Denifanstat, a Fatty Acid Synthase Inhibitor, Increased Circulating Polyunsaturated Triglycerides and Decreased LDL-Cholesterol in MASH Patients with Advanced Fibrosis

- a Post-Hoc Analysis of FASCINATE-2 Study

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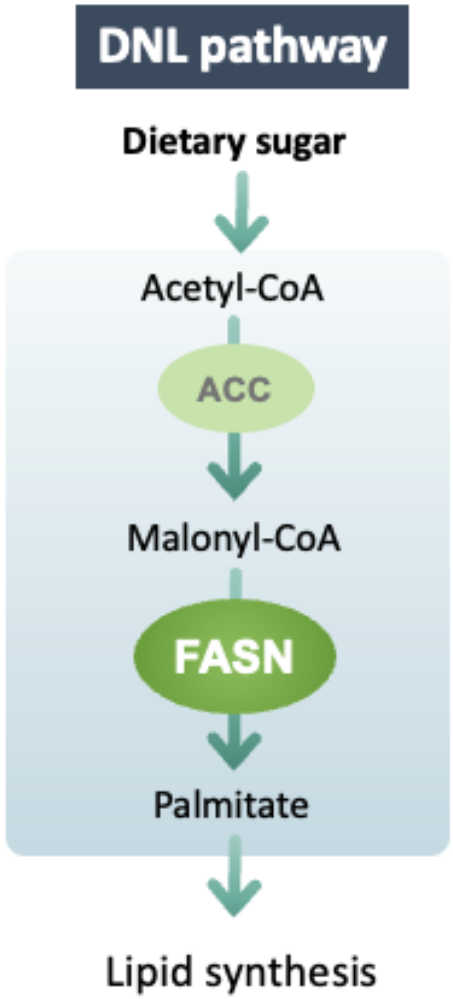
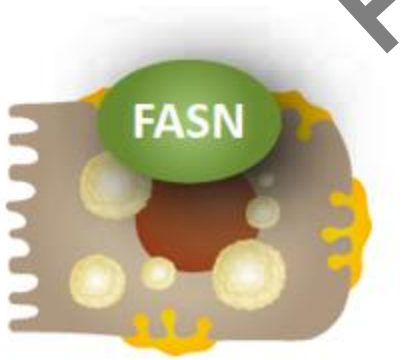
Outline

- Introduction of fatty acid synthase (FASN) and denifanstat MOAs
- Denifanstat phase 2b FASCINATE-2 study results in F2/F3 MASH
  - Histological endpoints
  - Lipidomic analysis of circulating lipids

FASN Plays a Key Role in Three Major Cell Types in MASH



Denifanstat

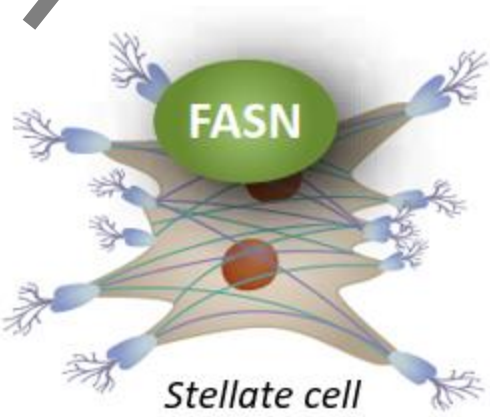


Hepatocyte

<u>Increases</u>
Fatty acids
Triglycerides
Lipotoxins

Immune cell

<u>Increases</u>
Cytokines
Chemokines
Cell activation



Stellate cell

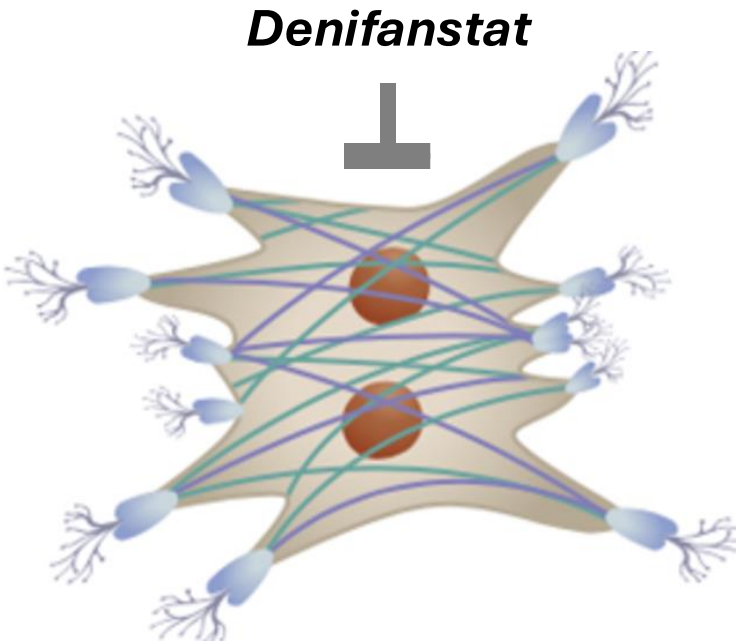
<u>Increases</u>
Fibrogenesis
Cell activation

DNL: *de novo* lipogenesis

FASN Inhibition Directly Blocks Human Liver Stellate Cell Function

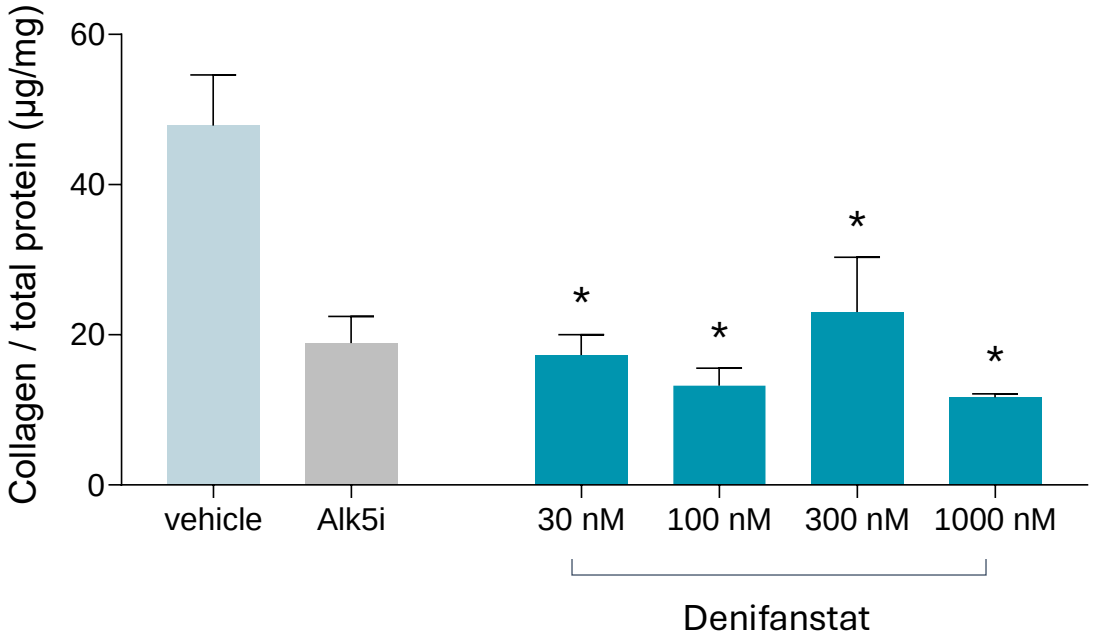
Stellate cells require DNL for fibrogenesis

Denifanstat blocks stellate cell activation



Primary human stellate cell assay

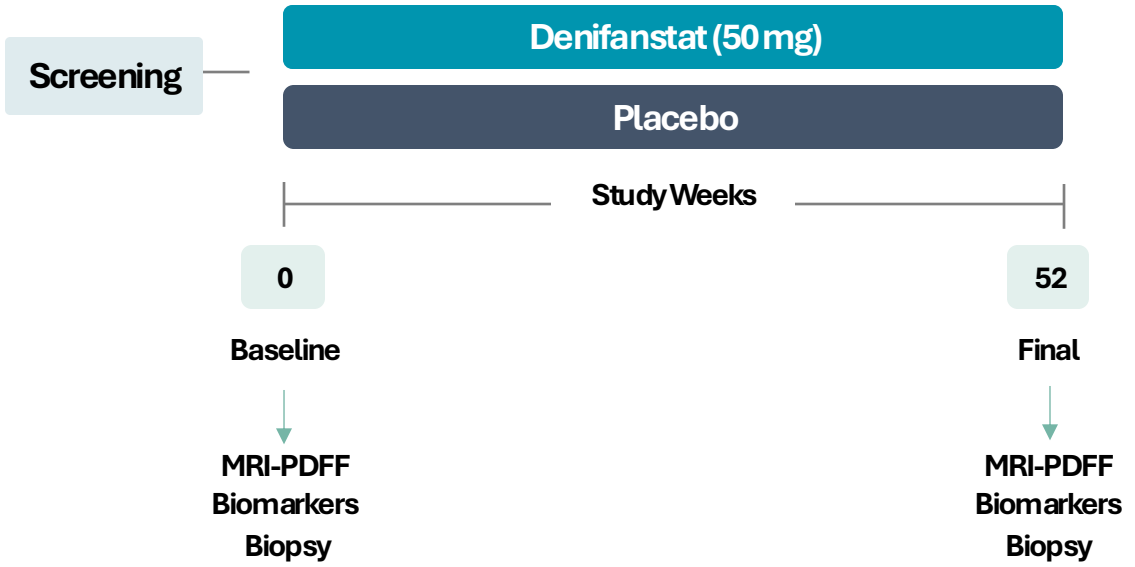
Denifanstat directly inhibits fibrogenic activity



- Stimulated by TGF-beta to activate fibrogenesis
- Denifanstat showed similar inhibition to positive control ALK5 inhibitor

*p<0.05. FASNi directly inhibits fibrosis. O'Farrell et al.,2022. Scientific Reports. 12:15661

FASCINATE-2: Biopsy Trial Design Focused on Histological Endpoints



- Biopsy confirmed F2-F3 MASH patients
- 52 weeks, 2:1 randomization to 50mg or placebo, double-blind
- Single pathology reader: Dr. Pierre Bedossa
- AI digital pathology: HistoIndex

Primary endpoints

- NAS ≥2 points improvement w/o worsening of fibrosis
- MASH resolution + NAS ≥2 improvement w/o worsening of fibrosis

Selected secondary endpoints

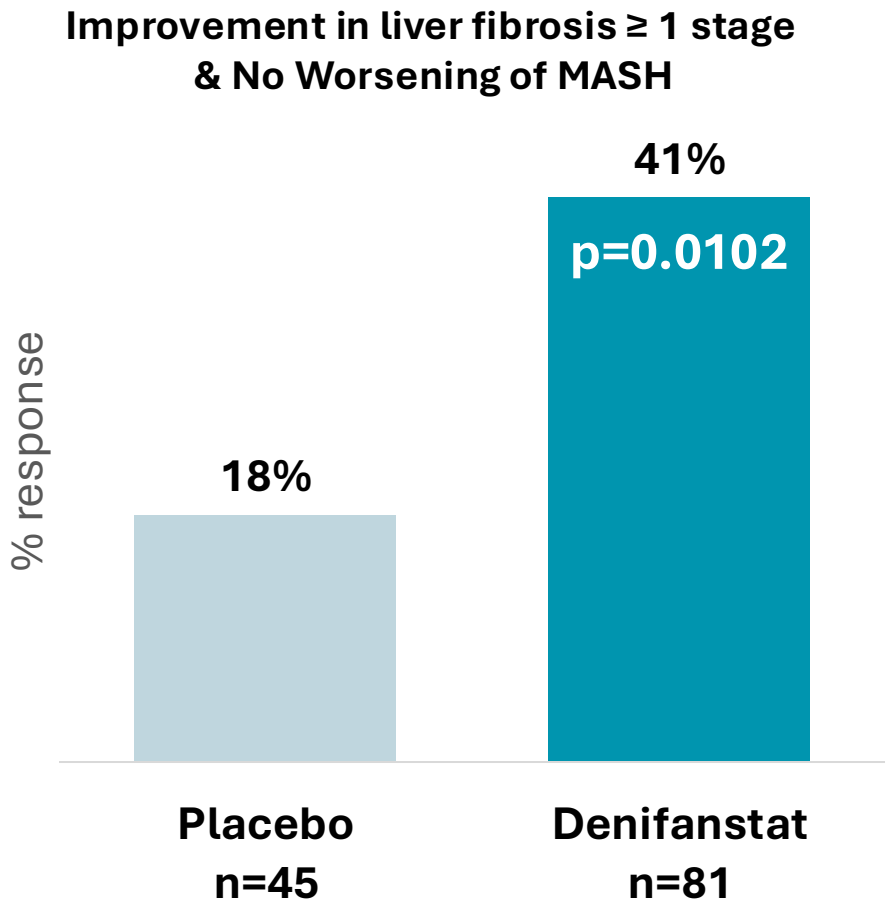
- **Improvement in liver fibrosis ≥1 stage without worsening of MASH as assessed by biopsy**
- **MASH resolution w/o worsening of fibrosis**
- Digital AI pathology
- MRI-PDFF: absolute decrease, % change from baseline, % pts ≥30% reduction from baseline (responders)

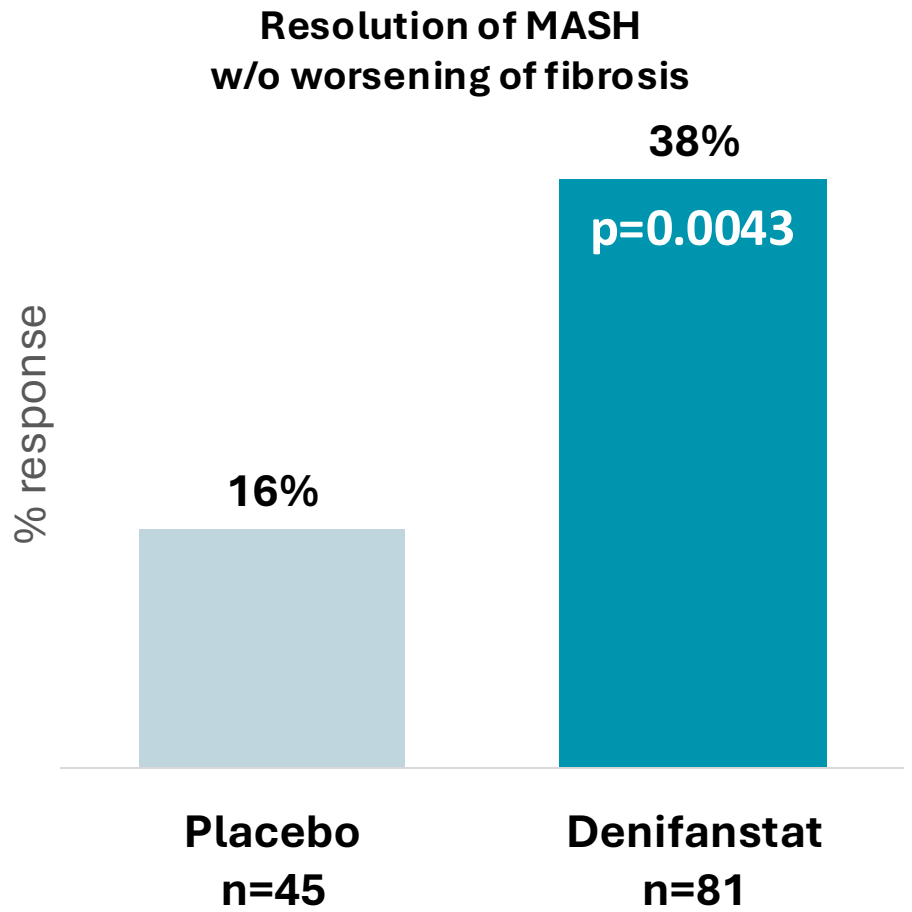
AI: Artificial Intelligence, MRI-PDFF; magnetic resonance imaging derived proton density fat fraction, NAS; NAFLD Activity Score.

Loomba et al., 2024. The Lancet Gastroenterology & Hepatology. doi:10.1016/S2468-1253(24)00246-2

Histology Endpoints of MASH Resolution and Liver Fibrosis at Week 52

Denifanstat Achieved Statistical Significance (Endpoints per FDA Draft Guidance 2020)





Cochran-Mantel-Haenszel Test – Two sided at the 0.05 significance level. mITT population. Statistical significance also reached for ITT population.

Additional Fibrosis Endpoint Analysis at Week 52

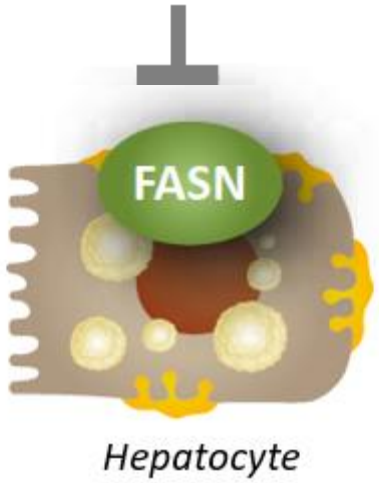
Denifanstat Achieved Strong Improvement in F3 Population

Fibrosis Endpoints	Subgroup	Placebo	Denifanstat	p-value
≥1 stage improvement in fibrosis w/o worsening of MASH	All pts	18%	41%	0.0102**
	F3 only	**13%**	**49%**	**0.0032****
≥2 stage improvement in fibrosis w/o worsening of MASH	**All pts**	**2%**	**20%**	**0.0065****
	F3 only	**4%**	**34%**	**0.0065****
Progression to cirrhosis (F4)	**All pts**	**11%**	**5%**	**0.0386***

mITT population; *One sided at the 0.05 significance level, **Two sided at the 0.05 significance level .

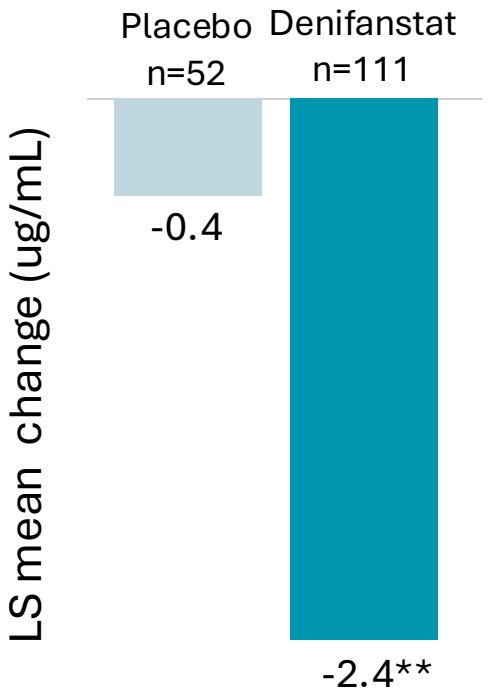
Denifanstat Rapidly Reduced De Novo Lipogenesis and Decreased Liver Fat

Denifanstat



Fatty Acids
Triglycerides
Lipotoxins

Tripalmitin
Change from Baseline



Week 4

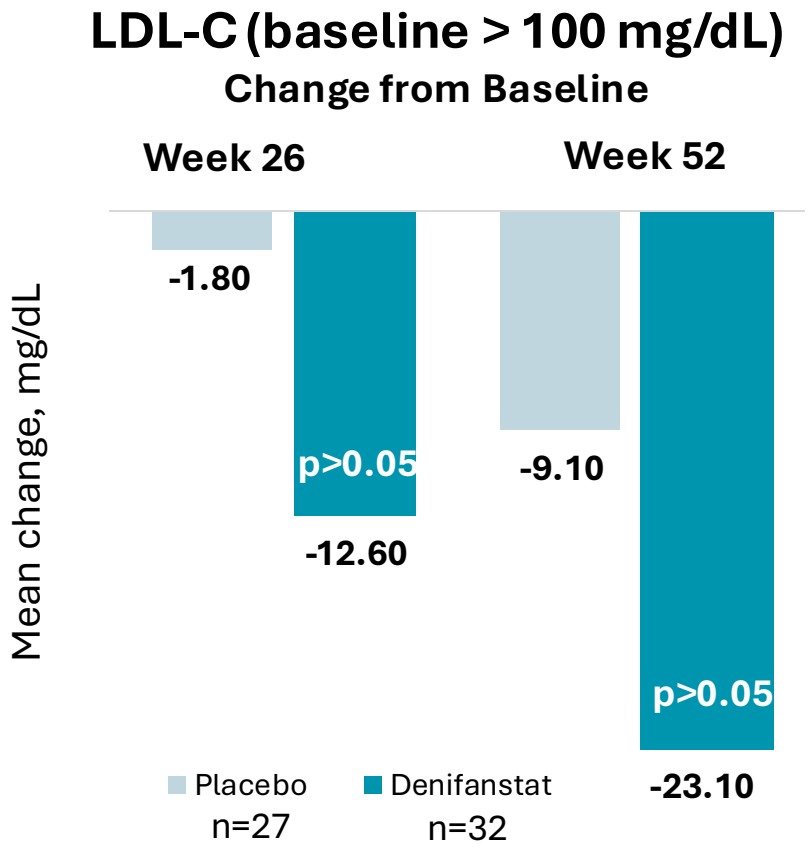
Liver Fat
Change from Baseline

Liver fat by MRI-PDFF	Placebo n=38	Denifanstat n=69	
Relative decrease Week 26	+5%	-23%	p=0.0036
Relative decrease Week 52	-8%	-31%	p=0.0008
≥ 30% relative decrease Week 52	21%	65%	p<0.0001

Two sided at the 0.05 significance level, ITT population; ** P < 0.01

Loomba et al., 2024. The Lancet Gastroenterology & Hepatology. doi:10.1016/S2468-1253(24)00246-2

Denifanstat Decreased LDL-Cholesterol in MASH Patients



Denifanstat Significantly Decreased LDL-Cholesterol and LDL Particle Number

Lipoprotein fractionation by NMR spectroscopy

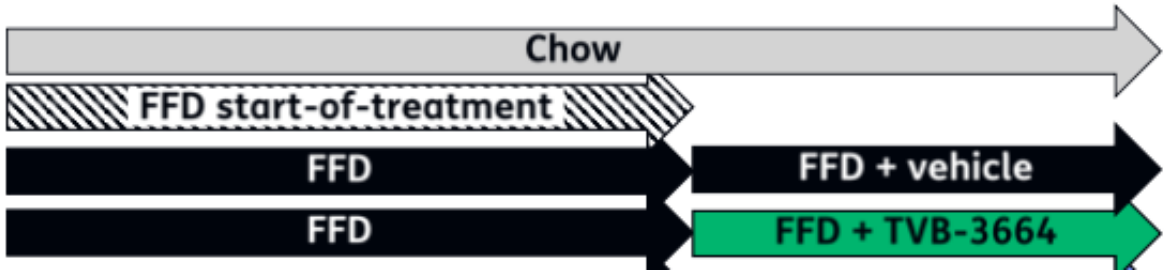
			Baseline		Week 26		Week 52	
Lipoprotein	**Variable**	**Size (nm)**	**Placebo (n=44)**	**Denifanstat (n=73)**	**Placebo % change**	**Denifanstat % change**	**Placebo % change**	**Denifanstat % change**
Cholesterol (mg/dL)	**IDL-C**	25-35	11.92	11.44	-4.11	-8.2 (NS)	-3.58	-2.89 (NS)
	LDL-C	18-25	112.99	108.77	2.5	**-6.54 (**)**	-0.24	**-4.86 (*)**
	HDL-C	5-12	48.52	48.32	-1.54	1.73 (NS)	-0.47	**2.98 (**)**

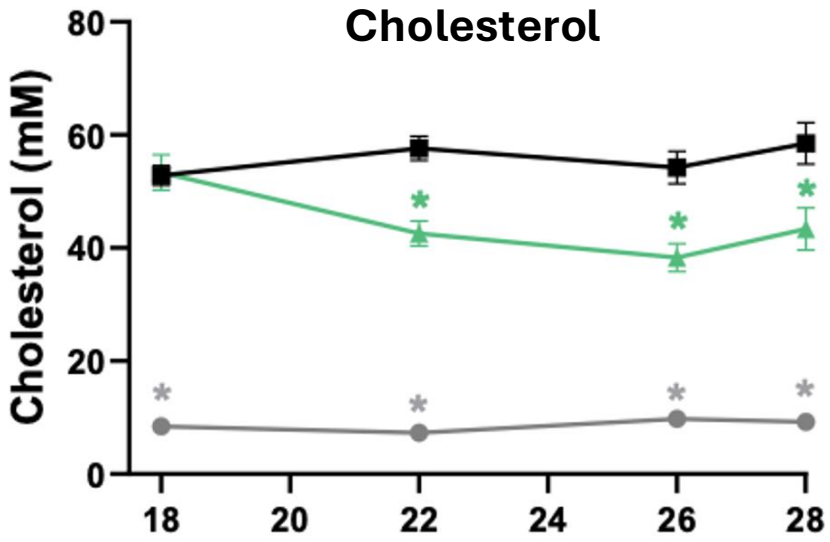
			Baseline		Week 26		Week 52	
Lipoprotein	**Particles**	**Diameter Size (nm)**	**Placebo (n=44)**	**Denifanstat (n=73)**	**Placebo % change**	**Denifanstat % change**	**Placebo % change**	**Denifanstat % change**
LDL-P (nmol/L)	**Large LDL-P**	26.5-28.5	176.96	168.21	3.04	**-8.55 (***)**	-1.71	**-4.62 (*)**
	Medium LDL-P	25.6-26.5	319.92	278.9	-0.85	**-12.25 (***)**	6.34	**-6.51 (**)**
	Small LDL-P	22-25.5	642.79	657.05	-2.43	0.28 (NS)	-2.36	-1.8 (NS)

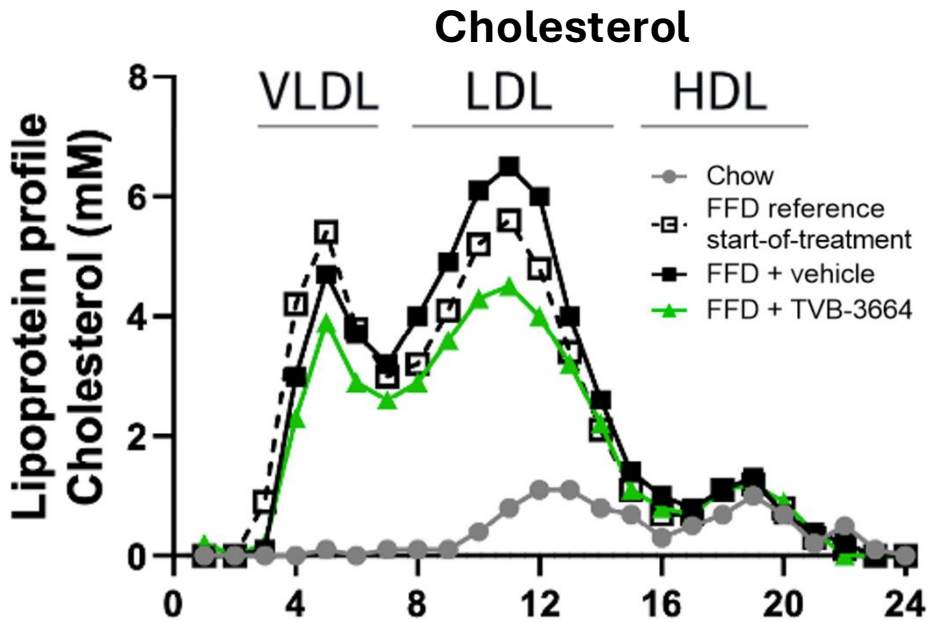
mITT population, all units are in median. Wilcoxon signed-rank test p-values: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; NS $p > 0.05$

FASN Inhibition Reduced LDL-Cholesterol in Ldlr-/- MASH Mice

Ldlr-/- mice fed with fast food diet (FFD) for 18 weeks, then treated with FASN inhibitor (TVB-3664) for 10 weeks





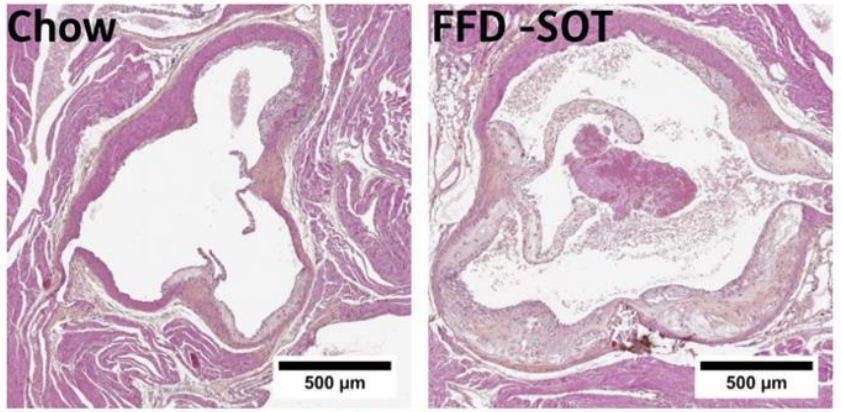


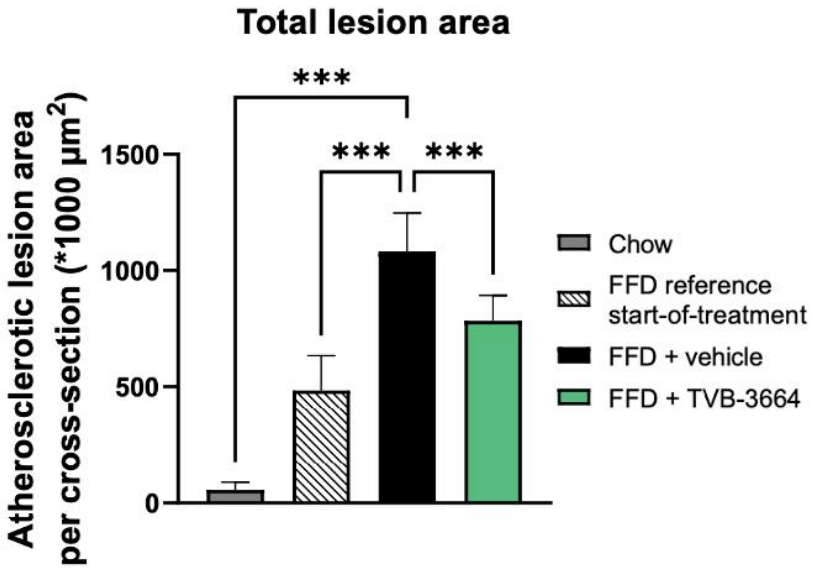
TNO innovation for life

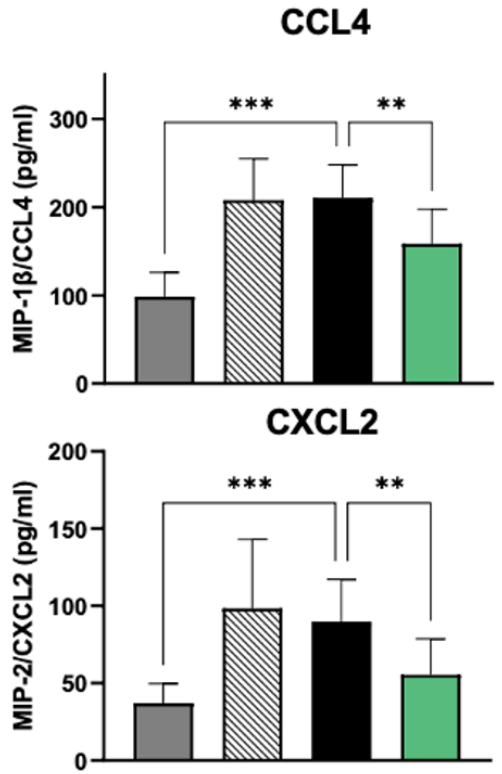
Data mean ± SD, * p<0.05; Ldlr-/-.Leiden mice fed with high fat (41%), high fructose (44%) diet (FFD)

FASN Inhibition Reduced Total Atherosclerotic Lesion Area of Aortic Root in Ldlr-/- MASH Mice

FASN inhibitor (TVB-3664) inhibited inflammation by reducing chemokines







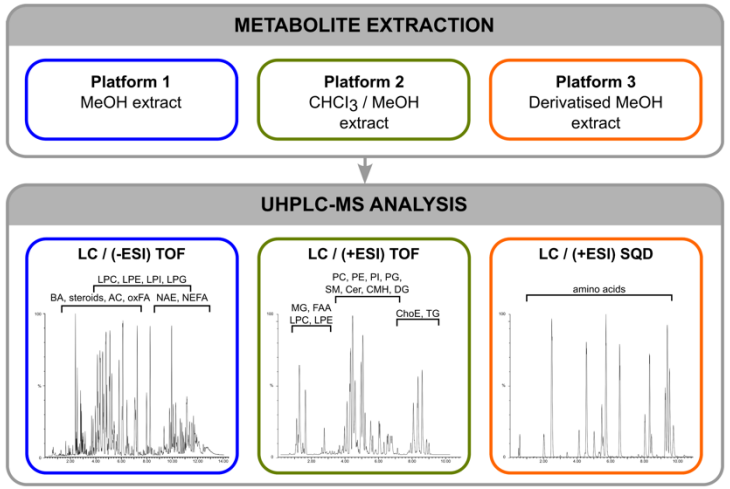
The Ldlr-/- mice developed severe type V plaque lesions and increased atherosclerotic lesion area with 28-week FFD feeding

Graphs represent data mean + SD, ** p<0.01, *** p<0.001

Denifanstat Changed Circulating Lipidomic Profiles Over Time in MASH Patients

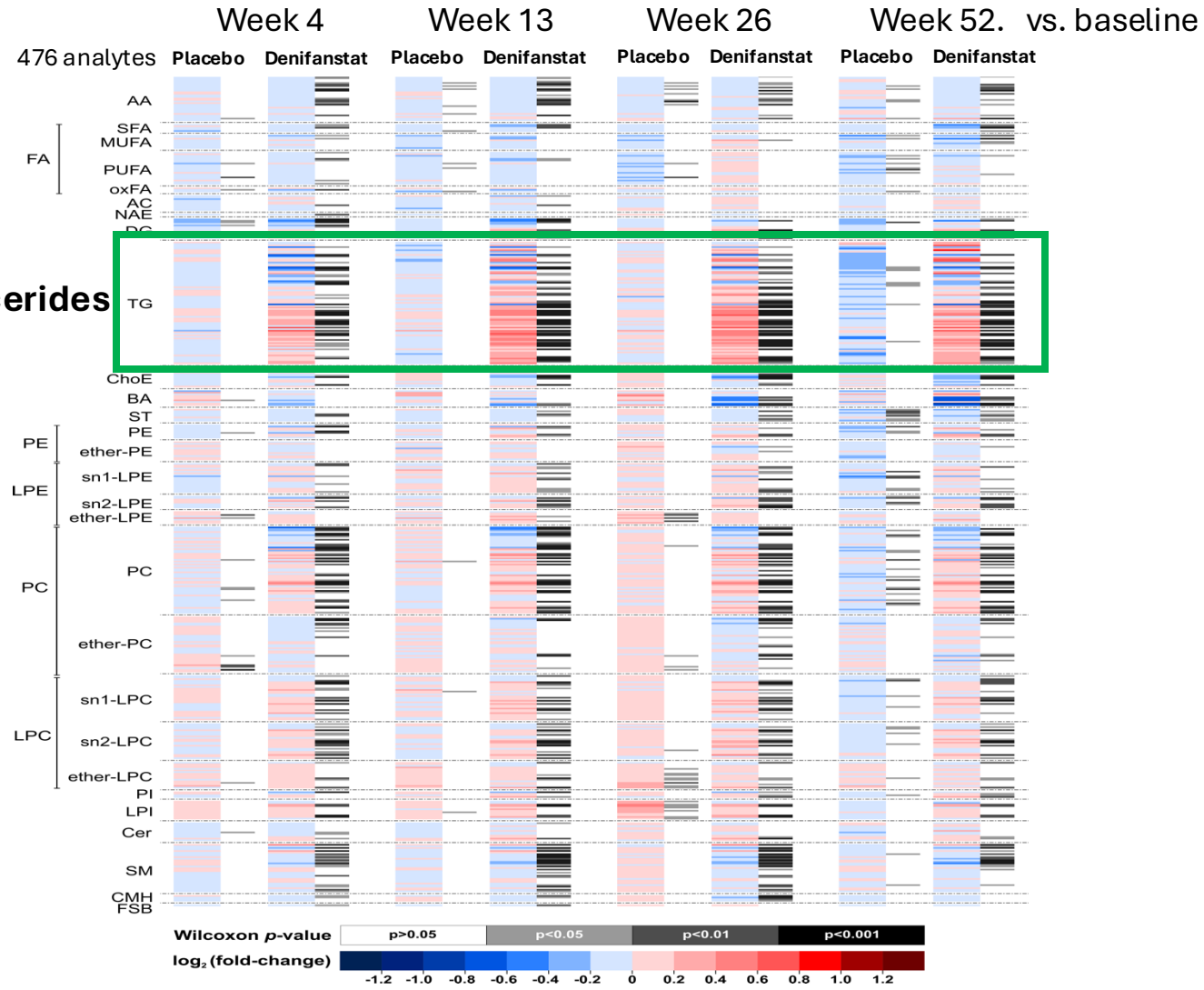
Plasma lipidomics

Mass Spectrometry analytic platform



owl

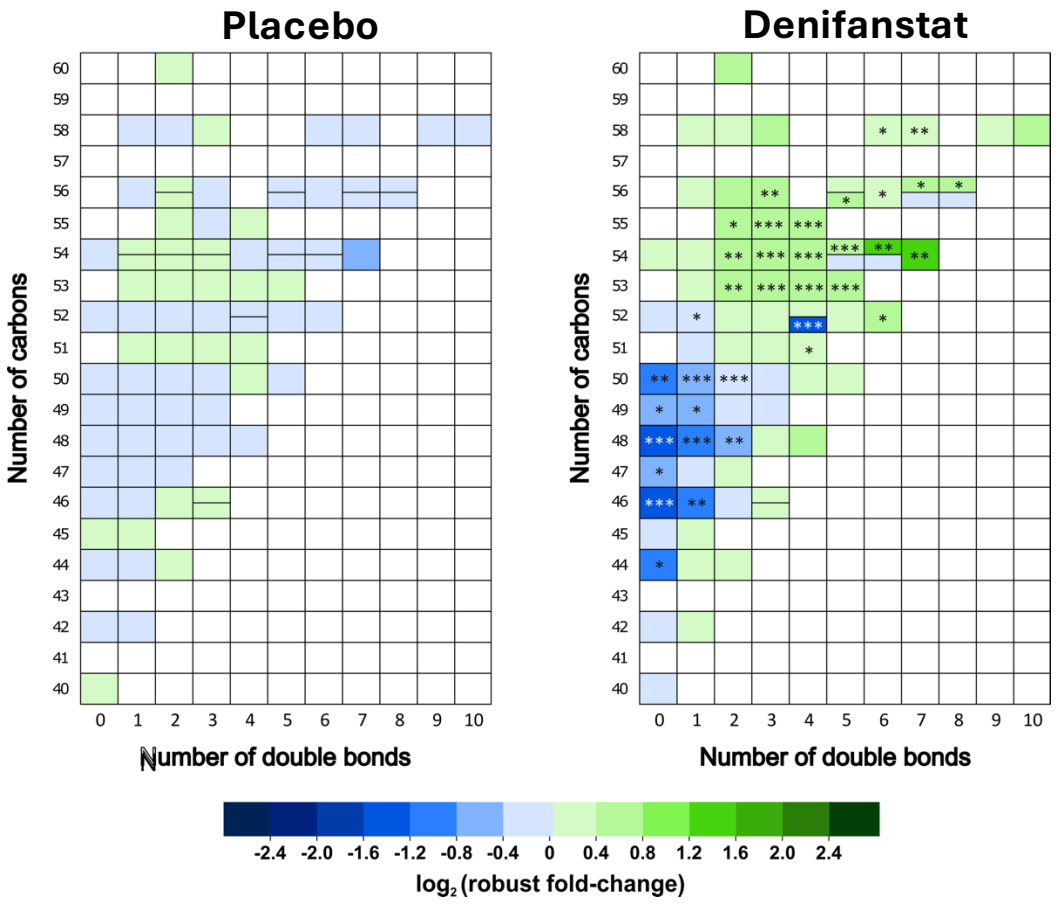
Triglycerides



Denifanstat Rapidly Increased Polyunsaturated TG and Decreased Saturated TG

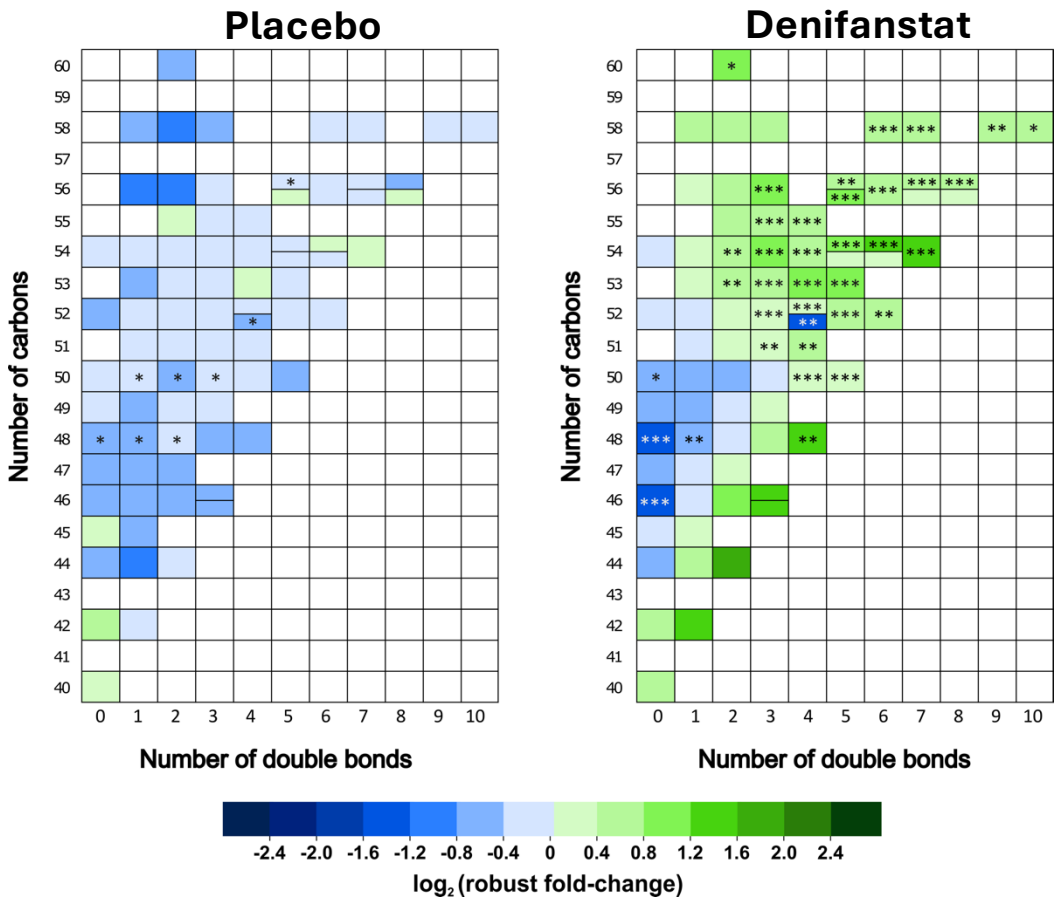
Triglycerides composition

Week 4 vs. baseline



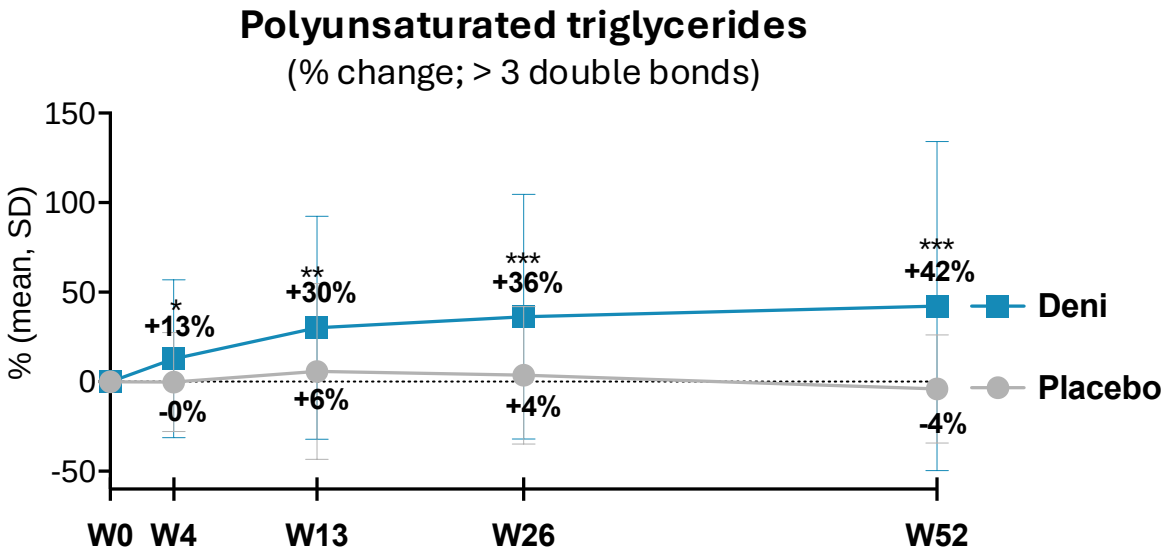
Triglycerides composition

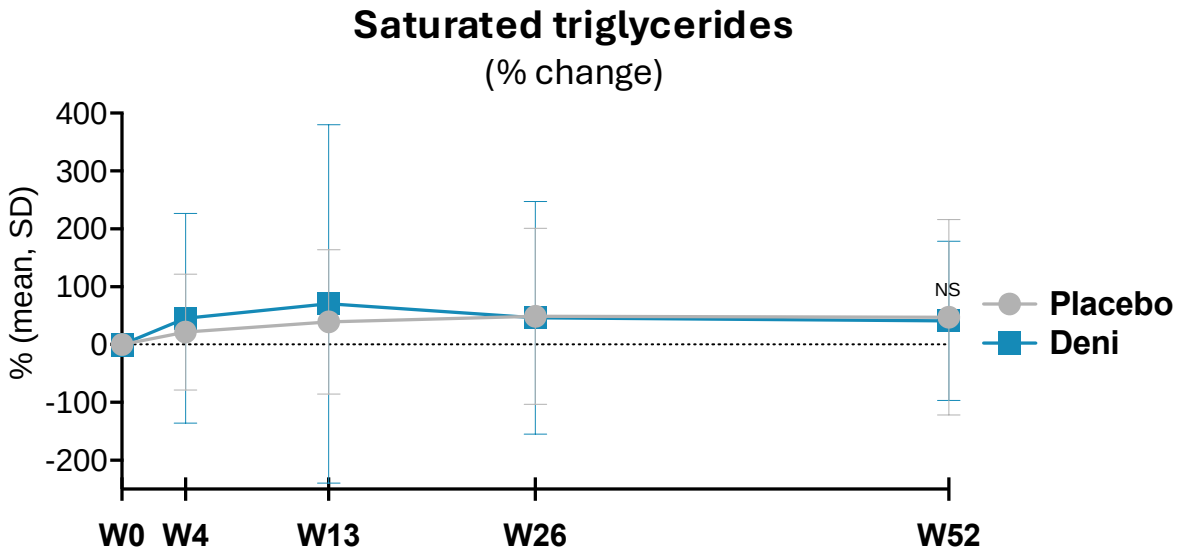
Week 52 vs. baseline



*p<0.05, **p<0.01, ***p<0.001

Denifanstat Increased Circulating Polyunsaturated Triglycerides Over Time





*p<0.05, **p<0.01, ***p<0.001, NS: p > 0.05

Denifanstat Provides a Differentiated Mechanism of Action in MASH

- Denifanstat is not only a liver fat blocker, but acts directly on stellate cells -> tackles both “initiating” (liver fat synthesis) and “progressing” (fibrosis) events
- Denifanstat showed significant improvement in MASH resolution and fibrosis in Phase 2b FASCINATE-2 study
- Denifanstat lowered LDL-cholesterol and increased circulating polyunsaturated TG in MASH patients, providing potential cardiovascular benefits
- FASN inhibition (TVB-3664) prevented progression of atherosclerosis in Ldlr-/- MASH mice by reducing LDL-cholesterol and chemokines

Acknowledgements

- Investigators, sites and patients involved in FASCINATE-2 studies
- Sagimet Team
- Sagimet Advisors
- OWL Metabolomics Team
- TNO Metabolic Health Research Team