Antisense Inhibition of Glucagon Receptor by IONIS-GCGR$_{Rx}$ Improves Type 2 Diabetes Without Increase in Hepatic Glycogen Content in Patients With Type 2 Diabetes on Stable Metformin Therapy

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OBJECTIVE

To evaluate the safety and efficacy of IONIS-GCGR$_{Rx}$, a 2′-*O*-methoxyethyl antisense oligonucleotide targeting the glucagon receptor (GCGR), and the underlying mechanism of liver transaminase increases in patients with type 2 diabetes on stable metformin therapy.

RESEARCH DESIGN AND METHODS

In three phase 2, randomized, double-blind studies, patients with type 2 diabetes on metformin received weekly subcutaneous injections of IONIS-GCGR$_{Rx}$ (50–200 mg) or placebo for 13 or 26 weeks.

RESULTS

Significant reductions in HbA$_{1c}$ were observed after IONIS-GCGR$_{Rx}$ treatment versus placebo at week 14 (−2.0% 200 mg, −1.4% 100 mg, −0.3% placebo; $P < 0.001$) or week 27 (−1.6% 75 mg, −0.9% 50 mg, −0.2% placebo; $P < 0.001$). Dose-dependent increases in transaminases were observed with IONIS-GCGR$_{Rx}$, which were attenuated at lower doses and remained mostly within the normal reference range at the 50-mg dose. There were no other significant safety observations and no symptomatic hypoglycemia or clinically relevant changes in blood pressure, LDL cholesterol, or other vital signs. At week 14, IONIS-GCGR$_{Rx}$ 100 mg did not significantly affect mean hepatic glycogen content compared with placebo (15.1 vs. −20.2 mmol/L, respectively; $P = 0.093$) but significantly increased hepatic lipid content (4.2 vs. −2.7%, respectively; $P = 0.005$) in the presence of transaminase increases.

CONCLUSIONS

IONIS-GCGR$_{Rx}$ is a potent inhibitor of hepatic glucagon receptor expression with a potential to improve glycemic control at low weekly doses in combination with metformin. Significant reductions in HbA$_{1c}$ occurred across the full-dose range tested, with minimal transaminase elevations at lower doses. Furthermore, novel results suggest that despite inhibition of glycogenolysis after GCGR antagonism, IONIS-GCGR$_{Rx}$ did not increase hepatic glycogen content.

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Dysregulation of glucagon secretion resulting in overproduction of hepatic glucose contributes to hyperglycemia in type 2 diabetes (1,2). Because of the progressive nature of type 2 diabetes, its comorbidities, and complexity of treatment, including the potential for severe hypoglycemia, there remains a demand for new safe and effective therapies to help these patients to achieve their target hemoglobin A_{1c} (HbA_{1c}) goals (3–5).

Although type 2 diabetes is characterized, in part, by impaired insulin secretion, the imbalance in the glucagon-insulin ratio favors elevated glucagon levels, resulting in hyperglycemia. Clinical data from randomized controlled studies investigating small-molecule inhibitors targeting the glucagon receptor (GCGR) show that inhibiting GCGR in patients with type 2 diabetes reduces fasting plasma glucose (FPG) and HbA_{1c}, thus validating GCGR as a therapeutic target for controlling hyperglycemia (6–8). However, reversible, dose-dependent increases in circulating lipids, transaminases, liver fat, and blood pressure have been reported (9,10).

Antisense technologies offer a third therapeutic platform with several advantages over other approaches for drug discovery and development, including target specificity (11,12). IONIS-GCGR$_{Rx}$ is a second-generation 2′-*O*-methoxyethyl (2′-MOE) modified antisense oligonucleotide (ASO) designed to selectively bind to the human *GCGR* pre-mRNA and promote RNase H1-mediated degradation of the target RNA (13). IONIS-GCGR$_{Rx}$ is the second of two 2′-MOE ASO inhibitors of similar pharmacokinetic disposition (14,15) that was selected for its higher potency (16,17). As observed with the first 2′-MOE ASO ISIS 325568 (18), IONIS-GCGR$_{Rx}$ produced a dose-dependent increase in plasma glucagon and total GLP-1 levels in healthy volunteers (16). Although mild liver transaminase increases were observed in the upper dose range (200 and 300 mg/week), no clinically significant effect on lipids or blood pressure was observed.

Although previous studies have shown that increases in hepatic transaminases are linked to increases in hepatic fat upon inhibition of GCGR signaling (9), it is still unknown whether other factors also contribute to increased liver transaminases. Because glucagon can stimulate glycogenolysis, inhibiting glucagon action could potentially cause glycogen accumulation in the liver, leading to increased transaminase levels. However, this relationship has not yet been investigated in humans.

We conducted three randomized placebo-controlled studies in patients with type 2 diabetes on stable doses of metformin. Two of these studies focused on safety and efficacy of IONIS-GCGR$_{Rx}$, and the third study was the first, to our knowledge, to specifically address the effect of IONIS-GCGR$_{Rx}$ on hepatic lipid and glycogen content in relationship to hepatic transaminase levels. The results from these studies serve both to identify the minimal efficacious dose of IONIS-GCGR$_{Rx}$ and to understand the effect of GCGR inhibition on hepatic lipid and glycogen content.

RESEARCH DESIGN AND METHODS

Clinical Trial Design and Participants

Three phase 2, double-blind, randomized placebo-controlled trials were conducted to evaluate the safety, tolerability, and efficacy of IONIS-GCGR$_{Rx}$ and the mechanism by which it increases transaminases in patients with type 2 diabetes on stable doses of metformin. First, a 3-month trial (the Initial study) was conducted between August 2013 and April 2014 at 15 sites in two countries (U.S. and South Africa). Subsequently, a 6-month dose-optimization trial (the Dose Optimization study) was conducted between September 2015 and March 2017 at 20 sites in the U.S. Finally, a third trial (the Mechanistic study) was conducted to evaluate the effect of IONIS-GCGR$_{Rx}$ on hepatic lipid and glycogen content at four sites in Austria and Hungary between April 2016 and May 2017. All studies were performed in compliance with the 2002 Declaration of Helsinki and the International Conference on Harmonisation Tripartite Guideline on Good Clinical Practice. Study protocols were approved by each site's institutional review board or independent ethics committee, and written informed consent was obtained from all patients before study participation.

Eligible participants were 18–75 years old with type 2 diabetes, a fasting C-peptide of $\geq$500 pmol/L (Initial and Dose Optimization studies only), HbA_{1c} $\geq$7.5% (58 mmol/mol) and $\leq$10.5% (91 mmol/mol), and BMI $\geq$25 kg/m^2 (and $<$36 kg/m^2 for the Mechanistic study) and were on a stable dose of metformin $\geq$1,000 mg/day ($\geq$1,500 mg/day for the Initial study) for at least 3 months before screening and continued through the study. Patients agreed to maintain their current diet and exercise regimen, conduct daily morning home FPG testing with a study glucometer, and abstain from alcoholic beverages at least 48 h before clinic visits. Exclusions included clinically significant abnormalities in screening laboratory parameters (including transaminases greater than the upper limit of normal [ULN]), complications of diabetes (e.g., painful neuropathy, nephropathy, proliferative retinopathy, foot ulcers), FPG reduction of $>$40 mg/dL during the pretreatment period compared with screening, or treatment with other antidiabetic drugs within 3 months of screening. In addition, in the Mechanistic study, participants were excluded who were contraindicated for MRS procedures (conducted at the Medical University of Vienna).

Each study consisted of four periods: screening, pretreatment, treatment, and posttreatment follow-up (Supplementary Fig. 1). During the pretreatment period, patients conducted daily glucometer assessments at home. Patients in the Mechanistic study also underwent MRS procedures to assess baseline hepatic lipid and glycogen content (overnight fast of at least 10 h was required and confirmed by glucometer). Eligible patients in both the Initial and the Dose Optimization studies were randomized 1:1 to one of two dose cohorts (100 or 200 mg in the Initial study and 50 or 75 mg in the Dose Optimization study) and then randomized 2:1 to receive IONIS-GCGR$_{Rx}$ or placebo in each cohort. Eligible patients in the Mechanistic study were randomized 2:1 to receive IONIS-GCGR$_{Rx}$ 100 mg or placebo. Doses were administered subcutaneously for 13 (Initial and Mechanistic studies) or 26 (Dose Optimization study) weeks. Randomized patients in the Initial and Mechanistic studies received loading doses on days 1, 3, and 5 followed by once-weekly dosing (days 3 and 5 dosing discontinued 2 months after the start of the Initial study for patients randomized to the 200-mg group, and subsequent randomized patients were dosed once weekly).

The loading dose period was designed to rapidly achieve tissue steady-state levels (11,19).

End Point Assessments

The primary efficacy end points for the Initial and Dose Optimization studies were change from baseline (defined as the nonmissing last value before the first dose) to week 14 in fructosamine or week 27 in plasma HbA_{1c}, respectively. Key secondary end points included change from baseline in plasma glucagon, GLP-1, FPG, plasma insulin, and C-peptide after fasting or an oral glucose tolerance test (OGTT).

The pharmacodynamic end points for the Mechanistic study included change from baseline in fasting hepatic lipid and glycogen content, fasting plasma glucagon, and fasting plasma total GLP-1 at week 6, week 14, or posttreatment assessments in the presence of elevated serum transaminase levels versus baseline. The key efficacy end point was change in HbA_{1c} from baseline to week 14.

Hepatic lipid and glycogen content were determined by ^{1}H- and ^{13}C-nuclear MRS at 7 Tesla, respectively (20,21). The coefficient of variation for glycogen at this setting was the same as previously reported at 3 Tesla, mean 10% (20,22,23).

Safety

Safety assessments included adverse events (AEs), vital signs, clinical laboratory tests (Medpace Reference Laboratories, Cincinnati, OH), daily glucose measurements, physical examination, electrocardiogram, and use of concomitant medications. Severe hypoglycemia was defined as FPG $<$60 mg/dL and requiring assistance of another person to obtain treatment for the event. Deteriorating glycemic control was defined as FPG levels $>$270 mg/dL after 6 weeks (Mechanistic study) or 8 weeks (Initial and Dose Optimization studies) or $>$240 mg/dL after 14 weeks (Dose Optimization study) confirmed on two consecutive weekly visits with no other explanations for the elevations.

Statistical Analysis

The sample size for the Initial study was determined to provide 80% power to detect a 40 μmol/L difference in reduction of serum fructosamine between each dose group and pooled placebo group at an α-level of 0.05, with 20 patients in the pooled placebo group and in each IONIS-$GCGR_{Rx}$ dose group. Sample size for the Dose Optimization study was determined to provide 85% power to detect a 1% difference in mean change of HbA_{1c} between each dose group and pooled placebo group at an α-level of 0.05, with 20 patients in the pooled placebo group and 20 patients in each IONIS-$GCGR_{Rx}$ dose group. The sample size of the Mechanistic study was determined to detect differences of $\geq$7% in hepatic glycogen and $\geq$5% in hepatic lipids between pretreatment and posttreatment assessments, with eight patients in the IONIS-$GCGR_{Rx}$ treatment group (24,25).

The safety population for each study consisted of patients who received at least one dose of study drug. For the Initial and Mechanistic studies, the per-protocol population consisted of patients who received at least 75% of their doses without significant misses in dosing early in the treatment period (dose-load patients) or at least 90% of their doses (non–dose-load patients), and for the Mechanistic study, patients completed protocol-required MRS procedures. In the Dose Optimization study, the per-protocol population consisted of patients who received at least 80% of their doses without significant misses in dosing early in the treatment period. The primary analysis compared absolute change from baseline to week 14 or 27 (or early termination) between the IONIS-$GCGR_{Rx}$–treated groups and placebo group for each respective study. The data were analyzed using ANOVA, van Elteren test, or the Wilcoxon rank sum test as appropriate.

RESULTS

Patients

Baseline characteristics and demographics of the three phase 2 studies are shown in Supplementary Table 1. There were no meaningful differences in the demographics between dose groups in each respective study. Baseline HbA_{1c} levels were relatively consistent in each study between their respective dose groups, with a mean range of 8.6–8.9% (71–74 mmol/mol) in the Initial study, 8.8–8.9% (73–74 mmol/mol) in the Dose Optimization study, and 7.9–8.7% (63–72 mmol/mol) in the Mechanistic study. In the Mechanistic study, patients treated with placebo had higher baseline mean fasting hepatic lipid content (23.0%) than IONIS-$GCGR_{Rx}$–treated patients (13.7%) and higher HbA_{1c} levels (8.7% vs. 7.9%, respectively), whereas both dose groups had comparable mean fasting hepatic glycogen content (211.5 vs. 208.9 mmol/L, respectively) (Supplementary Table 1).

In the Initial study, 77 patients were randomized, 75 received study drug, and 61 (81%) completed treatment (Supplementary Fig. 2). Two patients voluntarily withdrew before receiving any study drug (one placebo, one 200 mg). A total of 14 patients discontinued dosing prematurely (3 [11%] placebo, 3 [13%] 100 mg, 8 [31%] 200 mg). All discontinuations because of AEs occurred in IONIS-$GCGR_{Rx}$–treated patients and included elevated transaminases without increased total bilirubin (one 100 mg, two 200 mg), AEs at the injection site (one 200 mg), hypertriglyceridemia (one 100 mg), proteinuria (one 200 mg), and splenomegaly (one 200 mg). The events of hypertriglyceridemia, proteinuria, and splenomegaly were all considered unrelated to study drug. The patient with hypertriglyceridemia had elevated triglyceride levels at screening, which remained high throughout the study.

In the Dose Optimization study, 79 patients were randomized, and 60 (76%) completed treatment (Supplementary Fig. 3). Of the 19 discontinuations, 5 (2 placebo, 3 50 mg) were because of deteriorating glycemic control, with most events occurring after week 14 when the stopping rules became more stringent. Other reasons for discontinuations included voluntary withdrawals/loss to follow-up, noncompliance, AEs at the injection site, or investigator judgment.

In the Mechanistic study, a total of 15 patients were randomized, and 13 (87%) completed treatment (Supplementary Fig. 4). Two patients discontinued dosing prematurely: one because of protocol deviation (patient failed to maintain concomitant statin therapy during treatment period) and the other because of an unrelated AE of hypertriglyceridemia. The patient who discontinued because of hypertriglyceridemia had elevated triglycerides at screening that remained high throughout the study.

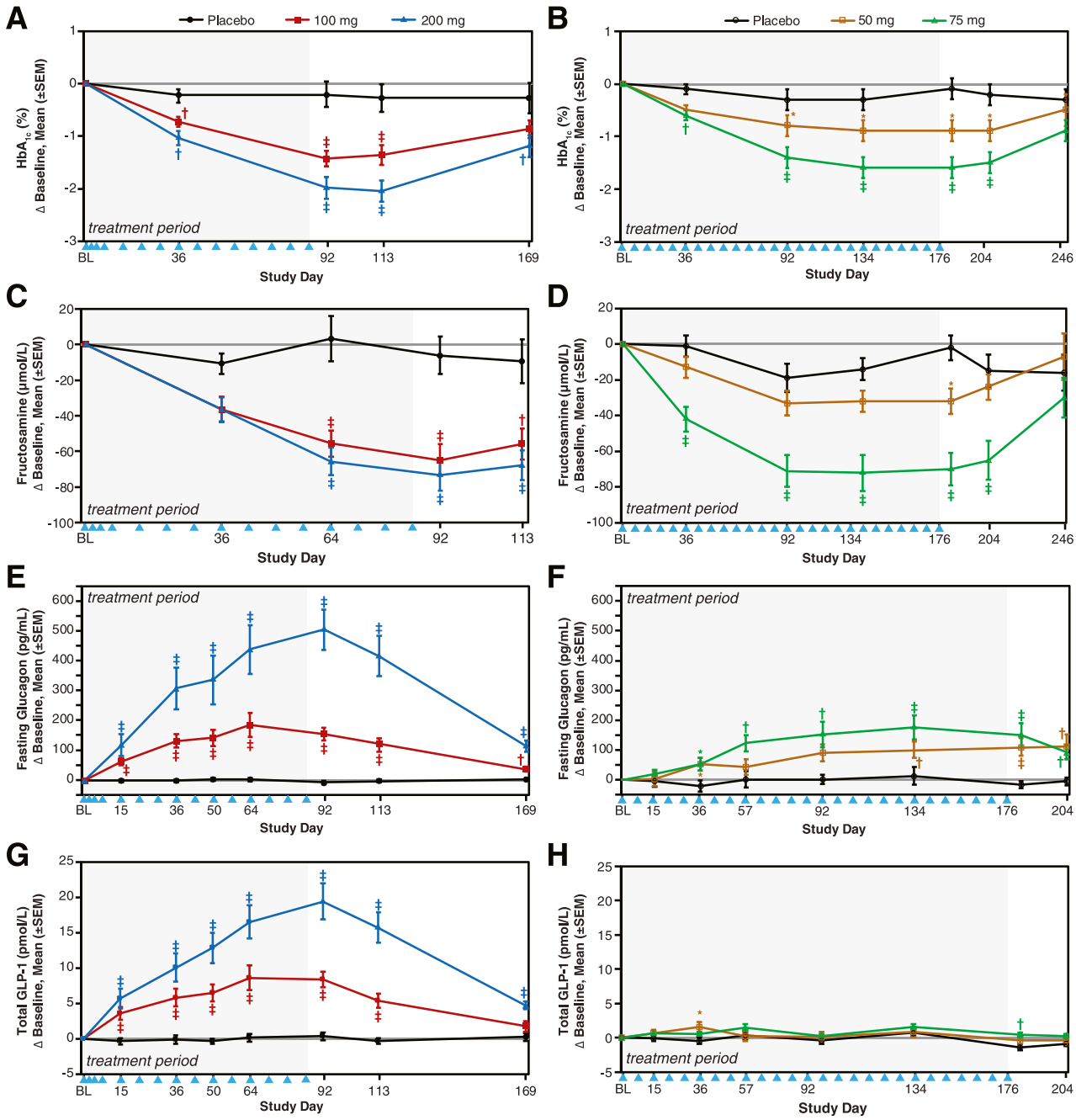

Figure 1—Primary and select secondary end points for the two phase 2 studies of IONIS-GCGR$_{Rx}$. Time course for mean change from baseline in HbA$_{1c}$ (*A* and *B*), fructosamine (*C* and *D*), fasting glucagon (*E* and *F*), and fasting total GLP-1 (*G* and *H*) for the Initial study (*A*, *C*, *E*, and *G*) and Dose Optimization study (*B*, *D*, *F*, and *H*). For the Initial study, dose loading (day 3 and 5 dosing) was discontinued for the 200-mg dose group 2 months after the study began, and every subsequent patient received once-weekly dosing. Light blue triangles represent days that a patient received the study drug. Error bars are the SEM. *$P < 0.05$; †$P < 0.01$; ‡$P < 0.001$. BL, baseline.

Glycemic Parameters

Both the Initial and Dose Optimization studies met their respective primary efficacy end points (Supplementary Table 2), including significant dose-dependent reductions in HbA$_{1c}$ in all dose groups after 13 and 26 weeks of treatment. In the Initial study, significant mean (SD) reductions from baseline in HbA$_{1c}$ were observed at week 14 in the IONIS-GCGR$_{Rx}$ 100-mg (−1.4% [0.7%] [−15.3 (7.7) mmol/mol]) and 200-mg (−2.0% [0.9%] [−21.9 (9.8) mmol/mol]) groups versus placebo (−0.3% [1.2%] [−3.3 (13.1) mmol/mol]; both $P < 0.001$) and remained decreased by the end of the study (Fig. 1*A* and Supplementary Table 2). In the Dose Optimization study, statistically significant reductions from baseline in mean (SD) HbA$_{1c}$ were observed at week 27 in the IONIS-GCGR$_{Rx}$ 50-mg (−0.9% [0.8%] [9.8 (8.7) mmol/mol]) and 75-mg (−1.6% [1.0%] [−17.5 (10.9) mmol/mol]) groups versus placebo (−0.2% [0.8%] [2.2 (8.7) mmol/mol]; $P = 0.017$ and $P < 0.001$, respectively) (Fig. 1*B* and Supplementary Table 2).

Glycemic efficacy assessments were consistent between the two studies,

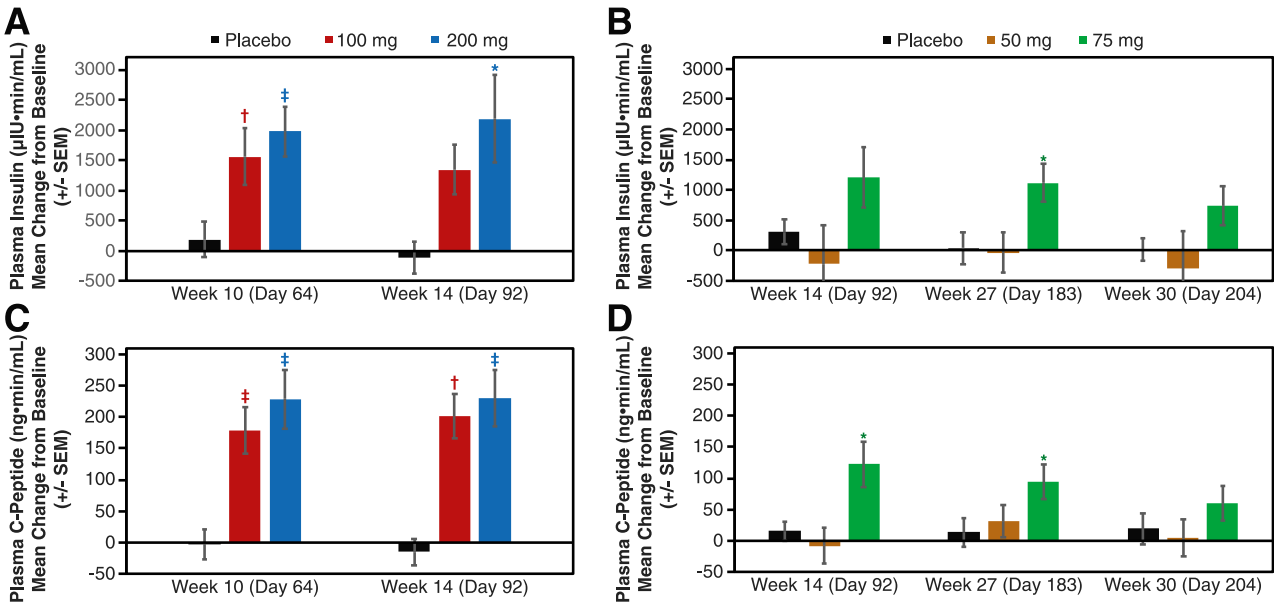

Figure 2—Incremental AUC analysis after a 2-h OGTT for the two phase 2 studies. Plasma insulin (*A* and *B*) and plasma C-peptide (*C* and *D*) for the Initial study (*A* and *C*) and the subsequent Dose Optimization study (*B* and *D*). Error bars are the SEM. **P* < 0.05; †*P* < 0.01; ‡*P* < 0.001. IU, international units.

with a dose-dependent response observed for each parameter. In the Initial study, significant mean (SD) reductions in serum fructosamine from baseline were observed in the IONIS-GCGR$_{Rx}$ 100-mg (−62 [40] μmol/L) and 200-mg (−73 [35] μmol/L) groups compared with placebo (−7.6 [49] μmol/L; both $P < 0.001$) (Fig. 1*C* and Supplementary Table 2). Significant reductions from baseline in mean (SD) serum fructosamine were also observed in the Dose Optimization study at week 27 for both the IONIS-GCGR$_{Rx}$ 50-mg (−32 [33] μmol/L) and 75-mg (−70 [44] μmol/L) groups compared with placebo (−3 [34] μmol/L; $P = 0.016$ and $P < 0.001$, respectively) (Fig. 1*D* and Supplementary Table 2). Mean weekly FPG and self-monitored plasma glucose weekly average also showed a dose-dependent improvement over time (Supplementary Fig. 5).

Glucagon increased in a dose-dependent manner in both studies and reversed after cessation of study drug administration (Fig. 1*E* and *F* and Supplementary Table 2). Although an increase from baseline in fasting total GLP-1 was also observed in the Initial study at both 100 mg (8.38 pmol/L; $P < 0.001$) (Fig. 1*G*) and 200 mg (19.39 pmol/L; $P < 0.001$) (Fig. 1*G*), this effect was diminished at 75 mg (0.40 pmol/L; $P = 0.018$) (Fig. 1*H*), and no significant change was observed at 50 mg (−0.41 pmol/L; $P = 0.280$) (Fig. 1*H* and Supplementary Table 2).

To assess the postprandial glycemic control, OGTT was performed in the Initial and Dose Optimization studies. Consistent with dose-dependent increases in fasting total GLP-1, statistically significant increases in the incremental area under the curve (AUC) for plasma insulin and C-peptide were observed during OGTT at week 10 (day 64) and week 14 (day 92) in the Initial study (Fig. 2*A* and *C*) and week 27 (day 183) in the Dose Optimization study (Fig. 2*B* and *D*). However, the incremental AUC for plasma glucose remained relatively unchanged compared with placebo (Supplementary Table 3).

Serum Transaminase Levels

Dose-dependent and reversible increases in transaminase levels were observed after treatment with IONIS-GCGR$_{Rx}$ at doses of 50–200 mg (Fig. 3), consistent with the pharmacology of GCGR inhibition. There were no cases of associated increases in bilirubin or alkaline phosphatase and no cases of Hy's law. No stopping rules for increased transaminases were met in any study; however, some elevations led to dose adjustments (e.g., interruption, reducing dose or frequency, discontinuation). In the Initial study, 13 of 26 (50%) patients treated with IONIS-GCGR$_{Rx}$ 200 mg and 3 of 23 (13%) treated with IONIS-GCGR$_{Rx}$ 100 mg experienced confirmed ALT >3× ULN (Supplementary Table 4). In the Dose Optimization study, 3 of 26 (12%) patients treated with IONIS-GCGR$_{Rx}$ 75 mg and none treated with IONIS-GCGR$_{Rx}$ 50 mg reported confirmed ALT >3× ULN (Supplementary Table 4). No patients in the Mechanistic study reported confirmed increases >3× ULN in either ALT or AST levels (Supplementary Table 5).

Hepatic Lipid and Glycogen Content

In the Mechanistic study, we assessed the effect of IONIS-GCGR$_{Rx}$ on liver glycogen and fat in relationship to the increase in ALT. As expected on the basis of the Initial study, IONIS-GCGR$_{Rx}$ 100-mg treatment for 13 weeks reduced HbA$_{1c}$ levels by a mean of 1% (Fig. 4*A*), with a mean ALT increase of 22 units/L at week 14 (Fig. 4*B*). By MRS, treatment with IONIS-GCGR$_{Rx}$ 100 mg did not show a significant change from baseline in hepatic glycogen content compared with placebo at week 6 (mean [SD] increase of 23.7 [24.6] vs. 24.2 [30.1] mmol/L, respectively; $P = 0.833$) or week 14 (mean [SD] change of 15.1 [39.3] vs. −20.2 [34.0] mmol/L, respectively; $P = 0.093$) (Fig. 4*C*). However, mean (SD) hepatic lipid content significantly increased from baseline with IONIS-GCGR$_{Rx}$ treatment compared with placebo at both week 6 (3.0% [3.0%] vs. −2.4% [2.5%], respectively; $P = 0.012$) and week 14 (4.2%

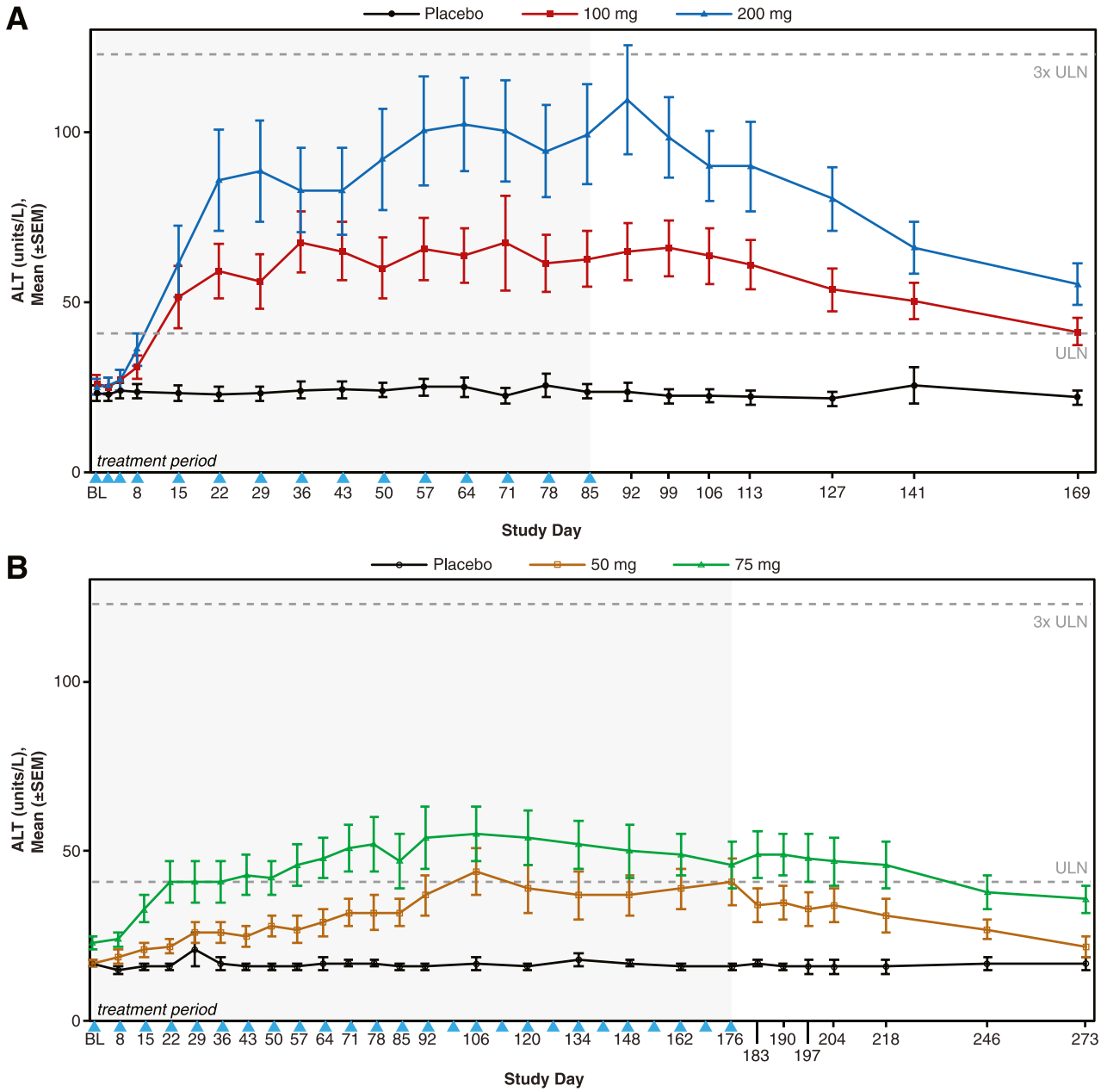

Figure 3—Mean ALT levels over time in the Initial study (*A*) and Dose Optimization study (*B*). Light blue triangles represent days that a patient received the study drug. ULN (41 units/L) is represented by the bottom dashed line. Top dashed line represents 3× ULN (123 units/L). Error bars are the SEM. BL, baseline.

[3.7%] vs. −2.7% [3.5%], respectively; P = 0.005) (Fig. 4*D* and Supplementary Table 6). Univariate regression analysis showed a strong association between changes in hepatic lipid content at week 14 and maximum fold change in ALT level (IONIS-GCGR$_{Rx}$ r = 0.906; P = 0.002), whereas no association was observed between changes in hepatic glycogen content and maximum fold change in ALT levels or hepatic lipid content (IONIS-GCGR$_{Rx}$ r = −0.420 [P = 0.301] and −0.369 [P = 0.369], respectively) (Supplementary Table 7).

Safety

A total of six serious AEs (SAEs) were observed across studies, with one in each treatment group and two in the placebo group. No SAEs led to discontinuation. Two SAEs were reported in the Initial study: one patient treated with IONIS-GCGR$_{Rx}$ 100 mg experienced a moderate SAE of hepatitis B, which was considered unrelated to the study drug, and one patient treated with IONIS-GCGR$_{Rx}$ 200 mg experienced a moderate worsening of depression, which was considered possibly related. Three SAEs were reported in the Dose Optimization study: one patient in the placebo group experienced on-treatment unstable angina, one treated with IONIS-GCGR$_{Rx}$ 50 mg experienced on-treatment hypotension, and one treated with IONIS-GCGR$_{Rx}$ 75 mg was diagnosed with recurrent endometrial cancer during posttreatment follow-up, all considered unrelated to the study drug. In the Mechanistic study, one unrelated SAE of depression was reported in a patient treated with placebo who had ongoing depression and recovered after psychiatric rehabilitation.

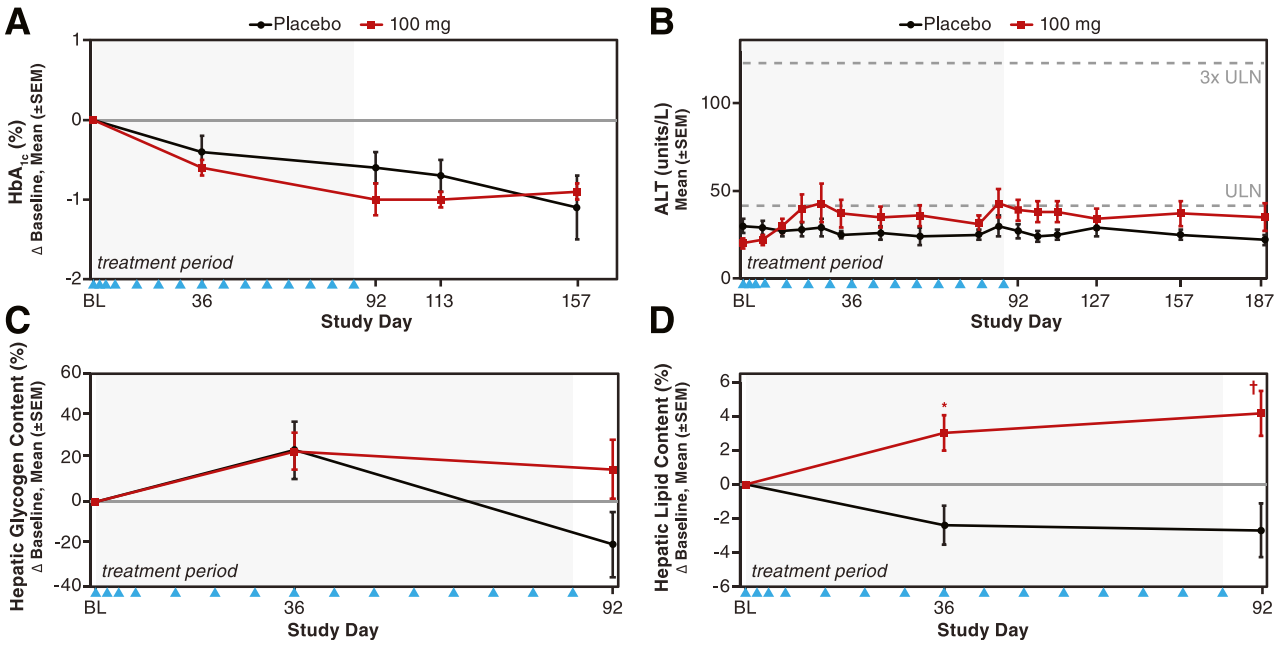

Figure 4—Results from the Mechanistic study. Time course for mean (SE) change from baseline in HbA_{1c} (*A*), ALT (*B*), fasting hepatic glycogen content (*C*), and fasting hepatic lipid content (*D*). Triangles represent days that a patient received the study drug. Error bars are the SEM. *$P < 0.05$; †$P < 0.01$. BL, baseline.

AEs at the injection site occurred in a mean of 6.4%, 4.4%, and 6.7% of IONIS-$GCGR_{Rx}$ injections in the Initial, Dose Optimization, and Mechanistic studies, respectively (Supplementary Tables 4 and 5). No flu-like reactions (defined as influenza-like illness or pyrexia plus at least two of the following: chills, myalgia, or arthralgia that started on or the day after receiving study drug injection) were reported in any study.

Outside of AEs at the injection site and increases in transaminases, urinary tract infection was the most common AE in the Initial and Dose Optimization studies, with similar incidences between the placebo and IONIS-$GCGR_{Rx}$–treated groups (Supplementary Table 4), whereas diarrhea and nasopharyngitis were most common in the Mechanistic study (Supplementary Table 5).

There were no cases of severe or symptomatic hypoglycemia in any of the studies; however, one patient (100-mg group) in the Initial study experienced unrelated mild asymptomatic hypoglycemia that resolved after self-treatment with an oral glucose source. Five patients experienced hyperglycemia in the Dose Optimization study, with four moderate in severity (two placebo, two 50 mg) and one mild (one 75 mg). Four of the five patients recovered spontaneously or after additional glucose-lowering treatment. No clinically relevant changes in blood pressure, body weight, lipids, hematology, or other vital signs were observed (Supplementary Tables 8–10).

CONCLUSIONS

Inhibition of GCGR with IONIS-$GCGR_{Rx}$ in patients with type 2 diabetes on metformin led to statistically significant dose-dependent improvements in markers of glycemic control, including HbA_{1c} and serum fructosamine without increasing the risk of symptomatic hypoglycemia. No unexpected safety findings or tolerability issues were observed with IONIS-$GCGR_{Rx}$ treatment in any of the studies. However, like other GCGR antagonists, we observed reversible and dose-dependent elevations in liver transaminases, which upon further investigation, were found to be directly associated with increases in hepatic fat content. This study also demonstrated for the first time in our knowledge that hepatic glycogen levels are unaffected by GCGR inhibition, suggesting that an increase in hepatic lipid content, but not glycogen, likely contributes to transaminase elevations after GCGR antagonism.

Dose-dependent increases in liver transaminase levels during IONIS-$GCGR_{Rx}$ treatment normalized posttreatment in all three studies. Although similar reversible increases in ALT and AST have been observed in previous clinical studies of GCGR inhibition by small molecules (6–8), we were able to improve the hepatic safety profile by reducing doses to as low as 50 mg and still maintain efficacy as evidenced by a significant 0.8% decrease in HbA_{1c} over placebo ($P = 0.017$). Previous studies showed that these increases are accompanied by an increase in hepatic fat content after treatment with a small-molecule inhibitor of GCGR (9).

Inhibition of GCGR could also decrease glycogenolysis and lead to glycogen accumulation in the liver. Therefore, to test whether hepatic glycogen content also contributed to liver transaminase increases, we chose a dose (100 mg) that would increase the serum ALT levels versus baseline values for the Mechanistic study. As anticipated, HbA_{1c} reductions were accompanied by increases in ALT after 13 weeks of treatment with IONIS-$GCGR_{Rx}$. Despite glucagon causing a robust effect on glycogenolysis, the absolute changes seen in liver glycogen levels after inhibition of GCGR were well within those observed under physiological conditions (20,22). In contrast, significant mean increases in

hepatic lipid content were observed, consistent with previously published results for the small-molecule antagonist LY2409021 (9). Although a limited data set, these results suggest that the liver has the ability to autoregulate, and thereby prevent, an increase in hepatic glycogen content.

IONIS-GCGR$_{Rx}$ was generally well-tolerated, with no evidence of severe or symptomatic hypoglycemia. No flu-like reactions were reported in any study. Rates of AEs at the injection site were low ($<$10%) in all studies compared with other second-generation 2′-MOE ASOs (26–28). Discontinuations as a result of AEs at the injection site occurred in the first 2 months of treatment, with no dose-dependent association. Most AEs leading to discontinuation were due to increases in hepatic transaminases, a known pharmacological side effect of GCGR inhibition. Although the initial 2′MOE ASO targeting GCGR showed no increase in hepatic transaminase levels (18), dose-dependent increases were observed with IONIS-GCGR$_{Rx}$ treatment. This effect is attributed to an increase in potency of IONIS-GCGR$_{Rx}$ (16).

There has been continued interest in developing an antagonist of GCGR for diabetes management, and multiple antagonists have been tested in humans with variable nonglycemic effects (29,30). Understandably, there are drug-specific and mechanism-specific differences between antagonists of GCGR. For instance, among the small molecules and antibodies that bind to GCGR, there can be differential effects as a result of binding specificity for GCGR as well as of other G-protein–coupled receptors, especially the highly homologous GLP receptors in the GCGR subfamily (31). IONIS-GCGR$_{Rx}$, on the other hand, is a very specific inhibitor of GCGR and unlikely to affect structurally similar proteins. Furthermore, IONIS-GCGR$_{Rx}$ does not appear to completely attenuate GCGR expression as evidenced by the absence of a plateau effect with increases in glucagon levels up to the highest dose tested in the current phase 2 studies. Because IONIS-GCGR$_{Rx}$ only partially inhibits GCGR (primarily hepatic), it may not be enough to elicit the compensatory increase in sympathetic activity, as observed in GCGR knockout mice (32), and therefore, does not increase blood pressure. Small-molecule GCGR inhibitors also increase absorption of cholesterol in the gastrointestinal tract (33), which may also explain an increase in serum lipids as well as a secondary increase in body weight. Because systemically administered second-generation ASOs are poorly distributed to the gut (11,34), we would not expect an increased absorption of cholesterol with IONIS-GCGR$_{Rx}$ and consequent increase in lipids or body weight. The absence of these side effects represents a potential improvement over previous small-molecule approaches to GCGR inhibition (7,10). Additional studies are needed, however, to confirm these initial results.

IONIS-GCGR$_{Rx}$ 100 mg and 200 mg produced robust and dose-dependent increases in fasting total GLP-1 in patients with type 2 diabetes and healthy volunteers without significantly modifying fasting active GLP-1. The incretin increase correlated with significant increases in plasma insulin and C-peptide during OGTT at week 10 and week 14. However, this effect was diminished when the IONIS-GCGR$_{Rx}$ dose was lowered to 50 mg in the Dose Optimization study, suggesting that hepatic glucose production is the main mechanism by which IONIS-GCGR$_{Rx}$ improves glycemia. Of note, despite the increase in insulin, incremental glucose AUC during OGTT did not show a significant decrease in any dose group after treatment with IONIS-GCGR$_{Rx}$. Although this result suggests a lack of postprandial action at the lowest dose tested, it is in line with findings from studies on the small-molecule antagonist LGD-6972 (6) and may reflect large glucose variations at the individual level. Thus, larger studies are needed to fully understand the action of IONIS-GCGR$_{Rx}$ in the postprandial state.

Previous studies in mouse models showed that reducing GCGR expression with GCGR ASOs resulted in α-cell hyperplasia similar to GCGR knockout mice and in combination with the increase in pancreatic preproglucagon expression, led to elevated levels of GLP-1 (32,35). However, in cynomolgus monkeys treated with GCGR ASOs, GLP-1 levels were increased by three- to fivefold, similar to those observed in clinical studies, without pancreatic α-cell expansion or hyperplasia at doses up to five times higher than the highest dose tested in humans (T. Zanardi, unpublished data). Furthermore, knocking in only 30–35% of GCGR back into the livers of GCGR knockout mice was enough to reverse the GLP-1/glucagon increase, suggesting that this feedback increase occurs at a higher magnitude of hepatic GCGR reduction. Because the increase in GLP-1 levels is a feedback effect secondary to hepatic GCGR inhibition, the lowest dose of IONIS-GCGR$_{Rx}$ likely did not cause the magnitude of hepatic GCGR reduction required to initiate this feedback effect.

Limitations of these findings include the relatively small sample size in each study, especially in the exploratory Mechanistic study. In addition, conclusions from the Mechanistic study are limited by the imbalance between hepatic fat at baseline and a strong placebo effect. An increasing effect from the placebo group has been observed in diabetes trials over the past couple of decades (36). Therefore, larger studies on IONIS-GCGR$_{Rx}$ are needed to further define the role of hepatic glycogen content on hepatic transaminase increases as a result of GCGR antagonism. Nevertheless, the absolute changes in liver glycogen content were within physiological levels, including those seen between fed and fasted conditions. Despite these limitations, results from the current phase 2 studies are generally consistent in glycemic improvement and ALT elevations observed with other pharmacologic agents antagonizing GCGR (7,8,30).

Longer studies with adjustable dosing of IONIS-GCGR$_{Rx}$ on the basis of glucose and ALT response will help to assess the risk-benefit ratio of the drug because glycemic goals should be individualized in patients with diverse risk factors, concomitant medications, and comorbidities. In addition, metformin could potentially be leading to underestimations of the effects of IONIS-GCGR$_{Rx}$ as an add-on therapy; therefore, future studies that assess IONIS-GCGR$_{Rx}$ as monotherapy or concomitantly with other diabetes medications may be useful.

In conclusion, IONIS-GCGR$_{Rx}$ is a potent and well-tolerated inhibitor of hepatic GCGR action and represents a novel approach toward improving glycemic control in patients who remain uncontrolled on currently available therapies. Robust and sustained reductions in HbA_{1c}, fructosamine, and other markers of glycemic control were observed at all doses tested without any cases of symptomatic hypoglycemia. No unexpected

safety findings were reported, and liver transaminase increases were monitorable and manageable by dose adjustment. Although glucagon stimulates glycogenolysis among other functions, we found that antisense inhibition of GCGR with IONIS-GCGR$_{Rx}$ did not lead to significant increases in hepatic glycogen content compared with placebo but did lead to an increase in hepatic lipid content. These findings represent a novel addition to our understanding of the mechanism for liver transaminase elevations after GCGR antagonism.

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