Preservation of beta cell function with anti-IL-21 antibody and liraglutide in adults with recent-onset type 1 diabetes

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Abstract

Background: Type 1 diabetes (T1D) is characterised by loss of functional beta-cell mass necessitating insulin treatment. We hypothesise that combining low-grade and transient immune-modulation with a therapy to improve beta-cell function could enable beta-cell survival with a lower risk of the complications associated with traditional immuno-modulation.

Methods: 308 adults recently diagnosed with T1D and residual C-peptide secretion were randomly assigned 1:1:1:1 to treatment in a double-blind, double-dummy manner with either anti-interleukin (IL)-21 plus liraglutide, anti-IL-21, liraglutide or placebo, all as adjunct to insulin. The trial comprised a 54-week treatment period followed by a 26-week observation period. For the primary endpoint (ratio of baseline versus week 54 meal-stimulated C-peptide secretion), there was 80% power to detect a decline of 2% with combination treatment compared to 35% with placebo.

Findings: Stimulated C-peptide secretion decreased across groups; compared with placebo (-39%), the decrease from baseline at week 54 was significantly smaller (p=0·0017) with the combination (-10%) but not with anti-IL-21 (-25%) or liraglutide alone (-32%). Combination treatment (vs placebo) reduced insulin requirements (p=0.0006), glycated haemoglobin levels and the risk of hypoglycaemia. Benefits diminished upon treatment cessation. Changes in immune cell subsets across groups were transient and mild (<10% change over time). Generally, treatments were well-tolerated with no safety concerns.

Interpretation: The novel combination of anti-IL-21 and liraglutide could preserve beta-cell function and improve glycaemic control in recently diagnosed T1D. The efficacy of this regimen appears comparable with that seen in other disease-modifying trials whilst providing a seemingly better side-effect profile.

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Introduction

Type 1 diabetes (T1D) is an autoimmune disease characterized by progressive loss of functional beta-cell mass. The incidence of T1D is increasing by 3% annually in high-income countries[1]; yet, the only currently available treatment for most people is life-long administration of exogenous insulin.

Disease-modifying therapeutic interventions in T1D have focused on disease prevention in high-risk individuals and the preservation of residual beta-cell function soon after diagnosis.[2-9] Both strategies have the potential to lead to better long-term glycaemic control, with lower rates of both hypoglycaemia and diabetes-related complications. Beta-cell function can be estimated based on C-peptide levels and patients with higher C-peptide levels have better glycaemic control and fewer and less pronounced long-term complications.[10,11] Clinical trials have confirmed that the rate of post-diagnosis decline of C-peptide levels can be attenuated; [2-4,8,9] in all trials, however, the benefit has lasted for the duration of treatment. While this indicates that prolonged treatment is necessary, it carries the advantage that, unless the intervention induces irreversible changes to the immune system, it may be possible to avoid that potential long-term benefits come at the expense of frequent side effects related to immune suppression such as infections[12] including Epstein-Barr virus (EBV) reactivation[13].

We hypothesize that a disease-modifying therapy combining milder immune modulation with a beta-cell-focused agent to improve cell function as well as prevent beta-cell apoptosis under immune stress[14] could ensure beta-cell survival with a lower risk of complications compared with traditional immunomodulation in newly diagnosed T1D.

Anti-interleukin (IL)-21 is a promising candidate for immunotherapy in T1D because the IL-21 pathway has been linked to diabetes progression both in animal models[15,16] and humans[17,18], putatively owing to the central role of the cytokine in promoting the trafficking of CD8+ lymphocytes from the lymph nodes and/or exocrine pancreas to the islets[19]. Further, non-clinical investigations found a minor impact of IL-21 blockade on the immune repertoire[19].

Glucagon-like peptide-1 receptor agonists (GLP-1 RA) such as liraglutide have been proposed to relieve beta-cell stress and prevent apoptosis[20], protect against cytokine-mediated inhibitory effects on glucose-stimulated insulin secretion[14] and ameliorate the proinsulin-to-insulin processing defects seen in T1D and type 2 diabetes.[21] Moreover, GLP-1 RAs have proven benefits on glycaemic control, body weight and cardiovascular risk.[22,23] Thus, pursuing the concept of a combination therapy integrating immuno-modulation and a component aimed at another mechanistically distinct target[24,25], the combination of IL-21 blockade and a GLP-1 RA had shown promising results in a mouse-model[26].

To elucidate these aspects in the clinical setting, we investigated the disease-modifying potential of anti-IL-21 and liraglutide in combination and as monotherapies, all as adjuncts to insulin, in a placebo-controlled trial in adults recently diagnosed with T1D.

Methods

Trial design

This was an 80-week (54-week treatment period followed by a 26-week off-drug observation period), randomized, parallel-group, placebo-controlled, double-dummy, double-blind, efficacy, safety and clinical proof-of-principle trial in adults with recently-diagnosed T1D and residual beta-cell function. The trial was carried out at 95 sites in 17 countries. The trial is registered with ClinicalTrials.gov (NCT02443155), was approved by Independent Ethics Committees and Institutional Review Boards at each participating country/site, and was undertaken in accordance with ICH Good Clinical Practice guidelines and the Declaration of Helsinki. Investigators, participants and sponsor (Novo Nordisk) personnel were blinded throughout the treatment period. After all participants had completed the treatment period, Novo Nordisk personnel became unblinded due to an internal analysis aimed to inform on the future clinical development programme. Data were collected by site investigators via electronic case report forms.

Participants

Eligible individuals had been diagnosed with T1D within 20 weeks before screening with a peak mixed-meal tolerance test (MMTT)-derived C-peptide level ≥0·2 nmol/L and with antibodies against glutamic acid decarboxylase (GAD), islet antigen[IA]-2 and/or zinc-transporter-8 (ZnT8). Individuals were not enrolled if their T1D was considered unstable (an episode of severe diabetic ketoacidosis within 2 weeks of enrolment) or if they were at risk of infectious or chronic disease manifestations such as tuberculosis or hepatitis. All individuals provided written informed consent before eligibility screening.

Procedures

At week 0 (baseline), participants were randomly assigned 1:1:1:1 to treatment with one of the following: combination of anti-IL-21 and liraglutide, anti-IL-21, liraglutide or placebo. Randomisation was stratified according to the peak

MMTT-derived C-peptide level at baseline (≥0·2 nmol/L to ≤0·6 nmol/L or >0·6 nmol/L). Anti-IL-21 at a dose of 12 mg/kg and placebo for anti-IL-21 were administered intravenously every 6 weeks during visits to the trial sites. Liraglutide and placebo for liraglutide were self-administered as once-daily subcutaneous injections; there were no indications that treatment non-compliance differed relevantly across groups. The liraglutide dose was escalated from 0·6 mg to a target of 1·8 mg/day in steps of 0·6 mg every 2 weeks. In the case of intolerable AEs related to dose escalation, the dose could be reduced to 1·2 mg.

Participants received treatment for 54 weeks and were subsequently observed for 26 weeks following treatment cessation. All participants were on a treat-to-target insulin regimen throughout the trial. Participants were withdrawn from the trial if treatment was discontinued.

Outcomes

The primary outcome evaluated the decline in C-peptide secretion from baseline to week 54 and was assessed as the MMTT-stimulated C-peptide area under the concentration-time curve (AUC) over a 4-hour period at week 54 relative to baseline ($AUC_{0\text{-}4h,C\text{-}peptide,week\ 54}/AUC_{0\text{-}4h,\ C\text{-}peptide,\ baseline}$). MMTTs were scheduled at baseline and at weeks 12, 24, 36, 54, 65 and 80. Other key efficacy outcomes included change from baseline to weeks 54 and 80 in MMTT-derived C-peptide and plasma glucose, insulin use, glucose metabolism (glycated haemoglobin and glucagon) and pharmacokinetics (PK). Auto-antibodies against insulin, GAD, ZnT8 and IA2 were evaluated at the start of the trial and monitored throughout using commercial RIA, EIA and ELISA assay kits. The frequency and phenotype of circulating immune cell subsets (B, T, natural killer, and myeloid cells) were measured using peripheral blood mononuclear cells and flow cytometry.

Key safety outcomes evaluated through week 80 included numbers of treatment-emergent AE, diabetic ketoacidosis, and hyperglycaemic and hypoglycaemic episodes. ‘Severe’ hypoglycaemic episodes were defined according to the ADA classification[27]; ‘severe or blood-glucose-confirmed symptomatic’ episodes also included those that were symptomatic and associated with a plasma glucose value <3.1 mmol/l (56 mg/dl).

Statistical analysis

Sample size calculation

The trial was powered for the combination treatment vs placebo comparison for the primary endpoint, for which the assumed ratio to baseline was 0·98 (-2%) and 0·65 (-35%) for the combination and placebo treatments, respectively, corresponding to an assumed treatment ratio of 1·50 (treatment effect of 33 %-points). The standard deviation on the log-transformed primary endpoint was assumed to be 0·5 for the combination treatment and 1·0 for placebo. With 60 subjects completing the trial in each of these two groups, the power would be 80·4% to detect a statistically significant treatment ratio. To account for non-completers, 77 subjects were randomly assigned to each treatment. The sample size for the anti-IL-21 and liraglutide groups was set to 77 subjects.

Primary endpoint

$AUC_{0\text{-}4h,\ C\text{-}peptide,t}$ / $AUC_{0\text{-}4h,\ C\text{-}peptide,\ baseline}$ at available timepoints (t) from baseline (week 0) to end-of-treatment (week 54) were log-transformed and analysed using a mixed model for repeated measurements combined for all four treatments and including all available assessments for subjects in the full analysis set with a least one available observation (intention-to-treat approach). Missing data were assumed to be missing at random. In the model, treatment, C-peptide stratum and sex were factors, and log($AUC_{0\text{-}4h,\ C\text{-}peptide,\ baseline}$) and age at baseline were covariates; further, the interaction between all variables and time was included as a fixed effect. Estimated geometric mean ratios to baseline (presented as %-changes) and treatment ratios with 95% confidence intervals were derived from the model. In a subgroup analysis of the primary endpoint, the interaction between C-peptide stratum and treatment was added to the model.

Secondary endpoints

A model similar to the one used for the primary endpoint was applied in the analysis of the other efficacy endpoints. Changes from baseline in glycated haemoglobin and immune biomarkers were analysed on the original scale, and the change from baseline in total daily insulin dose (average of the doses reported on the three days prior to the trial-site visits) was analysed using a normal linear regression model with treatment, stratum and sex as factors, and the baseline value and age at baseline as covariates.

The total number of treatment-emergent 'severe or blood-glucose-confirmed symptomatic' and nocturnal 'severe or blood glucose-confirmed symptomatic' hypoglycaemic episodes were analysed using a negative binominal regression model with a log link function, with the logarithm of the exposure time as an offset. The model included treatment, C-peptide stratum and sex as factors, and age as a covariate. In a post-hoc analysis, the total number of treatment-emergent hyperglycaemic episodes (PG values >16·7 mmol/l (300 mg/dl) were analysed using a similar negative binominal regression model.

No multiplicity correction was applied in any of the analysis, which were all performed using SAS version 9.4.

Role of the funding source

The trial was sponsored by Novo Nordisk who designed the trial, monitored trial sites, and collected, analysed and interpreted the data. Medical writing and editorial support was funded by Novo Nordisk. The trial did not have a data monitoring committee. All authors had access to trial data upon request. The manuscript was drafted by the corresponding author under the guidance of all authors. The authors assume responsibility for the accuracy and completeness of the manuscript and the decision to submit it for publication.

Results

Between November 2015 through February 2019, 553 adults were assessed for eligibility. Of these, 308 participants were randomly assigned to treatment, which was received by 307 participants; one participant did not receive treatment and was excluded from the full analysis set. A total of 212 individuals screened for eligibility were not enrolled because they did not meet one or more eligibility criteria; the most common reasons for ineligibility were absence of islet-specific autoantibodies (56 individuals), presence of certain laboratory abnormalities at screening (46 individuals), stimulated peak C-peptide ⩾0.2 nmol/L (45 individuals) as well positive test result for tuberculosis (32 individuals) or hepatitis B (22 individuals).

The trial profile is presented in **Figure 1**. The full analysis set and the safety analysis set each comprised the 307 participants. The PK analysis set comprised 84 participants, which was considered sufficient to obtain an adequate precision for the PK outcomes. Across the four 77-participant groups, 8 to 12 participants did not complete the treatment period.

In line with the randomised group assignment, participant characteristics (including age, gender, BMI, duration of diabetes, glycated haemoglobin levels and total daily insulin dose) were similar across treatment groups at baseline (**Table 1**). Across groups, the glycated haemoglobin level was 7·0% to 7·3% (53 to 56 mmol/mol) and most participants (58 to 65%) had a C-peptide level >0·6 mmol/L.

With the combination therapy and with liraglutide alone, an initial increase in MMTT-derived stimulated C-peptide secretion ($AUC_{0\text{-}4h}$) was observed; however, by end-of-treatment (week 54) and at the end of the off-drug observation period (week 80), the secretion had decreased in all groups (**Figure 2a**). The estimated decrease in C-peptide secretion from baseline to week 54 (primary endpoint) was statistically significantly smaller with the combination of anti-IL-21 and liraglutide (10% decrease) than with placebo (39% decrease) with an estimated treatment ratio (ETR) of 1·48 $[1·16;1·89]_{95\%CI}$ (p=0·0017) (**Figure 2b**). At week 54, the MMTT-stimulated C-peptide secretion was around 48% greater with the combination treatment ($AUC_{0\text{-}4h}$ of 1·84 nmol×h/L) than with placebo ($AUC_{0\text{-}4h}$ of 1·24 nmol×h/L) (**Figure 2a**). The change from baseline to week 54 in MMTT-stimulated C-peptide secretion did not differ statistically significantly between the monotherapies (anti-IL-21 or liraglutide alone) and placebo (**Figure 2b**). Change from baseline in MMTT-stimulated C-peptide secretion were comparable whether based on 4-hour (**Figure 2a-b**) or 2-hour AUCs **(Supplementary Table S1**). Further, the findings were corroborated by stimulated plasma glucose values, which were reduced by the liraglutide-containing regimens (**Supplementary Table S1**) with a statistically significant difference at week 54 between the combination treatment and placebo (ETR for $AUC_{0\text{-}4h}$ of 0·88 $[0·79;0·98]_{95\%CI}$). Analysis of fasting C-peptide also supported the above results (**Figure 3a-b**): At week 54, fasting C-peptide levels largely had not changed in the combination therapy group; in contrast, the secretion had decreased by 36% in the placebo group; the change difference was statistically significant (p=0·0003; **Figure 3b**).

A subgroup analysis demonstrated statistically significant (p=0·0214 for a test of interaction between treatment and C-peptide stratum) differential treatment effects depending on the baseline C-peptide level (>0·2 nmol/L to ≤0·6 nmol/L or >0·6 nmol/L) (**Supplementary Figure S1a-f**) as exemplified by participants with a baseline value >0·6 nmol/L, in whom the change from baseline in stimulated C-peptide secretion at week 54 was similar between the combination therapy and liraglutide alone.

With the combination of anti-IL-21 and liraglutide, the required total daily insulin dose decreased from baseline to week 54 by 12% (0·04 U/kg body weight) (**Supplementary Figure S2a-b**); the decrease was statistically significant (p=0·0006) compared with the change with placebo (dose increase of 28% [0·09 U/kg]). Despite the greater insulin use in the placebo group in this treat-to-target trial (**Supplementary Figure S2**), the decrease in glycated haemoglobin at week 54 was greater with all active treatments (-0·50 %-points across groups) than with placebo (-0·10 %-points) (**Figure 4a-b**) although the treatment differences were not statistically significant. Fasting glucagon levels did not change relevantly in any of the groups.
Body weight (protocol-defined safety endpoint) decreased from baseline to week 54 with the combination treatment and with liraglutide alone; compared with placebo, the decreases were statistically significant (**Supplementary Table S4**).

At week 80 (i.e. after the 26-week off-treatment observation period), the changes from baseline in stimulated C-peptide secretion (**Figure 2c**, **Supplementary Figure S1c** and **S1f, Supplementary Table S2**), glycated haemoglobin (**Figure 4c**) and total daily insulin dose (**Supplementary Figure S2c**) did not differ statistically significantly between the active treatments and placebo; the one notable exception was that the stimulated C-peptide secretion had decreased statistically significantly more with liraglutide alone than with placebo (-65% vs -49%; p=0·0065) (**Figure 2c**).

There were no treatment-related differences in the number of patients with insulin autoantibodies or autoantibodies against GAD, ZnT8 and IA2 throughout the trial (data not shown). Overall, there were only minor and generally transient changes (<10%) in the frequency of conventional T cells (including regulatory), natural killer cells and myeloid cells with little impact on follicular T helper cells or B cells across timepoints and between treatment groups (**Supplementary Figure S3**); no safety issues related to these minor changes were identified.

Treatment with liraglutide did not affect anti-IL-21 PK properties (**Supplementary Table S3**).

All active treatments as well as placebo were generally safe and well-tolerated. Overall, AE and tolerability profiles observed for the active trial treatments (**Table 2** and **Supplementary Table S6**) were consistent with previous reports on the use of anti-IL-21 treatment in humans and with the well-established profiles of GLP-1 RAs, including liraglutide in type 2 diabetes. Accordingly, the most frequently reported types of AE included gastrointestinal disorders, which are recognised as a known class effect with exogenous GLP-1 RAs. Apart from the gastrointestinal disorders, the most

frequently reported AEs did not differ relevantly between the active treatments and placebo (**Supplementary Figures S4 to S6**)

One participant died during the trial (while on treatment with liraglutide alone) in connection with the reported 3 AEs with fatal outcome ('hypoglycaemic coma', which after a marked drop in blood glucose in the early morning led to hospitalisation the same evening where 'pneumonia' and 'brain oedema' ensued); the 3 events were considered unlikely to be related to trial treatment and another potential causality was identified.

AEs leading to participants withdrawing from the trial were infrequent across groups (2·7 to 3·7 and 1·8 events per 100 years of exposure with the active treatments and placebo, respectively; **Table 2**). Of the 13 AEs leading to withdrawal, two were severe ('hypoglycaemic coma' and 'brain oedema' in the participant who died). Of the participants completing the treatment period, the large majority (72% to 82% for the active treatments and 88% for placebo) were dosed with liraglutide 1.8 mg; the remaining participants were on liraglutide 1.2 mg or had no information available.

No safety concerns related to hypersensitivity reactions, injection/infusion site reactions, development of anti-drug antibodies, neoplasms, pancreatitis or thyroid disease were identified.

At baseline across groups, 60·6% of the participants were positive for cytomegalovirus (CMV) immunoglobulin (Ig)G and 76·2% were positive for EBV-IgG. During the trial, there was no recurrence of CMV infections, whereas 5 EBV-IgG-positive participants developed EBV-IgM antibodies (1 participants on combination treatment, 1 on anti-IL-21 alone and 3 on placebo). All AEs related to positive EBV IgM samples were mild.

A total of 3 participants (1 on combination treatment and 2 on anti-IL-21 alone) reported severe hypoglycaemic episodes during the treatment period; none were nocturnal (**Table 2**). In the treatment period, the rate of severe or blood glucose-confirmed symptomatic hypoglycaemic episodes was lower with the active trial treatments (418·9 to 525·3 episodes per 100 years of exposure) than with placebo (675·2 episodes per 100 years of exposure) (**Table 2**); for liraglutide, the difference vs placebo was statistically significant with an ETR of 0·50 $[0·30; 0·85]_{95\%CI}$; **Supplementary Figure S7 and Supplementary Table S5Error! Reference source not found.**), whereas there were no differences upon trial treatment cessation in the observation period (**Supplementary Table S5Error! Reference source not found.**).

During treatment, the rate of hyperglycaemic episodes was similar or lower with the active treatments compared with placebo (288·6 to 397·9 vs 390·6 episodes per 100 years of exposure, respectively); no events of diabetic ketoacidosis were observed (**Table 2, Supplementary Table S5 and Supplementary Figure S8**). In the observation period, the rate

of hyperglycaemic episodes was significantly higher for the combination and liraglutide groups compared with placebo (**Supplementary Tables S5 and S6Error! Reference source not found.**).

No unexpected or clinically important differences across groups were observed in clinical laboratory parameters or vital signs. As expected based on the well-known class effects of GLP-1 RAs, blood pressure, pulse rate and amylase and lipase levels changed during treatment with the combination treatment and liraglutide alone (**Supplementary Table S4**).

Discussion

The trial was successful in showing that one year of treatment with the combination of a monoclonal anti-IL-21 antibody and the GLP-1 RA liraglutide was significantly better than placebo in preserving endogenous insulin secretion, measured by a 48% higher MMTT-stimulated C-peptide secretion after 54 weeks. The effect was accompanied by an almost complete maintenance of fasting baseline C-peptide secretion and a reduction in exogenous insulin need by almost one third. A trend showing a 34% reduction in hypoglycaemia was accompanied by reduced glycated haemoglobin levels, despite the treat-to-target trial design. Of note, the benefit of the combination therapy appeared more pronounced in the participants with a lower baseline C-peptide level (≤0·6 nmol/L) likely reflecting the beneficial effect of the anti-IL-21 component in preserving remaining beta-cell function. In those with a higher baseline C-peptide level reflecting more residual beta-cell function, the combination treatment and liraglutide alone were equally beneficial, suggesting that in this group of participants, the effect of liraglutide did not depend on the beta-cell preserving effect of anti-IL-21. Additional investigations are warranted to explore the patient subgroups likely to benefit the most from the combination.

Importantly, no safety concerns were identified during the trial, with the treatments being well tolerated and with no indications of generalised immune suppression based on the assessed immune biomarkers, including multiple cell populations.

The overall purpose of the combination treatment investigated in the present trial is to achieve safe preservation of beta-cell function. It is well-recognized that even modest residual endogenous insulin secretion, as measured by low levels of C-peptide have important clinical benefits, including lower rates of hypoglycaemia and of diabetes-related

complications, such as retinopathy.[10,28-30] The benefits appear independent of, or at least additional to, improved glycated haemoglobin levels.[2-4,31]

The efficacy of the combination therapy seen in the current trial appear at least on par with the best seen in other disease-modifying trials[8,32] and also greater than each of the individual components (anti-IL-21 and liraglutide) given as monotherapy. Conversely, the observed benign safety profile of anti-IL-21 combined with liraglutide appears favourable compared with the safety profiles observed in previous attempts to preserve beta-cell function in recent-onset T1D, where side effects appeared more severe and where long-term (months to years) changes to the immune system were seen, resulting in risk of, for example, reactivation of EBV infection[12,13].

Although the effect was less pronounced, anti-IL-21 alone also preserved C-peptide and was associated with similar trends in key glycaemic parameters (reduced glycated haemoglobin alongside less frequent severe or glucose-confirmed hypoglycaemic episodes). This supports a future role for anti-IL-21 treatment either as monotherapy or in combination with other agents in disease modulation in T1D.

The identification of the exact mechanisms by which liraglutide plus anti-IL-21 leads to preservation of C-peptide secretion was not the purpose of the present trial and requires further study. However, evidence suggests that IL-21 blockade reduces the pancreatic influx of new CD8+ effector T cells, thereby modulating the inflammatory process in the pancreas[19]. Similarly, liraglutide has the potential to relieve beta-cell stress and ameliorate the proinsulin-to-insulin processing defects seen in T1D.[20,21] Thus, liraglutide might, in addition to evidently preserving glucose-induced insulin secretion under immune stress, also directly preserve beta-cell health. Of note, however, another GLP-1 RA, albiglutide, did not preserve β-cell function (vs placebo) during a recent 1-year trial in newly diagnosed T1D [33]. To elucidate these important aspects, additional studies are needed, likely on organoids because no human *in vivo* beta-cell mass assessments are currently available.

For the duration of the treatment period, the combination therapy was more efficacious than placebo in sustaining the endogenous C-peptide secretion capacity, with the observation for placebo likely reflecting the natural disease course. In contrast to most other comparable investigations, the present trial included an off-drug observation period of 26 weeks immediately after the 54-week treatment period. This allowed for the observation that, upon cessation of treatment, the beneficial effects diminished rapidly as the ongoing autoimmune process presumably resumed its course.

Arguably, this is a positive finding, considering that it indicates that the treatments did not induce permanent changes to the immune system as seen with other immuno-modulatory interventions.

Interestingly, there appeared to be a more rapid decline in C-peptide secretion during the observation period (i.e. after treatment cessation) in the combination therapy and liraglutide-alone groups, which might reflect the observed increase in hyperglycaemia after cessation of liraglutide (with or without anti-IL-21). The hyperglycaemia may have been due to a delayed up-titration or otherwise sub-optimal titration of the insulin dose in these groups in particular. This notion is corroborated by the well-established marked glycaemic efficacy of GLP-1 RAs such as liraglutide that may had required tighter insulin titration than the one specified in the trial protocol or applied at the trial sites. Whatever the cause, the observed hyperglycaemia might have led to increased glucose toxicity towards the beta cell and in turn to the observed more rapid decrease in C-peptide secretion in the liraglutide-containing regimens. Moreover, further damage to the beta cell could also arise from liraglutide withdrawal via an increase in self-presentation of the beta-cell autoantigen pro-insulin.

C-peptide and plasma glucose outcomes were assessed using 4-hour AUC values by default; 2-hour AUC values were also derived, and for the primary outcome (stimulated C-peptide secretion) the results and the statistical inferences were overall consistent across the two AUC approaches with the addition that anti-IL-21 alone was statistically significant better at preserving C-peptide secretion than placebo when evaluated using the 2-hour but not the 4-hour AUC. Of note, the 4-hour AUC was the default because evidence was available to suggest that C_{max} for C-peptide is reached around 2-hours after a meal.

Whether the benefits of the combination therapy can be sustained beyond the 54 weeks studied in the present trial will require further evaluation. Future trials should also address the period following treatment withdrawal with a specific focus on glycaemia.

Finally, the weight loss reduction seen with the liraglutide-containing regimens are in line with the licenced use of liraglutide in weight management and constitute an additional benefit of a T1D intervention, considering that T1D may be associated with excess body weight.

In conclusion, treatment with the combination of anti-IL-21 and liraglutide for 54 weeks was well-tolerated and resulted in sustained endogenous insulin secretion in response to an MMTT and improved glucose metabolism compared with placebo. The results imply that the combination of anti-IL-21 and liraglutide has the potential to offer a novel and valuable disease-modifying therapy for patients recently diagnosed with T1D.

Contributions

MVH and KC developed the ideas and concept for the study, and were substantially involved in the design and data interpretation of the trial as well as writing the manuscript. SS was the principal statistician and provided the data analyses and edited the manuscript, JOC was Novo Nordisk's International Medical Director on this trial. SCB, BB, LG, JG, TKH, ChM, CrM, OM, GT and TRP were clinical site heads who were instrumental in the success of this trial and all of whom have been involved in data analysis, and editing the manuscript.

Declaration of interests

MVH, JOC, KC and SS are employees of Novo Nordisk and SS also holds shares in the company. MVH and KC hold a patent related to this work that has been issued and is owned by Novo Nordisk. SCB reported receiving personal fees from AstraZeneca, Boehringer Ingelheim, Eli Lilly, Merck Sharp & Dohme, Novo Nordisk and Sanofi-Aventis (honoraria); Medscape (funding for the development of educational programmes); All-Wales Medicines Strategy Group and National Institute for Health and Care Excellence UK (providing expert advice) and is a shareholder of Glycosmedia. BB reported receiving grants and personal fees from Novo Nordisk for consulting and lectures. JG reported receiving personal fees from Eli Lilly, Boehringer Ingelheim, Sanofi-Aventis, Merck Sharp & Dohme, Merck, AstraZeneca, Mundipharma, Polfa Tarchomin, Bioton, Servier, Berlin-Chemie, Adamed, and Novo Nordisk. ChM reported receiving grants from Sanofi-Aventis, Eli Lilly, Novartis, Boehringer Ingelheim, and ActoBio Therapeutics and personal fees from Sanofi-Aventis, Eli Lilly, Novartis, Boehringer Ingelheim, ActoBio Therapeutics, AstraZeneca, Roche, Medtronic, and Pfizer. OM reported receiving grants from Novo Nordisk and AstraZeneca and personal fees from Novo Nordisk, AstraZeneca, Eli Lilly, Sanofi-Aventis, Merck Sharp & Dohme, Boehringer Ingelheim and Teva. TRP reported receiving grants from Novo Nordisk and AstraZeneca and personal fees from Adocia, Arecor, AstraZeneca, Eli Lilly, Novo Nordisk and Sanofi-Aventis. LG, TKH, CrM, GT reported no relevant conflicts of interest.

Data sharing

Individual participant data will be shared in data sets in a de-identified or anonymised format. Data sets from clinical research sponsored by Novo Nordisk and completed after 2001 for product indications approved in both the European Union (EU) and the USA will be shared. The study protocol and redacted Clinical Study Report will be available according to Novo Nordisk data sharing commitments. The data will be available

permanently after research completion and approval of product and product use in both the EU and USA. Data will be shared with bona fide researchers submitting a research proposal and requesting access to data. Data will be made available for analyses as approved by the Independent Review Board (IRB) according to the IRB Charter. The access request proposal form and the access criteria can be found on the Novo Nordisk Trials website (https://www.novonordisk-trials.com/en/how-access-clinical-trial-datasets/). The data will be made available on a specialised SAS data platform.

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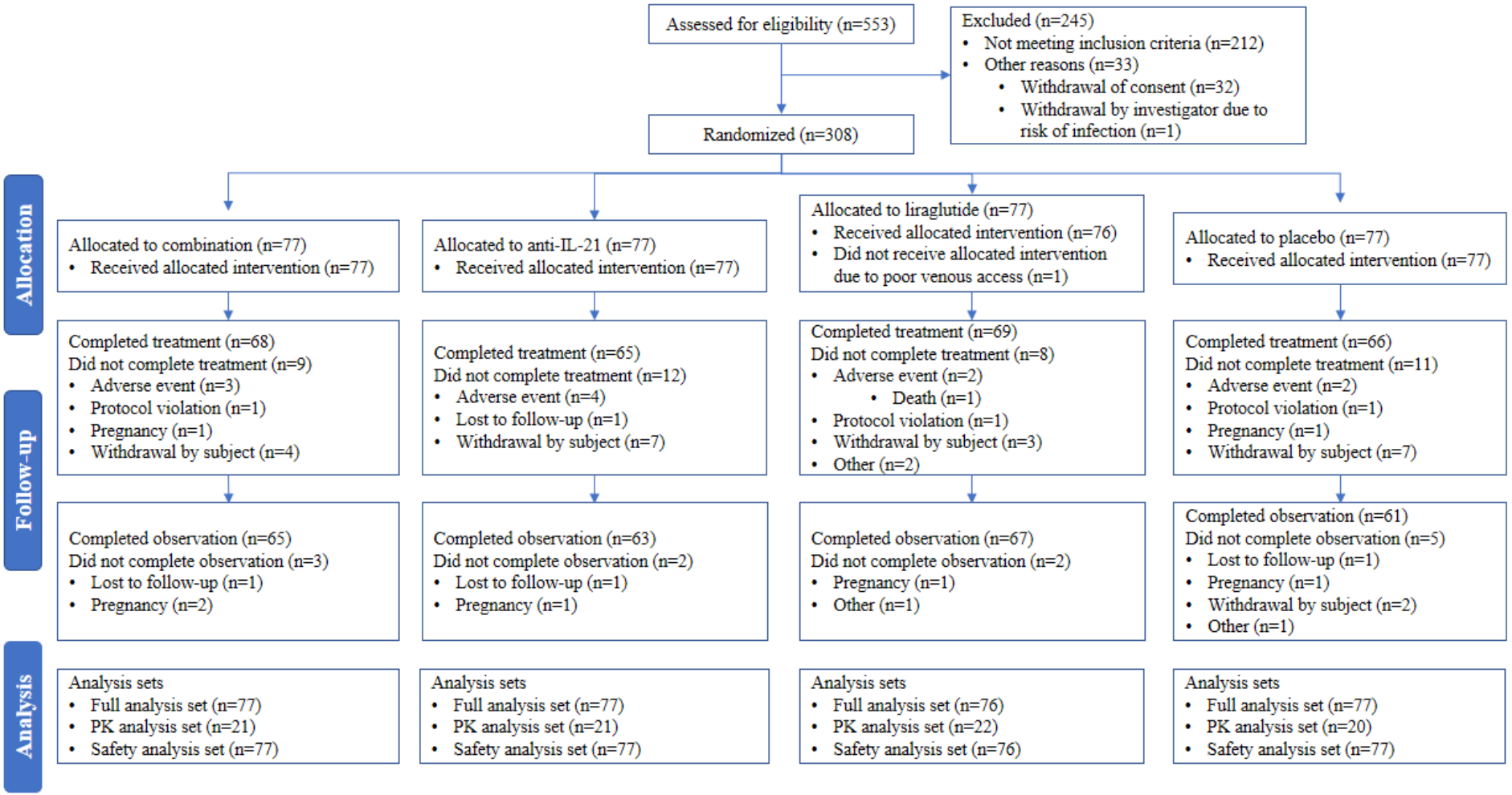
We thank the participants who took part in this trial, the investigators, all trial-site staff, and all Novo Nordisk employees involved in the trial. We thank Johnna D Wesley, PhD, of Novo Nordisk for scientific input and for overseeing the analysis of cell populations, and Bernt Johan von Scholten, DMSc, of Novo Nordisk, for scientific input. We also thank Kristine Grønning Kongsbak, PhD, and Frederik Flindt Kreiner, PhD, both of Novo Nordisk, and Eleanor Ling, PhD, as an independent contractor, for medical writing assistance and Izabel James of Watermeadow Medical, an Ashfield company, part of UDG Healthcare, for editorial assistance.

Figures and tables

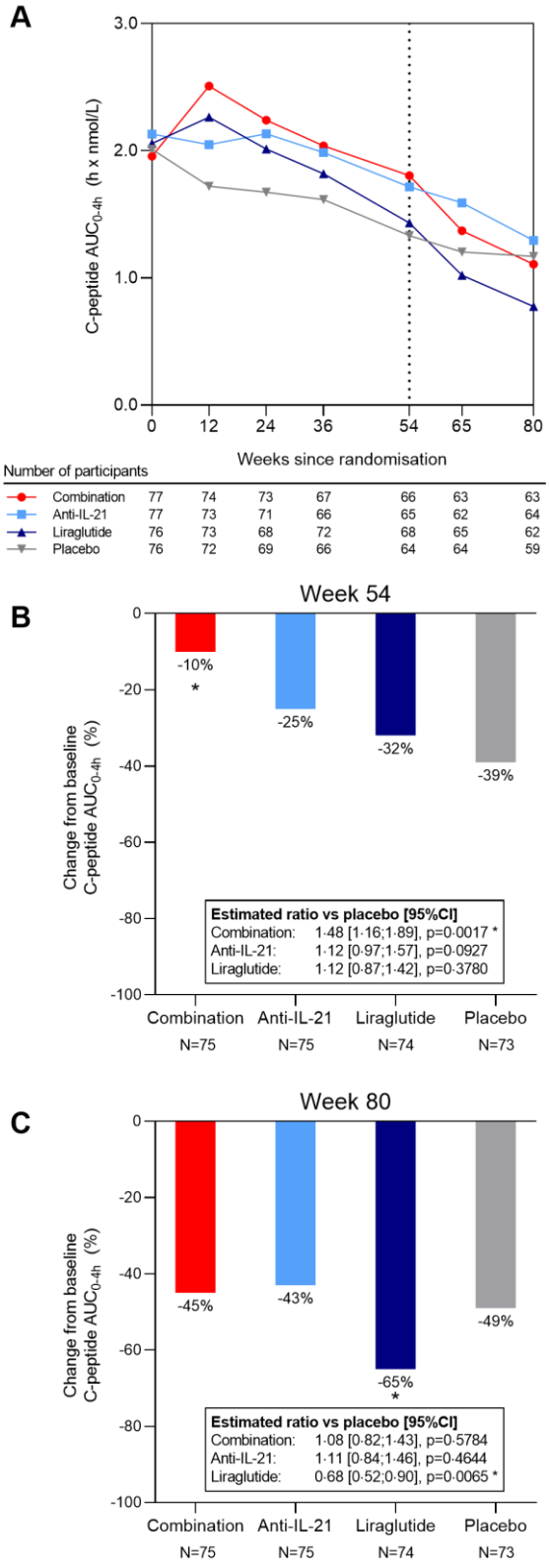
Figure 1 Trial profile



Analyses of efficacy and biomarker endpoints were based on the full analysis set, which includes all participants randomly assigned to a treatment group. Only in exceptional cases were participants excluded from the full analysis set; in such cases, the reason for exclusion was justified and

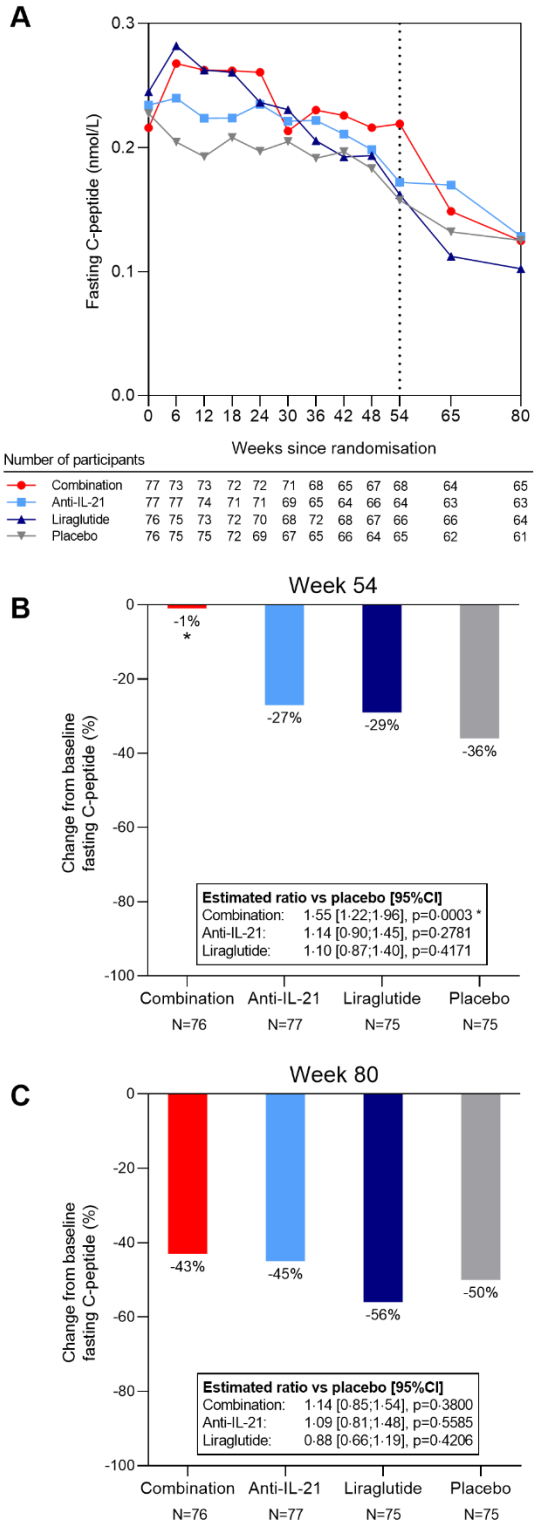
documented. For analyses based on the full analysis set, participants contributed according to the trial treatment assigned at randomisation. Analyses of the pharmacokinetic (PK) endpoints were based on the PK analysis set, which includes randomised participants with a full PK profile and with at least one valid PK measurement. Analyses of safety endpoints were based on the safety analysis set, which includes all participants who were exposed to trial treatment. For analyses based on the PK and safety analysis sets, participants contributed according to the trial treatment actually received. IL, interleukin; PK, pharmacokinetic.

Figure 2: MMTT-stimulated C-peptide secretion



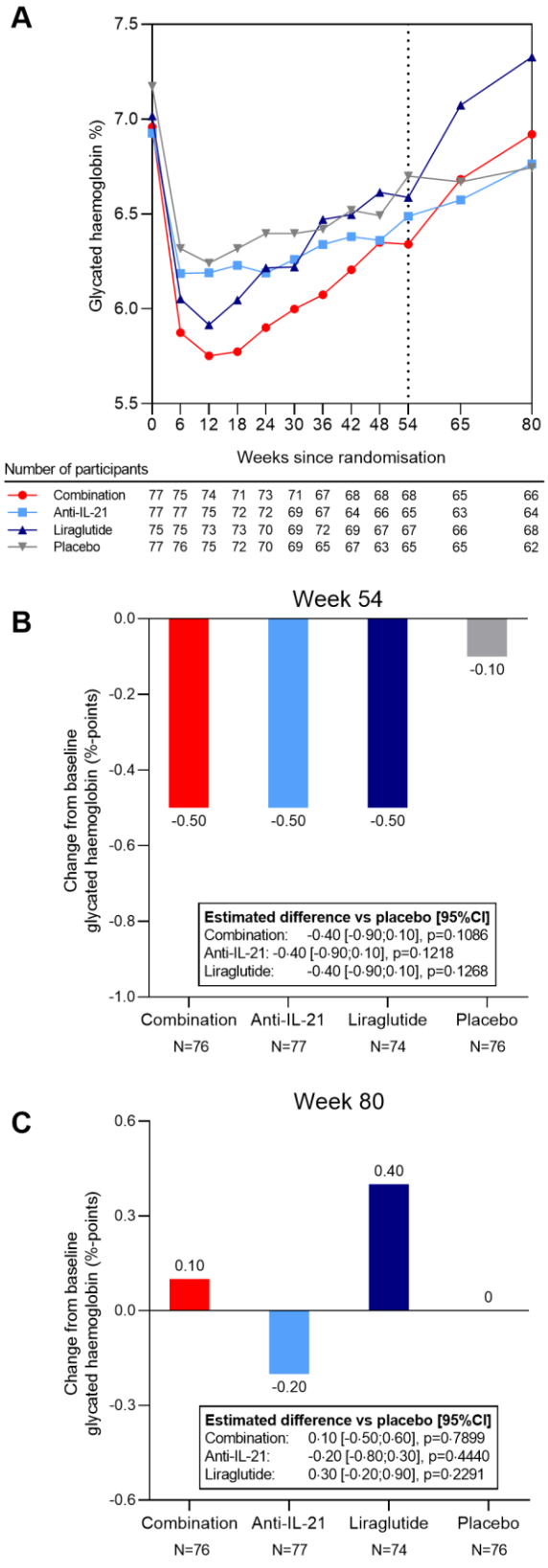
Data are observed geometric means by week (Panel A) and estimated geometric mean changes from baseline at end-of-treatment (week 54, Panel B) and end-of-trial (week 80, Panel C). Number of participants (N) contributing to the observed means (Panel A) or the analysis (Panels B and C) are presented. Data were log-transformed; in the statistical analysis, data were then analysed using a mixed model for repeated measures with treatment group, sex and C-peptide stratum as factors, and baseline value and age as covariates, as well as the interaction between all variables and time. Estimated changes from baseline calculated on the multiplicative scale as ratios to baseline are presented as %-changes from baseline; the estimated treatment contrasts and the associated 95% CI are presented as the ratio of the estimated ratios to baseline between active treatment and placebo. Participants with at least one post-baseline value contribute to the statistical analysis. *$p<0.05$ vs placebo. AUC, area under the time-concentration curve; CI, confidence interval; ETR, estimated treatment ratio; IL, interleukin; MMTT, mixed-meal tolerance test

Figure 1. Fasting C-peptide secretion



Data are observed geometric means by week (Panel A) and estimated geometric mean changes from baseline at end-of-treatment (week 54, Panel B) and end-of-trial (week 80, Panel C). Number of participants (N) contributing to the observed means (Panel A) or the analysis (Panels B and C) are presented. Data were log-transformed; in the statistical analysis, data were then analysed using a mixed model for repeated measures with treatment group, sex and C-peptide stratum as factors, and baseline value and age as covariates, as well as the interaction between all variables and time. Estimated changes from baseline calculated on the multiplicative scale as ratios to baseline are presented as %-changes from baseline; the estimated treatment contrasts and the associated 95% CI are presented as the ratio of the estimated ratios to baseline between active treatment and placebo. Participants with at least one post-baseline value contribute to the statistical analysis. *$p<0.05$ vs placebo. CI, confidence interval; ETR, estimated treatment ratio; IL, interleukin.

Figure 2: Glycated haemoglobin



Data are observed means by week (Panel A) and estimated mean changes from baseline at end-of-treatment (week 54, Panel B) and end-of-trial (week 80, Panel C). Data were analysed using a mixed model for repeated measures with treatment group, sex and C-peptide stratum as factors, and baseline value and age as covariates. Participants with at least one post-baseline value contribute to the statistical analysis.
CI, confidence interval; IL, interleukin.

Table 1: Demographics and baseline characteristics

	Combination (FAS = 77)	Anti-IL-21 (FAS = 77)	Liraglutide (FAS = 76)	Placebo (FAS = 77)
Age, years	28·0±7·5	28·6±7·9	28·0±7·1	29·0±7·0
Females, N	21 (27·3)	32 (41·6)	25 (32·9)	28 (36·4)
BMI, kg/m^2	22·9±3·8	23·7±3·4	24·2±3·8	24·0±5·0
Duration of type 1 diabetes, weeks	11·6±5·7	11·5±5·3	10·8±4·8	10·2±4·7
Glycated haemoglobin, %	7·1±1·5	7·0±1·3	7·2±1·5	7·3±1·3
Glycated haemoglobin, mmol/mol	54±16	53±14	55±16	56±14
Baseline MMTT-derived peak C-peptide				
≤0·6 nmol/L, N	31 (40·3)	27 (35·1)	32 (42·1)	30 (39·0)
>0·6 nmol/L, N	46 (59·7)	50 (64·9)	44 (57·9)	47 (61·0)
Baseline mean total daily insulin dose	0·32±0·21	0·33±0·19	0·30±0·17	0·32±0·21
Mean daily bolus insulin dose, U/kg	0·17±0·11	0·16±0·11	0·16±0·10	0·16±0·12
Mean daily basal insulin dose, U/kg	0·19±0·12	0·19±0·11	0·16±0·09	0·19±0·11
Number of severe hypoglycaemic episodes since diagnosis				
0 episodes, N	75 (97·4)	77 (100)	76 (100)	76 (98·7)
1 episode, N	2 (2·6)	0	0	1 (1·3)
Number of islet-specific auto-antibodies				
1 auto-antibody, N	24 (31.2)	20 (26.0)	22 (28.9)	28 (36.4)
2 auto-antibodies, N	22 (28.6)	27 (35.1)	21 (27.6)	24 (31.2)
3 auto-antibodies, N	31 (40.3)	30 (39.0)	33 (43.4)	25 (32.5)

Data are means (±SD) or participant counts (proportion relative to the FAS) for participants in the full analysis set. Baseline is defined as the time of randomisation or latest information prior to this. BMI, body mass index; FAS, full analysis set; IL, interleukin; MMTT, mixed-meal tolerance test; N, number of subjects; SD, standard deviation.

Table 2: Treatment-emergent adverse events and hypoglycaemic and hyperglycaemic episodes

	Combination			Anti-IL-21			Liraglutide			Placebo		
	N	%	R	N	%	R	N	%	R	N	%	R
Safety analysis set	77			77			76			77		
Patient-years of exposure	75·85			75·04			75·17			74·51		
All adverse events	59	76·6	572	64	83·1	436	65	85·5	545	63	81·8	489
Possibly or probably related to												
Anti-IL-21	25	32·5	88·3	20	26·0	72·0	22	28·9	125·0	24	31·2	87·2
Liraglutide	37	48·1	176·5	24	31·2	72·0	50	65·8	208·8	27	35·1	108·7
Serious AE	6	7·8	8	3	3·9	5	7	9·2	13	7	9·1	12
AE leading to withdrawal	3	3·9	3·6	4	5·2	3·7	2	2·6	2·7	2	2·6	1·8
Most frequently reported AE [a]												
Nasopharyngitis	18	23·4	35·6	23	29·9	57·3	22	28·9	54·5	22	28·6	47·0
Nausea	19	24·7	43·5	6	7·8	12·0	41	53·9	81·1	9	11·7	17·4
Vomiting	13	16·9	30·3	0	0·0	0·0	17	22·4	43·9	3	3·9	5·4
Diarrhoea	12	15·6	21·1	8	10·4	18·7	13	17·1	26·6	8	10·4	12·1
Headache	9	11·7	19·8	8	10·4	12·0	9	11·8	13·3	10	13·0	18·8
Oropharyngeal pain	10	13·0	14·5	11	14·3	20·0	3	3·9	6·6	7	9·1	10·7
Decreased appetite	14	18·2	19·8	2	2·6	2·7	11	14·5	17·3	1	1·3	1·3
Hypoglycaemic episodes												
ADA classification	73	94·8	4419·0	73	94·8	4560·0	69	90·8	4287·2	73	94·8	4578·5
Severe	1	1·3	1·3	2	2·6	2·7	0	0·0	0·0	0	0·0	0·0
Severe or blood glucose-confirmed symptomatic [b]	43	55·8	521·7	55	71·4	525·3	46	60·5	418·9	51	66·2	675·2
Hyperglycaemic episodes [c]	32	41·6	397·9	31	40·3	341·3	29	38·2	288·6	36	46·8	390·6
Diabetic ketoacidosis	0	0·0	0·0	0	0·0	0·0	0	0·0	0·0	0	0·0	0·0

[a] Events with an overall rate (R) of ≥10 events per 100 participant-years of exposure.

[b] 'Severe or blood glucose-confirmed symptomatic' hypoglycaemic episodes are either severe according to the ADA classification[27] or an episode confirmed by a plasma blood glucose <3.1 mmol/L (56 mg/dL) and with symptoms consistent with hypoglycaemia.

[c] Hyperglycaemic episodes were defined as and confirmed by plasma glucose values >16.7 mmol/l (300 mg/dl).

ADA, American Diabetes Association; AE, adverse event; IL, interleukin; N, number of participants with at least one event; %, proportion of participants with at least one event; R, events per 100 participant-years of exposure.

Supplementary Appendix

Table of contents

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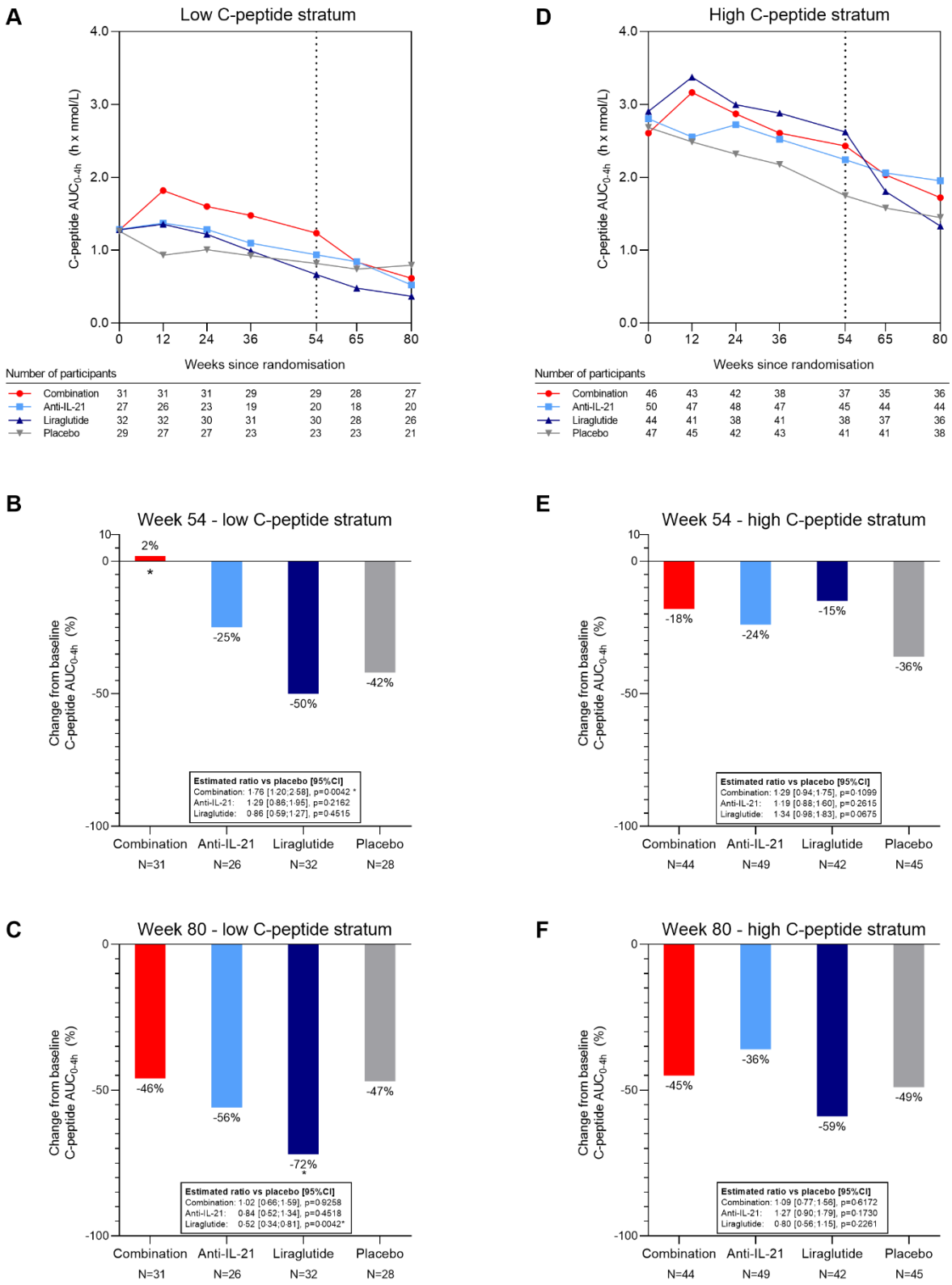
Supplementary Figures

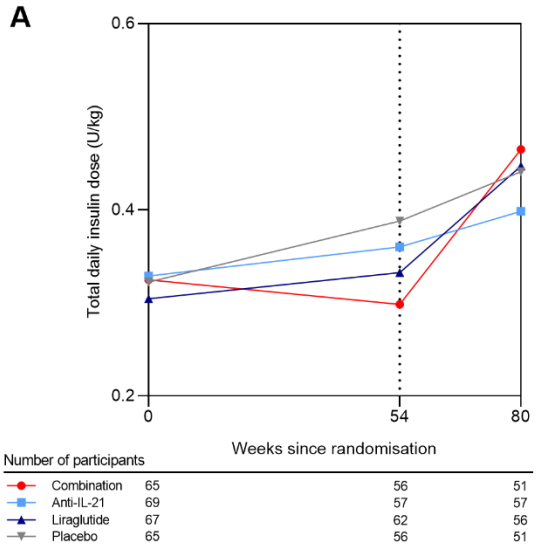
Supplementary Figure S1 Stimulated C-peptide secretion by baseline C-peptide stratum

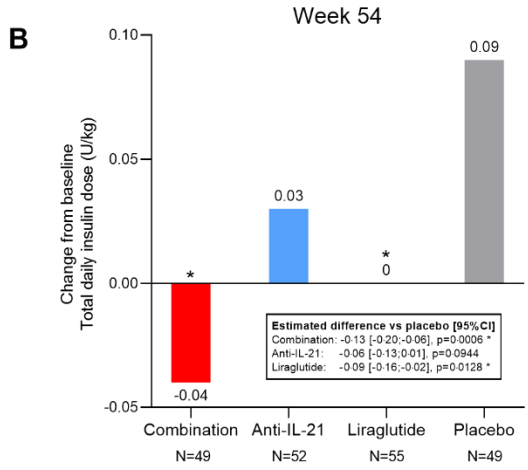


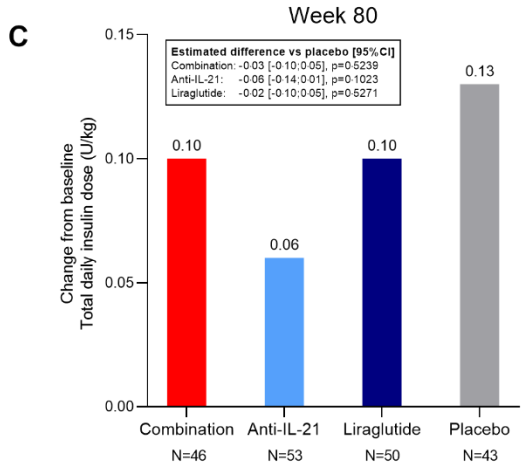
Treatment by stratum interaction p-value = 0.0214. Panels A-C: participants in the low baseline C-peptide stratum (0.2nmol/L ≤ MMTT-derived peak C-peptide ≤ 0.6nmol/L; N=120 (39.1%)). Panel D-F: participants in the high baseline C-peptide stratum (MMTT-derived peak C-peptide > 0.6nmol/L; N=187 (60.9%)).

Number of participants (N) contributing to the observed means (Panels A and D) or the analysis (Panels B, C, E and F) are presented. Data were log-transformed; in the statistical analysis, data were then analysed using normal linear regression with treatment group, sex and C-peptide stratum as factors and baseline value and age as covariates. Participants with data for baseline and week 54/80 contributed to analysis. *$p<0.05$ vs placebo. CI, confidence interval; ; IL, interleukin.

Supplementary Figure S2 Total daily insulin dose

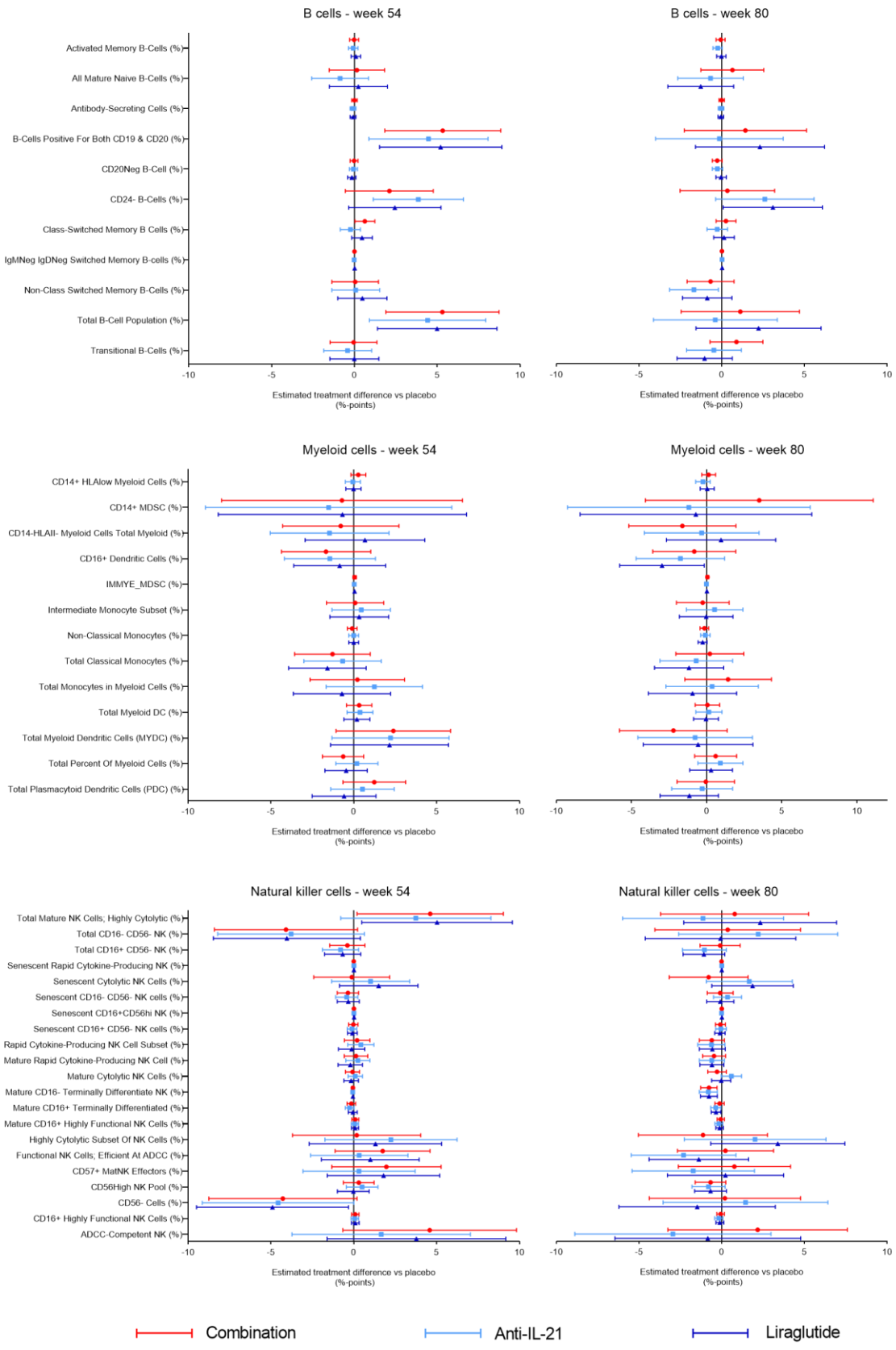


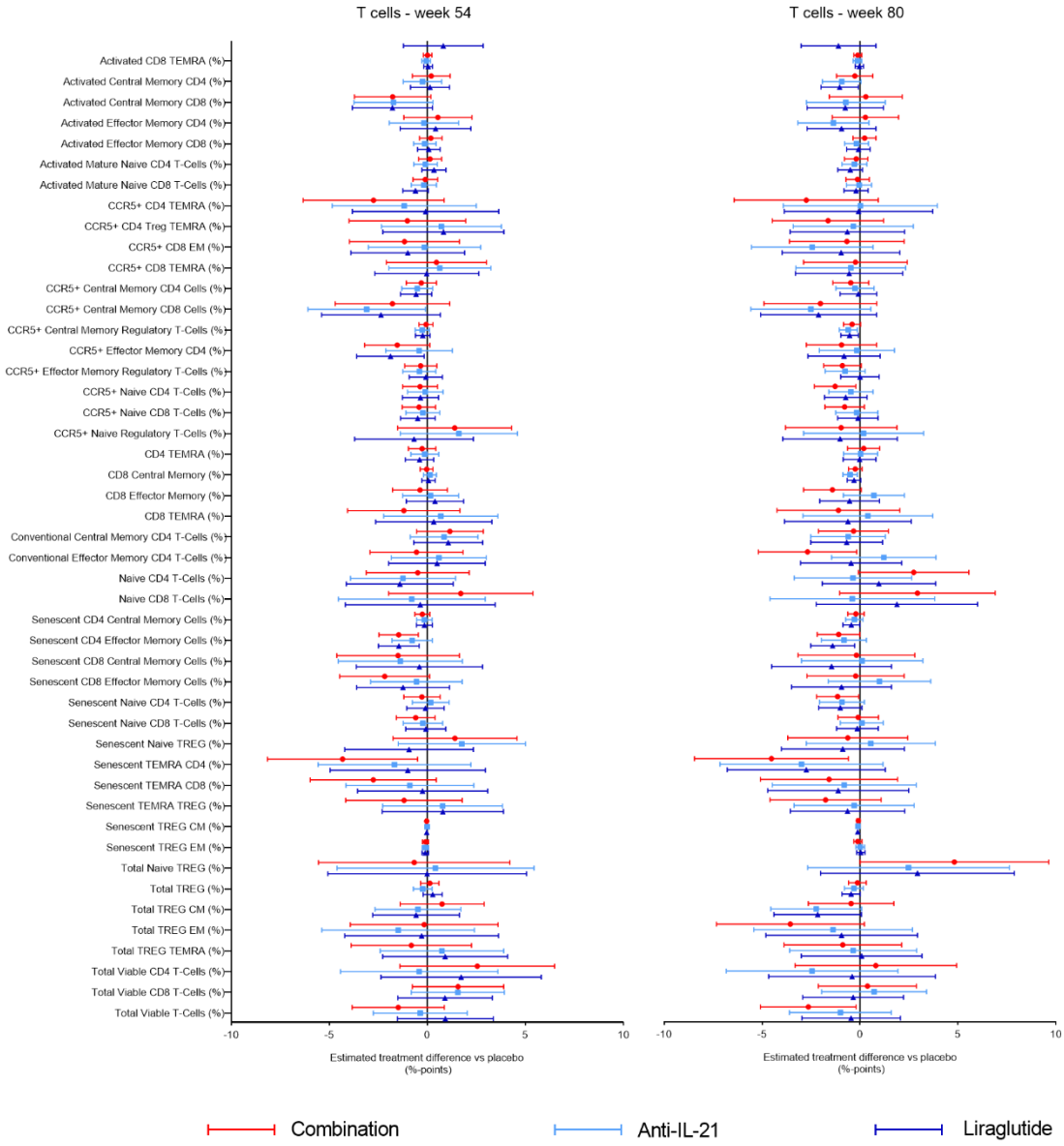


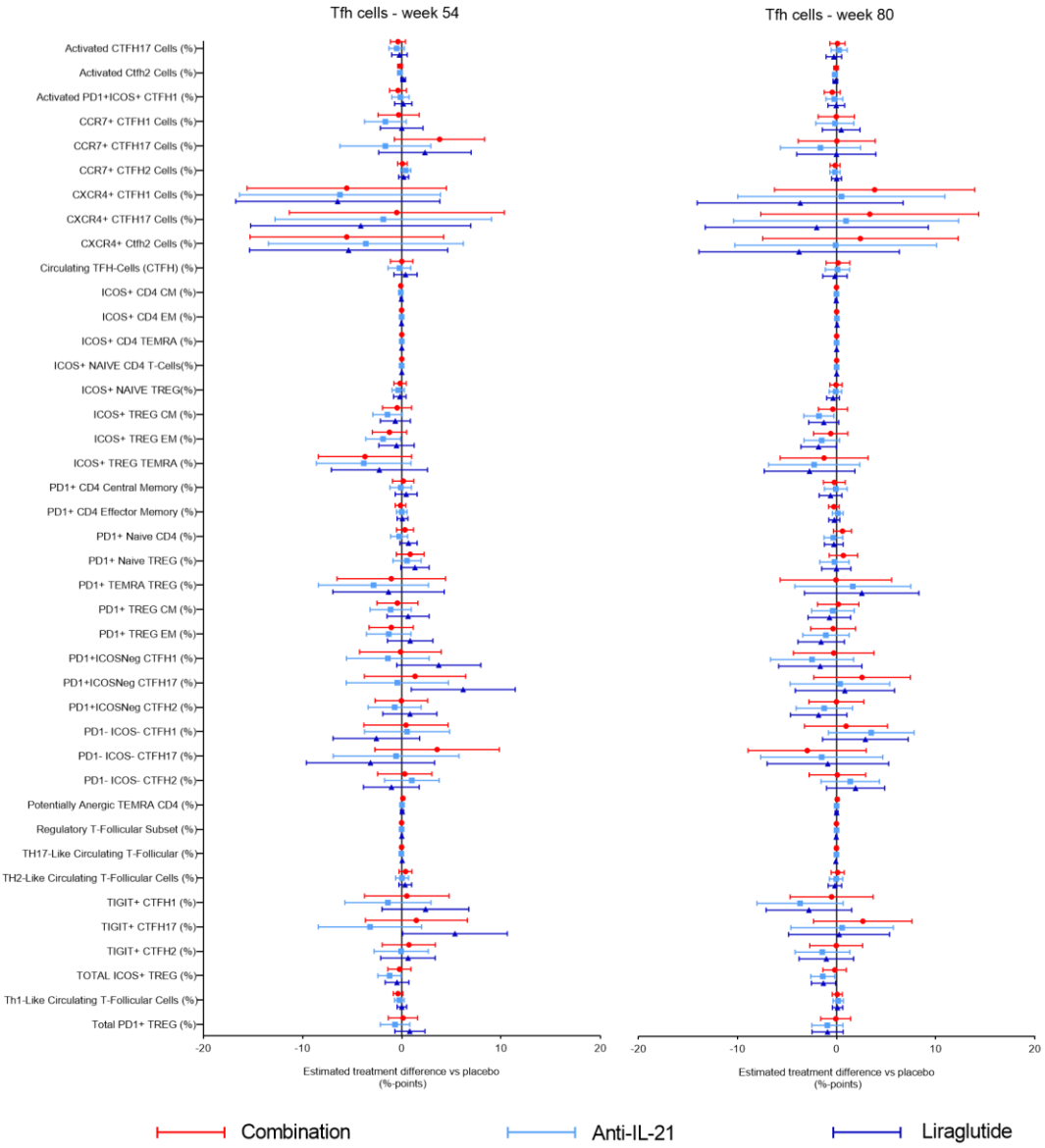


Data are observed means by week (Panel A) and estimated mean changes from baseline at end-of-treatment (week 54, Panel B) and end-of-trial (week 80, Panel C). Number of participants (N) contributing to the observed means (Panel A) or the analysis (Panels B and C) are presented. Data were log-transformed; in the statistical analysis, data were then analysed using normal linear regression with treatment group, sex and C-peptide stratum as factors and baseline value and age as covariates. Participants with data for baseline and week 54/80 contributed to analysis. *$p<0.05$ vs placebo. CI, confidence interval; ; IL, interleukin.

Supplementary Figure S3 Immune cell populations – change from baseline vs placebo




T cells - week 54
T cells - week 80
Activated CD8 TEMRA (%)
Activated Central Memory CD4 (%)
Activated Central Memory CD8 (%)
Activated Effector Memory CD4 (%)
Activated Effector Memory CD8 (%)
Activated Mature Naive CD4 T-Cells (%)
Activated Mature Naive CD8 T-Cells (%)
CCR5+ CD4 TEMRA (%)
CCR5+ CD4 Treg TEMRA (%)
CCR5+ CD8 EM (%)
CCR5+ CD8 TEMRA (%)
CCR5+ Central Memory CD4 Cells (%)
CCR5+ Central Memory CD8 Cells (%)
CCR5+ Central Memory Regulatory T-Cells (%)
CCR5+ Effector Memory CD4 (%)
CCR5+ Effector Memory Regulatory T-Cells (%)
CCR5+ Naive CD4 T-Cells (%)
CCR5+ Naive CD8 T-Cells (%)
CCR5+ Naive Regulatory T-Cells (%)
CD4 TEMRA (%)
CD8 Central Memory (%)
CD8 Effector Memory (%)
CD8 TEMRA (%)
Conventional Central Memory CD4 T-Cells (%)
Conventional Effector Memory CD4 T-Cells (%)
Naive CD4 T-Cells (%)
Naive CD8 T-Cells (%)
Senescent CD4 Central Memory Cells (%)
Senescent CD4 Effector Memory Cells (%)
Senescent CD8 Central Memory Cells (%)
Senescent CD8 Effector Memory Cells (%)
Senescent Naive CD4 T-Cells (%)
Senescent Naive CD8 T-Cells (%)
Senescent Naive TREG (%)
Senescent TEMRA CD4 (%)
Senescent TEMRA CD8 (%)
Senescent TEMRA TREG (%)
Senescent TREG CM (%)
Senescent TREG EM (%)
Total Naive TREG (%)
Total TREG (%)
Total TREG CM (%)
Total TREG EM (%)
Total TREG TEMRA (%)
Total Viable CD4 T-Cells (%)
Total Viable CD8 T-Cells (%)
Total Viable T-Cells (%)
-10
-5
0
5
10
Estimated treatment difference vs placebo (%-points)
Combination
Anti-IL-21
Liraglutide



Symbols are estimated changes from baseline (%) vs placebo at weeks 54 and 80 with 95% confidence intervals. Data were log-transformed; in the statistical analysis, data were then analysed using normal linear regression with treatment group, sex and C-peptide stratum as factors and baseline value and age as covariates. Participants with data for baseline and week 54/80 contributed to analysis. Cells populations were counted using flow cytometry. IL, interleukin.

Supplementary Figure S4 Adverse events for the combination versus placebo in the treatment period

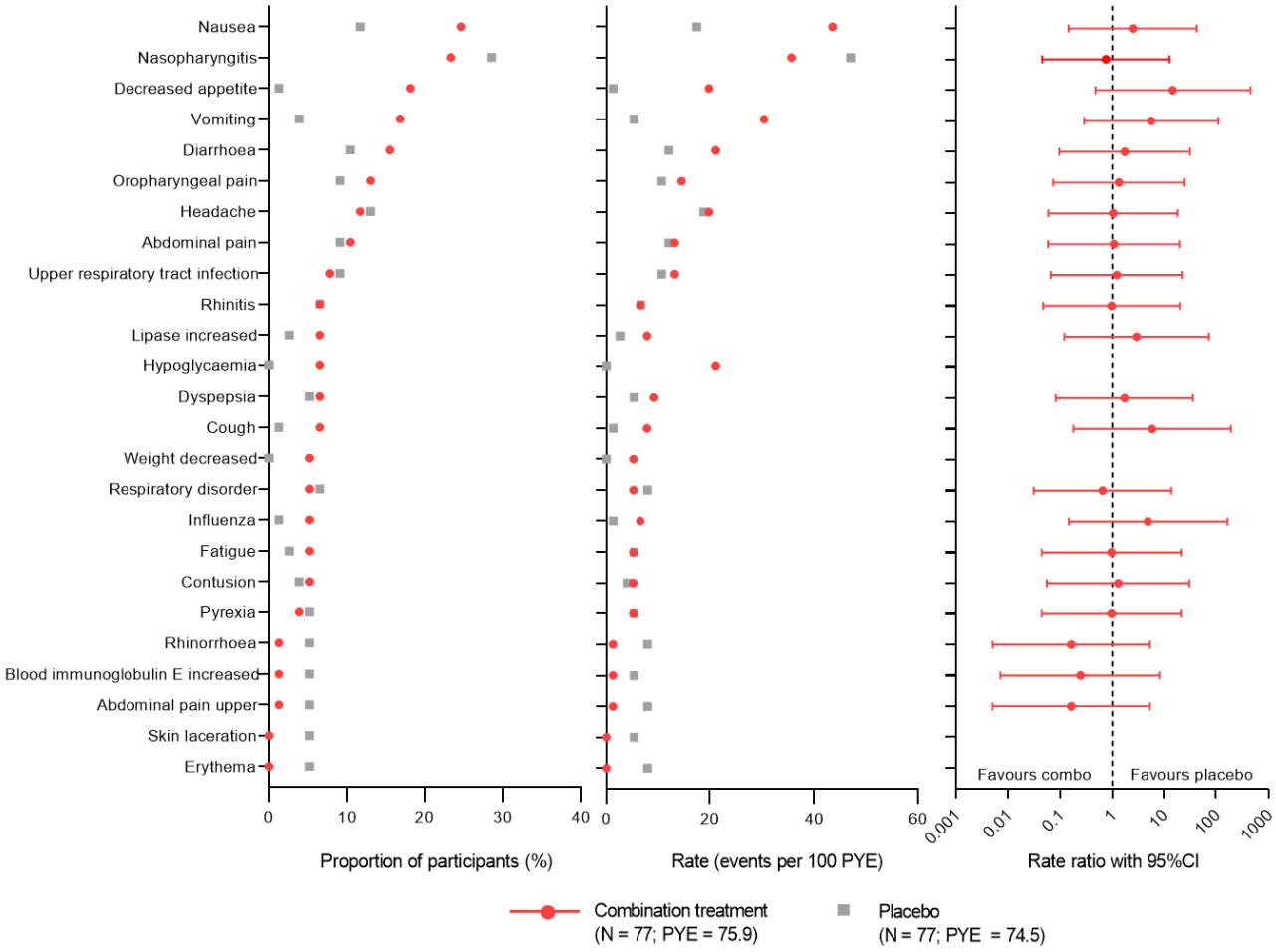


Figure includes events reported for at least 5% of the participants in either treatment group. The rate ratios were estimated using a negative binomial regression with treatment, stratum and sex as factors and age as covariate.
CI, confidence interval; N, number of participants; PYE, patient-years of exposure.

Supplementary Figure S5 Adverse events for anti-IL-21 versus placebo in the treatment period

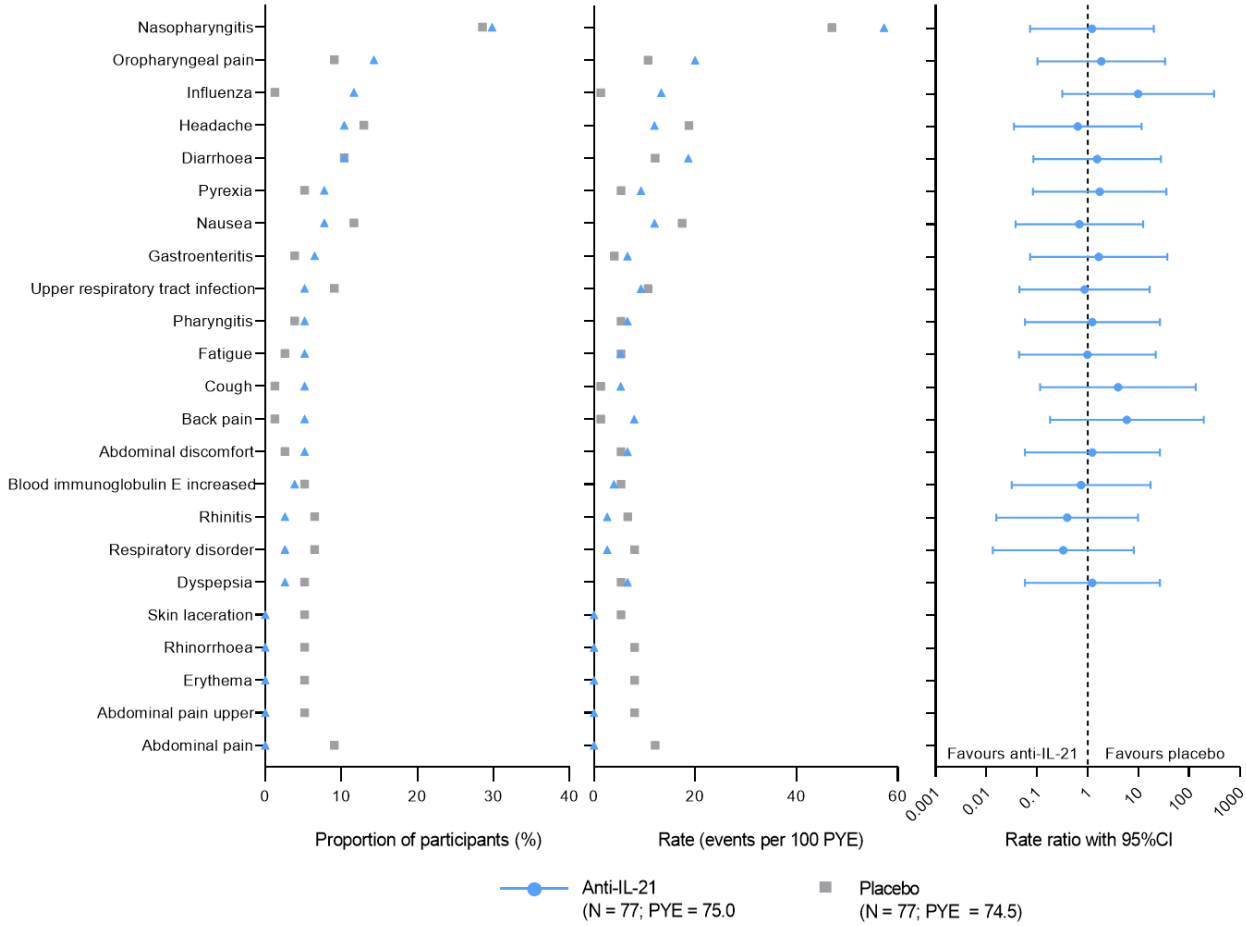


Figure includes events reported for at least 5% of the participants in either treatment group. The rate ratios were estimated using a negative binomial regression with treatment, stratum and sex as factors and age as covariate.

CI, confidence interval; IL, interleukin; N, number of participants; PYE, patient-years of exposure.

Supplementary Figure S6 Adverse events for liraglutide versus placebo in the treatment period

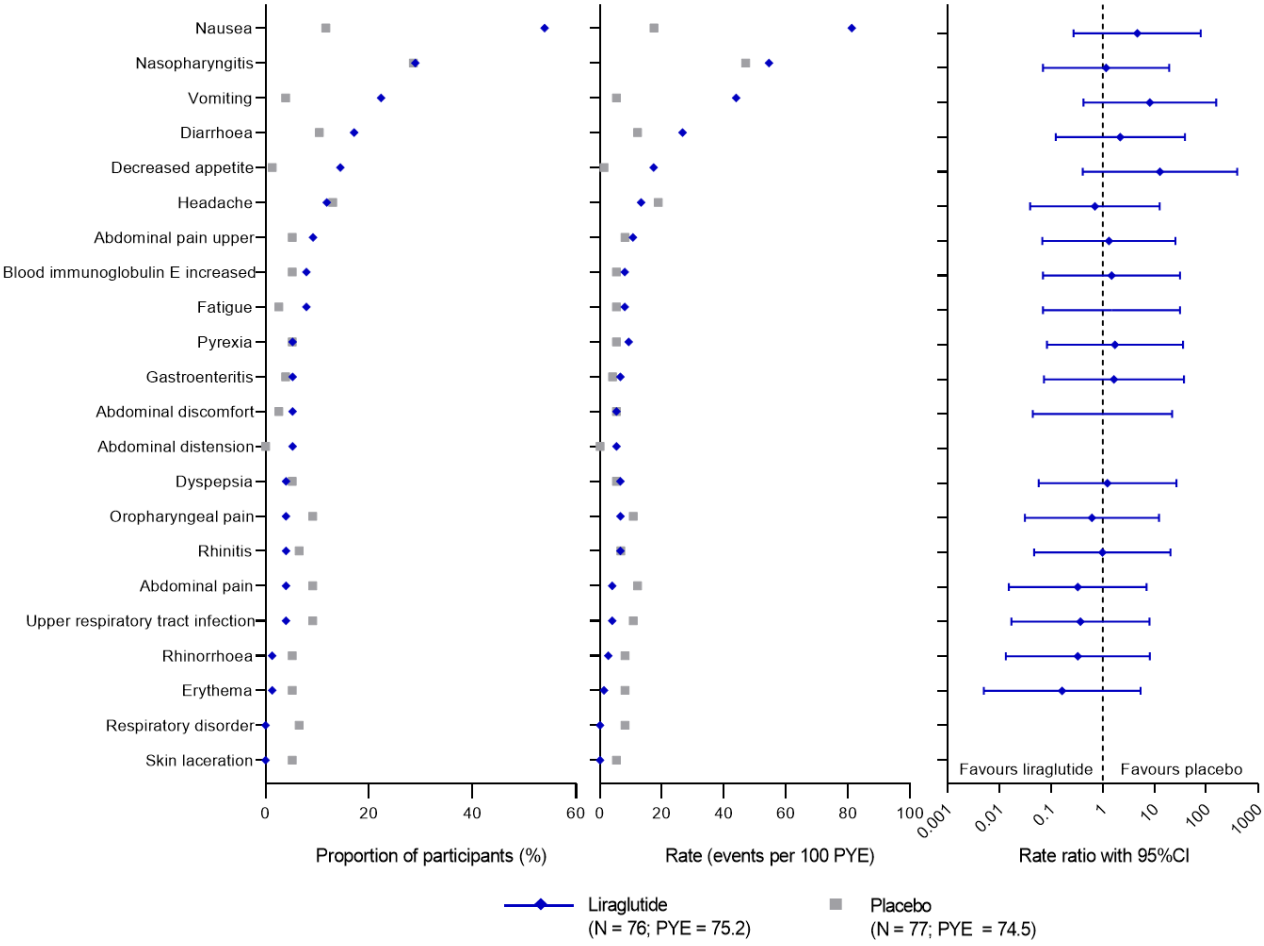
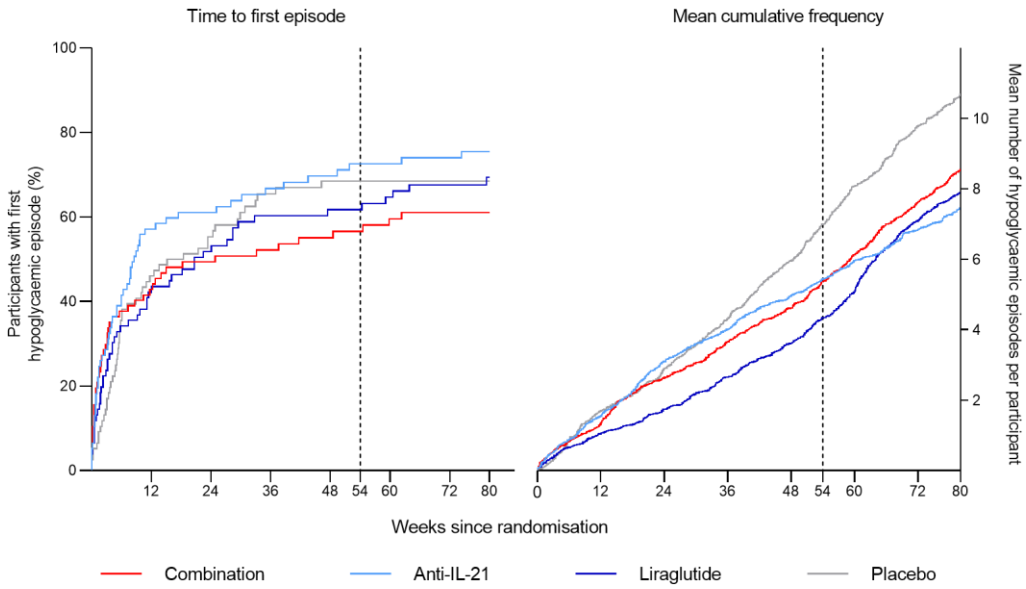


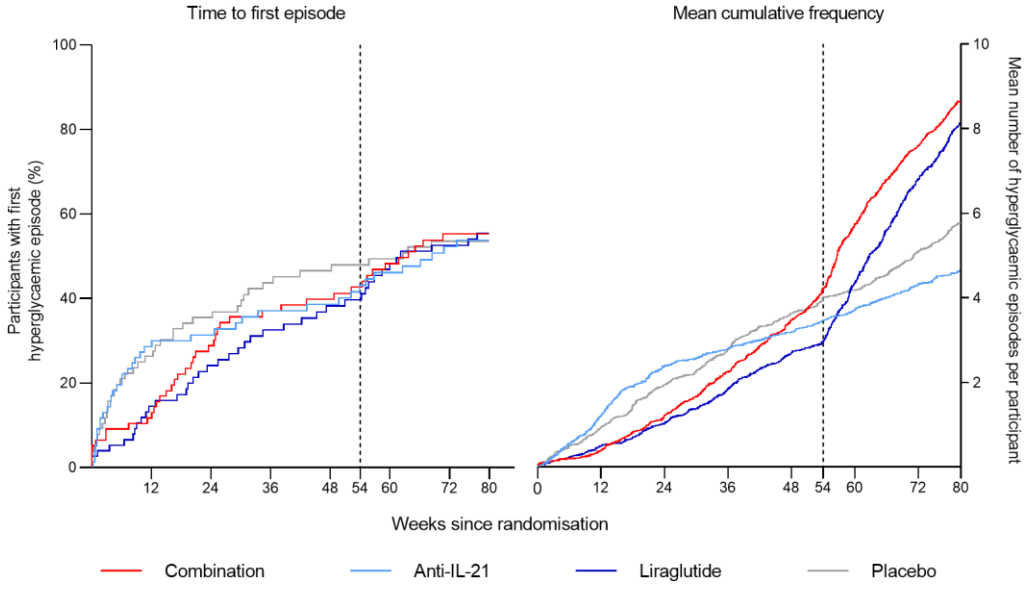
Figure includes events reported for at least 5% of the participants in either treatment group. The rate ratios were estimated using a negative binomial regression with treatment, stratum and sex as factors and age as covariate.
CI, confidence interval; N, number of participants; PYE, patient-years of exposure.

Supplementary Figure S7 Severe or blood glucose-confirmed symptomatic hypoglycaemia



Severe or blood-glucose confirmed hypoglycaemia is defined as episodes that are severe according to the ADA classification or blood-glucose confirmed by a plasma glucose value <3.1 mmol/l (56 mg/dl) with symptoms consistent with hypoglycaemia. ADA, American Diabetes Association; IL, interleukin.

Supplementary Figure S8 Hyperglycaemia



Hyperglycaemic episodes are defined as and confirmed by plasma glucose values >16.7 mmol/l (300 mg/dl). IL, interleukin.

Supplementary Tables

Supplementary Table S1 Other supportive secondary efficacy endpoints – week 54

Treatment group	N	Ratio to baseline	ETR (vs placebo)	95% CI
C-peptide $AUC_{0\text{-}2h}$				
Anti-IL-21 + liraglutide	75	0·93	1·56	[1·23; 1·99]
Anti-IL-21	75	0·79	1·32	[1·04; 1·68]
Liraglutide	74	0·63	1·06	[0·83; 1·35]
Placebo	73	0·60	-	-
Plasma glucose $AUC_{0\text{-}4h}$				
Anti-IL-21 + liraglutide	75	0·96	0·88	[0·79; 0·98]
Anti-IL-21	75	1·06	0·98	[0·88; 1·09]
Liraglutide	74	1·00	0·92	[0·83; 1·02]
Placebo	73	1·08	-	-
Plasma glucose $AUC_{0\text{-}2h}$				
Anti-IL-21 + liraglutide	75	0·98	0·94	[0·86; 1·03]
Anti-IL-21	75	1·05	1·00	[0·91; 1·10]
Liraglutide	74	0·99	0·95	[0·86; 1·04]
Placebo	73	1·04	-	-
Fasting plasma glucagon				
Anti-IL-21 + liraglutide	76	1·00	1·00	[0·91; 1·10]
Anti-IL-21	76	0·96	0·96	[0·87; 1·06]
Liraglutide	75	0·97	0·97	[0·88; 1·07]
Placebo	75	1·00	-	-

AUC, area under the curve; CI, confidence interval; ETR, estimated treatment ratio; IL, interleukin; N: number of patients contributing to the analysis.

Supplementary Table S2 Other supportive secondary efficacy endpoints - week 80

Treatment group	N	Ratio to baseline	ETR (vs placebo)	95% CI
C-peptide $AUC_{0\text{-}4h}$				
Anti-IL-21 + liraglutide	75	0·55	1·08	[0·82; 1·43]
Anti-IL-21	75	0·57	1·11	[0·84; 1·46]
Liraglutide	74	0·35	0·68	[0·52; 0·90]
Placebo	73	0·51	-	-
C-peptide $AUC_{0\text{-}2h}$				
Anti-IL-21 + liraglutide	75	0·58	1·25	[0·94; 1·67]
Anti-IL-21	75	0·58	1·26	[0·95; 1·68]
Liraglutide	74	0·35	0·75	[0·57; 1·00]
Placebo	73	0·46	-	-
Plasma glucose $AUC_{0\text{-}4h}$				
Anti-IL-21 + liraglutide	75	1·19	0·99	[0·89; 1·09]
Anti-IL-21	75	1·09	0·91	[0·83; 1·01]
Liraglutide	74	1·16	0·97	[0·87; 1·07]
Placebo	73	1·20	-	-
Plasma glucose $AUC_{0\text{-}2h}$				
Anti-IL-21 + liraglutide	75	1·16	1·02	[0·93; 1·11]
Anti-IL-21	75	1·06	0·93	[0·85; 1·01]
Liraglutide	74	1·10	0·96	[0·88; 1·04]
Placebo	73	1·14	-	-
Fasting plasma glucagon				
Anti-IL-21 + liraglutide	76	0·89	0·96	[0·86; 1·07]
Anti-IL-21	76	0·86	0·92	[0·82; 1·03]
Liraglutide	75	0·96	1·03	[0·93; 1·15]
Placebo	75	0·93	-	-

AUC, area under the curve; CI, confidence interval; ETR: estimated treatment ratio; IL, interleukin, N, number of patients contributing to the analysis.

Supplementary Table S3 Pharmacokinetic properties of anti-IL-21

	Anti-IL-21 + liraglutide N=21	Anti-IL-21 N=21
Endpoint (unit)	Geometric mean (CV%)	
$AUC_{\tau, \text{anti-IL-21}}$, area under the anti-IL-21 time-concentration curve over a dosing interval at steady state (day*ug/mL)	3969 (17·9)	4115 (23·1)
Terminal $t_{½}$, half-life after last dose of anti-IL-21 (days)	22·3 (17·9)	22·2 (16·5)
$V_{ss, \text{anti-IL-21}}$, the apparent volume of distribution of anti-IL-21 at steady-state (mL/kg)	83·7 (23·1)	79·3 (17·6)
$CL_{ss, \text{anti-IL-21}}$, clearance of anti-IL-21 at steady-state ([mL/day]/kg)	3·02 (17·9)	2·92 (23·1)
$MRT_{, \text{anti-IL-21}}$, the mean residence time of anti-IL-21 (days)	27·7 (24·5)	27·2 (22·6)
$R_{A,AUC, \text{anti-IL-21}}$, accumulation ratio of anti-IL-21 defined as $AUC_{48\text{-}54 \text{ weeks}}/AUC_{0\text{-}6 \text{ weeks}}$ (ratio)	1·24 (17·8)	1·26 (17·4)
Anti-IL-21 concentration prior to dosing of anti-IL-21 at steady-state (ug/L)	34·7 (42·3)	36·7 (36·8)
Anti-IL-21 concentration 1 h after dosing of anti-IL-21 at steady state (ug/L)	298·6 (16·8)	282·6 (56·4)

CV, coefficient of variation; IL, interleukin.

Supplementary Table S4 Vital signs and amylase and lipase measurements at weeks 54 and 80

Treatment group	N	Change from baseline	ETD (vs placebo)	95% CI
Systolic blood pressure (mmHg), week 54				
Anti-IL-21 + liraglutide	76	-0	-4	[-7;-0]
Anti-IL-21	77	2	-1	[-4;2]
Liraglutide	75	2	-1	[-5;2]
Placebo	76	3	-	-
Diastolic blood pressure (mmHg), week 54				
Anti-IL-21 + liraglutide	76	0	-0	[-3;2]
Anti-IL-21	77	2	2	[-1;4]
Liraglutide	75	1	1	[-2;3]
Placebo	76	1	-	-
Pulse rate (beats per minute), week 54				
Anti-IL-21 + liraglutide	76	-1	-1	[-4;2]
Anti-IL-21	77	-0	-1	[-4;2]
Liraglutide	75	2	1	[-2;4]
Placebo	76	1	-	-
Body weight (kg), week 54				
Anti-IL-21 + liraglutide	76	-1·8	-3·0	[-4·6; -1·4]
Anti-IL-21	77	1·3	0·1	[-1·5;1·7]
Liraglutide	75	-2·6	-3·8	[-5·4; -2·2]
Placebo	76	1·2	-	-
Body weight (kg), week 80				
Anti-IL-21 + liraglutide	76	1·8	-1·0	[-2·7 ; 0·7]
Anti-IL-21	77	1·8	-1·0	[-2·7 ; 0·7]
Liraglutide	75	0·3	-2·5	[-4·2 ; -0·8]
Placebo	76	2·8	-	-
		Ratio to baseline	ETR (vs placebo)	95% CI
Amylase (U/L), week 54				
Anti-IL-21 + liraglutide	76	1·04	1·01	[0·94;1·09]
Anti-IL-21	77	0·98	0·96	[0·89;1·03]
Liraglutide	75	1·04	1·02	[0·95;1·09]
Placebo	76	1·03	-	
Lipase (U/L), week 54				
Anti-IL-21 + liraglutide	76	1·06	1·08	[0·97;1·20]
Anti-IL-21	77	0·92	0·95	[0·86;1·06]
Liraglutide	75	1·02	1·05	[0·95;1·16]
Placebo	76	0·98	-	

N, number of participants contributing to the analysis. ETD, estimated treatment difference; ETR, estimated treatment ratio; IL, interleukin.

Supplementary Table S5 Analysis of hypoglycaemic and hyperglycaemic endpoints

Treatment group	N	Estimated rate (events/100 years)	Treatment ratio (vs placebo)	95% CI
Treatment period				
Severe or blood glucose-confirmed symptomatic hypoglycaemia				
Anti-IL-21 + liraglutide	77	468·6	0·66	[0·39; 1·12]
Anti-IL-21	77	490·0	0·69	[0·41; 1·16]
Liraglutide	76	355·7	0·50	[0·30; 0·85]
Placebo	77	708·3	-	-
Nocturnal severe or blood glucose-confirmed symptomatic hypoglycaemia				
Anti-IL-21 + liraglutide	77	54·9	0·89	[0·38; 2·12]
Anti-IL-21	77	43·3	0·70	[0·29; 1·71]
Liraglutide	76	42·5	0·69	[0·29; 1·63]
Placebo	77	61·5	-	-
Hyperglycaemia				
Anti-IL-21 + liraglutide	77	481·8	1·27	[0·59; 2·73]
Anti-IL-21	77	278·4	0·73	[0·34; 1·57]
Liraglutide	76	192·0	0·51	[0·23; 1·12]
Placebo	77	379·3	-	-
Observation period				
Severe or blood glucose-confirmed symptomatic hypoglycaemia				
Anti-IL-21 + liraglutide	77	558·1	0·80	[0·36; 1·77]
Anti-IL-21	77	355·9	0·51	[0·23; 1·13]
Liraglutide	76	575·3	0·83	[0·39; 1·77]
Placebo	77	695·4	-	-
Nocturnal severe or blood glucose-confirmed symptomatic hypoglycaemia				
Anti-IL-21 + liraglutide	77	117·8	1·74	[0·60; 5·01]
Anti-IL-21	77	25·4	0·37	[0·11; 1·24]
Liraglutide	76	75·6	1·12	[0·39; 3·16]
Placebo	77	67·7	-	-
Hyperglycaemia				
Anti-IL-21 + liraglutide	77	866·0	2·91	[1·18; 7·2]
Anti-IL-21	77	186·7	0·63	[0·25; 1·59]
Liraglutide	76	801·6	2·69	[1·10; 6·60]
Placebo	76	297·5	-	-

Nocturnal hypoglycaemic episodes: episodes occurring between 00:01 and 05:59 both inclusive. Estimated rates are weighed according to the distribution in the data. CI, confidence interval; IL, interleukin; N, number of participants contributing to analysis.

Supplementary Table S6 Adverse events in the observation period

	Combination			Anti-IL-21			Liraglutide			Placebo		
	N	%	R	N	%	R	N	%	R	N	%	R
Participants (N)	77			77			76			77		
Participant-years of observation	34·88			33·07			35·04			33·57		
All adverse events	34	44·2	201	36	46·8	221	31	40·8	223	31	40·3	259
Possibly or probably related to												
Anti-IL-21	1	1·3	2·9	2	2·6	12·1	4	5·3	17·1	3	3·9	8·9
Liraglutide	1	1·3	2·9	2	2·6	12·1	3	3·9	14·3	2	2·6	6·0
Serious adverse events	4	5·2	11	2	2·6	6	4	5·3	14	5	6·5	36
Most frequently reported adverse events [a]												
Nasopharyngitis	7	9·1	22·9	14	18·2	60·4	9	11·8	25·7	8	10·4	29·8
Headache	4	5·2	20·1	3	3·9	9·1	1	1·3	2·9	2	2·6	8·9
Hypoglycaemic episodes												
ADA classification	44	57·1	3229·2	49	63·6	2673·7	52	68·4	3777·1	46	59·7	2919·6
Severe	2	2·6	8·6	1	1·3	3·0	0	0·0	0·0	1	1·3	3·0
Severe or blood glucose-confirmed symptomatic [b]	27	35·1	618·9	27	35·1	392·7	37	48·7	697·1	23	29·9	714·3
Hyperglycaemic episodes [c]	28	36·4	876·8	20	26·0	223·6	31	40·8	1005·7	17	22·1	333·3
Diabetic ketoacidosis	0	0·0	0·0	0	0·0	0·0	1	1·3	0·9	1	1·3	0·9

[a] Events reported at an overall rate of ≥10 events per 100 participant-years of observation.

[b] Severe or blood glucose-confirmed symptomatic' hypoglycaemic episodes are either severe according to the ADA classification[27] or an episode confirmed by a plasma blood glucose value <3.1 mmol/L (56 mg/dL) and with symptoms consistent with hypoglycaemia. This hypoglycaemia classification was the endpoint used for the pre-specified statistical analysis of hypoglycaemia.

[c] Hyperglycaemic episodes are defined as and confirmed by plasma glucose values >16.7 mmol/l (300 mg/dl). ADA, American Diabetes Association; IL, interleukin; N, number of participants with at least one event/episode; %, proportion of participants with at least one event/episode; R, events/episodes per 100 participant-years of exposure (treatment period) or observation (observation period).

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Research in context

Evidence before this study

Prevention of type 1 diabetes would require early modulation of the pathway that leads to autoimmune destruction of beta cells in the pancreatic islets of Langerhans. Recent insights into the mechanisms of disease in type 1 diabetes have led to clinical trials of several novel therapies. New strategies designed to regulate the immune system include the use of antigen-based immunotherapies and immunomodulatory or immunosuppressive agents. However, side-effects associated with systemic immune suppression and/or the partial nature of the observed efficacy have contributed to the lack of regulatory approval of these therapies so far. A single agent or approach seems unlikely to halt disease progression in all people with type 1 diabetes.

Added value of this study

We hypothesized that a compound with low-grade and transient immune-modifying effects combined with a therapy to improve beta-cell function would synergise to offer efficacy-related benefits on beta-cell survival but with lower risk of the complications generally associated with immune suppression. In this randomised, double-blind trial with 308 adult patients with recent-onset diabetes and residual C-peptide secretion, combination treatment of anti-IL-21 and liraglutide for 54 weeks was well-tolerated and resulted in sustained endogenous insulin secretion and improved glucose metabolism compared with placebo. To date, the present trial is the largest of its kind in adults and in contrast to most other investigations, it included an off-drug follow-up period.

Implications of all the available evidence

Combination therapy with agents such as anti-IL-21 and liraglutide could constitute a potential novel disease-modifying therapy by preserving endogenous beta-cell function. The beta-cell preservation observed in the present trial appears at least on par with what has been seen in other similar disease-modifying trials in type 1 diabetes. While reported adverse events were few and benign indicating a favourable safety profile, the long-term safety of the therapy remains to be evaluated. The results obtained from this study imply that beta-cell function could be preserved longer if treatment had been continued, ensuring added benefit for patients. Moreover, prevention of type 1 diabetes might become a reality if this regimen were to be given to high-risk patients with Stage-1 or Stage-2 type 1 diabetes.