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Discovery and Nonclinical Profile of AMB-131, a Novel, Oral Small Molecule GIP Receptor Antagonist

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Introduction

Putative loss of function variants of GIPR are strongly associated with protection from obesity in large-scale human genomics studies. This phenotype is reproducible in preclinical models, as GIPR knockout mice resist diet-induced obesity (DIO). GIPR deletion or GIPR antagonism induces greater weight loss when combined with a GLP-1R agonist than from GLP-1R agonist monotherapy.

The most advanced clinical GIPR antagonist, maridebart cafraglutide, is a GIPR antagonist antibody conjugated to GLP-1 peptides. Despite their efficacy and favorable pharmacokinetics (PK), biologics are costly and create challenges for distribution and administration. Unimolecular polypharmacology also poses challenges for titrating receptor modulation. It is therefore desirable to develop small molecule, oral GIPR antagonists that could be combined with GLP-1R agonists to provide more effective and personalized treatments for clinical obesity. The only small molecule, oral GIPR antagonist that has advanced to the clinic is PF-07976016. AMB-131 is being developed as novel, oral small molecule GIP receptor antagonist.

Key In Vitro Characteristics

	PF-07976016	AMB-131
GIPR Antagonist Cell Line IC_{50} @ 37 °C, cAMP, nM	2.9	3.7
Microsome Stability CL_{int}, mL/min/kg (Human, Monkey, Mouse)	4, 8, 24	<4, 7, <11
Hepatocyte Stability CL_{int}, mL/min/kg (Human, Monkey, Mouse)	10, 55, 9	<3, 19, 24
Permeability MDCKII, A:B P_{app} (10^{-6} cm/s)	13 (High)	32 (High)
Therm. Solubility (FaSSIF pH 6.5 / PBS, pH 7.4, µg/mL)	15 / 74	8 / 122
Plasma Protein Binding (Human, Monkey, Mouse, %fu)	1.5, 1.6, 0.4	0.7, 1.2, 0.5

In Vitro Methods

Cell Potency
CHO-K1 cells (Fremont, CA) expressing hGIPR were incubated with compounds for 1 h at 37 °C. Cells were then stimulated by addition of hGIP(1-42) (10 nM). Antagonism of the pathway was determined by quantifying the production of cAMP, a secondary messenger, using a competitive immunoassay. Data was fit to a 4-parameter logistic curve, allowing calculation of an IC_{50}.

ADME Assays
Thermodynamic Solubility: Substrates (1 mg/mL) were incubated in FaSSIF (BioRelevant) or PBS buffer for 24 hours at 37 °C. After incubation, substrate concentrations were measured using HPLC-UV.

Stability Assays: Liver microsomes (BioIVT, 0.5 mg/mL) were incubated with substrate (1 µM) and NADPH for 40 min at 37 °C. Hepatocytes (BioIVT, 0.5 x 10^6 cells/mL) were incubated with substrate (1 µM) for 120 min at 37 °C. Substrate disappearance was measured over time by LC-MS/MS to calculate half-life and intrinsic clearance.

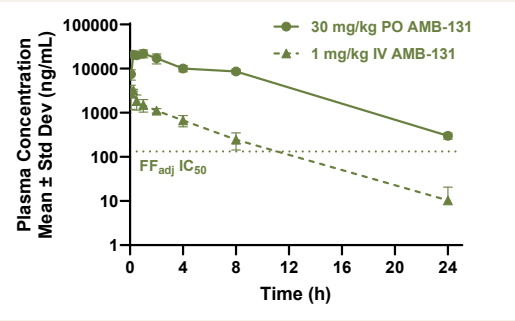
Permeability: MDCKII cKO mock cells (Charles River Laboratories) were seeded in 96-well Transwell HTS plates (Corning). Once confluent, substrates (1 µM) were incubated for 2 hours on a plate shaker at 37 °C, 5% CO_2, and controlled humidity. After incubation, the apparent permeability was measured by LC-MS/MS and calculated. Lucifer yellow permeability and transepithelial electrical resistance (TEER) were used to assess monolayer integrity.

Protein Binding: Substrates were incubated in human and mouse plasma at a concentration of 1 µM for 5 hours at 37 °C, 5% CO_2, and controlled humidity using an HTD equilibrium dialysis device. Percent bound was determined by measuring drug concentrations in each dialysis compartment using LC-MS/MS.

Results

AMB-131 PK: Plasma Concentrations in the Lean Mouse

AMB-131 demonstrated higher exposure and target coverage at 24 h than PF-07976016

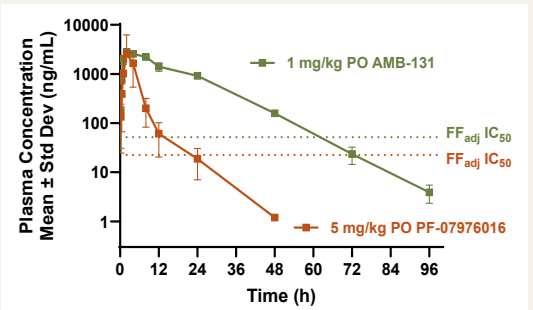


	PF-07976016	AMB-131
IV dose	*1 mg/kg*	*1 mg/kg*
$t_{1/2}$ (h)	4.1 ± 0.4	2.5 ± 0.1
CL (mL/min/kg)	9.3 ± 2.2	1.9 ± 0.2
Vss (L/kg)	1.4 ± 0.2	0.4 ± 0.1
PO dose	*30 mg/kg*	*30 mg/kg*
$AUC_{(0-\infty)}$ (h*ng/mL)	8,900 ± 8,200	176,000 ± 23,000
%F	16 ± 15	67 ± 9
Target Coverage	IC_{39} @ 24 h	IC_{69} @ 24 h

PF-07976016 or AMB-131 (1 mg/kg for the IV groups and 30 mg/kg for the PO groups) was administered to male C57BL/6J mice, 7-9 weeks. Solution formulation for IV: 10% DMSO/40% PEG300/5% Tween 80/45% saline; suspension formulation for PO: 0.5% HPMC in water. FF_{adj}: free fraction-adjusted. Plasma concentrations were measured using an AB Sciex 5500 mass spectrometer. Pharmacokinetic parameters were calculated using non-compartmental analysis with the WinNonLin, Version 8.0 software (Pharsight, CA).

AMB-131 PK: Plasma Concentrations in the Lean Cynomolgus Monkey

A 1 mg/kg oral dose of AMB-131 exhibited multiple days of IC_{50} target coverage

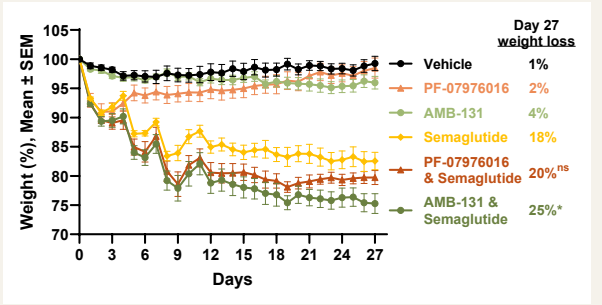


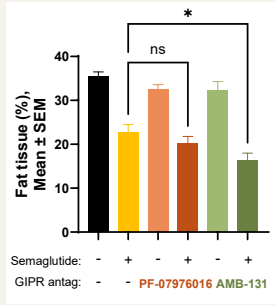
	PF-07976016	AMB-131
IV dose	*1 mg/kg*	*1 mg/kg*
$t_{1/2}$ (h)	6.0 ± 0.8	9.0 ± 0.5
CL (mL/min/kg)	5.8 ± 1.6	0.3 ± 0.0
Vss (L/kg)	0.9 ± 0.3	0.2 ± 0.1
PO dose	*5 mg/kg*	*1 mg/kg*
$AUC_{(0-\infty)}$ (h*ng/mL)	11,400 ± 9,100	54,900 ± 2,600
%F	76 ± 61	107 ± 5
Target Coverage	IC_{45} @ 24 h	IC_{95} @ 24 h

AMB-131 (1 mg/kg for both the IV and PO groups) or PF-07976016 (1 mg/kg for the IV group; 5 mg/kg for the PO group) was administered to lean cynomolgus monkeys. Formulation all groups: 10% DMSO/40% PEG300/5% Tween 80/45% saline. Plasma concentrations and PK parameters were determined as described in the mouse PK experiment.

AMB-131 Promotes Weight Loss in Mice with Diet-Induced Obesity

AMB-131 enhanced the weight loss induced by semaglutide and promoted a leaner body composition

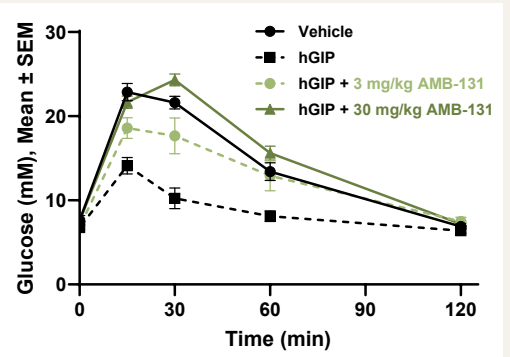


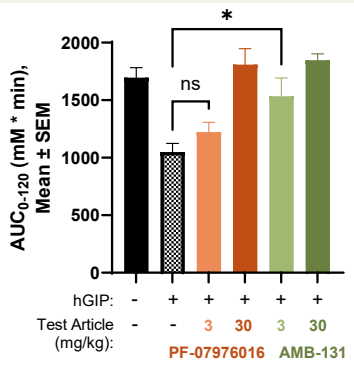


Male B-hGIPR mice, 7-9 weeks, were established with diet-induced obesity with a high-fat diet for 16 weeks. The mice were sham-dosed with vehicle (4 days) and then randomized (N = 8/group). On days 0, 4, and 7, mice were dosed with 30 nmol/kg semaglutide, SC; from day 11 through the end of treatment, mice received 3 nmol/kg semaglutide, SC, QD. Mice were dosed PO with either vehicle, PF-07976016 (30 mg/kg), or AMB-131 (30 mg/kg) as a suspension formulation (0.5% HPMC). At the end of the study, the body composition of all groups was determined by qMRI. The D27 weight data and the fat tissue (%) were analyzed by a one-way ANOVA in GraphPad Prism comparing all groups to the semaglutide-only group with Dunnett's correction for multiple comparisons. Only select comparisons are shown for clarity. $P < 0.05$ was considered statistically significant; * = $P < 0.05$; ns = not significant.

AMB-131 Blunts the Effects of GIP Administration During an IPGTT in Mice

AMB-131 opposes the action of GIP in vivo, thereby demonstrating target engagement of the GIPR





Male C57BL/6J with deleted mouse GIPR and a knocked-in humanized GIPR (B-hGIPR mice) were obtained at an age of 9-11 weeks. 1 h prior to glucose challenge, mice were dosed with either vehicle (0.5% HPMC in water) or test article. 15 min prior to glucose challenge, mice were dosed s.c. with hGIP(1-42) (1 nmol/kg, formulated in 0.9% saline). Mice were challenged with glucose (2 g/kg, i.p.) at 0 min, and glucose measurements were collected out to 120 min with a handheld glucometer (Roche, 1784892). The areas under the curve from 0-120 min (AUC_{0-120}) were calculated in Graphpad Prism. The AUC_{0-120} were analyzed by a one-way ANOVA in GraphPad Prism comparing all groups to the hGIP group with Dunnett's correction for multiple comparisons. Only select comparisons are shown for clarity. $P < 0.05$ was considered statistically significant; * = $P < 0.05$; ns = not significant.

Conclusions

AMB-131, a novel oral small molecule GIP receptor antagonist, has a desirable in-vitro profile with improved target coverage in both mice and non-human primates compared to PF-07976016. Enhanced weight loss in DIO mice was observed when combined with a GLP-1R agonist. This profile suggests that AMB-131 or a similar compound could be a once-daily oral small molecule GIPR antagonist for more effective treatment of clinical obesity. Studies are ongoing for potential development candidate nomination.