Impact of polyethylene glycol loxenatide on cardiovascular outcomes in patients with type 2 diabetes: study protocol for a multicentre, randomised, double-blind, placebo-controlled trial (BALANCE-3)

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ABSTRACT

Introduction Recent cardiovascular outcomes trials have demonstrated that glucagon-like peptide 1 receptor agonist (GLP-1RA) decreases the incidence of major adverse cardiovascular events (MACEs) in individuals with type 2 diabetes mellitus (T2DM). Polyethylene glycol loxenatide (PEG-Loxe) is a once-weekly GLP-1RA obtained by modifying exendin-4. No clinical trials have been designed to assess the impact of PEG-Loxe on cardiovascular (CV) outcomes in individuals with T2DM. This trial aims to test the hypothesis that PEG-Loxe treatment does not result in an unacceptable increase in CV risk in individuals with T2DM.

Methods and analysis This study is a multicentre, randomised, double-blind, placebo-controlled trial. Patients with T2DM who fulfilled the inclusion criteria were randomly divided to receive weekly administration of either PEG-Loxe 0.2mg or placebo (1:1 ratio). The randomisation was stratified according to utilisation of sodium-glucose cotransporter 2 inhibitors, history of CV disease and body mass index. The research period is expected to be 3 years, with a 1-year recruitment period and a 2-year follow-up period. The primary outcome is the occurrence of the first MACE, described as CV death, non-fatal myocardial infarction or non-fatal stroke. The statistical analyses were undertaken on the intent-to-treat patient. The primary outcome was evaluated using a Cox proportional hazards model with treatment and randomisation strata as the covariates.

Ethics and dissemination The current research has been authorised by the Ethics Committee of Tianjin Medical University Chu Hsien-I Memorial Hospital (approval number: ZXYJYYXMEC2022-2). Researchers must acquire informed consent from every participant before conducting any protocol-associated procedures. The findings of this study will be published in a peer-reviewed journal.

Trial registration number ChiCTR2200056410.

INTRODUCTION

According to the 10th edition of the International Diabetes Federation Diabetes Atlas, approximately 536.6 million adults between the ages of 20 and 79 years were predicted to have diabetes worldwide in 2021, with the majority experiencing type 2 diabetes mellitus (T2DM). In 2045, the number of adults with diabetes is anticipated to rise to 783.2 million.1 T2DM is a serious cardiovascular disease (CVD) risk factor, and CVD is the paramount cause of mortality in individuals with T2DM. A recent systematic review involving 4.5 million patients with T2DM from 25 countries revealed that the overall incidence of CVD among individuals with T2DM was 32.2%. In patients with T2DM, CVD accounts for approximately half of the mortality.2 CVD has been established as the primary contributor to the costs of T2DM.3

Since 2008, evidence of cardiovascular (CV) safety has been required for all new antidiabetic drugs to be approved for use, as mandated by the US Food and Drug Administration (FDA). Thus far, three classes of antidiabetic medications, sodium-glucose cotransporter 2 inhibitors (SGLT2i),
dipeptidyl peptidase 4 inhibitors (DPP-4i) and glucagon-like peptide 1 receptor agonist (GLP-1RA), have met FDA’s predesignated safety CV endpoints with non-inferiority compared with placebo. Furthermore, meta-analyses of CV outcomes trials (CVOTs) with GLP-1RA and SGLT-2i found a significant reduction in major adverse cardiovascular event (MACE) incidence in individuals with T2DM.4

GLP-1RA possesses multiple glucose-lowering mechanisms, such as glucose-dependent insulin secretion, inhibiting glucagon secretion, enhancing β-cell function, delaying stomach emptying and providing appetite suppression.5 In patients with T2DM, GLP-1RA treatment is correlated with a significant reduction of glycated haemoglobin A1c (HbA1c), weight loss, and a modest improvement of hypertension and hyperlipidaemia.5 Recent CVOTs have demonstrated that some GLP-1RAs, such as dulaglutide, liraglutide, semaglutide and efpeglatide, decrease the incidence of MACEs in individuals with T2DM.7–10

Polyethylene glycol loxenatide (PEG-Loxe) is a once-weekly GLP-1RA obtained by double modification of exendin-4, including amino acid substitution at four positions and PEG modification at N-terminus.11 These modifications make PEG-Loxe easier to be absorbed, increase the half-life of PEG-Loxe and reduce immunogenicity of PEG-Loxe.12 Two 24-week randomised controlled phase 3 trials demonstrated glycaemic benefits with acceptable safety and tolerability when PEG-Loxe was administered at doses of 0.1–0.2 mg.13 14 This trial examines the hypothesis that PEG-Loxe treatment does not result in an unacceptable increase in CV risk in individuals with T2DM.

METHODS AND ANALYSIS

Design
The BALANCE-3 Study is an investigator-initiated, multicentre, randomised, double-blind, placebo-controlled trial comparing PEG-Loxe with placebo as an adjunct to the standard of care for preventing MACE in individuals with high CV risk or established CVD. To verify the CV safety of PEG-Loxe, this trial selected placebo as control by referring to other CVOTs.7–10 The recruitment period runs from July 2022 to June 2023. The research period is expected to be 3 years, with a 1-year recruitment period and a 2-year follow-up period. Qualified participants were randomly assigned to receive weekly PEG-Loxe 0.2 mg or placebo injections in a 1:1 ratio. This event-driven research concludes when about 122 individuals experience a primary endpoint event.15 Figure 1 depicts the flow chart of this investigation.

Participants
Participants were recruited by the investigators from outpatient clinics in mainland China. Patients are recruited mainly through local advertising and identification in the local patient registry (database). All participants who satisfied the eligibility and exclusion criteria at screening were registered.

Inclusion criteria
1. Written informed permission has been signed.
2. T2DM with 7.0%≤HbA1c≤9.0%, or fasting plasma glucose (FPG)≥7 mmol/L.
3. Individuals satisfying at least one CVD criterion or CV risk factor (Table 1).
4. Body mass index (BMI)≥25 kg/m².
5. Female participants agreed to follow contraceptive guidance for up to 5 weeks after the intervention.

Exclusion criteria
1. Type 1 diabetes mellitus.
2. A clinically significant history of gastrointestinal disease related to protracted nausea and vomiting within 6 months before screening (e.g., gastroparesis and gastro-oesophageal reflux illness).
3. A previous diagnosis of chronic pancreatitis or acute idiopathic pancreatitis.
4. Personal or familial history of medullary thyroid carcinoma (MTC) or genetic predispositions to MTC.
5. Systolic blood pressure >180 mm Hg and/or diastolic blood pressure >100 mm Hg at screening.
6. Emergency hospitalisation for hypertension within 3 months before randomisation.
7. Planning coronary, carotid, peripheral arterial revascularisation, electrophysiological device implantation or cardiac surgery.
8. A previous solid organ transplant or the need for a solid organ transplant.
9. Known or suspected hypersensitivity to research medicine or similar items.
10. Individuals diagnosed with serious non-proliferative diabetic retinopathy, diabetic macular oedema or proliferative diabetic retinopathy; or treatment with intravitreal injections or laser or vitrectomy surgery scheduled within 3 months before allocation and during the research.
11. Individuals with a short life expectancy, in the opinion of the investigator, which makes implementation of the protocol or interpretation of research findings challenging, because of severe anaemia, congestive heart failure (New York Heart Association III/IV) or other significant systemic diseases.
12. A history of medication or alcohol misuse within 6 months prior to screening.
13. Treated with any GLP-1RA product or DPP-4i 3 months preceding the screening.
14. Use of drugs known to cause considerable weight loss in the past 3 months (e.g., prescription weight loss medications).
15. Participation in a prior PEG-Loxe clinical trial within 3 months before screening.
17. Laboratory findings at the screening: (1) estimated glomerular filtration rate (eGFR) <60 mL/min/1.73 m²; (2) total bilirubin >1.5 times the upper limit of normal (ULN); (3) alanine transaminase or aspartate transaminase >3 times the ULN (unless Gilbert’s syndrome); (4) amylase and/or lipase >3 times the ULN.

18. The following CV events occurred within 3 months before randomisation: cerebrovascular disease (cerebral haemorrhage, cerebral infarction, subarachnoid haemorrhage and transient ischaemic attack); new or recurrent non-fatal coronary artery disease (myocardial infarction and unstable angina); arteriosclerotic disease hospitalisation (aneurysm, arterial dissection and arteriosclerotic occlusion).

**Randomisation and blinding**

Individuals were randomised using the Interactive Web Response System, which employs central randomisation and concealment, thus keeping the centre unaware of upcoming assignments. The randomisation was stratified according to the following three stratification variables: (1) using SGLT2i (ongoing use, future potential use, and neither current nor future potential use; ‘future potential use’ is defined as not currently used but may be used during the study at the discretion of the investigator); (2) history of CVD (yes or no) and (3) BMI (25 kg/m² ≤ BMI < 30 kg/m², BMI ≥ 30 kg/m²).

This is a double-blind study in which neither the investigator nor the participant knows which of the two therapies
(PEG-Loxe or placebo) was taken by the participant. The research drug and placebo are identical in form and colour. The investigator might only reveal a participant’s therapy assignment in the event of an urgent or critical medical issue.

**Interventions**

Eligible participants randomly received PEG-Loxe 0.2 mg or a placebo. A study drug (PEG-Loxe or matching placebo) was added to each participant’s T2DM treatment regimen. The research drug was injected once weekly via subcutaneous injection. Research drug may be administered at any time of day, regardless of diet. Preferably, this must be injected once a week on the same day. Drug accountability was performed during clinic visits.

During the treatment term of the trial, the researcher managed glycaemia according to clinical guidelines at their discretion. Investigators monitored glycaemic status during the investigation. To prevent hypoglycaemia in individuals with 7.5% HbA1c at the assessment period, who are managed with insulin, sulfonylureas or glinides, their doses of antidiabetic therapy can be lowered at allocation. The investigator may prescribe any other antihyperglycaemic drug, except for GLP-1RA or DPP-4i if intensification is deemed necessary after increasing the dose of background antihyperglycaemic therapy. As required, additional drugs that are unlikely to be incompatible with the research drug or trial protocols are permitted and must be documented.

**Data collection and follow-up**

Researchers must acquire informed consent from every participant before conducting any protocol-associated procedures. At assessment (visit 1), participants should provide medical history information, undergo a physical examination, record their vital signs and ECG, and provide specimens for laboratory testing (figure 2). Before randomisation, women of reproductive potential underwent a pregnancy test.

Eligible participants proceeded to randomisation (visit 2). Participants can be randomly assigned to one of two treated arms. At each appointment after randomisation, concurrent drugs, adverse events and compliance with the study medication were assessed. New endpoint events (eg, CV) were gathered and documented. ECG and vital signs were recorded every 3 months. Specimens for laboratory analysis were collected (figure 2). Individuals who cannot tolerate the research medicine may temporarily discontinue its use. Whether or not individuals continue to take the research medicine, they remain monitored for adverse events and study outcomes.

All clinical data were collected and managed using the electronic data capture system. Each patient will be identified by a study identifier and an initial without the full name to ensure the confidentiality of the data. The primary investigator and designated teammates will be given access to the final dataset. Relevant documents will be stored at Tianjin Medical University Chu Hsien-I Memorial Hospital and reserved for 10 years after the trial.

**Measurement of outcomes**

**Primary outcome**

The primary outcome was the occurrence of the first MACEs, which include CV death, non-fatal myocardial infarction or non-fatal stroke (3-point MACE).

**Secondary outcomes**

1. The occurrence of the first expanded MACE (MACE plus coronary revascularisation and unstable angina requiring hospitalisation).
2. The first incidence of composite renal endpoint, described as initiation or advancement to macroalbuminuria with a ≥30% increase in urine albumin-
to-creatinine ratio (UACR) from baseline, maintained a 40% decline in eGFR from baseline (for 1 month), chronic dialysis, renal transplant and prolonged eGFR <15 mL/min/1.73 m² (≥30 days).

3. Changes in HbA1c, FPG, body weight, lipid profile, blood pressure, pulse, eGFR and UACR.

Safety outcomes
1. Adverse events and serious adverse events.
2. Changes in laboratory safety results and ECG.

Exploratory outcomes
1. The first occurrence of each expanded MACE constituent.
2. The first incidence of every constituent of the renal composite endpoint.
3. The occurrence of the first fatality for any reason.
4. The first occurrence of retinal photocoagulation, therapy with intravitreal drugs, vitreous haemorrhage or diabetes-associated blindness (described as Snellen visual acuity of 20/200 (6/60) or below, or a visual field of fewer than 20°, in the better eye).

Endpoint Adjudication Committee and Data Monitoring Committee
An independent Endpoint Adjudication Committee (EAC) composed of three clinicians is responsible for adjudicating endpoint events. The EAC is blinded to...
treatment allocation. An independent Data Monitoring Committee (DMC) composed of two clinicians and one statistician will monitor patient safety throughout the trial. The trial could be stopped early based on the safety review of the DMC. The members of the EAC and DMC are independent of the sponsor and competing interests.

### Statistical hypotheses
For the primary outcome, the following hypothesis was designed to test for the non-inferiority of PEG-Loxe compared with placebo (5% two-sided α level).

- **H0**: HR ≥ 1.8 vs Ha: HR < 1.8
- If non-inferiority is established, the superiority hypotheses for the primary outcome will be evaluated.

**H0**: HR ≥ 1.0 vs Ha: HR < 1.0

### Sample size calculation
Estimates of sample size were made based on the following assumptions: (1) 0.05 significance level for two-sided tests; (2) 90% power for the primary endpoint; (3) after the last randomised individual, the duration of the trial is anticipated to be roughly 2-year follow-up; (4) annual incidence rate of 3.37% for the primary endpoint in the placebo group and (5) a loss to follow-up rate of no more than 10%. Using these assumptions, a sample size of about 2006 participants (the first MACE experienced by 122 participants) is required to reveal the non-inferiority of PEG-Loxe compared with placebo with an upper bound (UB) of the 95% CI for HR of 1.8, which is recommended by the FDA. The sample size is computed using the PASS program (V.15.0.5, NCSS, Kaysville, Utah, USA).

### Statistical analysis
The statistical analyses were undertaken on the intent-to-treat patient. The primary outcome was evaluated using a Cox proportional hazards model with treatment and randomisation strata as the covariates. Kaplan-Meier curves will be used to estimate cumulative incidence by treatment group. Non-inferiority test was determined if the UB of the two-sided 95% CI was < 1.8. If non-inferiority is established, the superiority hypotheses for the primary outcome will be evaluated. The superiority is determined if the UB of the two-sided 95% CI is < 1. Subgroup analyses were performed with respect to age, sex, BMI, duration of T2DM, baseline HbA1c, CVD history and SGLT2i use. Per-protocol analysis will be performed for the primary endpoint as a sensitivity analysis.

The analysis of the remainder time-to-event outcomes was similar to that of the primary outcome. The changes between the baseline to the last measurement in HbA1c, FPG, weight, lipid profile, blood pressure, pulse, eGFR and UACR were analysed employing an analysis of variance with therapy. Adverse events were evaluated using descriptive statistics. For quantitative variables, the missing data were handled by using the mixed-effects model for repeated measures. All statistical evaluations were conducted using SAS V.9.4; a p value of <0.05 is a measure of statistical significance.

### Patient and public involvement
Patients and the public were not directly involved in the design, recruitment and conduct of the trial. The trial results will be disseminated to them through social media.

### ETHICS AND DISSEMINATION
Our research has been authorised by the Ethics Committee of Tianjin Medical University Chu Hsien-I Memorial Hospital (approval number: ZXYJNYHMEC2022-2 and trial registration number: ChiCTR2200056410). Researchers must acquire informed consent from every participant before conducting any protocol-associated procedures. Important protocol modifications will be communicated to the Ethics Committee of Tianjin Medical University Chu Hsien-I Memorial Hospital. The Asia-Pacific Property & Casualty Insurance company has insurance to cover for harm associated with research medicine or related products. Investigators who have contributed to the design, conduct, statistical analysis, interpretation and reporting will be eligible for authorship. This study's findings will be published in a peer-reviewed journal.

### DISCUSSION
CV benefits provided by GLP-1RA have gained immense attention in the field of diabetes. Currently, seven CVOTs in GLP-1RA have been published, with results demonstrating CV safety for lixisenatide (ELIXA), long-acting exenatide (EXSCEL) and oral semaglutide (PIONEER 6),16–18 while liraglutide (LEADER), dulaglutide (REWIND), semaglutide (SUSTAIN-6) and efpeglenatide (AMPLITUDE-O) presented CV benefits.7–10 Such inconsistent results made it unclear whether the CV-protecting impact of GLP-1RA is a class effect. Caruso et al revealed that the time of exposure to GLP-1RA was negatively correlated with MACE HR.26 Another study found that the dose of exposure to GLP-1RA was negatively linked to MACE HR.27 These studies support the notion that CV protection of GLP-1RA is a class effect. AMPLITUDE-O trial that used an exendin-based GLP-1RA, efpeglenatide, reported a significantly reduced MACE risk.10 The structure of PEG-Loxe is similar to that of efpeglenatide, both of which are once-weekly GLP-1RA derived from exendin. Furthermore, PEG-Loxe improved endothelial cell function in patients with T2DM.45 These studies raise hope for possible CV benefits of PEG-Loxe.

In general, most non-inferiority trials use active comparator. However, to rule out additional CV risks associated with new glucose-lowering drugs, the 2008 FDA guidance allowed the use of placebos as an ‘add on’ to the standard care, compared with an experimental drug.22 The SURPASS-CVOT was designed as a non-inferiority trial against an active comparator instead of a placebo; both the experimental drug (tirzepatide) and active comparator (dulaglutide) are manufactured by Eli Lilly, which enabled the trial to adopt a double-blind study design.23 If
the present trial used an active comparator from another company, it would have to use an open-label study design due to the difference in the injection device. Thus, this trial was designed as a non-inferiority trial against a placebo.

The non-inferiority margin is the key parameter in a non-inferiority trial. Previous studies used the FDA-recommended margin of 1.8 or 1.3. For example, 1.8 was chosen by the SUSTAIN-6 trial,9 while a more conservative margin of 1.3 was chosen by the EMPAREG trial.24 The choice of margin affects sample size; a wider margin of non-inferiority indicates a smaller sample size. The 2020 FDA-drafted guidance recommends that trials looking at CV safety of new glucose-lowering drugs require a minimum of 600 patients with established CVD. Additionally, there is no absolute requirement for CVOTs.25 Thus, 1.8 was chosen as the non-inferiority margin in this trial.

This trial demonstrates the CV safety of PEG-Loxe by evaluating the non-inferiority of PEG-Loxe versus placebo in terms of 3-point MACE. If non-inferiority is determined, then superiority can be evaluated. To obtain the expected results, it is necessary to reduce any potential disparity among treated groups using concurrent drugs that have been found to influence CV risk in the patient population. In the current trial, individuals who use SGLT2i are permitted to participate. This study refers to the randomisation method of AMPLITUDE-O Study,10 both baseline use and future expected usage of SGLT2i were addressed in the randomisation stratification. In addition, increasing the proportion of SGLT2i used in the participants may reduce the overall incidence of MACEs, resulting in a longer study period.

The foreseeable risks in this study were mainly gastrointestinal adverse effects. In the two phase 3 trials of PEG-Loxe, the incidence of gastrointestinal events was 10%–25%, and most were mild and transient. In addition, the incidence of hypoglycaemia is low at 0.6%–2.2%.13 14

GLP-1RA-containing treatment regimens have emerged as an important cardioprotective approach in patients with T2DM. The BALANCE-3 trial expands the understanding of this medication category while determining CV impacts of PEG-Loxe in patients with T2DM.

TRIAL STATUS

When the manuscript is submitted, the trial is in the recruitment phase. The recruitment is anticipated to conclude in June 2023.

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