A liver-targeted structurally engineered fatty acid, icosabutate, potently reduces hepatic pro-fibrotic gene expression and improves glycemic control in an obese diet-induced mouse model of NASH

David A Fraser¹, Ditte Denker Thorbek², Brittany Allen³, Sebastian W Thrane⁴, Tore Skjaeret⁵, Scott L Friedman⁶, Michael Feigh⁷ (1) Northsea Therapeutics, 1411 DC Naarden, The Netherlands (2) Gubra, 2970 Hørsholm, Denmark (3) Ichein School of Medicine at Mount Sinai, NY 10029, USA

Introduction
It was recently shown that icosabutate (C₂₄H₂₃O₃), a novel structurally-engineered eicosapentaenoic acid (EPA) derivative, induced potent anti-inflammatory and anti-fibrotic effects in a rodent NASH model without insulin resistance (COAA model). As a significant proportion of NASH patients have type 2 diabetes, we investigated the effects of delayed onset treatment with icosabutate on hepatic fibrosis in 2 separate studies in an obese diet-induced mouse model (ob/ob) of NASH along with its effects upon glycemic control. To assess whether anti-fibrotic effects were dependent on paracrine signals from hepatocytes and/or macrophages, we also evaluated the proliferative effects of icosabutate in human stellate (LX-2) cells.

Methods
Study 1: 30 male B6.V-Lepob/Jrj (ob/ob) mice (5 weeks old) were fed a diet high in fat (40% total calories, containing 18% trans-fat), 20% fructose, 2% cholesterol (AMLN diet) for 15 weeks then randomised into 3 ob/ob-NASH groups of 10 mice to receive either 112mg/kg icosabutate PO, 30mg/kg pioglitazone PO or no treatment (vehicle) for a further 4 weeks. An oral glucose tolerance test (GTT) was also performed at 3 weeks and assessment of hepatic fibrogenic gene expression was performed at 4 weeks.

Study 2: 36 male B6.V-Lepob/Jrj (ob/ob) mice (5 weeks old) were fed the AMLN diet for 18 weeks then, after biopsy confirmation of fibrotic NASH, randomised into 3 ob/ob-NASH groups of 12 mice to receive either 135mg/kg icosabutate PO, 30mg/kg obeticholic (OCA) acid PO or no treatment (control) for a further 8 weeks. Assessment of multiple NASH relevant parameters and fibrosis was made at study end.

Study 3: 30 male B6.V-Lepob/Jrj (ob/ob) mice (5 weeks old) were fed a diet high in fat (40% total calories, containing 18% trans-fat), 20% fructose, 2% cholesterol (AMLN diet) for 15 weeks then randomised into 3 ob/ob-NASH groups of 10 mice to receive either 112mg/kg icosabutate PO, 30mg/kg pioglitazone PO or no treatment (vehicle) for a further 4 weeks. Assessment of multiple NASH relevant parameters and fibrosis was made at study end.

Study 3: Icosabutate reduces proliferative responses in human stellate cells

Below. Icosabutate significantly reduces hepatic transcripts of pivotal genes regulating fibrogenesis in AMLN fed (4-weeks) ob/ob mice. **p<0.01, ***p<0.001 vs vehicle.

Conclusions
Therapeutics, (1) a robust anti-inflammatory and anti-fibrotic effect in LX-2 cells in vitro confirm a direct anti-fibrotic effect of icosabutate independent of effects on hepatocytes and macrophages

Liver injury (plasma ALT) and hepatic inflammation (galectin-3, immunohistochemical determination) and hepatic collagen both as total and relative content. **p<0.01, ***p<0.001 vs vehicle.

Study 3: Icosabutate reduces proliferative responses in human stellate cells

(a) Icosabutate (20, 50 and 75μM) significantly reduced proliferation of LX-2 cells by 25-34% at 24h whereas oleic acid (OA) was without effect: *p<0.005. ****p < 0.0001 vs. vehicle. (b) A non-significant increase in cell viability (MTS assay) demonstrating the anti-proliferative effects are not secondary to cytotoxicity. Results are presented as normalised mean values ± S.E.M. of 5 independent experiments performed in triplicate for icosabutate and 2 independent experiments for OA.

Results
Study 1: Treatment with icosabutate or pioglitazone in AMLN fed (4-weeks) ob/ob mice

Above. icosabutate improves both glucose (AUC) in response to an oral glucose load (middle) and fasting glucose (right) at 4 weeks. Unlike the PPARγ agonist, pioglitazone, the improvements in glycemic control with icosabutate are not associated with an increase in bodyweight. **p<0.01, ***p<0.001 vs vehicle.

Conclusions
In a biopsy confirmed fibrotic NASH model, oral icosabutate reduced hepatic fibrosis in association with a 45% decrease in myofibroblast content in pre- versus post-treatment liver biopsies

Robust anti-proliferative effects in human stellate (LX-2) cells in vitro confirm a direct anti-fibrotic effect of icosabutate independent of effects on hepatocytes and macrophages

Liver injury (plasma ALT) and hepatic inflammation (galectin-3) are also reduced after icosabutate treatment.

With established improvements in atherogenic lipids and glycemic control in humans, the direct and robust anti-proliferative and anti-fibrotic effects of icosabutate further underline its clinical potential for the treatment of human NASH.

CONTACT INFO: David A. Fraser, CSO, david.fraser@northseatherapeutics.com