

STUDY PROTOCOLS

INnoVative trial design for testing the Efficacy, Safety and Tolerability of 6-month treatment with incretin-based therapy to prevent type 1 DIAbetes in autoantibody positive participants: A protocol for three parallel double-blind, randomised controlled trials (INVESTDIA)

Jukka Kero^{1,2,3} | Jaakko J. Koskenniemi^{1,2,3} | Sara Karsikas² | Tytti Pokka^{4,5} | Olivia Lou⁶ | Jorma Toppari^{1,3} | Riitta Veijola^{4,5} 

¹Research Centre for Integrative Physiology and Pharmacology, Institute of Biomedicine, University of Turku, Turku, Finland

²Department of Paediatrics, Turku University Hospital, Turku, Finland

³Centre for Population Health Research, University of Turku and Turku University Hospital, Turku, Finland

⁴Department for Children and Adolescents, Oulu University Hospital, Oulu, Finland

⁵Department of Paediatrics, PEDEGO Research Unit, MRC Oulu, University of Oulu, Oulu, Finland

⁶JDRF, New York, New York, USA

Correspondence

Riitta Veijola, Department of Paediatrics, University of Oulu and Oulu University Hospital, PO Box 5000, FIN-90014, University of Oulu, Oulu, Finland.

Email: riitta.veijola@oulu.fi

Funding information

JDRF International, Grant/Award Number: 3-SRA-2014-301-M-R, 3-SRA-2016-142-M-R and 3-SRA-2016-143-M-R; Novo Nordisk

Abstract

Aims: β -cell stress and dysfunction may contribute to islet autoimmunity and progression to clinical type 1 diabetes. We present a protocol of three randomised controlled trials assessing the effects of glucagon-like peptide 1 (GLP-1) analogue liraglutide in three early stages of type 1 diabetes.

Methods: We will test 10- to 30-year-old people with multiple islet autoantibodies for their glucose metabolism and randomise participants with stage 1 (multiple islet autoantibodies and normoglycaemia), stage 2 (multiple islet autoantibodies and dysglycaemia) and early stage 3 (clinical diagnosis) type 1 diabetes, 10–14 persons in each, to a 6-month intervention with liraglutide or placebo with 6-month follow-up in the stage 2 and stage 3 trials and 18-month follow-up in the stage 1 trial. Primary efficacy outcome in the stage 1 and stage 2 trials is a first-phase insulin response in an intravenous glucose tolerance test and C-peptide area under the curve in a 2-h mixed-meal tolerance test in the stage 3 trial. In addition, safety and tolerability of liraglutide treatment will be assessed.

Conclusions: Most prevention trials of type 1 diabetes have targeted the immune system. Treatment with GLP-1 analogue liraglutide supports the pancreatic β -cells, which should likewise attenuate islet autoimmunity. Our innovative study design allows simultaneous investigation of an intervention in three groups of people who represent various early stages of type 1 diabetes and maximises the eligibility to participate.

Jukka Kero and Jaakko J. Koskenniemi shared first authorship.

Combined description of three study protocols: Stage 1 trial: Version 6, Date: July 29, 2018;

Stage 2 trial: Version 5, Date: July 29, 2018;

Stage 3 trial: Version 5, Date: July 29, 2018

This is an open access article under the terms of the [Creative Commons Attribution-NonCommercial](https://creativecommons.org/licenses/by-nc/4.0/) License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.

© 2022 The Authors. *Diabetic Medicine* published by John Wiley & Sons Ltd on behalf of Diabetes UK.

Trial registration: NCT02611232 (stage 1 trial), NCT02898506 (stage 2 trial), NCT02908087 (stage 3 trial).

KEYWORDS

diabetes prevention, glucagon-like peptide 1 analogue, islet autoantibody, liraglutide, type 1 diabetes

1 | INTRODUCTION

Type 1 diabetes is a chronic disease, which requires lifelong insulin replacement therapy and careful glucose monitoring to prevent hyperglycaemia-induced complications and avoid hypoglycaemia.¹ Type 1 diabetes results from immune-associated dysfunction of insulin-producing β -cells in the pancreas.

When multiple autoantibodies against islet-derived peptides are observed in childhood, at least 70% of children progress to clinical diabetes within 10 years through identifiable disease stages, including stage 1 (multiple islet autoantibodies and normoglycemia in an oral glucose-tolerance test [OGTT]), stage 2 (multiple islet autoantibodies and dysglycemia in an OGTT) and stage 3 (clinical type 1 diabetes fulfilling WHO and ADA criteria).^{2–6} However, stage 3 type 1 diabetes includes a very heterogeneous group of people from an early-diagnosed asymptomatic hyperglycaemia observed in an OGTT to late-diagnosed severe ketoacidosis. Thus, careful definition of eligibility criteria according to the two presymptomatic stages of type 1 diabetes, and strict criteria within stage 3, can identify homogeneous study groups.⁴ This would improve the power to observe clinically meaningful differences between trial arms even with small sample sizes.

Recent findings suggest that β -cell stress may contribute to the immune attack against β -cells.^{7,8} Glucagon-like peptide-1 (GLP-1) analogue exendin-4 has been shown to decrease β -cell stress⁹ and delay the onset of diabetes in NOD mouse model of type 1 diabetes.¹⁰ GLP-1 is produced by intestinal epithelial L-cells in response to nutrient intake, and it stimulates insulin secretion, suppresses glucagon secretion and thus helps to maintain the postprandial glucose concentrations in the normal range.¹¹

A recent, randomised controlled study by von Herrath and co-workers showed that an anti-interleukin-21 antibody (anti-IL-21) and liraglutide together slowed down the decrease in mixed meal stimulated C-peptide in people with newly diagnosed type 1 diabetes, although neither liraglutide nor anti-IL-21 alone were effective.¹² Similarly, in adults with newly diagnosed type 1 diabetes monotherapy with albiglutide, a long-acting GLP-1 analogue, was not effective in preservation of β -cell function.¹³ In clinical practice, GLP-1 analogues play an important role

in treatment of type 2 diabetes and obesity in adults.^{14,15} The safety profile of liraglutide is well known, and it is considered safe, generally well tolerated, and efficient also among children and adolescents with type 2 diabetes.^{16,17}

Despite these promising results, no randomised controlled trials on the use of GLP-1 analogues among children and adolescents with early-diagnosed or presymptomatic type 1 diabetes to delay the disease process have been published. Here, we present a description of three protocols randomising participants at stage 1, 2 or 3 type 1 diabetes to a 6-month treatment with self-administered subcutaneous liraglutide or placebo.

2 | METHODS

2.1 | Hypothesis

We hypothesise that liraglutide treatment is superior to placebo in preservation of β -cell function among participants with multiple islet autoantibodies with or without dysglycaemia, prevents or delays the development of insulin deficiency and that recruitment at different stages of type 1 diabetes can be effectively done using an innovative trial design.

2.2 | Objectives and end points

The main objective of the study is to assess whether daily administered 6-month treatment with liraglutide improves endogenous insulin production and glucose metabolism. Secondary objectives include determination of safety and tolerability of liraglutide treatment among participants at different stages of type 1 diabetes as well as testing of feasibility of parallel recruitment into three simultaneous type 1 diabetes stage-based trials. (Table 1).

2.3 | Trial design

INVESTDIA consists of three double-blinded, parallel group, two-arm, randomised controlled superiority trials with 1:1 allocation ratio to liraglutide or placebo (Supplementary

Table S1). In all trials, similar 6-month interventions are performed in parallel. Screening in islet autoantibody positive young people to stage 1, 2 and 3 trials is performed by a 6-point OGTT, which together with glycated haemoglobin

(HbA1c) directs eligibility to different trials. Therefore, the screening OGTT and other study assessments at baseline visit are valid for all three trials. The innovative design and recruitment scheme is shown in Figure 1.

TABLE 1 Primary and secondary end points of three type 1 diabetes prevention trials testing incretin-based therapy in islet autoantibody positive participants

| | Stage 1 trial | Stage 2 trial | Stage 3 trial |
|--|---|---|---|
| Primary end points (after 6-month treatment with liraglutide or placebo) | FPIR in IVGTT | FPIR in IVGTT | C-peptide AUC in MMTT |
| Secondary end points | Glycaemic variability* HbA1c C-peptide AUC in OGTT Insulin AUC in 10 min IVGTT Progression to dysglycaemia or type 1 diabetes • Proportion of participants • Time to the end point from the start of intervention | Glycaemic variability* HbA1c C-peptide AUC in OGTT Insulin AUC in 10 min IVGTT Progression to type 1 diabetes or reversal to normoglycaemia • Proportion of participants • Time to the diagnosis from the start of the intervention • Time to the diagnosis from seroconversion to ≥ 2 autoantibodies | Glycaemic variability* HbA1c IDAA1c Total daily insulin dose IU/kg/day (3-day average) |

CGM variables include mean glucose; SD; CV%; day mean glucose; night mean glucose; time (%) of glucose <3.0 mmol/L, 3.9 – 7.8 mmol/L, >7.8 mmol/L, >11.0 mmol/L, and >13.9 mmol/L; mean amplitude of glucose excursions (MAGE).

Abbreviations: *glycaemic variability will be assessed with CGM, continuous glucose monitoring; AUC, area under the curve; FPIR, first-phase insulin response; HbA1c, haemoglobin A1c; IDAA1c, insulin dose-adjusted A1c; IVGTT, intravenous glucose tolerance test; MMTT, mixed meal tolerance test; OGTT, oral glucose tolerance test.

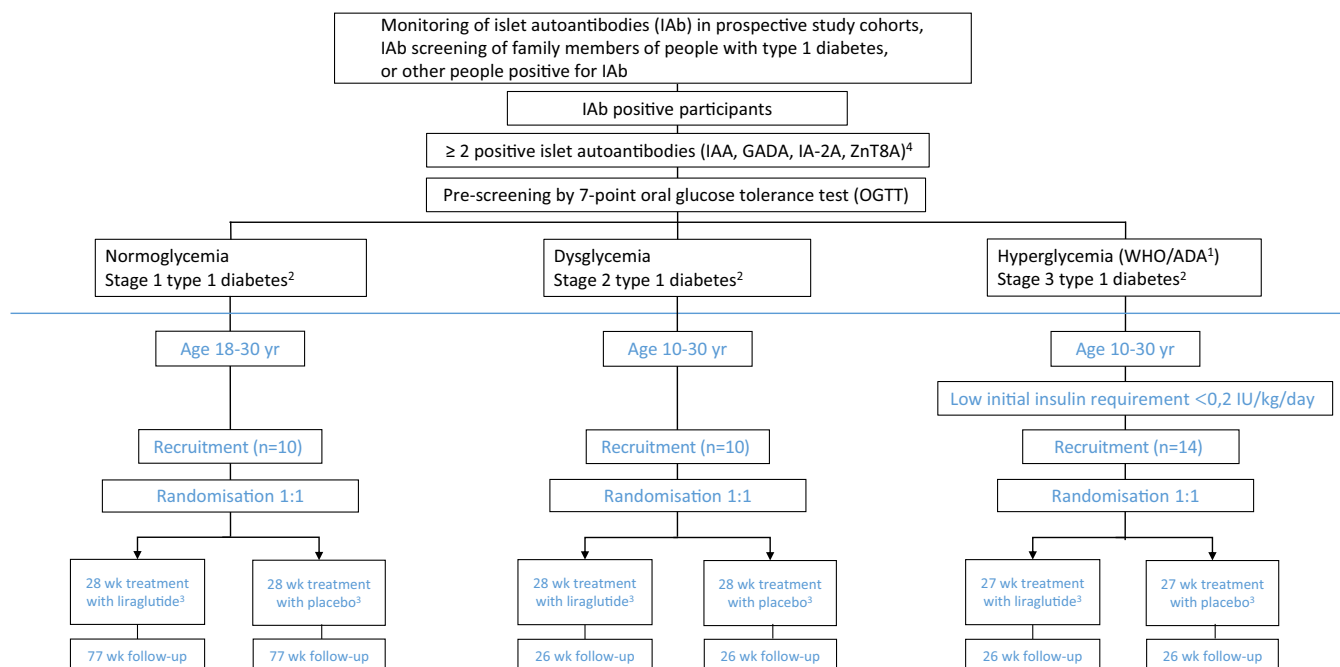


FIGURE 1 Overview of the design and recruitment for three parallel type 1 diabetes prevention trials testing incretin-based therapy in islet autoantibody positive participants at various stages of type 1 diabetes. ¹See ref [5,6]; ²See ref [4]; ³Primary endpoint assessed before and at the end of intervention; ⁴IAA (insulin autoantibody), GADA (glutamic acid decarboxylase antibody), IA-2A (insulinoma-associated protein 2 antibody), ZnT8A (zinc transporter 8 antibody).

2.4 | Recruitment and enrolment

The majority of the islet autoantibody multipositive participants are recruited from The Finnish Type 1 Diabetes Prediction and Prevention (DIPP) Study, a population-representative birth cohort study ongoing in three university hospitals in Finland.¹⁸ In addition, autoantibody positive participants identified otherwise, for example, from other follow-up studies such as the TrialNet study,¹⁹ The Environmental Determinants of Diabetes in the Young (TEDDY) Study²⁰ or family members of people with type 1 diabetes in the Finnish Pediatric Diabetes Register²¹ may be recruited.

Enrolment and follow-up takes place in three university hospitals (Oulu, Turku and Tampere), which have extensive experience on clinical follow-up studies and clinical trials aiming at prediction and prevention of type 1 diabetes.

2.5 | Eligibility and exclusion criteria

Specific inclusion and exclusion criteria of all three trials are listed in [Tables 2](#) and [3](#), respectively. In general, all participants have to be positive for two or more islet autoantibodies (insulin autoantibodies, IAA; autoantibodies against glutamic acid decarboxylase, GADA; insulinoma-associated protein 2, IA-2A; or zinc transporter 8, ZnT8A). The glycaemic state defined during an OGTT determines whether a person is eligible for stage 1, stage 2, or stage 3 trial. All participants for stage 2 and stage 3 trials must be 10–30 years old and for stage

1 trial 18–30 years. Stage 1 trial was limited to adults based on limited information about the risk–benefit balance of liraglutide in the minors. Those to be randomised in stage 3 trial must have early diagnosis of diabetes with low insulin requirement of <0.2 IU/kg/day and randomised within 2 months from diagnosis. The main exclusion criteria include treatment with any antidiabetic drug prior to the study (except for insulin in stage 3 trial), other severe disease or pregnancy.

2.6 | Intervention

After baseline visit at visit 2, participants are randomised 1:1 for placebo or liraglutide (liraglutide solution [6 mg/ml], Victoza, Novo Nordisk) in all three trials. The starting dose of liraglutide is 0.6 mg once per day, and the dose will be increased every 2 weeks to 1.2 mg/day and then to maximal dose 1.8 mg/day if no significant side effects are reported and if participant's body mass index (BMI) is >20 (stage 1 trial) or body weight ≥ 30 kg (required for the 1.2 mg dose) or ≥ 45 kg (required for the 1.8 mg dose) for stage 2 and 3 trials. In case of significant side effects, the participants are asked to contact the study personnel to consider the adjustment of the dose to the maximum tolerable dose. The participants are advised to inject the study drug prior to bedtime to minimise the potential gastrointestinal side effects. Compliance is monitored by recording the consumption of the study product (drug/placebo). The participants

TABLE 2 Inclusion criteria for three type 1 diabetes prevention trials testing incretin-based therapy in islet autoantibody positive participants

| Inclusion criteria (trial specific) | | |
|--|---|---|
| Stage 1 trial | Stage 2 trial | Stage 3 trial |
| Age 18–30 years | Age 10–30 years | Age 10–30 years |
| Positivity for ≥2 islet autoantibodies (GADA, IA-2A, IAA, ZnT8A) at least twice in consecutive samples | Positivity for ≥2 islet autoantibodies (GADA, IA-2A, IAA, ZnT8A) at least twice in consecutive samples | |
| | Glucose intolerance or dysglycaemia documented during the preceding 12 months defined as having IGT, or IFG, or 10% rise in HbA1c, or HbA1c ≥ 5.9% (41 mmol/mol) within the last 12 months, or p-gluc ≥ 11.1 mmol/L at 30, 60, or 90 min timepoints during OGTT | Early diagnosis of type 1 diabetes defined as 1. Fasting p-gluc ≥ 7.0 mmol/L and/or 2-h p-gluc ≥ 11.1 mmol/L in an OGTT or 2. Two diabetic p-gluc values in a single OGTT, or in two OGTTs performed at least 1 week apart, and 3. Initial insulin requirement < 0.2 IU/kg/day |
| Inclusion criteria (common) | | |
| Informed consent | | |
| Negative pregnancy test in female participants | | |

Abbreviations: GADA, glutamic acid decarboxylase antibodies; HbA1c, haemoglobin A1c; IA-2A, insulinoma associated protein 2 antibodies; IAA, insulin autoantibodies; IFG, impaired fasting glucose; IGT, impaired glucose tolerance; OGTT, oral glucose tolerance test; ZnT8A, zinc transporter 8 antibodies.

TABLE 3 Exclusion criteria of three type 1 diabetes prevention trials testing incretin-based therapy in islet autoantibody positive participants

| Exclusion criteria (trial specific) | | |
|--|-----------------|---------------|
| Stage 1 trial | Stage 2 trial | Stage 3 trial |
| Type 1 diabetes | Type 1 diabetes | |
| Dysglycaemia ^a | | |
| Exclusion criteria (common): | | |
| Allergy to liraglutide or other ingredients of Victoza® | | |
| Ketoacidosis | | |
| Previous treatment in the last 3 months with any antidiabetic medication | | |
| Impaired liver or kidney function or on dialysis | | |
| Severe heart failure | | |
| Severe gastrointestinal problem resulting in gastroparesis, or IBD | | |
| Past or current history of pancreatitis | | |
| Serum calcitonin value above normal (>50 ng/L or ≥3.4 pmol/L) | | |
| Presence or history of any significant metabolic, hematologic or malignant disease | | |
| Pregnant female participants and female participants of child-bearing potential using inadequate contraception | | |
| Breastfeeding | | |

^aDefined as having IFG (impaired fasting glucose) or IGT (impaired glucose tolerance), or 10% rise in haemoglobin A1c (HbA1c) or HbA1c ≥5.9% (41 mmol/mol) measured within the last 12 months, or plasma glucose ≥11.1 mmol/L at 30, 60, or 90 min timepoints during oral glucose tolerance test. IBD, inflammatory bowel disease.

are asked to return all unused and used pens and vials at the next study visit, and the remaining liraglutide/placebo volume will be measured. If the participant temporarily or permanently discontinues the study product before the end of the 6-month treatment phase, he or she will be encouraged to continue the study visits according to the protocol. The investigator can decide in collaboration with the principal investigator and Data and Safety Monitoring Board (DSMB) to discontinue the participation of a person if deemed necessary to protect the health and well-being of the participant. Study treatment may be terminated prematurely by the investigator for reasons such as allergic reactions to the study product, significant repeated increase in serum calcitonin or serum/urine amylase levels, other significant deviation in the metabolic laboratory tests, development of type 1 diabetes (in stage 1 and 2 trial), severe repeated hypoglycaemia, pregnancy or diagnosis of a new significant disease during the intervention period.

2.7 | Concomitant medication

The permissibility of other medication during the study is evaluated by the investigator who will consult the

principal investigator and DSMB when necessary. All concomitant medications and therapies are recorded throughout the study.

2.8 | Outcomes

Primary end points of this study are first-phase insulin response (FPIR) during a 10-min intravenous glucose tolerance test (IVGTT) in stage 1 and 2 trials, and the serum C-peptide area under the curve (AUC) during a 2-h mixed-meal tolerance test (MMTT) in stage 3 trial between liraglutide-treated and placebo groups after 6 months' treatment (Table 1). Primary outcomes FPIR in 10-min IVGTT and C-peptide AUC in 2-h MMTT are widely used tests to study the β -cell function before and after the onset of type 1 diabetes, respectively.^{22–24} FPIR is a sensitive marker, which has been shown to decrease among multiple autoantibody positive people several years before the diagnosis of stage 3 type 1 diabetes.²²

Secondary end points include HbA1c and plasma glucose variability during continuous glucose monitoring (CGM) (all three trials), serum C-peptide AUC during a 2-h oral glucose tolerance test (OGTT) and serum insulin AUC during a 10-min IVGTT (stage 1 and 2 trials), total daily insulin dose (TDD) IU/kg/day, and insulin dose-adjusted A1c (IDAA1c) as defined by Mortensen and co-workers²⁵ (stage 3 trial). In addition, proportion of participants who develop dysglycaemia or clinical type 1 diabetes, and those with reversal to normoglycaemia during the follow-up are analysed, as well as the time to the development of dysglycaemia or type 1 diabetes from the start of the study product.

Safety and tolerability are closely monitored with frequent study calls, visits (specifically addressing to detect any gastrointestinal symptoms, hypoglycaemia, hyperglycaemia or weight loss), and laboratory measurements including full blood count, serum and urine amylase, serum lipase and serum calcitonin.

2.9 | Trial visits and examinations

The study outlines are described in Tables 4–6. At screening, participants are assigned to one of the three concurrent trials based on OGTT results: those with normoglycaemia or dysglycaemia to stage 1 and 2 trials, respectively, and those with ADA/WHO defined clinical type 1 diabetes to stage 3 trial. Randomisation at baseline is followed by a 28-, 27- and 27-week intervention and 77-, 24- and 26-week follow-up periods in stage 1, 2 and 3 trials, respectively. Altogether, there will be 7–13 visits with assessment and measurements and between-visit

TABLE 4 Study contacts and procedures in the stage 1 trial

| | Visit | | Phone | | | Visit | | | | | Phone | Visit | | | | | | |
|--|----------------|---|-------|-----|-----|------------------|------------------|------------------|------------------|------------------|------------------|-------|----|----|----|-----|-----|---|
| | 1 | 2 | 1 | 2 | 3 | 3 | 4 | 5 | 6 | 7 | 4 | 8 | 9 | 10 | 11 | 12 | 13 | |
| Timepoint (weeks ^A) | −1 | 0 | 1 | 2 | 3 | 4 | 8 | 16 | 26 | 27 | 28 | 39 | 52 | 53 | 78 | 104 | 105 | |
| Intervention period | R ^B | | | | | | | | | | | | | | | | | |
| Dose of the study drug (mg/day) or placebo | 0.6 | | 0.6 | 1.2 | 1.2 | 1.8 ^C | 1.8 ^C | 1.8 ^C | 1.8 ^C | 1.8 ^C | 1.8 ^C | | | | | | | |
| Informed consent and medical history | X | | | | | | | | | | | | | | | | | |
| Food record ^D | X | X | | | | X | | | | X | | | X | X | | | X | X |
| SMBG ^E | X | | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X |
| AE/SAE ^F | X | | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X |
| HbA1c | X | | | | | X | X | X | X | | | | X | X | | | X | X |
| Islet autoantibodies | X | | | | | | X | X | X | | | | X | X | | | X | X |
| Thyroid function tests | X | | | | | | | | | X | | | | X | | | | |
| Safety parameters ^G | X | | | | | X | X | X | X | | | | X | X | | | X | X |
| IVGTT | X | | | | | | X | | | | X | | | | X | | | |
| 7-point OGTT | X | | | | | X | | | X | X | | | X | X | | | X | X |
| 7-day CGM | X | | | | | | | | X | | | | X | | | | X | |
| Physical examination | X | | | | | | X | | | X | | | X | X | | | X | X |
| Samples for mechanistic studies ^E | X | | | | | | X | X | X | | | | X | X | | | X | X |

Note: ^Aweeks from randomisation visit, ^Brandomisation, ^Cif BMI ≥ 20 , ^D24-h food record interview, ^Eself-monitored blood glucose (SMBG) once a day will be requested for 3 days before the next contact, ^Fassessment for any adverse event (AE) or serious adverse event (SAE), ^Gsafety parameters include full blood count, serum and urine amylase, serum lipase, serum calcitonin. ^Esamples for mechanistic studies include fecal sample, serum sample and collections of peripheral blood mononuclear cells and whole blood RNA. Intervention period is marked by a light blue box.

Abbreviations: CGM, continuous glucose monitoring; HbA1c, haemoglobin A1c; IVGTT, intravenous glucose tolerance test; OGTT, oral glucose tolerance test.

telephone contacts to ensure the safety and compliance of the study participants.

2.10 | Sample size

Based on reference values reported earlier, we expected that in stage 1 and stage 2 trials, 6 months after randomisation, FPIR mean would be 46 (SD 15) and 40 (SD 15) mU/L in placebo groups, respectively.²⁴ For stage 3 trial, C-peptide AUC in 2-h MMTT was assumed to be 1.0 nmol/L/120 min (SD 0.3) after 6 months from early diagnosis of type 1 diabetes in the placebo group. This was based on C-peptide AUC data in 2-h OGTT at diagnosis in the DPT-1 study.²⁶ Thus, sample sizes of 42, 54 and 14 participants in stage 1, 2 and 3 trials, respectively, would give an 80% probability to observe a 30% higher FPIR in 10-min IVGTT and 50% higher C-peptide AUC in 2-h MMTT in the liraglutide groups as compared with the placebo groups when using two-sided t-tests given alpha 0.05 and 10% dropout. However, our early observations from still blinded stage 1 and 2 trials indicated that some participants had marked increases of 100% to 360% in FPIR

whereas others had stable or declining FPIR. Therefore, we supposed that the remarkable increase in FPIR had occurred in the participants receiving active investigational product and recalculated the sample size under the assumption of 100% increase after liraglutide treatment, and sample sizes of 10 participants to be randomised in stage 1 and 2 trials were found to be sufficient to detect a clinically significant difference even when assuming a dropout rate of 20%. The participants who withdraw from the study before visit 4 will be replaced and randomised as a new participant. However, data from all randomised participants will be included in the intention to treat analyses.

2.11 | Allocation, concealment and blinding

The participants will be randomised with a ratio of 1:1 by a computer-generated pseudo-random number generator (StatsDirect version 3, StatsDirect Ltd, England). Randomisation lists for each study site are produced by the study statistician in the coordinating study centre (Oulu), kept in sealed envelopes and sent to the local

TABLE 5 Study contacts and procedures in the stage 2 trial

| | Visit | | Phone | | Visit | | Phone | | Visit | | Phone | | Visit | | Phone | | Visit | | Phone | | | | |
|--|----------------|-----|-------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|-------|----|-------|----|----|----|----|
| | 0 ^A | 1 | 2 | 1 | 2 | 3 | 4 | 5 | 3 | 4 | 6 | 7 | 5 | 6 | 8 | 9 | 10 | 7 | 11 | 12 | 8 | 9 | 13 |
| Timepoint (weeks) | -1 | 0 | 1 | 1 | 2 | 3 | 4 | 5 | 6 | 12 | 16 | 20 | 26 | 27 | 28 | 30 | 34 | 39 | 43 | 47 | 52 | 53 | 54 |
| Intervention period | R ^B | | | | | | | | | | | | | | | | | | | | | | |
| Dose (study drug mg/day or placebo) | | 0.6 | 0.6 | 1.2 ^C | 1.2 ^C | 1.8 ^D | 1.8 ^D | 1.8 ^D | 1.8 ^D | 1.8 ^D | 1.8 ^D | 1.8 ^D | 1.8 ^D | 1.8 ^D | 1.8 ^D | 1.8 ^D | | | | | | | |
| Informed consent and medical history | X | (X) | | | | | | | | | | | | | | | | | | | | | |
| Food record ^E | X | X | | | | | | | X | X | | | X | | | | | X | | | | | |
| SMBG ^F | | | | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X |
| AE/SAE ^G | | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X |
| HbA1c | X | X | | | | | | | X | X | | | X | | | | | X | | | | | |
| Islet autoantibodies | X | | | | | | | | | X | | | X | | | | | X | | | | | |
| Thyroid function tests | X | | | | | | | | | | | | X | | | | | X | | | | | |
| Safety parameters ^H | X | | | | | | | | X | X | | | X | | | | | X | | | | | |
| IVGTT | | X | | | | | | | | | | | | X | | | | | | | | X | |
| 7-point OGTT | X | (X) | | | | | | | X | X | | | X | | | | | X | | | | | |
| 7-day CGM | X | | | | | | | | | X | | | X | | | | | X | | | | | |
| Physical examination | X | | | | | | | | X | X | | | X | | | | | X | | | | | |
| Samples for mechanistic studies ^I | X | | | | | | | | | X | | | X | | | | | X | | | | | |

Note: ^Ascreening visit performed if necessary. If the participant has a history of impaired glucose tolerance (IGT) or impaired fasting glucose (IFG) or dysglycaemia in oral glucose tolerance test (OGTT); plasma glucose ≥ 11.1 mmol/L at 30, 60, or 90 min timepoints) or 10% rise in HbA1c or HbA1c $\geq 5.9\%$ (41 mmol/mol) measured within the last 12 months, screening visit is not needed. If the OGTT or HbA1c criterion is met, the participant proceeds to Visit 1. If the OGTT or HbA1c criterion is not met, screening visits will continue every 3 months (HbA1c is analysed every 3 months and 7-point OGTT is repeated every 6 months). ^BRandomisation, ^Cdose 1.2 mg if body weight (bw) ≥ 20 kg, ^Ddose 1.8 mg if bw ≥ 45 kg, ^E24 h food record interview, ^Fself-monitored blood glucose (SMBG) requested for 3 days before the next contact, ^Gassessment for any adverse event (AE) or serious adverse event (SAE), ^Hsafety parameters including full blood count, serum and urine amylase, serum lipase and serum calcitonin. ^ISamples for mechanistic studies including fecal sample, serum sample and collections of peripheral blood mononuclear cells and whole blood RNA. Intervention period is marked by a light blue box.

Abbreviations: CGM, continuous glucose monitoring; HbA1c, haemoglobin A1c; IVGTT, intravenous glucose tolerance test.

TABLE 6 Study contacts and procedures in the stage 3 trial

| | Visit | | Phone | | Visit | | Phone | | Visit | | Phone | | Visit | | Phone | | Visit | | Phone | |
|--|----------------|-----|-------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|-------|----|-------|----|
| | 1 | 2 | 1 | 2 | 3 | 4 | 5 | 6 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 6 | 11 | 12 | 7 |
| Timepoint (weeks) | −1 | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 12 | 12 | 16 | 20 | 26 | 27 | 30 | 34 | 39 | 43 | 47 | 52 |
| Intervention period | R ^A | | | | | | | | | | | | | | | | | | | |
| Dose (study drug mg/day or placebo) | 0.6 | 0.6 | 0.6 | 1.2 ^B | 1.2 ^B | 1.8 ^C | 1.8 ^C | 1.8 ^C | 1.8 ^C | 1.8 ^C | 1.8 ^C | 1.8 ^C | 1.8 ^C | 1.8 ^C | 1.8 ^C | 1.8 ^C | | | | |
| Informed consent and medical history | X | | | | | | | | | | | | | | | | | | | |
| Food record ^D | X | X | | | | | | X | X | | | | | X | | | X | | | X |
| SMBG ^E | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X |
| AE/SAE ^F | | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X |
| HbA1c | X | | | | | | | X | X | X | | | | X | | | X | | | X |
| Islet autoantibodies | X | | | | | | | | X | | | | | X | | | X | | | X |
| Thyroid function tests | X | | | | | | | | | | | | | X | | | | | | X |
| Safety parameters ^G | X | | | | | | | X | X | X | | | | X | | | X | | | X |
| 7-day CGM | X | | | | | | | | X | X | | | | X | | | X | | | X |
| Physical examination | X | | | | | | | X | X | X | | | | X | | | X | | | X |
| 2-h MMTT | X | | | | | | | | X | X | | | | X | | | X | | | X |
| Insulin dose record | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X |
| Samples for mechanistic studies ^H | X | | | | | | | | X | | | | | X | | | X | | | X |

Note: ^A randomisation, ^B dose 1.2 mg if body weight (bw) ≥30 kg, ^C dose 1.8 mg if bw ≥45 kg, ^D 24 h food record interview, ^E self-monitored blood glucose (SMBG) requested for 3 days before the next contact, ^F assessment for any adverse event (AE) or serious adverse event (SAE), ^G safety parameters including full blood count, serum and urine amylase, serum lipase and serum calcitonin. ^H samples for mechanistic studies including fecal sample, serum sample and collections of peripheral blood mononuclear cells and whole blood RNA. Intervention period is marked by a light blue box.

Abbreviations: CGM, continuous glucose monitoring; HbA1c, haemoglobin A1c; MMTT, mixed-meal tolerance test.

hospital pharmacists. The allocation sequence is generated by a study statistician, enrolment by study physicians and local pharmacists assign the enrolled participants to an intervention according to the randomisation list (liraglutide or placebo) at each study site. After the recalculation of sample sizes on June 19, 2019, the local randomisation lists have been changed to centralised lists for all three trials that are kept in the coordinating hospital pharmacy. The local pharmacies receive allocation for a new study participant from the coordinating pharmacy by phone. More details of the randomisation procedures will be given upon request after the blinding has ended.

The liraglutide and placebo preparations (pre-filled pens or vials) cannot be distinguished. Thus, the study doctors, nurses and study participants are blinded until all randomised participants have completed the trial and the codes are opened. Before the end of the trials, the blinding of an individual participant can be opened only by the request of the DSMB and will be performed by a person who does not belong to the study staff (e.g. DSMB statistician). Another exception is a case of emergency, during which the opening of the code is considered if necessary for safety.

2.12 | Data collection

OGTT, MMTT and IVGTT are performed by trained study personnel according to standard protocols as described earlier.^{5,23,27} All primary end point variables are measured at the coordinating study centre (Oulu) to minimise variability. Furthermore, duplicate samples are collected to be able to repeat the measurements when necessary. Loss of earnings arising from attending the study visits may be reimbursed to the participant, and travel costs will be reimbursed based on actual costs according to receipts. All available data are used in the intention to treat analysis.

2.13 | Data management

All data will be recorded to Case Report Forms (CRF) which are pseudonymised by unique study ID. The data recorded in CRF are entered to a centralised electronic database via the study portal by trained study personnel. The principles of Good Clinical Practice (GCP) are followed in all data collection, records and completion of the CRFs.

2.14 | Statistical methods

The continuous primary and secondary outcomes of each of the three trials is analysed with linear mixed effects

models. Differences in categorical secondary outcomes, such as proportions of participants who progress to type 1 diabetes in stage 1 and 2 trials, between liraglutide and placebo groups are tested using standardized normal deviate test. Time to event analyses are modelled using Cox proportional hazards regression models. The analyses will be performed in two sets considering all participants that were randomised (intention-to-treat set) and those who self-administered most of the study drug (per protocol set). Continuous primary and secondary outcomes are compared between liraglutide and placebo groups using estimated marginal means within the linear mixed effect model. Missing data will not be imputed. Initially, we planned to adjust for sex and age in all analyses. However, because recalculated sample sizes were considerably decreased, we will not adjust for sex and age.

2.15 | Monitoring and auditing

An external DSMB is nominated and will monitor the study regularly. The main objectives of the DSMB are to assess the progress and safety of the study to assure continued feasibility and the safety of study participants. Further details on the responsibilities and the structure of the DSMB are available in the DSMB charter, which is available upon request. External monitors will audit the study sites regularly 3–4 times during the study to ensure data recording and adherence to GCP.

2.16 | Harms

All adverse events (AEs) are recorded during the trials. The investigators will report serious adverse events (SAEs) to the PI and DSMB within 24 h from being notified of the SAE. The PI and DSMB will make the decision whether the investigational product will be continued or not.

2.17 | Ethics

The study is performed in accordance with Declaration of Helsinki and GCP. Institutional review board (IRB), the Regional Ethics Committee of the Northern Ostrobothnia Hospital District and the Finnish Medicines Agency (Fimea) have approved the study protocols with the following licence numbers: 85/2014 and 133/2015; 67/2015 and 170/2015; 68/2015 and 177/2015 for stage 1, stage 2 and stage 3 trials; respectively. Possible protocol amendments are approved by the Ethics Committee before coming into effect. Fimea will be notified of all amendments.

Oral and written information about the trials is given to the candidates and their legal guardians, as appropriate. Before any study procedures, participants younger than 15 years and their guardians are asked to give a written assent and consent, respectively, and a written informed consent is required from participants who are 15 years or older. Participants who progress to type 1 diabetes during stage 1 or 2 trial are referred to standard clinical care, and the use of the investigational product is discontinued. Participants who remain nondiabetic after completion of the study are given an opportunity to continue follow-up in the DIPP study. All study participants are covered against treatment injury by insurance of the local university hospitals.

2.18 | Confidentiality

Each study site maintains a person register. Personal data are managed according to the EU general data protection regulation (GDPR) and the Finnish data protection act. The data will be archived in the local study centres for at least 15 years after study completion. Only study personnel and study investigators have access to CRFs and the final pseudonymised data set. External researchers are granted an access to full study protocol, pseudonymised data and statistical code upon reasonable request.

3 | DISCUSSION

Increasing evidence suggests that type 1 diabetes develops as an interplay between β -cells and the immune system.^{7,8} We aim to support the β -cells by treatment with liraglutide and thereby alleviate β -cell stress and dysfunction that is likely to contribute to the immune assault. This intervention avoids compromising the immune system.

Earlier trials in adults with newly diagnosed type 1 diabetes and conventional insulin treatment have not demonstrated that treatment with GLP-1 analogues would preserve β -cell function.^{12,13} Von Herrath et al. noticed that after cessation of liraglutide the decrease in C-peptide secretion was more rapid compared with participants not receiving liraglutide and speculated that the explanation may be delayed up-titration of insulin and increased glucose levels after liraglutide withdrawal rather than an effect of liraglutide treatment as such.¹² Of note, the present trials have different eligibility criteria and include young individuals with early-stage type 1 diabetes with the purpose to investigate the putative beneficial effect of liraglutide on insulin secretion capacity in the early stages of the disease. Furthermore, surveillance of β -cell health by measuring for example the proinsulin-to-C-peptide ratio during and after the liraglutide treatment gives insight

whether liraglutide alleviates β -cell stress and affects β -cell preservation.²⁸ Liraglutide is known to decrease body weight and this will be carefully monitored in the individuals randomised to the present trials as they are typically normal weight or even underweight. However, overweight individuals are also eligible and liraglutide may alleviate their insulin resistance by lowering the weight.

In practice, clinical type 1 diabetes prevention trials often suffer from insufficient number of islet autoantibody positive participants available, and increasing this number requires substantial investments in autoantibody screening. We present here a blueprint to maximise the return from the investment to trial screening. Our study design allows simultaneous efficient recruitment for three parallel trials and assesses the effect of an intervention in various early stages of type 1 diabetes. This increases the homogeneity of the study groups within the three trials, and thereby improves the statistical power to detect associations and decreases required sample sizes. Simultaneously, it allows most multipositive people to be eligible to participate independent on their glycaemic status, which maximises trial screening efficiency. This study design could serve as a good model for future trials with other investigational products to prevent type 1 diabetes. If GLP-1 treatment is found to be efficacious in the planned trials with 6-month treatment period, future trials with longer treatment should investigate whether GLP-1 analogue could maintain its effect or even cure the disease in the long run.

ACKNOWLEDGEMENTS

These are investigator-initiated clinical trials. The work is supported by JDRF International, a non-profit foundation for type 1 diabetes research (grant numbers 3-SRA-2014-301-M-R, 3-SRA-2016-142-M-R and 3-SRA-2016-143-M-R). JDRF also follows the progress of the study. Study drug and placebo are donated by Novo Nordisk for the stage 1 trial. The design, management, analysis and reporting of the study are entirely independent of Novo Nordisk. Neither JDRF nor Novo Nordisk have any role in the data interpretation and reporting. We are grateful to the Data and Safety Monitoring Board (Markku Savolainen, Jukka Hakkola, Jarmo Jääskeläinen, Tapani Rönkä and Pentti Nieminen) for their valuable comments.

CONFLICT OF INTEREST

The authors declare no competing interests.

ORCID

Riitta Veijola  <https://orcid.org/0000-0002-6557-270X>

REFERENCES

- DiMeglio LA, Evans-Molina C, Oram RA. Type 1 diabetes. *Lancet*. 2018;391(10138):2449-2462. doi:10.1016/S0140-6736(18)31320-5

2. Ziegler AG, Rewers M, Simell O, et al. Seroconversion to multiple islet autoantibodies and risk of progression to diabetes in children. *JAMA*. 2013;309(23):2473-2479. doi:10.1001/jama.2013.6285
3. Anand V, Li Y, Liu B, et al. Islet autoimmunity and HLA markers of Presymptomatic and clinical type 1 diabetes: joint analyses of prospective cohort studies in Finland, Germany, Sweden, and the U.S. *Diabetes Care*. 2021;44(10):2269-2276. doi:10.2337/dc20-1836
4. Insel RA, Dunne JL, Atkinson MA, et al. Staging presymptomatic type 1 diabetes: a scientific statement of JDRF, the Endocrine Society, and the American Diabetes Association. *Diabetes Care*. 2015;38(10):1964-1974. doi:10.2337/dc15-1419
5. World Health Organization, International Diabetes Federation. *Definition and Diagnosis of Diabetes Mellitus and Intermediate Hyperglycaemia: Report of a WHO/IDF Consultation*. World Health Organization; 2006.
6. American Diabetes Association. Diagnosis and classification of diabetes mellitus. *Diabetes Care*. 2010;33(Suppl 1):S62-S69. doi:10.2337/dc10-S062
7. Roep BO, Thomaidou S, van Tienhoven R, Zaldumbide A. Type 1 diabetes mellitus as a disease of the β -cell (do not blame the immune system?). *Nat Rev Endocrinol*. 2021;17(3):150-161. doi:10.1038/s41574-020-00443-4
8. Mallone R, Eizirik DL. Presumption of innocence for beta cells: why are they vulnerable autoimmune targets in type 1 diabetes? *Diabetologia*. 2020;63(10):1999-2006. doi:10.1007/s00125-020-05176-7
9. Cunha DA, Ladrière L, Ortis F, et al. Glucagon-like peptide-1 agonists protect pancreatic β -cells from lipotoxic endoplasmic reticulum stress through upregulation of BIP and JunB. *Diabetes*. 2009;58(12):2851-2862. doi:10.2337/db09-0685
10. Hadjiyanni I, Baggio LL, Poussier P, Drucker DJ. Exendin-4 modulates diabetes onset in nonobese diabetic mice. *Endocrinology*. 2008;149(3):1338-1349. doi:10.1210/en.2007-1137
11. Holst JJ. The physiology of glucagon-like peptide 1. *Physiol Rev*. 2007;87(4):1409-1439. doi:10.1152/physrev.00034.2006
12. von Herrath M, Bain SC, Bode B, et al. Anti-interleukin-21 antibody and liraglutide for the preservation of β -cell function in adults with recent-onset type 1 diabetes: a randomised, double-blind, placebo-controlled, phase 2 trial. *Lancet Diabetes Endocrinol*. 2021;9(4):212-224. doi:10.1016/S2213-8587(21)00019-X
13. Pozzilli P, Bosi E, Cirkel D, et al. Randomized 52-week phase 2 trial of albiglutide versus placebo in adult patients with newly diagnosed type 1 diabetes. *J Clin Endocrinol Metab*. 2020;105(6):e2192-e2206. doi:10.1210/clinem/dgaa149
14. Pi-Sunyer X, Astrup A, Fujioka K, et al. A randomized, controlled trial of 3.0 mg of liraglutide in weight management. *N Engl J Med*. 2015;373(1):11-22. doi:10.1056/NEJMoa1411892
15. Htike ZZ, Zaccardi F, Papamargaritis D, Webb DR, Khunti K, Davies MJ. Efficacy and safety of glucagon-like peptide-1 receptor agonists in type 2 diabetes: a systematic review and mixed-treatment comparison analysis. *Diabetes Obes Metab*. 2017;19(4):524-536. doi:10.1111/dom.12849
16. Klein DJ, Battelino T, Chatterjee DJ, et al. Liraglutide's safety, tolerability, pharmacokinetics, and pharmacodynamics in pediatric type 2 diabetes: a randomized, double-blind, placebo-controlled trial. *Diabetes Technol Ther*. 2014;16(10):679-687. doi:10.1089/dia.2013.0366
17. Tamborlane WV, Barrientos-Pérez M, Fainberg U, et al. Liraglutide in children and adolescents with type 2 diabetes. *N Engl J Med*. 2019;381(7):637-646. doi:10.1056/NEJMoa1903822
18. Nääntö-Salonen K, Kupila A, Simell S, et al. Nasal insulin to prevent type 1 diabetes in children with HLA genotypes and autoantibodies conferring increased risk of disease: a double-blind, randomised controlled trial. *Lancet*. 2008;372(9651):1746-1755. doi:10.1016/S0140-6736(08)61309-4
19. Battaglia M, Anderson MS, Buckner JH, et al. Understanding and preventing type 1 diabetes through the unique working model of TrialNet. *Diabetologia*. 2017;60(11):2139-2147. doi:10.1007/s00125-017-4384-2
20. Rewers M, Hyöty H, Lernmark Å, et al. The environmental determinants of diabetes in the young (TEDDY) study: 2018 update. *Curr Diab Rep*. 2018;18(12):136. doi:10.1007/s11892-018-1113-2
21. Parviainen A, But A, Siljander H, Knip M. Finnish pediatric diabetes register. Decreased incidence of type 1 diabetes in young Finnish children. *Diabetes Care*. 2020;43(12):2953-2958. doi:10.2337/dc20-0604
22. Koskinen MK, Helminen O, Matomäki J, et al. Reduced β -cell function in early preclinical type 1 diabetes. *Eur J Endocrinol*. 2016;174(3):251-259. doi:10.1530/EJE-15-0674
23. Greenbaum CJ, Mandrup-Poulsen T, McGee PF, et al. Mixed-meal tolerance test versus glucagon stimulation test for the assessment of beta-cell function in therapeutic trials in type 1 diabetes. *Diabetes Care*. 2008;31(10):1966-1971. doi:10.2337/dc07-2451
24. Heinze E, Holl RW. Tests of Beta-cell function in childhood and adolescence. In: Ranke MB, ed. *Diagnostics of Endocrine Function in Children and Adolescents*. Karger; 2003:318-338. doi:10.1159/000073557
25. Mortensen HB, Hougaard P, Swift P, et al. New definition for the partial remission period in children and adolescents with type 1 diabetes. *Diabetes Care*. 2009;32(8):1384-1390. doi:10.2337/dc08-1987
26. Sosenko JM, Skyler JS, Herold KC, Palmer JP, Type 1 Diabetes TrialNet and Diabetes Prevention Trial-Type 1 Study Groups. The metabolic progression to type 1 diabetes as indicated by serial oral glucose tolerance testing in the diabetes prevention trial-type 1. *Diabetes*. 2012;61(6):1331-1337. doi:10.2337/db11-1660
27. Bingley PJ, Colman P, Eisenbarth GS, et al. Standardization of IVGTT to predict IDDM. *Diabetes Care*. 1992;15(10):1313-1316. doi:10.2337/diacare.15.10.1313
28. Sims EK, Evans-Molina C, Tersey SA, Eizirik DL, Mirmira RG. Biomarkers of islet beta cell stress and death in type 1 diabetes. *Diabetologia*. 2018;61(11):2259-2265. doi:10.1007/s00125-018-4712-1

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Kero J, Koskenniemi JJ, Karsikas S, et al. INnoVative trial design for testing the Efficacy, Safety and Tolerability of 6-month treatment with incretin-based therapy to prevent type 1 DIAbetes in autoantibody positive participants: A protocol for three parallel double-blind, randomised controlled trials (INVESTDIA). *Diabet Med*. 2022;39:e14913. doi: [10.1111/dme.14913](https://doi.org/10.1111/dme.14913)