**Icosabutate induces a potent reduction in hepatic oxidative stress in multiple rodent models of metabolic stress and fibrosing NASH**

### Introduction

- Long-chain n-3 fatty acids and their oxygenated metabolites act as ligands and substrate for beneficial signaling pathways regulating hepatic inflammation and metabolism.
- However, their accumulation into cellular membranes and proneness to peroxidation could reduce their therapeutic potential in conditions associated with excessive oxidative stress, such as NASH.
- Icosabutate is a structurally-engineered eicosapentaenoic acid (EPA) derivative designed to resist both incorporation into complex cellular lipids (including cell membranes) and (oxidation).
- Potent anti-fibrotic effects were observed after oral icosabutate treatment in multiple rodent models of NASH, whereas no effects were seen with either EPA, a FXR agonist (obeticholic acid) or a PPARα agonist (rosiglitazone).
- Effects on hepatic oxidative stress and the arachidonic cascade as a potential differentiating factor between treatments was investigated.

### Methods

- Partitioning of [14C]icosabutate or [14C]EPA into cellular lipids in Huh7 cells was investigated over 24h in 3 separate experiments.
- Oxidised (GSSG) and reduced (GSH) glutathione along with oxygenated arachidonic acid (AA) metabolites were measured in liver samples collected from 4 mouse models: (1) High-fat diet-12 week high-fat diet high (31% total calories) choline-sufficient diet fed mice treated last 6 weeks with 112 mg/kg icosabutate or 91 mg/kg EPA or 91 mg/kg icosabutate or 91 mg/kg EPA; (2) ob/ob-NASH diet induced and biopsy-confirmed model of fibrotic NASH treated with icosabutate (135mg/kg) or OCA (30mg/kg) for 8 weeks; (4) APoE*3Leiden.CETP and other time points for icosabutate (p<0.0001) vs baseline. Placebo was reduced at t=4 only (p<0.01).

### Results

- Cellular partitioning of icosabutate vs. EPA in hepatocytes in vitro

### Conclusions

- Icosabutate does not accumulate in hepatocytes and acts as a potent hepatic antioxidant in both mild metabolic overload (HFH) and NASH (CDAA, ob/ob-NASH, APOE*3L.CETP models).
- Icosabutate increases the generation of hepatic oxygenated omega-3 (icosabutate) metabolites with concomitant reductions in detrimental oxygenated AA metabolites.
- A reduction in hepatic oxidative stress and/or pro-inflammatory arachidonic acid metabolites may contribute to the potent anti-fibrotic effects of icosabutate observed in multiple rodent NASH models and differentiates icosabutate from unmodified EPA, FXR and PPARα agonists.

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