ESM Methods

GLP-1 receptor cAMP accumulation CHO cell assay

Stable Chinese hamster ovary (CHO) cell lines expressing human glucagon-like peptide-1 (GLP-1) receptor were generated in-house. DNA coding for the GLP-1 analogue peptide in fusion with an amino acid linker was generated by gene synthesis (GeneArt, Thermo Fisher Scientific, Waltham, MA, USA). Fusion with the human Fc(γ4) fragment was done by overlapping PCR before cloning into a mammalian expression vector as previously described [1]. The construct was transiently expressed in CHO cells using a polyethylenimine based transfection method. The supernatant was purified by affinity chromatography using a Protein A column followed by a size exclusion chromatography step. Purified compound was analysed for integrity by SDS-PAGE, size-exclusion chromatography-HPLC and liquid chromatography/mass spectrometry. EndoC-βH1cells were kindly supplied by Prof. R Scharfmann (Endocells, Paris, France). All cell lines were authenticated in-house as mycoplasm-free.

CHO cell lines were dispensed at $1x10^5$ cells/ml and EndoC-βH1 cells were dispensed at 1.6 $x10^6$ cells/ml in assay buffer (Hanks Balanced Salt Solution, containing 0.1% bovine serum albumin and 0.5mM 3-isobutyl-1-methylxanthine, Sigma-Aldrich, St Louis, MO, USA). The cells were dispensed in 384-well assay plates (5μL per well) before adding serial dilutions of tested molecules prepared using non-contact acoustic dispensing. After 30 minutes of incubation, cAMP levels were measured using the cAMP dynamic 2 homogeneous Time Resolved Fluorescence (HTRF) kit (Cisbio, Bedford, MA, USA). Fluorescence emissions at 665nm and 620nm following excitation at 320nm were detected using an EnVision reader (PerkinElmer, Waltham, MA, USA) and data were transformed to % Delta F as described in manufacturer's guidelines.

Data represent n>5 experiments and are expressed as means ± SEM. Samples were not randomised or blinded.

ESM References

[1] Persic L, Roberts A, Wilton J, et al. (1997) An integrated vector system for the eukaryotic expression of antibodies or their fragments after selection from phage display libraries. Gene 10:9-18

ESM Tables

ESM Table 1 Change from baseline to day 36 in exploratory efficacy outcomes assessed in Part B of the study

Parameter		MEDI4166 50 mg (*n*=9)	MEDI4166 200 mg (*n*=18)	MEDI4166 400 mg (*n*=21)	Placebo (*n*=15)
Body weight, kg		–2.5	–1.4	–1.5	–1.3
	95% CI	–3.4, –1.5	–2.0, –0.7	–2.1, –0.8	–2.0, –0.5
	p value[a]	0.0585	0.9027	0.7474	—
HbA1c, %		–0.7	–0.8	–0.4	–0.5
	95% CI	–1.0, –0.3	–1.0, –0.5	–0.6, –0.2	–0.7, –0.2
	p value[a]	0.3668	0.1334	0.6938	—
FPG, mmol/l		–3.33	–2.20	–1.47	–2.66
	95% CI	–4.84, –1.82	–3.23, –1.17	–2.42, –0.51	–3.79, –1.53
	p value[a]	0.4887	0.5480	0.1101	—
Insulin $AUC_{0–4h}$, pmol-h/l		–58.6	–18.6	139.7	245.9
	95% CI	–510.1, 393.0	–328.5, 291.2	–189.2, 468.5	–94.3, 586.1
	p value[a]	0.2826	0.2516	0.6572	—
Fructosamine, µmol/l		–13.8	–22.1	–20.7	–21.2
	95% CI	–35.9, 8.2	–37.6, –6.6	–35.0, –6.5	–38.1, –4.3
	p value[a]	0.5999	0.9355	0.9685	—

All data are LS means

[a]*p* values are vs placebo

FPG, fasting plasma glucose; LS, least squares

ESM Table 2 Potency for MEDI4166 and GLP-1Fc for cAMP accumulation in transfected receptors expressed in CHO cell lines expressing human GLP-1 receptor and in endogenous receptor populations in the human EndoC-βH1-human insulinoma line

	MEDI4166	**GLP-1Fc**
CHO-Human GLP-1 Receptor		
EC_{50}, pmol/l	2560	10.4
	(1920–3410)	(7.8–14.0)
n[a]	14	20
Human EndoC-βH1		
EC_{50}, pmol/l	1180000	2470
	(749000–1850000)	(1530–3980)
n[a]	5	13

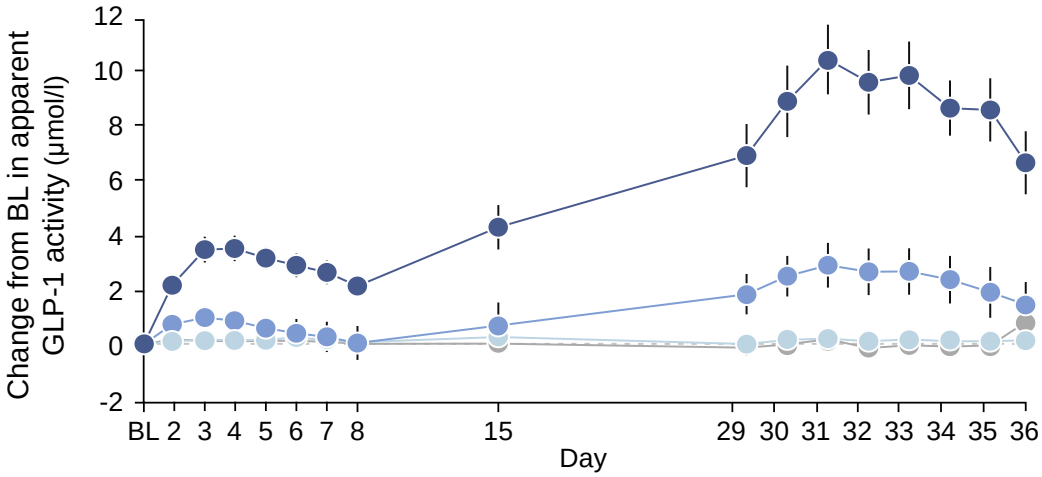
Data are geometric means (95% CI)

[a]Number of experiments conducted in the presence of 0.1% bovine serum albumin

CHO, Chinese hamster ovary; EC_{50}, half maximal effective concentration; GLP-1, glucagon-like peptide-1

ESM Figures

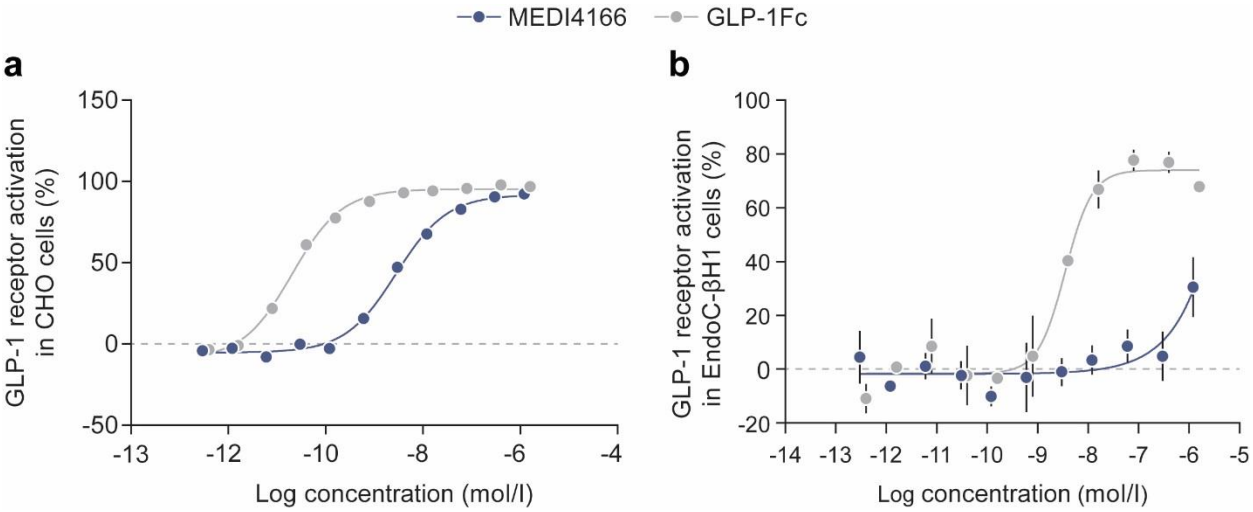
ESM Fig. 1 Change from baseline in apparent GLP-1 activity over time in Part B of the study with MEDI4166 at 50 mg (*n*=9), 200 mg (*n*=18) or 400 mg (*n*=21) or placebo (*n*=15)



Data are means ± SEM

BL, baseline

ESM Fig. 2 Concentration–response curves for MEDI4166 and GLP-1Fc in (**a**) CHO cell lines expressing human glucagon-like receptor 1 (n>14 experiments; representative data from duplicate data points within a single experiment are fitted with 4-parameter logistic fit to determine EC_{50} relative to 70 nM maximum GLP-1 {7-36 amide} peptide control) or (**b**) EndoC-βH1 cell line (n>5 experiments; representative data from duplicate data points within a single experiment are fitted with 4-parameter logistic fit to determine EC_{50} relative to 14nM maximum GIP {1-42} peptide control)



All data are mean ±SEM and are plotted on a logarithmic scale (base 10)

CHO, Chinese hamster ovary; EC_{50}, half maximal effective concentration; GIP, gastric inhibitory polypeptide; GLP-1, glucagon-like peptide-1