An Engineered Fatty Acid, Icosabutate, Targets Free-Fatty Acid Receptor 4 (GPR120) via the β-arrestin-2 Internalisation Pathway

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Introduction

- Icosabutate, a semi-synthetic eicosapentaenoic acid (EPA) derivative, is currently in Phase 2b clinical development for the treatment of NASH (NCT04052516).
- Icosabutate is designed to target the liver and to achieve therapeutic efficacy beyond what is possible with oral dosing of natural EPA by overcoming the inherent drawbacks of unmodified EPA. Icosabutate is engineered to:

  (1) remain in a free acid form by resisting incorporation into complex cellular lipids through an ethyl group in the α-position.

  (2) minimize its metabolism through β-oxidation via the incorporation of an oxygen atom in the β-position

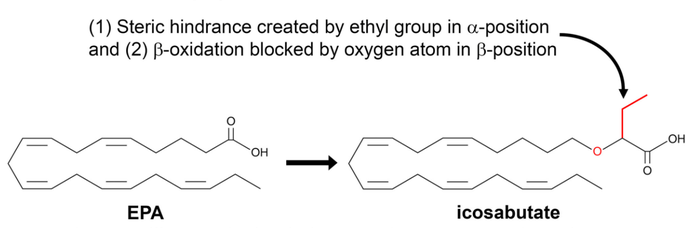


Figure 1. Differences in chemical structure between EPA and Icosabutate

- Preclinically, we have shown potent hepatic anti-inflammatory and anti-fibrotic effects with icosabutate in multiple rodent NASH models (1,2).
- Despite icosabutate's lack of PPAR-γ activity, preclinical and clinical studies also demonstrate significant improvements in glycemic control (2, 3).
- The combination of anti-inflammatory effects and improvements in glycemic control support a potential role for activation of FFAR4 (GPR120), an omega-3 fatty acid receptor expressed on pro-inflammatory macrophages and Kupffer cells (5).

Objectives

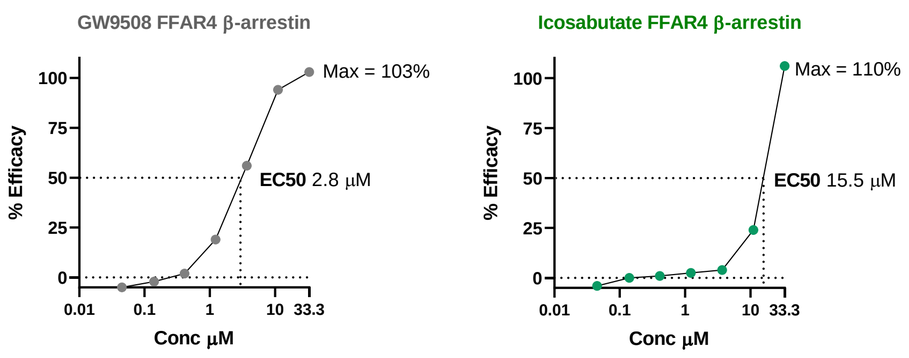
- To determine if icosabutate is a FFAR4(s) and/or FFAR1 (β-arrestin-2 pathway for both receptors) agonist *in vitro.*
- Provide clinical and preclinical *in vivo* data to support FFAR target engagement by icosabutate.

Methods

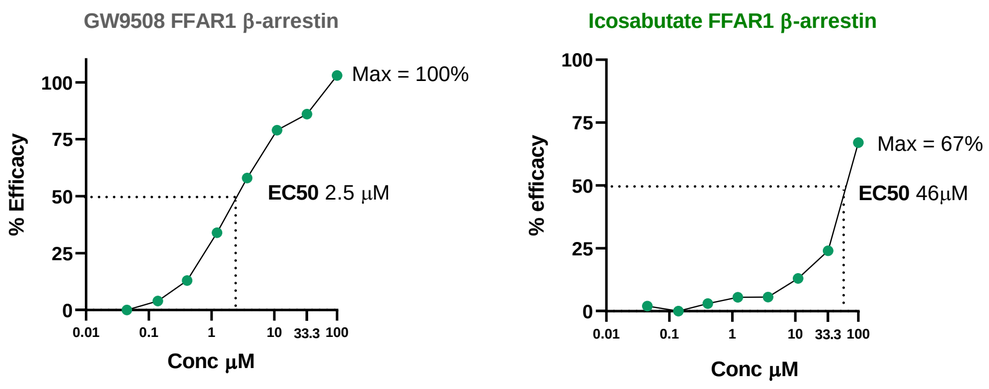
- **Human FFAR1 and FFAR4 (short isoform) activation by icosabutate** was determined *in vitro* using the PathHunter® β-arrestin 2 assay (Eurofins DiscoverX, CA, USA). PathHunter cell lines (CHO and DLD1 background for FFAR4 and 1 respectively) were expanded from freezer stocks and cells were seeded in AssayComplete™ Cell Plating 0 Reagent (25 Reagent for FFAR1) according to standard SOPs prior to incubating with icosabutate or GW9508 (positive control) for 90 minutes at 37ºC in a 10-point concentration curve from 0.001 to 100 µM. Compound activity was determined via chemiluminescence using β-galactosidase as the functional reporter and using CBIS data analysis suite (ChemInnovation, CA, USA). Percentage activity was calculated as: % Activity=100% x (mean RLU of test sample-mean RLU of vehicle control) / (mean MAX control ligand-mean RLU of vehicle control).
- The effect of oral Icosabutate [total 165 mg/kg given as 2 doses (91 and 74 mg/kg) separated by a 5 hr interval] on plasma **active GLP-1 concentrations** was determined in healthy, lean SPD rats 19-24h post-dosing. Corn oil was used as a control and a DPP4 inhibitor (linagliptin, 3 mg/kg per oral) was added to prevent rapid degradation of active GLP-1 in both treated and vehicle groups.
- The effects of icosabutate on **inflammation and glucose control** in patients with NASH (F1-3, NAS≥4, MRI-PDFF≥10%) were determined as part of an interim analysis of the Phase 2b ICONA trial. Patients (n=90) were randomized (1:1:1) to icosabutate (300 or 600 mg) or placebo and treated for 16 weeks. Biomarkers for inflammation, fibrogenesis, glycemic control and lipid metabolism were assessed at baseline and week 16.

Results

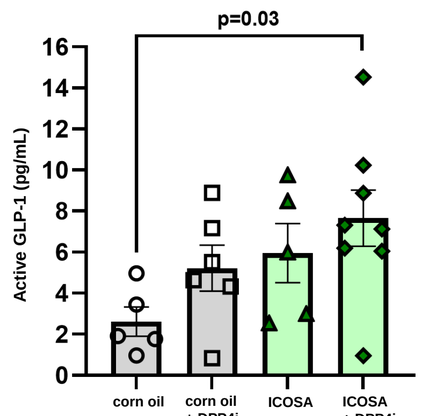
1) Icosabutate is a full FFAR4 and partial FFAR1 agonist



The concentration-response curves show that icosabutate maximally activates FFAR4 (above) and partially activates FFAR1 (below). Notably, the EC50 (~15 µM) for FFAR4 β-arrestin-2 activation is ~3-fold lower than the established plasma C_{max} (~ 50µM) in humans dosed at 600 mg/day.



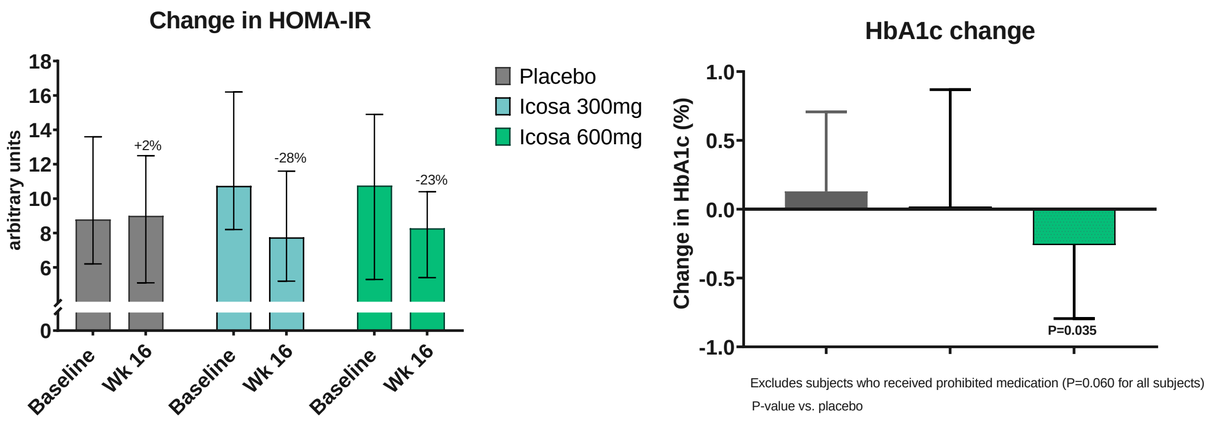
2) Icosabutate increases active GLP-1 concentrations in lean, healthy rats



- FFAR4/1 are highly expressed in enteroendocrine cells and pancreatic islet cells respectively , with activation increasing GLP-1 secretion (4).
- Icosabutate (ICOSA), but not corn oil, increases plasma concentrations of active plasma GLP-1 in rats 19-24 hours post-dose (165 mg/kg).

Values expressed as mean ± SEM, Dunnett's test one-factor linear model

3) Icosabutate improves glycemic control in patients with NASH



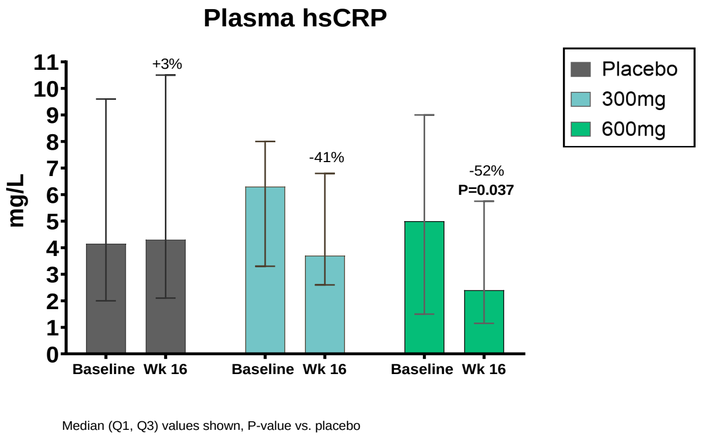
Median (Q1, Q3) values

Excludes subjects who received prohibited medication (P=0.060 for all subjects)

P-value vs. placebo

As evidence of FFAR4 and/or 1 engagement *in vivo,* oral once-daily icosabutate (600 mg q.d.) for 16 weeks improves glycemic control in patients with NASH.

4) Icosabutate has potent anti-inflammatory effects in patients with NASH



Median (Q1, Q3) values shown, P-value vs. placebo

- FFAR4 negatively regulates inflammation via both toll-like receptor and TNF-α/inflammasome activation (5-6).
- Icosabutate treatment for 16 weeks results in a marked reduction (-52% with 600 mg once daily) in plasma hsCRP concentrations in patients with NASH

Summary

- FFAR4 (GPR120) is an omega-3 fatty acid responsive receptor highly expressed on pro-inflammatory macrophages and Kupffer cells
- As activation induces potent anti-inflammatory effects alongside improving glycemic control, FFAR4 is an attractive target for NASH therapy
- Icosabutate is a full FFAR4 (β-arrestin-2) agonist *in vitro* with an ~3-fold lower than the established plasma C_{max} (~ 50µM) in humans at a therapeutic dose (600mg/d).
- Icosabutate also targets FFAR1 (GPR40) via the β-arrestin-2 pathway.
- Multiple lines of evidence in both pre-clinical (increase in active GLP-1 in rats) and clinical studies (improvements in inflammation and glycemic control) support on target activation of FFAR4 by icosabutate.

Conclusions

- FFAR4 (GPR120) activation via the β-arrestin-2 signalling pathway may underlie both the potent anti-inflammatory effects and improvments in glycemic control seen in response to icosabutate therapy.
- The ability to activate FFAR4 *in vivo,* in contrast to high-dose unmodified EPA, is likely driven by icosabutate's ability to (a) target the liver and (b) avoid incorporation into complex lipids and usage as an energy source.

References

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