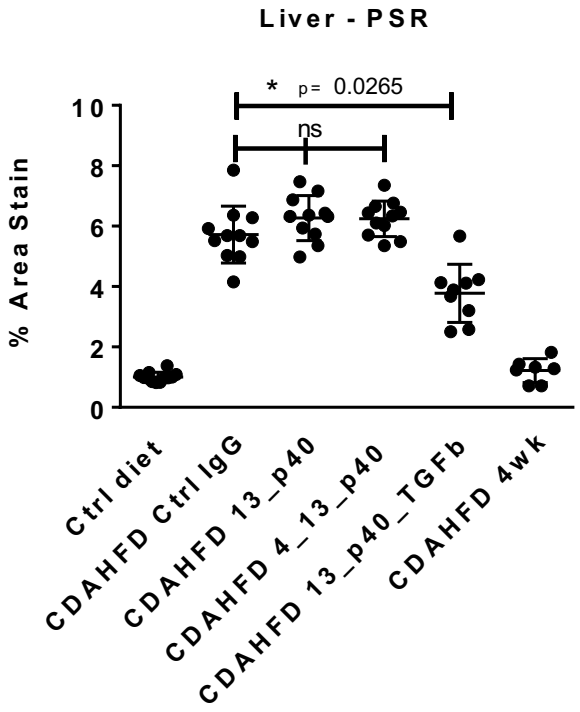
Real-time shear wave elastography measurement of liver fibrosis (mice with cirrhotic liver excluded)



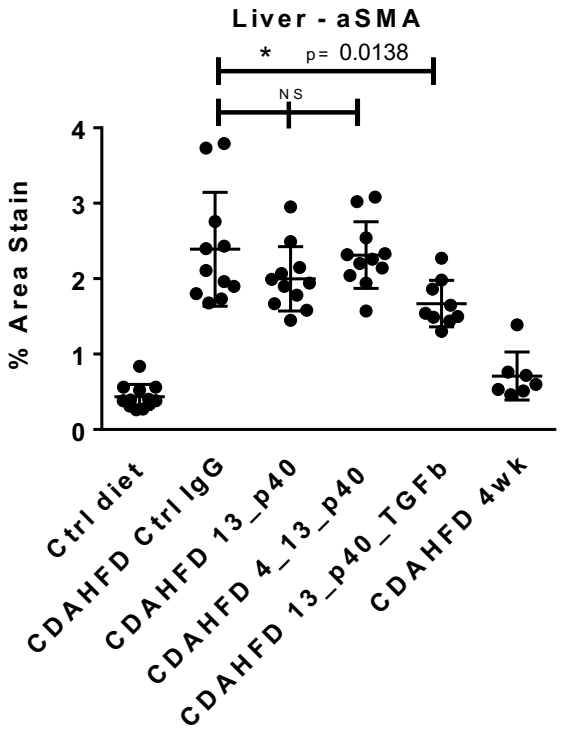
Histology results and interpretations – Kate Hammerman

- The triple (TGFb) combination significantly reduced PSR, aSMA staining and increased Iba1 staining.

- There were small foci of hepatic necrosis and multifocal hepatocellular vacuolation in liver from individual control chow-fed animals; the necrosis may impact liver enzyme data in this group.



Statistics: GraphPad Prism, Kruskal-Wallis ANOVA with multiple comparisons (TA vs CDAHFD/IgG); NS= p>0.05



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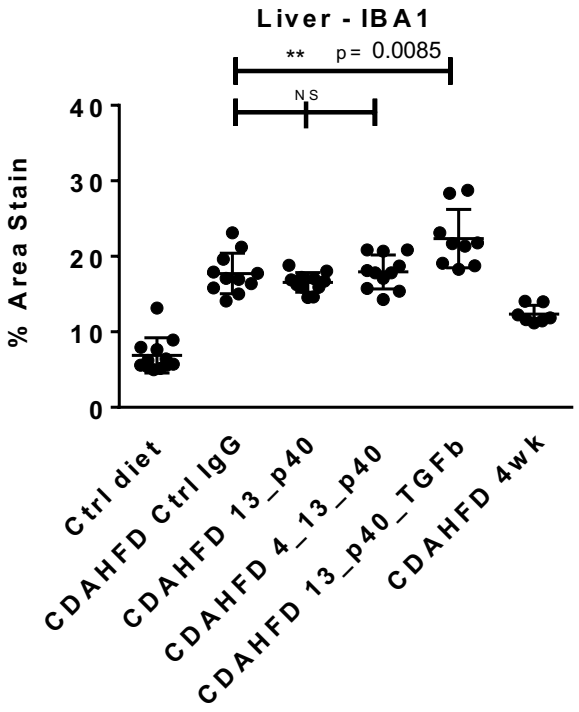

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Cirrhotic liver excluded

Summary

- Both triple combination treatments reduced liver index at 12-wk time point.
- No effect on liver enzymes from any treatments.
- All treatments (dual, both IL4 and TGFb triple combination) showed reduction of SWE signal at 12-week time point, whereas histologically, only TGFb triple combination showed significantly attenuated PSR and a-SMA.

Samples selected for RNAseq

- 6 samples from each group 1 to 5.
- Sample ID TBD.

Group ID	Animal ID	Diet	Compound	route	Duration
1	1_12	chow	none		12 wk
2	14_24	CDAHFD	mIgGs		12 wk
3	25_36	CDAHFD	sIL13Ra2, anti-p40		12 wk
4	37_44, 49_52	CDAHFD	anti-IL4, sIL13Ra2, anti-p40		12 wk
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