Ambrosia Biosciences
Boulder, Colorado

Discovery and Nonclinical Profile of AMB-895, a Long Acting and Novel, Oral Small Molecule GLP-1R Agonist

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Introduction

GLP-1 receptor agonists (GLP-1RA) have revolutionized the treatment paradigm for people living with obesity and Type 2 diabetes and are now also showing promise for other cardiometabolic disorders. Currently approved therapeutics are predominately injectable peptides that are dosed once weekly, with recent approvals for both peptidic and small molecule daily oral therapies. However, there are no approved once-weekly, oral, small molecule GLP-1RA. Many patients currently taking GLP-1RA desire a more sustainable long-term maintenance therapy, while others are unable to access these medicines due to economic pressure. A once-weekly, oral, small molecule therapy could fill these gaps in patient care options, amongst other applications. Ambrosia has discovered AMB-895, a novel, oral small molecule GLP-1RA with the potential for highly efficacious once-weekly dosing.

Key In Vitro Characteristics

	Orforglipron	Aleniglipron	AMB-895
GLP-1R Agonist Cell Line EC_{50} @37° C, cAMP, nM	1.1	0.9	0.6
GLP-1R Low Expression Cell Line EC_{50} @37° C, cAMP, nM (% Max Resp.)	9.8 (39%)	3.2 (16%)	13 (114%)
β-arrestin 2 Cell EC_{50} @37° C, cAMP, nM	>1,000	>1,000	>1,000
Microsome Stability CL_{int}, mL/min/kg (Human, Monkey, Mouse)	<4, 6, 15	11, 13, 16	5, <4, 11
Permeability MDCKII, A:B P_{app} (10^{-6} cm/s)	20 (High)	10 (High)	10 (High)
Therm. Solubility (PBS, pH 7.4, µg/mL)	<1	9	3
Plasma Protein Binding (Human, Monkey, Mouse; % fu)	0.03, 0.05, 0.08	0.4, 0.3, 0.17	0.1, 0.1, 0.1

fu=percent fraction unbound

In Vitro Methods

Cell Potency
CHO-K1 cells (Fremont, CA) expressing hGLP-1R were incubated with the compounds. Agonism of the pathway was measured by quantifying the amount of cAMP, a secondary messenger, using a competitive immunoassay. Data was fit to a 4-parameter logistic curve, allowing calculation of an EC_{50}.

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

Microsome Stability: Liver microsomes (BioIVT) were used at a final protein concentration of 0.5 mg/mL and incubated with 1 µM substrate and NADPH for 40 minutes at 37° C. Substrate disappearance was measured over time by LC-MS/MS to calculate half-life and intrinsic clearance.

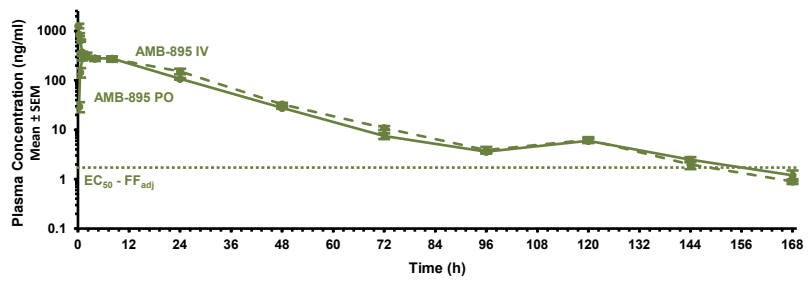
Permeability: MDCKII cKO mock cells (Charles River Laboratories) were seeded in 96-well Transwell HTS plates (Corning). Once confluent, substrates at 1 µM were incubated for 2 hours on a plate shaker at 37° C, 5% CO_2, and controlled humidity. After incubation, the apparent permeability was measured 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-895 PK: Plasma Concentration in Mouse

AMB-895 demonstrated one week of >EC_{40} target coverage at a single 1 mg/kg oral dose



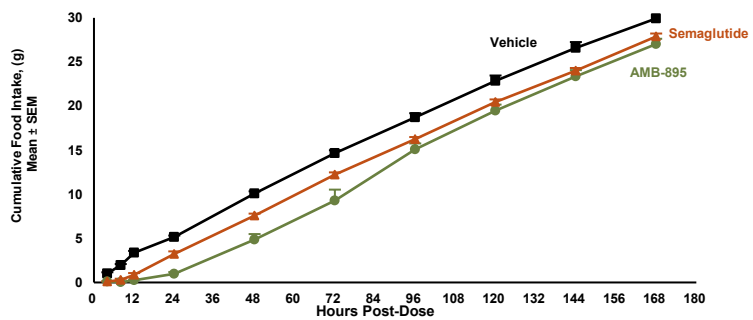
Male C57BL/6J mice, 7-9 weeks were used for the intravenous and oral pharmacokinetic studies. Solution formulation: 10% DMSO/40% PEG300/5% Tween 80/45% Saline. FFadj: free fraction-adjusted. Plasma concentrations were measured using an AB Sciex 5500 mass spectrometer. Pharmacokinetic parameters were calculated using non-compartmental analysis with the WinNonLin, Version 8.0 software (Pharsight, CA). † Calculated using AUC_{last} (hr*ng/mL)

Mouse PK Parameters (± SD)

	1 mg/kg Dose IV			1 mg/kg Dose PO			
	$t_{1/2}$ (h)	CL (mL/min/kg)	Vss (L/kg)	$t_{1/2}$ (h)	$AUC_{(0-\infty)}$ (h*ng/mL)	%F	ECx Coverage
Orforglipron	11.8 ± 1.6	2.7 ± 0.6	2.8 ± 0.6	7.5 ± 0.4	5,815 ± 230	92 ± 4	EC_{42} @ 24 hr
Aleniglipron	2.0 ± 0.2	76.8 ± 1.9	0.9 ± 0.3	2.5 ± 0.5	1,276 ± 88 †	56 ± 3	BLOQ @ 24hr
AMB-895	17.3 ± 1.8	1.8 ± 0.2	2.2 ± 0.2	21.4 ± 7.4	7,719 ± 328	82 ± 3	EC_{99} @ 24hr EC_{42} @ 168hr

AMB-895: Food Intake in Mouse

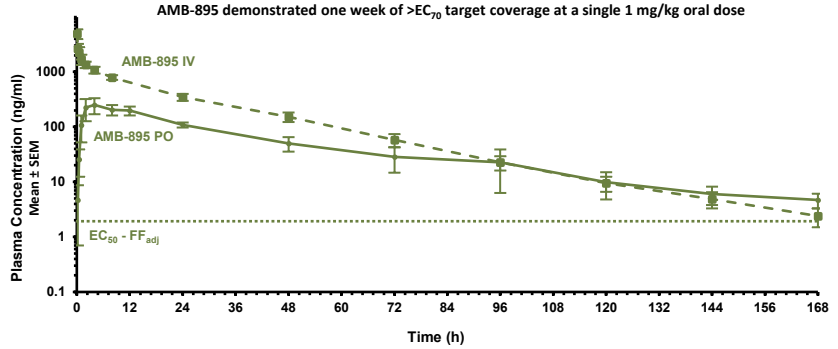
AMB-895 1 mg/kg oral dose demonstrated superior food intake suppression compared to 0.123 mg/kg SC semaglutide



On day 1 a single oral dose of AMB-895 at 1 mg/kg and a single SC dose of semaglutide at 0.123 mg/kg were administered to transgenic humanized GLP-1R mice after an overnight fast. Food was returned 15 minutes after dosing and food intake was measured for 168 hours.

AMB-895 PK: Plasma Concentration in Lean Cynomolgus Monkey

AMB-895 demonstrated one week of >EC_{70} target coverage at a single 1 mg/kg oral dose



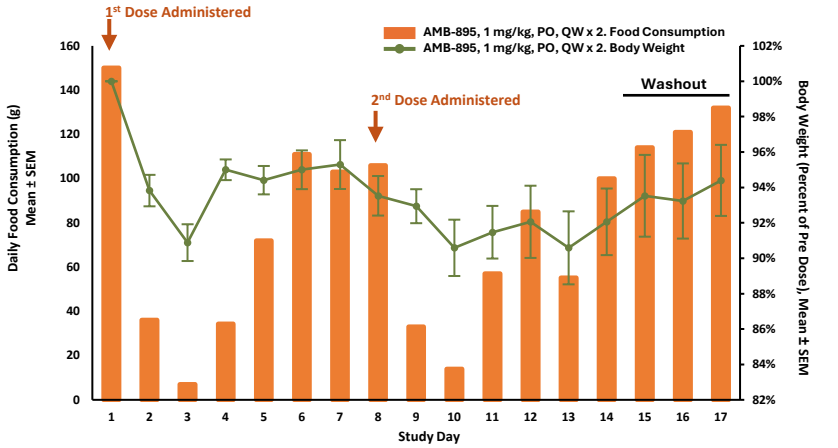
LLOQ: 1 ng/mL; solution formulation: 10% DMSO/40% PEG300/5% Tween 80/45% saline; FFadj: free fraction-adjusted

Dosing	$t_{1/2}$ (h)	$AUC_{(0-\infty)}$ (h*ng/mL)	CL (mL/min/kg)	ER (%)	Vss (L/kg)
AMB-895 (IV 1mg/kg)	24.5 ± 5.2	29,771 ± 1,665	0.6 ± 0	1.3 ± 0.1	0.7 ± 0.1

Dosing	$t_{1/2}$ (h)	$AUC_{(0-\infty)}$ (h*ng/mL)	T_{max} (h)	C_{max} (ng/mL)	Oral Bio (%F)	ECx Coverage
AMB-895 (PO 1mg/kg)	30.1 ± 7.4	8,624 ± 2,568	6.8 ± 4.8	281 ± 137	29 ± 9	EC_{72} @ 168hr

AMB-895: Two Week Body Weight Loss Study in Lean Cynomolgus Monkey

AMB-895 dosed QW resulted in significant, sustained body weight loss and decreased food intake



AMB-895 was dosed at 1 mg/kg on days 1 and 8; body weight and food intake were measured as part of 14-day PK study

Conclusions

AMB-895, a novel oral small molecule GLP-1RA, possesses a desired in-vitro profile, and demonstrated efficacy dosing once per week in both rodent and monkey. This nonclinical profile suggests that AMB-895 has the potential to be a once-weekly oral small molecule GLP-1RA therapeutic for obesity and cardiometabolic disorders. Ongoing studies are in progress for potential development candidate nomination.