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ORIGINAL ARTICLE

A first-in-human pharmacodynamic and pharmacokinetic study of a fully human anti-glucagon receptor monoclonal antibody in normal healthy volunteers

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Aims: Glucagon receptor (GCGR) blockers are being investigated as potential therapeutics for type 1 and type 2 diabetes. Here we report the safety, tolerability, pharmacokinetics (PK) and pharmacodynamics (PD) of REGN1193, a fully human glucagon receptor blocking monoclonal antibody from a first-in-human healthy volunteer randomized double-blinded trial.

Methods: Healthy men and women received single ascending doses of REGN1193 ranging from 0.05 to 0.6 mg/kg (n = 42) or placebo (n = 14) intravenously. Safety, tolerability and PK were assessed over 106 days. The glucose-lowering effect of REGN1193 was assessed after induction of hyperglycaemia by serial glucagon challenges.

Results: REGN1193 was generally well tolerated. There were small (<3× the upper limit of normal) and transient dose-dependent increases in hepatic aminotransferases. No increase in LDL-C was observed. Hypoglycaemia, assessed as laboratory blood glucose ≤70 mg/dL, occurred in 6/14 (43%) subjects on placebo and 27/42 (57%) on REGN1193 across all dose groups. All episodes of hypoglycaemia were asymptomatic, >50 mg/dL, and did not require treatment or medical assistance. Concentration-time profiles suggest a 2-compartment disposition and marked nonlinearity, consistent with target-mediated clearance. REGN1193 inhibited the glucagon-stimulated glucose increase in a dose-dependent manner. The 0.6 mg/kg dose inhibited the glucagon-induced glucose area under the curve for 0 to 90 minutes ($AUC_{0\text{-}90\ minutes}$) by 80% to 90% on days 3 and 15, while blunting the increase in C-peptide. REGN1193 dose-dependently increased total GLP-1, GLP-2 and glucagon, with plasma levels returning to baseline by day 29 in all dose groups.

Conclusion: REGN1193, a GCGR-blocking monoclonal antibody, produced a safety, tolerability and PK/PD profile suitable for further clinical development. The occurrence of transient elevations in serum hepatic aminotransferases observed here and reported with several small molecule glucagon receptor antagonists suggests an on-target effect of glucagon receptor blockade. The underlying mechanism is unknown.

KEYWORDS

GCGR, glucagon stimulation, phase 1, REGN1193

1 | INTRODUCTION

Glucagon secreted from α-cells of the pancreas in response to fasting and low glucose concentrations acts primarily on glucagon receptors in the liver to increase hepatic glucose output to maintain an adequate supply of fuel to vital organs.[1] Glucagon is also secreted in response to autonomic stimulation and to circulating amino acids.[2] Hyperglucagonaemia is a common feature of diabetes and is thought to be a direct result of loss of insulin-induced suppression of glucagon secretion.[3–5] Based on the fact that hyperglucagonaemia contributes to fasting and

postprandial hyperglycaemia in people with type 2 diabetes (T2D), glucagon and the glucagon receptor have been investigated as potential targets for diabetes control.[6] Clinical trials with small molecule glucagon receptor antagonists in patients with T2D treated for up to 24 weeks have demonstrated a significant decrease in fasting glucose, postprandial glucose and HbA1c, without significant hypoglycaemia.[7–10] Reversible increases in LDL-cholesterol and elevated serum hepatic aminotransferases levels have also been reported.[7,9,11] Modest increases in systolic and diastolic blood pressure (1.3-2.3 mm Hg) measured by 24-hour ambulatory blood pressure monitoring have recently been reported in patients with T2 diabetes after 6 weeks of treatment with a small molecule GCGR blocker.[9]

We developed REGN1193, a human monoclonal GCGR-blocking antibody as a potential therapeutic for diabetes to determine if the safety and efficacy profile could be improved compared with small molecule glucagon receptor blockers. Preclinical studies with REGN1193 in diabetic monkeys provided evidence of a rapid glucose-lowering effect, but no increase in LDL-C or liver enzymes after single doses of 5 and 20 mg/kg.[12] Thus, the current phase 1 study (NCT01933763) was conducted as part of a full development programme.

In this single-dose healthy volunteer study, the main objective was to assess the safety and tolerability profile of REGN1193. We also sought to determine the PK/PD profile of REGN1193 and to assess if the adverse laboratory effects reported with small molecule GCGR antagonists, ie, increases in hepatic aminotransferases and LDL-C, were specific to small molecule GCGR antagonists.

2 | METHODS

This single-centre, phase I, single ascending dose, randomized, double-blinded study was conducted at Covance Clinical Research Unit in Dallas, TX, and sponsored by Regeneron Pharmaceuticals Inc., Tarrytown, NY. All patients provided written informed consent, and the study was conducted in accordance with the International Conference on Harmonization Good Clinical Practice guidelines and all applicable local regulatory requirements and laws.

2.1 | Patient eligibility

Eligible subjects were healthy men and women, 18 to 45 years of age (inclusive), with a body mass index (BMI) ranging from 18.0 to 30.0 kg/m^2 (inclusive), and with no history of change in body weight greater than 10% over 6 months prior to screening. Sexually active men or women of childbearing potential were required to practice adequate contraception and not become pregnant (or have their partner[s] become pregnant) during and for up to 3 months after participation in the study. Female subjects were screened for gynaecological cancers as recommended by the American Cancer Society. Other inclusion criteria were HbA1c ≤5.5%, fasting plasma glucose >70 and ≤110 mg/dL, systolic blood pressure between 90 and 140 mm Hg, diastolic blood pressure between 60 and 90 mm Hg, heart rate between 45 and 90 bpm, and clinically normal standard 12-lead electrocardiogram (ECG).

Exclusion criteria were any clinical abnormalities or significant concomitant illnesses that would preclude subjects from safely completing the study, and more specifically, a history of high blood pressure, abnormal fasting blood glucose or diabetes, unprovoked sweating, palpitations, frequent nausea, panic attacks, insulinoma, glucagonoma, pheochromocytoma, hypersensitivity to glucagon or lactose, adrenal hormone disorder, hypoglycaemia, renal hormone disorder, autonomic nervous system dysfunction, a hypersensitivity reaction to doxycycline or similar compound, malignancy (including carcinoma in situ) and family history of any type of multiple endocrine neoplasia (MEN) or any other genetic cancer predisposition. Also exclusionary were fasting triglyceride concentrations >300 mg/dL, catecholamine levels >2× the upper limit of normal (ULN) in a 24-hour urine test and blood donation of any volume within 1 month prior to administration of study drug.

2.2 | Antibody properties

REGN1193, derived using Regeneron's Velocimmune technology platform,[13] is a fully human monoclonal antibody that has a human IgG4 constant region with a stabilizing mutation in the hinge region (serine to proline in position 108 in accession P01861) to minimize half-antibody formation. REGN1193 demonstrates high affinity binding to mouse, rat, monkey and human GCGR and inhibits glucagon signaling through human GCGR in the picomolar range (EC_{50} of 68 pM, using a cell-based reporter assay).[12]

2.3 | Study design and treatment

The study was a randomized, single-dose, placebo-controlled, double-blinded design in 4 sequential ascending dose cohorts of 12 subjects enrolled at the following REGN1193 dose levels: 0.05, 0.1, 0.3 and 0.6 mg per kilogram of body weight. Dose rationale was based on glucose-lowering efficacy, toxicology and PK in preclinical animal models. The study drug was administered by IV infusion over 30 minutes. The REGN1193 drug product was supplied as a lyophilized powder in a 20 mL glass vial for IV administration.

The study design consisted of a screening period (day −28 to day −3) and a treatment and observation period (day 1 [baseline] to day 106) (Figure 1). All subjects were randomly assigned in a 3:1 ratio to receive either REGN1193 or placebo on day 1. Dose escalation cohorts consisted of two randomly assigned groups: Group A subjects (n = 4) did not undergo glucagon stimulation tests and group B subjects (n = 8) underwent glucagon stimulation tests on day −1 (~24 hours prior to study drug administration), day 3, day 8, day 15 and day 22. Subjects in group A were admitted to the clinic on day −1 and subjects in group B on day −2; all subjects were discharged from the clinic after the day 4 safety assessments were completed. Subjects in the ascending dose cohorts were observed for at least 15 days after receiving REGN1193 before opening enrollment in the next dose cohort.

Dose escalation was discontinued at the 0.6 mg/kg dose level because that dose level inhibited ≥50% of the mean percent change in glucose in response to stimulation with glucagon on study day 15, as defined by a pre-specified efficacy stopping rule. Inhibition of the glucagon response was determined by the area under the curve (AUC) for glucose change from pre-glucagon stimulation and by the mean percent increase in glucose from pre-glucagon stimulation in

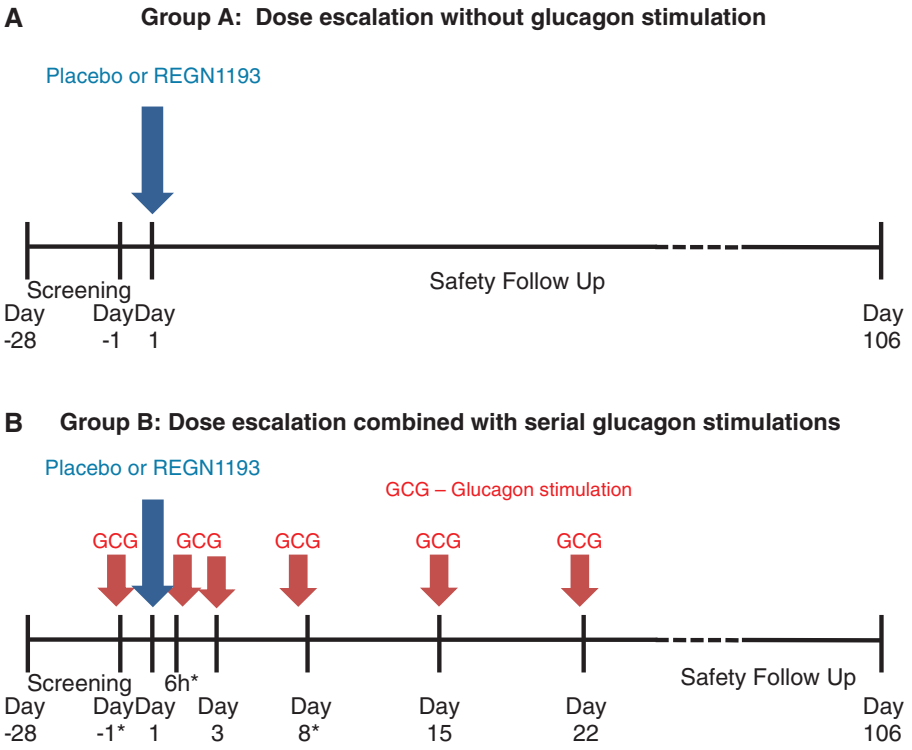

FIGURE 1 Study design schema. Dose escalation cohorts treated with placebo or REGN1193 (at 0.05, 0.1, 0.3 and 0.6 mg/kg) in group A (A) and group B combined with serial glucagon stimulations on day −1, day 3, day 8, day 15 and day 22 (B) as well as an "early onset" cohort treated with placebo or REGN1193 (at 0.3 mg/kg) with glucagon challenges on day −1, day 1 at 6 hours post-dosing and day 8 (B*). Subjects in group A were admitted to the clinic on day −1 and subjects in group B on day −2. All subjects were discharged on day 4

the REGN1193 group compared with placebo. The totality of the data was considered (including the variance of the different measures) when the decision was made to discontinue dose escalation.

An additional cohort consisting of eight subjects was enrolled after the dose escalation portion of the study was completed to assess time to the onset of action of REGN1193 at the 0.3 mg/kg dose level. Subjects in this cohort underwent glucagon stimulation tests on day −1 (~24 hours prior to study drug administration), day 1 (~6 hours after study drug administration), and day 8.

Blood was drawn for evaluation of plasma glucose levels during screening, at baseline and on days 1, 2, 3, 4, 8, 11, 15, 22, 29, 43, 64, 85 and 106. Safety assessments were performed on the same days and included an evaluation of vital signs, a physical examination, and laboratory tests. ECG was performed pre-infusion, and at 1, 2, 3, 4, 6, 9 and 12 hours after initiation of infusion. Twenty-four-hour ECG telemetry was performed from entry into the clinic until 24 hours following study drug administration. Adverse events (AEs) were collected from the timing of signing the informed consent through study day 106. All AEs reported in this study were coded using the Medical Dictionary for Regulatory Activities (MedDRA) version 16.1.

Subjects had periodic blood glucose monitoring and recording from the time of entry to the clinic until discharge from the clinic. Testing was performed every 2 hours overnight (until the morning of day 4). During the daytime, testing was performed whenever indicated by subject signs or symptoms. Hypoglycaemia was defined as a blood glucose level <70 mg/dL (measured by venipuncture) accompanied by symptoms assessed to be due to hypoglycaemia. Any blood glucose measurement of <70 mg/dL was confirmed by a plasma glucose sample. Blood chemistries, lipid panels, haematology, pregnancy tests, and urine analyses were performed at Medpace Central Labs LLC (Cincinnati, Ohio).

2.4 | Glucagon stimulation test

Serial glucagon stimulation tests were utilized to test the principal PD effect of REGN1193 on glucose regulation. The glucagon stimulation test was conducted on fasting subjects while recumbent. Recombinant glucagon (Eli Lilly LLC, Indianapolis, Indiana) was administered intravenously at a dose of 0.8 mg. Blood samples were drawn from an indwelling catheter prior to and at 6, 15, 20, 25, 30, 45, 60, 75, 90, 120, 135, 150, 165, 180, 195, 210, 225 and 240 minutes after administration of glucagon for determination of plasma glucose levels. Additional blood samples were collected for assessment of endocrine hormones and other potential PD biomarkers as described below.

2.5 | Pharmacokinetic analysis

Blood samples for analysis of functional (ie, free and partially bound) REGN1193 concentration in serum were collected on day 1 at pre-infusion, end of infusion, and 1, 2, 4 and 8 hours after the end of infusion; and then on days 2, 3, 4, 8, 11, 15, 22, 29, 43, 64, 85 and 106 (end of study), or at the time of early termination. Concentrations of functional REGN1193 were determined in human serum using a validated enzyme-linked immunosorbent assay (ELISA). The lower limit of quantitation (LLOQ) of functional REGN1193 in unaltered (neat) human serum is 0.078 mg/L. Concentration-time profiles of functional REGN1193 were evaluated, and the results of non-compartmental analysis were reported. Non-compartmental analyses were performed using WinNonlin (Phoenix 6.4). Figures were prepared with R software version 3.3.0 (http://www.r-project.org).

2.6 | Immunogenicity

Blood samples for analysis of anti-REGN1193 antibodies were collected prior to treatment on day 1, days 15, 29, 64, 85 and 106 (end

of study), or at the time of early termination. Anti-REGN1193 antibodies (anti-drug antibodies; ADA) were assessed using an electrochemiluminescence bridging immunoassay. The minimal detectable titre was 30. Anti-drug antibody status (positive or negative) and titre category were reported (Glossary S1).

2.7 | Pharmacodynamic biomarker analysis

As subjects in group B received serial glucagon stimulation tests, the PD effect of REGN1193 was explored separately in group A and group B subjects. Blood samples in both groups were collected on days −1, 1, 2, 3, 4, 8, 11, 15, 22, 29, 43, 64, 85 and 106 for measurement of the following PD markers: glucagon, total and active glucagon-like peptide 1 (GLP-1), glucagon-like peptide 2 (GLP-2), gastric inhibitory polypeptide (GIP), C-peptide and insulin. In group B subjects, additional blood samples were collected before and during the glucagon stimulation tests. The administration of the exogenous glucagon was taken into consideration in the analysis of glucagon levels in group B. PD biomarker measurements were performed by validated assays at Pacific Biomarkers, Seattle, Washington. Reference ranges were 1.3 to 16.9 pmol/L, <13.9 pmol/L, <2.89 pmol/L, 2.7 to 7.5 ng/mL, <22.2 pmol/L, 1.1 to 4.4 ng/mL, and 2.6 to 24.9 U/mL for glucagon, total and active GLP-1, GLP-2, GIP, C-peptide, and insulin, respectively.

2.8 | Pharmacodynamic efficacy analysis

The primary efficacy variables, percent change of $AUC_{0\text{-}90\ minutes}$ in plasma glucose from pre-glucagon stimulation and percent change of peak plasma glucose from pre-baseline visit (day −1) to post-baseline visits (days 3, 8, 15 and 22) were analyzed using descriptive statistics. The interval of 0 to 90 minutes was chosen based on the time-course of glucose increase induced by glucagon stimulation. The peak in glucose was observed within 30 to 60 minutes of glucagon administration and by approximately 90 minutes, glucose levels returned to the baseline. A second transient increase in glucose was observed at 150 to 160 minutes after glucagon stimulation. As it was not mechanistically clear what caused the second increase and as the glucose levels remained close to baseline values thereafter, the analysis was restricted to the changes in $AUC_{0\text{-}90\ minutes}$. The mean percent change in $AUC_{0\text{-}90\ minutes}$ from pre-baseline to each of the post-baseline visits per treatment group was calculated and so was the mean percent change in peak plasma glucose from pre-baseline visit. Differences between REGN1193 treatment groups and placebo in mean percent change in $AUC_{0\text{-}90\ minutes}$ and mean percent changes in peak plasma glucose were also determined by post-baseline visit. These post-baseline differences in mean percent change in $AUC_{0\text{-}90\ minutes}$ and mean percent change in peak plasma glucose were used to determine the inhibition of glucagon response and the decision of dose escalation.

2.9 | Pharmacokinetic-pharmacodynamic (PK-PD) analysis

The relationship between functional REGN1193 concentration and REGN1193 efficacy was assessed by plotting functional REGN1193 concentrations in serum at the time of glucagon stimulation against the percent change in glucose AUC through 90 minutes following the stimulation. A sigmoid E_{max} model was used to analyze the relationship between functional REGN1193 concentrations and REGN1193 efficacy. A sigmoid $E_0 - I_{max}$ inhibitory model can be described as:

$$E = E_0 - \frac{I_{max} \cdot C^{Gamma}}{C^{Gamma} + IC_{50}^{Gamma}}$$

in which C is the systemic concentration of functional REGN1193, E is the percent change from pre-baseline (day −1) of plasma glucose AUC (up to 90 minutes) for GCG-stimulated glucose, E_0 is the percent change of glucose AUC when C is 0, I_{max} is the theoretical maximal drug effect (inhibitory), gamma is the slope parameter of the PD curve, and IC_{50} is the C when 50% E is inhibited. The analysis did not account for the difference in the treatment effects between subject, dose or time post-dose.

This sigmoid PK-PD relationship was characterized by nonlinear mixed-effects modeling using NONMEM (7.3, ICON Development Solutions, Ellicott City, Maryland). Figures were prepared with R software version 3.3.0 (http://www.r-project.org).

3 | RESULTS

3.1 | Patient characteristics and disposition

In total, 342 subjects were screened, 56 of whom were enrolled in the study (16 and 40 subjects in groups A and B, respectively) between August 2013 and September 2014. Screen failures were due primarily to subjects not meeting inclusion criteria related to HbA1c values, BMI and vital signs. Demographics and baseline characteristics of the subjects are provided in Table 1.

3.2 | Pharmacokinetic (PK) profile

Evaluation of the functional REGN1193 concentration-time profiles in serum suggests two-compartment disposition and marked nonlinearity, consistent with target-mediated clearance (Figure 2). Mean clearance of functional REGN1193 was markedly dose dependent, declining from 272 mL/d/kg at 0.05 mg/kg to about 5 mL/d/kg at 0.6 mg/kg (see Table 2). Due to the nonlinearity in clearance, half-life estimates of functional REGN1193 in serum were dose and concentration-dependent, ranging from 0.5 to ~4 days. While the mean peak concentration (C_{max}) of functional REGN1193 increased greater than dose proportionally in the lower dose cohorts (ie, 0.05 and 0.1 mg/kg), it increased dose proportionally in the higher dose cohorts (ie, 0.3 and 0.6 mg/kg). Highest C_{max} (~20 mg/L) was achieved at the highest dose group of 0.6 mg/kg dose, immediately after IV administration. Based on limited data, there is no consistent difference in functional REGN1193 concentration-time profiles of group A (no glucagon stimulation) and group B (glucagon stimulation) across the dose cohorts (0.1 mg/kg to 0.6 mg/kg), indicating PK profiles of potential patient populations with elevated glucagon levels may not significantly differentiate from those observed in heathy volunteers.

TABLE 1 Demographics and baseline characteristics of study subjects

Characteristic	Placebo (N = 14)	0.05 mg/kg (N = 9)	0.1 mg/kg (N = 9)	0.3 mg/kg (N = 15)	0.6 mg/kg (N = 9)
Age (years); Mean (SD); Range	30.9 (4.94); 22-39	30.4 (8.44); 19-44	33.2 (9.59); 19-45	29.2 (7.66); 19-44	29.8 (6.42); 19-38
Male sex (%)	11 (78.6)	8(88.9)	7 (77.8)	10 (66.7)	7 (77.8)
Race (%)					
White	5 (35.7)	2(22.2)	2 (22.2)	11 (73.3)	4 (44.4)
Black	6 (42.9)	2(22.2)	5 (55.6)	4 (26.7)	5 (55.6)
Asian	1 (7.1)	0	1 (11.1)	0	0
American Indian or Alaska Native	0	2 (22.2)	0	0	0
Other	2 (14.3)	3 (33.3)	1(11.1)	0	0
Body mass index (kg/m^2); Mean (SD); Range	24.9 (2.57); 20.9-29.8	25.0 (4.31); 19.8-29.8	26.0 (2.98); 21.2-29.8	25.4 (3.26); 19.1-29.8	24.8 (3.19); 19.9-28.9
Fasting plasma glucose (mg/dL)	92.3 (6.11)	93.1 (7.37)	93.4 (6.86)	93.5 (6.48)	95.7 (5.89)

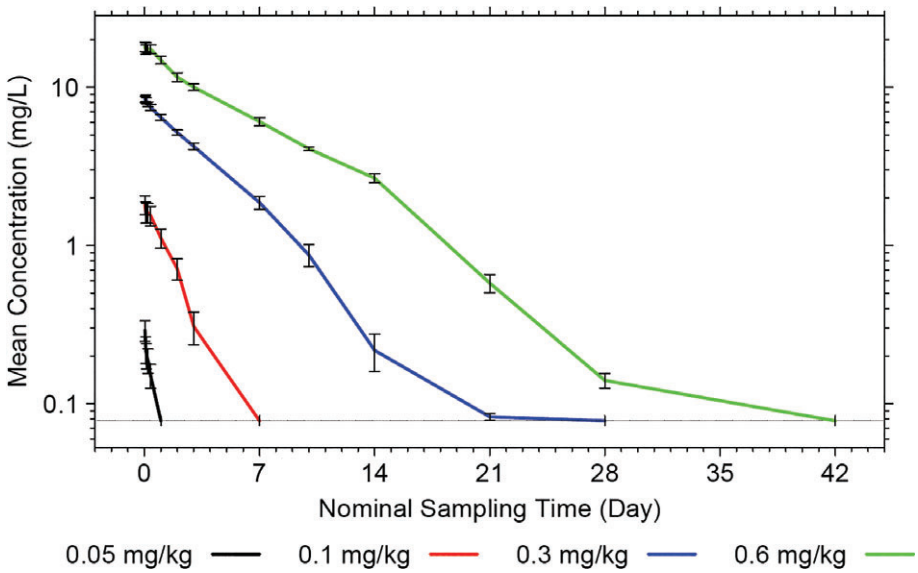

FIGURE 2 Mean (± standard error [SE]) time-concentration profile of functional REGN1193 following a single IV dose of REGN1193 in all study subjects (regardless of glucagon stimulation)

TABLE 2 Summary of observed pharmacokinetic parameters of functional REGN1193 by dose group following a single IV dose of REGN1193 in healthy adult volunteers

Parameter	Units	0.05 mg/kg IV				0.1 mg/kg IV				0.3 mg/kg IV				0.6 mg/kg IV			
		N[a]	Mean	Median	SD	N[b]	Mean	Median	SD	N	Mean	Median	SD	N	Mean	Median	SD
$t_{½}$	day	6	0.5	0.449	0.217	8	1.19	1.23	0.226	15	2.04	1.8	0.532	9	3.69	3.25	1.35
CL	L/d/kg	6	0.272	0.215	0.154	8	0.0269	0.0288	0.00622	15	0.00841	0.00831	0.00184	9	0.00517	0.0052	0.000537
V_{ss}	L/kg	6	0.161	0.139	0.0416	8	0.0454	0.0399	0.00949	15	0.0307	0.0311	0.00317	9	0.0341	0.0322	0.00768
C_{max}	mg/L	9	0.291	0.294	0.131	8	2.05	2.05	0.413	15	8.99	8.94	1.38	9	19.3	19.8	3.6

Abbreviations: CL, clearance; C_{max}, maximum concentration; N, number of subjects; SD, standard deviation; $t_{1/2}$, half-life; V_{ss}, steady-state volume of distribution.

[a] Several parameters were not calculated for three subjects (group A of 0.05 mg/kg group) who had only one quantifiable drug concentration-time point.

[b] One subject from the 0.1 mg/kg group was excluded from analysis for non-quantifiable drug concentrations at all time points.

3.3 | Pharmacodynamic (PD) effects

Treatment with REGN1193 caused an increase in glucagon, total GLP-1 and GLP-2. In group A, glucagon, total GLP1-1 and GLP-2 demonstrated a dose-response increase with greater increases in the 0.3 and 0.6 mg/kg dose groups compared with the two lower dose groups (Figure 3). Levels returned to baseline by day 106 in all subjects. In group A, following administration of REGN1193, levels of active GLP-1 and total GIP were variable with no consistent change over time for any treatment subgroup (data not shown).

As expected, administration of glucagon IV (0.8 mg) to subjects in group B (prior to treatment with REGN1193 or placebo) resulted in an increase in plasma glucose levels. Although the glucose levels were periodically measured for 240 minutes post-stimulation with glucagon, the efficacy analysis was focused on the first 90 minutes,

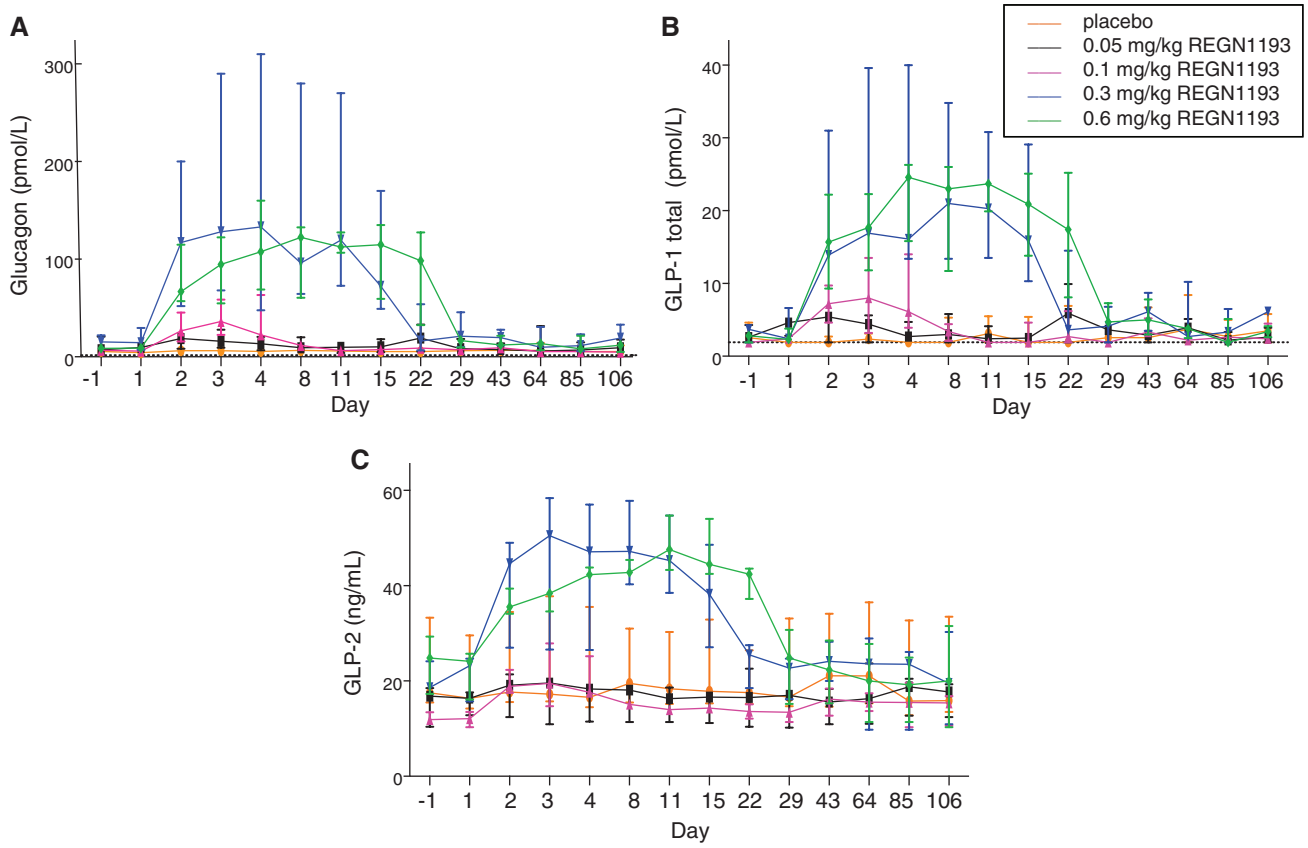

FIGURE 3 Effect of REGN1193 treatment in group A on glucagon, total GLP-1, and GLP-2 (A, B, and C, respectively) in the dose escalation portion of the study. X-axes show discreet time points, y-axes show median ± range; Reference ranges are 1.3 to 16.9, <13.9, and <22.2 pmol/L, for glucagon, total GLP-1, and GLP-2, respectively

which encompassed the peak glucose levels and the return to pre-stimulation levels. No differences were seen at baseline for any of the cohorts on day −1. On day 3, a significant reduction in the mean glucose AUC and the mean peak glucose induced by glucagon stimulation was observed in subjects treated with 0.1, 0.3 and 0.6 mg/kg REGN1193 compared with placebo, while no effect was observed in subjects treated with 0.05 mg/kg REGN1193. By day 8, the inhibitory effect of 0.1 mg/kg REGN1193 was no longer evident. In contrast, the effect of the 0.3 and 0.6 mg/kg doses of REGN1193 on the mean glucose AUC and the mean peak glucose levels induced by glucagon stimulation was maintained on day 8 and day 15, but reduced on day 22. Assessments of the changes in the mean glucose AUC and the mean glucose peak values in the 0.3 mg/kg REGN1193 cohort of subjects who received glucagon stimulation test on days −1 and 1 indicated REGN1193 diminished the glucose response to glucagon stimulation as early as 6 hours post-drug administration (Figure S2F).

During the glucagon stimulation test conducted prior to study drug administration, glucagon administration resulted in a rapid rise in plasma C-peptide levels that peaked at 6 minutes and returned

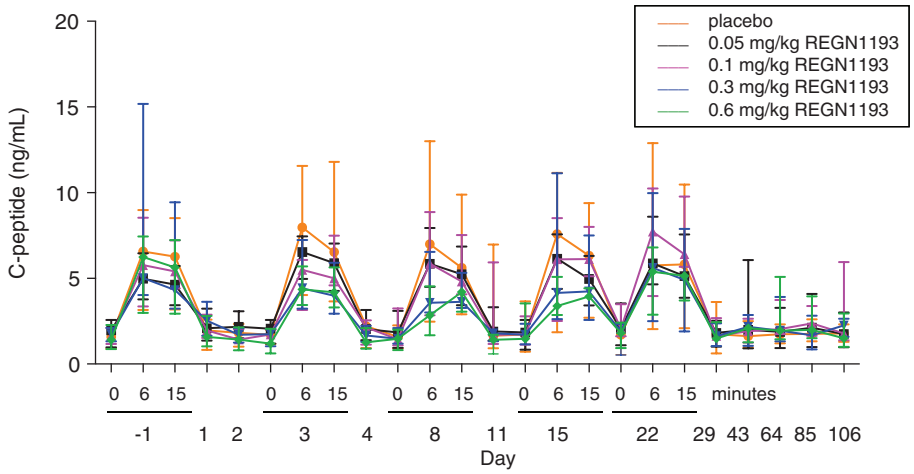

FIGURE 4 Effect of REGN1193 treatment in group B on C-peptide in the dose escalation portion of the study. X-axis shows discreet time points (0, 6 and 15 indicating pre-glucagon stimulation, 6 and 15 minutes post-glucagon stimulation, respectively), y-axis shows median ± range, the reference range is 1.1 to 4.4 ng/mL

to baseline at 90 minutes. REGN1193 at doses of 0.3 and 0.6 mg/kg reduced glucagon-stimulated C-peptide elevations on days 3, 8 and 15. However, the 0.05 and 0.1 mg/kg doses had little inhibitory effect on glucagon-stimulated increases in C-peptide levels (Figure 4).

3.4 | PK-PD analysis

Time-dependent pharmacokinetics-pharmocodynamics (PK/PD) of functional REGN1193 in serum versus mean % change in $AUC_{0\text{-}90\ minutes}$ glucose in group B subjects (with glucagon stimulation) in the 0.3 and 0.6 mg/kg dose cohorts is shown in Figure 5A. A temporal fast response was observed after study drug IV administration. With increasing dose levels from 0.3 to 0.6 mg/kg, further glucose reduction was achieved and the duration of inhibition was prolonged.

The sigmoid E_{max} model showed that glucose AUC declined monotonically with increasing functional REGN1193 concentration, reaching a maximum effect of ~90% glucose reduction (Figure S1). At a single dose of 0.6 mg/kg, at least 80% reduction was maintained for about 2 weeks before returning to its baseline (Figure 5A). In addition, the IC_{50} value of REGN1193 was estimated to be 0.422 ± 0.066 mg/L.

Time-dependent PK/PD of functional REGN1193 in serum versus glucagon, GLP-1 and GLP-2 in group A subjects (without glucagon stimulation) in 0.3 and 0.6 mg/kg dose cohorts is shown in Figure 5B to D. A temporal fast on and slow off response was observed for each of the proglucagon products.

3.5 | Safety

All 56 subjects received a single dose of REGN1193 or placebo (42 and 14 subjects, respectively). Most subjects (92.9%) completed the study. Four subjects prematurely withdrew from the study, including two in the placebo group who were lost to follow-up, one in the 0.1 mg/kg group who was noncompliant with the protocol, and one in the 0.6 mg/kg group who withdrew consent due to change in work schedule that conflicted with study visits (Table S1). In total, 36/56 subjects (64.3%) experienced at least 1 treatment emergent adverse event (TEAE) during the study. The overall TEAE profile was similar between treatment groups (57.1% and 66.7% subjects in the placebo and in the combined REGN1193 treatment groups, respectively) (Table S1). No differences were observed in frequency of TEAEs among treatment cohorts. No infusion or injection site reactions were reported. One male subject with baseline bradycardia in the 0.6 mg/kg REGN1193 cohort developed an accelerated idioventricular rhythm on day 1 that resolved spontaneously on day 2. The diagnosis was confirmed by two independent cardiologists and was concluded to be of no clinical significance.

Symptomatic hypoglycaemic events were not reported and the observed frequency of asymptomatic hypoglycaemic events was infrequent and similar in the placebo and REGN1193 treatment groups (Table 3). There were no clinically meaningful changes in vital signs and laboratory tests by REGN1193. No subject treated with REGN1193 had alanine transaminase (ALT) levels above 3× the ULN. One subject had aspartate transaminase (AST) >3× but <5× the ULN

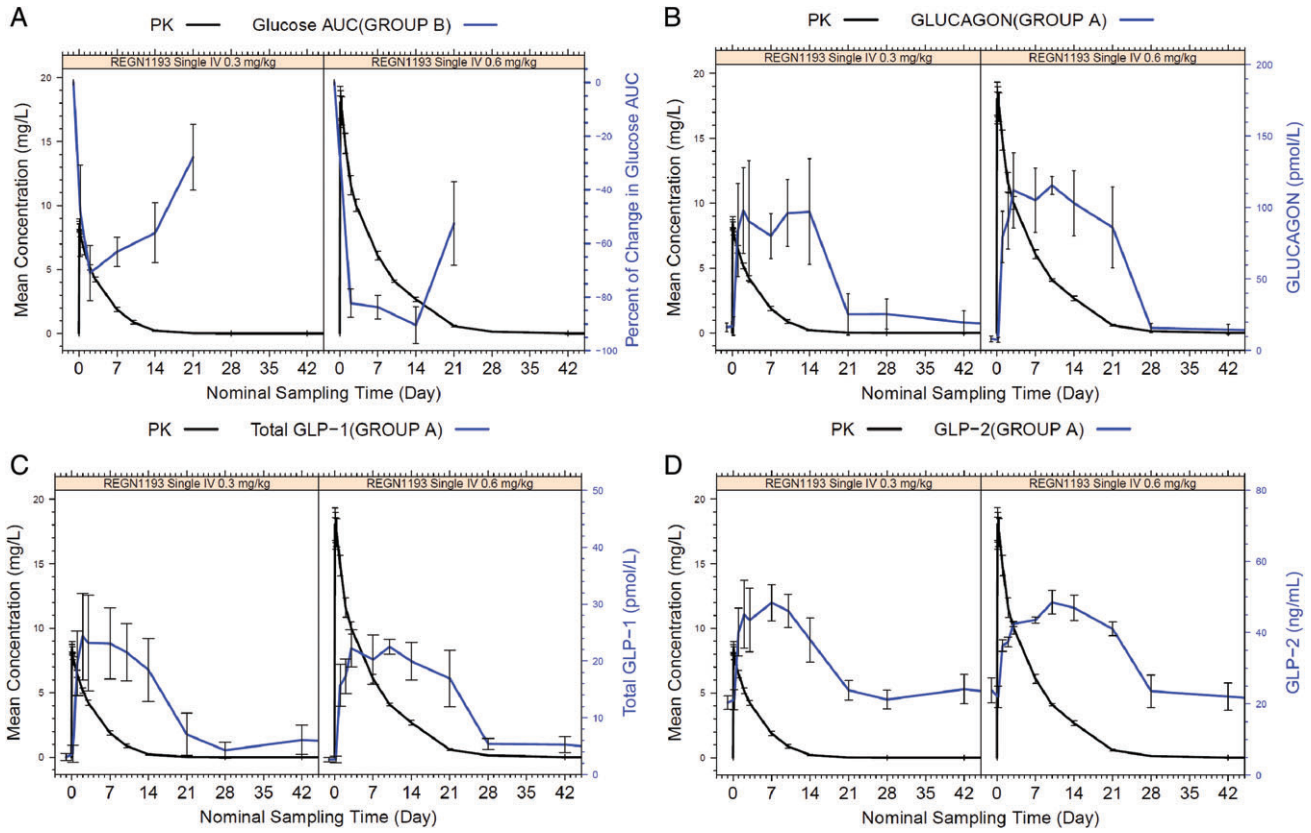

FIGURE 5 Time-dependent pharmacokinetics-pharmocodynamics (PK/PD) of REGN1193 concentrations versus glucose-lowering effect after glucagon challenge in group B for the 0.3 and 0.6 mg/kg dose cohorts (A). Time-dependent pharmacokinetics-pharmocodynamics (PK/PD) of REGN1193 concentrations versus glucagon (B), total GLP-1 (C) and GLP-2 (D) in group A for the 0.3 mg/kg and 0.6 mg/kg dose cohorts

TABLE 3 Number (%) of subjects with hypoglycaemia in the post-treatment period

		0.05 mg/kg (N = 9)	0.1 mg/kg (N = 9)	0.3 mg/kg (N = 15)	0.6 mg/kg (N = 9)
Blood glucose; 61 to 70 mg/dL	5 (36)	1 (11)	2 (22)	9 (60)	3 (33)
Blood glucose; 51 to 60 mg/dL	2 (14)	0	2 (22)	4 (27)	1 (11)

Normal range: 74 to 118 mg/dL.

(Table S2). The changes in ALT and AST were reversible as the values returned to the normal range after day 22 (Figure S3).

Two of the 42 REGN1193-treated subjects (<5%) exhibited a treatment-emergent ADA response: one exhibited a transient response and the other exhibited a treatment-persistent response. All ADA titres were low (at the minimum dilution, 1:30). Neither of the two positive ADA responses was considered to have affected the systemic exposure to functional REGN1193 and these two subjects did not display hypersensitivity or injection site reactions. No subjects in the placebo group had a positive ADA response.

4 | DISCUSSION

Inhibitors of glucagon secretion and glucagon action have been studied as potential treatments for diabetes for over 50 years[14,15] More recent clinical trials with small molecule glucagon receptor blockers have demonstrated >1% HbA1c lowering in patients with T2D after 12 to 24 weeks.[7,9,10,16] However, none of the molecules has advanced to market authorization or to phase 3 clinical trials. The reasons for this apparent lack of success vary from lack of efficacy, potential safety concerns, or for business reasons. The current study with our potent and selective human glucagon receptor blocking monoclonal antibody in healthy subjects shows that a single dose of REGN1193 is well tolerated, and rapidly lowers glucose elevations induced by a glucagon challenge. This glucose-lowering effect occurred without symptomatic or significant hypoglycaemia. However, statistical comparisons were not performed for effect of REGN1193 versus placebo. In addition, single IV administration of REGN1193:

1. Demonstrated a nonlinear PK profile consistent with target-mediated clearance.
2. Resulted in 90% blockade of glucagon action at the dose of 0.6 mg/kg.
3. Induced no change in total cholesterol, LDL-C, HDL or triglycerides.
4. Induced a small increase in ALT and AST that returned to baseline and was not associated with other laboratory or clinical events.

The experimental challenge of glucagon-induced hyperglycaemia used here has been tested by other investigators[17] and increased glucose levels above 150 mg/mL to mimic levels in patients with diabetes. The magnitude of the glucose-lowering effect of REGN1193 was nearly complete at the highest dose tested, 90%, and the effect of a single dose lasted for up to 15 days. Previous studies in preclinical models demonstrated that the glucose-lowering effect of REGN1193 was attributable to blocking glucagon-mediated hepatic glucose output, potential weight loss, and enhanced GLP-1 secretion.[12] However, weight loss was not observed in this single-dose study of healthy volunteers.

Studies in glucagon receptor knockout mice rendered insulin deficient by streptozotocin have firmly demonstrated the metabolic benefits of absence of glucagon action without insulin administration.[18–20] In addition, we have shown in mice with near absence of insulin receptor signaling that REGN1193 treatment is able to lower glucose to near normal ranges.[21] Thus, patients with insulin receptor loss of function mutations, manifested as severe insulin resistance and diabetes, ie, Rabson-Mendenhall syndrome and other related diseases, may benefit from anti-glucagon receptor therapy. In this study, the effects of REGN1193 on C-peptide elevation observed post-glucagon challenge suggested that the glucose-lowering effect is independent of insulin secretion and action, as C-peptide was significantly lower at the top two doses. This property of glucagon receptor blockade suggests REGN1193 may be a viable treatment of hyperglycaemia and other metabolic perturbation not only for patients with T2D but also for patients with T1D, particularly those with low levels of β-cell insulin secretion.

The most consistent adverse finding in clinical trials of glucagon receptor blockers has been a mild increase in ALT and AST without clinical symptoms or signs that resolved upon discontinuation of the agent.[7,9,16,22,23] However, there has been a report of a single patient with a homozygous inactivating mutation (P86S) in the glucagon receptor, who had normal ALT and AST from 64.7 to 66.9 years of age.[24,25] As we observed similar increases in ALT and AST with our monoclonal antibody, the findings cannot be attributed to an off-target small molecule effect. In addition, we observed these changes in healthy volunteers who are unlikely to have occult liver disease or hepatic steatosis. The recent publication of Lilly's small molecule glucagon receptor blocker (LY2409021) reported in patients with T2D that there was an increase in hepatic fat fraction (3.7%) after 6 months of treatment that was significantly higher than placebo and sitagliptin.[26] Fourteen percent of patients on LY2409021 had a 10% or higher increase in hepatic fat. The increase in hepatic fat was associated with an increase in aminotransferases. Studies in large numbers of patients exposed to this class of agents for extended periods of time will need to be conducted to determine the potential consequences of the increase in hepatic aminotransferases.

Finding a dose level or dose regimen that lowers glucose and HbA1c, while having no significant effect on hepatic aminotransferases, is key to advancing this class of agents beyond phase 2. Alternatively, there are likely to be clinical scenarios in which the clinical benefits might outweigh the potential risks. For example, patients with severe insulin resistance syndromes who are unresponsive to high doses of insulin or patients with T2D who are not at goal despite treatment with multiple anti-hyperglycemic agents may benefit from GCGR blockade.

In conclusion, we demonstrated that REGN1193, a GCGR-blocking monoclonal antibody, has a safety, tolerability and PK/PD profile suitable for further clinical development. The occurrence of transient elevations in serum hepatic aminotransferases observed here and reported with several small molecule glucagon receptor antagonists suggests an on-target effect of glucagon receptor blockade. The underlying mechanism is not known.

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Conflict of interest

A. Kostic, F. Yang, K. Chan, J. Gromada, and J. B. Harp are employees and shareholders of Regeneron Pharmaceuticals, Inc. George D Yancopoulos is an officer and shareholder of Regeneron Pharmaceuticals, Inc. T.A. King received research funding from Regeneron Pharmaceuticals for clinical trial conduct and advisory board membership, paid to Covance, of which he is an employee.

Author contributions

A. K., K. C., G. D. Y., and J. H. did the experimental design. T. A. K. was involved with the study conduct. The analysis of data was done by A. K., F. Y., and K. C.

A. K., F. Y., K. C., G. D. Y., J. G., and J. B. H were involved with the data interpretation. All authors were involved in the writing process of the paper.

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REFERENCES

1. Unger RH, Aguilar-Parada E, Müller WA, Eisentraut AM. Studies of pancreatic alpha cell function in normal and diabetic subjects. *J Clin Invest*. 1970;49(4):837–848.
2. Gromada J, Franklin I, Wollheim CB. Alpha-cells of the endocrine pancreas: 35 years of research but the enigma remains. *Endocr Rev*. 2007;28(1):84–116.
3. Cooperberg BA, Cryer PE. Insulin reciprocally regulates glucagon secretion in humans. *Diabetes*. 2010;59(11):2936–2940.
4. Kawamori D, Kurpad AJ, Hu J, et al. Insulin signaling in alpha cells modulates glucagon secretion in vivo. *Cell Metab*. 2009;9(4):350–361.
5. Unger RH, Orci L. Paracrinology of islets and the paracrinopathy of diabetes. *Proc Natl Acad Sci U S A*. 2010;107(37):16009–16012.
6. Bagger JI, Christensen M, Knop FK, Vilsbøll T. Therapy for obesity based on gastrointestinal hormones. *Rev Diabet Stud*. 2011;8(3):339–347.
7. Kelly RP, Garhyan P, Raddad E, et al. Short-term administration of the glucagon receptor antagonist LY2409021 lowers blood glucose in healthy people and in those with type 2 diabetes. *Diabetes Obes Metab*. 2015;17(4):414–422.
8. van Dongen MG, Geerts BF, Morgan ES, et al. First proof of pharmacology in humans of a novel glucagon receptor antisense drug. *J Clin Pharmacol*. 2014;55(3):298–306.
9. Kazda CM, Ding Y, Kelly RP, et al. Evaluation of efficacy and safety of the glucagon receptor antagonist LY2409021 in patients with type 2 diabetes: 12- and 24-week phase 2 studies. *Diabetes Care*. 2016;39(7):1241–1249.
10. Vajda EG, Logan D, Lasseter K, et al. Pharmacokinetics and pharmacodynamics of single and multiple doses of the glucagon receptor antagonist LGD-6972 in healthy subjects and subjects with type 2 diabetes mellitus. *Diabetes Obes Metab*. 2017;19(1):24–32.
11. Guan HP, Yang X, Lu K, et al. Glucagon receptor antagonism induces increased cholesterol absorption. *J Lipid Res*. 2015;56(11):2183–2195.
12. Okamoto H, Kim J, Aglione J, et al. Glucagon receptor blockade with a human antibody normalizes blood glucose in diabetic mice and monkeys. *Endocrinology*. 2015;156(8):2781–2794.
13. Valenzuela DM, Murphy AJ, Frendewey D, et al. High-throughput engineering of the mouse genome coupled with high-resolution expression analysis. *Nat Biotechnol*. 2003;21(6):652–659.
14. Unger RH. Glucagon and the insulin: glucagon ratio in diabetes and other catabolic illnesses. *Diabetes*. 1971;20(12):834–838.
15. Gerich JE, Lorenzi M, Schneider V, et al. Effects of somatostatin on plasma glucose and glucagon levels in human diabetes mellitus. Pathophysiologic and therapeutic implications. *N Engl J Med*. 1974;291(11):544–547.
16. Kazierad DJ, Bergman A, Tan B, et al. Effects of multiple ascending doses of the glucagon receptor antagonist PF-06291874 in patients with type 2 diabetes mellitus. *Diabetes Obes Metab*. 2016;18(8):795–802.
17. Matsuda M, Defronzo RA, Glass L, et al. Glucagon dose–response curve for hepatic glucose production and glucose disposal in type 2 diabetic patients and normal individuals. *Metabolism*. 2002;51(9):1111–1119.
18. Lee Y, Wang MY, Du XQ, Charron MJ, Unger RH. Glucagon receptor knockout prevents insulin-deficient type 1 diabetes in mice. *Diabetes*. 2011;60(2):391–397.
19. Damond N, Thorel F, Moyers JS, et al. Blockade of glucagon signaling prevents or reverses diabetes onset only if residual beta-cells persist. *Elife*. 2016;5:e13828.
20. Neumann UH, Ho JS, Mojibian M, Covey SD, Charron MJ, Kieffer TJ. Glucagon receptor gene deletion in insulin knockout mice modestly reduces blood glucose and ketones but does not promote survival. *Mol Metab*. 2016;5(8):731–736.
21. Okamoto H, Cavino K, Na E, et al. Glucagon receptor inhibition normalizes blood glucose in severe insulin-resistant mice. *Proc Natl Acad Sci U S A*. 2017;114(10):2753–2758.
22. Kazda CM, Frias J, Foga I, et al. Treatment with the glucagon receptor antagonist LY2409021 increases ambulatory blood pressure in patients with type 2 diabetes. *Diabetes Obes Metab*. 2017;19(8):1071–1077.
23. Gumbiner B, Esteves B, Dell V, et al. Robust glucose and HbA1c lowering after a single dose of RN909 (PF-06293620) in type 2 diabetes (T2D). American Diabetes Association 76th Scientific Sessions; June 10–14, 2016; New Orleans, LA. American Diabetes Association. Abstract 110-LB.
24. Zhou C, Dhall D, Nissen NN, Chen CR, Yu R. Homozygous P86S mutation of the human glucagon receptor is associated with hyperglucagonaemia, alpha cell hyperplasia, and islet cell tumor. *Pancreas*. 2009;38(8):941–946.
25. Yu R, Nissen NN, Dhall D, Heaney AP. Nesidioblastosis and hyperplasia of alpha cells, microglucagonoma, and nonfunctioning islet cell tumor of the pancreas: review of the literature. *Pancreas*. 2008;36(4):428–431.
26. Guzman CB, Zhang XM, Liu R, et al. Treatment with LY2409021, a glucagon receptor antagonist, increases liver fat in patients with type 2 diabetes. *Diabetes Obes Metab*. 2017. https://doi.org/10.1111/dom.12958.

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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