A structurally engineered fatty acid, icosabutate, displays optimised absorption, distribution and metabolism properties for targeting hepatic inflammation and normalises elevated liver enzymes in dyslipidemic patients.

DA FRASER 1, X WANG 2, T SKJÆRET 1, JP KASTELEIN 3, D SCHUPPAN 2,4
1NorthSea Therapeutics, 1411 DC Naarden, The Netherlands, 2Institute of Translational Immunology, University Medical Center, Mainz, Germany, 3Department of Vascular Medicine, Academic Medical Center, Amsterdam, The Netherlands, 4Division of Gastroenterology, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA, USA.

INTRODUCTION
The use of unmodified fatty acids as efficacious oral drugs targeting inflammatory/fibrotic liver disease is inherently restricted by a marked dose-related increase in (auto-) oxidation as an energy source (1), systemic distribution and incorporation into complex lipids. Icosabutate is a structurally engineered fatty acid (SEFA) designed to overcome these inherent limitations and may offer a novel approach to treat necroinflammation and subsequent fibrosis in NASH.

AIMS
We tested the absorption, distribution, metabolism, excretion (ADME) properties of icosabutate, a structurally engineered fatty acid, in rodents. To further explore hepatoprotective properties we also evaluated the effects of 12 weeks icosabutate treatment (600mg QD) on elevated liver enzymes in patients with hypertriglyceridemia (HTG).

METHODS
ADME properties of icosabutate were assessed in rodents in vivo and human hepatocytes in vitro. Treatment effects [introduced from week 7-12 of a 12-week choline deficient or choline sufficient amino acid defined modestly high-fat (31% of total calories) diet (CDAA and CLAA, resp.) in 9-week old C56BL/6J mice] of low- and high-dose icosabutate (56mg/kg ICOSA-H and 112mg/kg ICOSA-H orally) vs a GLP-1 agonist (Bydureon, 0.4mg/kg weekly) on hepatic inflammatory gene expression were also assessed. The liver enzyme response to 12-weeks treatment with icosabutate 600mg QD or placebo was also measured in HTG patients with elevated 1- to 3 X ULN alanine aminotransferase (ALT) and gamma-glutamyl transferase (GGT) levels in a phase 2 study (CTN 4016 13201) investigating the hypolipidemic effects of icosabutate.

RESULTS
(1) STRUCTURE AND BACKGROUND
Icosabutate (C13:1CH3) (below) was selected from >30 in vivo tested SEFAs based on its potent and broad pharmacodynamic properties. The oxygen atom in the β-position and the ethyl group in the α-position serve to minimise β-oxidation and esterification with a goal of facilitating a high turnover of phospholipase A2 independent signalling substrate in liver cells. In a recent phase 2 study, icosabutate at 600mg QD in severe HTG patients achieved highly significant reductions in plasma triglycerides, fasting plasma insulin and HOMA-IR (2).

(2) ADME
(a) After a single 50mg/kg oral administration to Wistar rats, (14-C) icosabutate is adsorbed almost entirely (>99%) via the portal vein (below).

(b) Rapid hepatic accumulation, minimal systemic distribution (below), values µg equiv/g tissue and rapid excretion (95% complete at 48hrs, urinary 55.5%, faecal 39.5%) is observed after a 100mg/kg oral dose in rats.

(c) In Huh7 cells, minimal incorporation of [14C] icosabutate and its metabolites into complex lipids at 24hrs is reflected in the high percentage of radioactivity (>85%) in the most bioactive non-esterified fraction as compared to unmodified [14C] docosahexaenoic acid of which <10% is non-esterified (below).

(3) EFFECTS ON GLUCOSE METABOLISM IN OBSEZ ZUCKER RATS
Icosabutate (135mg/kg) significantly improves plasma insulin, glucose and HbA1c in obese Zucker rats (*p<0.05 versus baseline). A synthetic PPAR-α agonist, fenofibrate (100mg/kg), had no effect on glucose, insulin or HbA1c (below).

(4) EFFECTS UPON HEPATIC INFLAMMATION IN HIGH FAT FEED MICE
Icosabutate, introduced from week 7-12 of the CDAA diet (HFD), induced potent decreases in hepatic TNF-α (p=0.005 and <0.0001 for ICOSA-4 and ICOSA-8, respectively) and IL-1β (transcripts both p=0.01) and increased ARG-1 (92 KDa macrophase marker) expression (p=0.001 and p=0.005 for ICOSA-4 and HFD respectively vs control). The GLP-1 agonist Bydureon and eicosapentaenoic acid (EPA) at equal emic dose to ICOSA-H had no significant effect on any transcript (below).

(5) EFFECTS ON MARKERS OF FIBROSIS IN CHOLINE DEFICIENT DIET FED MICE
Icosabutate, introduced from week 7-12 of the CDAA diet (CON), significantly reduced both hepatic Coll1a1 transcripts (p=0.017 ICOSA-4 and p=0.0001 ICOSA-8) and collagen content (hydroxyproline, [HPr]) versus control CD mice (p=0.027 ICOSA-4 and p=0.013 ICOSA-8). Bydureon (BY) reduced Coll1a1 expression (p=0.024) but had no effect on collagen content, whilst eicosapentaenoic (EPA) had no effect on any parameter (below).

REFERENCES

ACKNOWLEDGEMENTS
Supported by a grant from NorthSea Therapeutics. Thanks to R&D Pronova Biopharma/BASF for their extensive work in the development of icosabutate.

CONCLUSIONS
Icosabutate overcomes the inherent ADME limitations of unmodified fatty acids as oral drugs targeting metabolic and inflammatory pathways in the liver and demonstrates potent anti-inflammatory, insulin sensitising and anti-fibrotic effects.

A 600mg/day dose normalised elevated liver enzymes in dyslipidemic patients.

Icosabutate may offer a novel, potent and differentiated therapeutic approach to inflammatory and fibrotic disorders of the liver, including NASH.

CONTACT INFORMATION
Rob de Ree, CEO. rob.deree@northseatherapeutics.com