STATISTICAL ANALYSIS PLAN FOR THE LOSEIT-X TRIAL

SECTION 1: ADMINISTRATIVE INFORMATION

TITLE

Effect of a liraglutide-based weight-loss intervention on radiographic knee osteoarthritis progression in patients with overweight: a secondary analysis of a randomized controlled trial

TRIAL ACRONYM

LOSEIT-X.

TRIAL REGISTRATIONS

ClinicalTrials.gov Identifier: NCT02905864.

SAP VERSION

v.2 (2021-10-01).

PROTOCOL VERSION

This document has been written based on information contained in the study protocol version 6, dated the 30th of January 2017, 15:30 {Gudbergsen, H, January 2017}.

SAP REVISIONS

None; currently not applicable.

ROLES AND RESPONSIBILITY .

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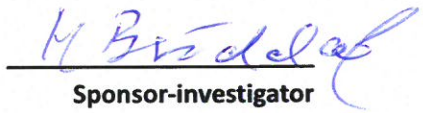

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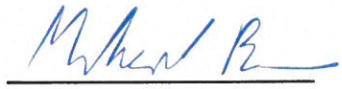

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SECTION 2: INTRODUCTION

BACKGROUND AND RATIONALE

Knee osteoarthritis (KOA) is the leading cause of pain and disability and a source of societal cost. With increasing prevalence of obesity and older age, health issues related to KOA will intensify, consequently driving the need for improved diagnostics and treatment [1].
Scientific literature links obesity to KOA; the two conditions share pathogenic phenotypes, and the progress of one condition increases the probability of the other, possibly activating a vicious cycle [2]. Obesity rates have risen dramatically over the past decades, creating a global need to tackle its impact on public health. While challenging, weight loss is the primary strategy for achieving and maintaining a healthy state in order to prevent and manage obesity-related disease. Consequently, international guidelines recommend weight loss as a fundamental part of managing these patients [3]. Nevertheless, the majority of patients achieving a successful weight loss regain their weight and the upholding of a clinically significant weight loss over time remains a challenge.
The lack of widely available and feasible means to manage patients with KOA and obesity warrants further investigations. Glucagon-like peptide 1 receptor agonists (GLP-1-RAs) are widely used in managing type 2 diabetes, and the body weight-lowering effects of GLP-1-RAs have prompted approval of the compound for the treatment of obesity. Exploiting an anti-obesity medication to maintain, or even extend, weight loss in patients with KOA and obesity represents an attractive opportunity to decrease knee related symptoms.
Pathologically, KOA is characterized by focal loss of articular cartilage in weight-bearing areas and new bone formation (osteophytes) at joint margins. As the disease progresses these changes become apparent on radiographs. The extent of cartilage loss can be estimated by measuring joint space width (JSW) on radiographs obtained in a weight-bearing position. Structural radiographic findings have been shown to predict joint space narrowing (JSN) in subjects with symptomatic KOA. The progression of KOA on radiographs – measured as JSN - in an unselected population is reported to be as low as 0.1 to 0.15 mm/year. In addition, in such a population as much as 40 % of a study population will not show any progression over 12-24 months. The radiograph is reported as an imprecise method of following KOA progressive in the clinical setting because small variations in x-ray beam angulation can lead to erroneous readings [4]. In trials, radiographs are performed with the knee in a fixed-flexion device, reducing the risk of incorrect angulation.
Radiological reading of radiographs is subjective by nature and standardized categorization is seldom used in routine clinical practice. Automated tools for KOA feature classification and measurement have emerged in recent years. Particularly, improvements in computational power has enabled the development of machine learning methods for KOA analysis with performance on par with field experts. Two recent papers, both using Convolutional Neural Networks (CNN), claimed state--of-the-art performance on automatic Kellgren-Lawrence (KL) grading of knee radiographs [5] and automatic Osteoarthritis Research Society International (OARSI) grading of osteophytes and joint space narrowing [6].

To address the challenge of detecting progression in KOA, we aim to apply a KOA analysis tool to precisely measure and subsequently detect any radiographic progression in patients with overweight/obesity and KOA subjected to weight loss.

OBJECTIVE

The objective of this investigation is to evaluate the value of structural imaging biomarkers assessed by a machine learning (ML) tool at baseline in predicting radiographic progression, measured as joint space width (JSW), in patients with obesity and KOA during a clinically significant weight loss intervention.

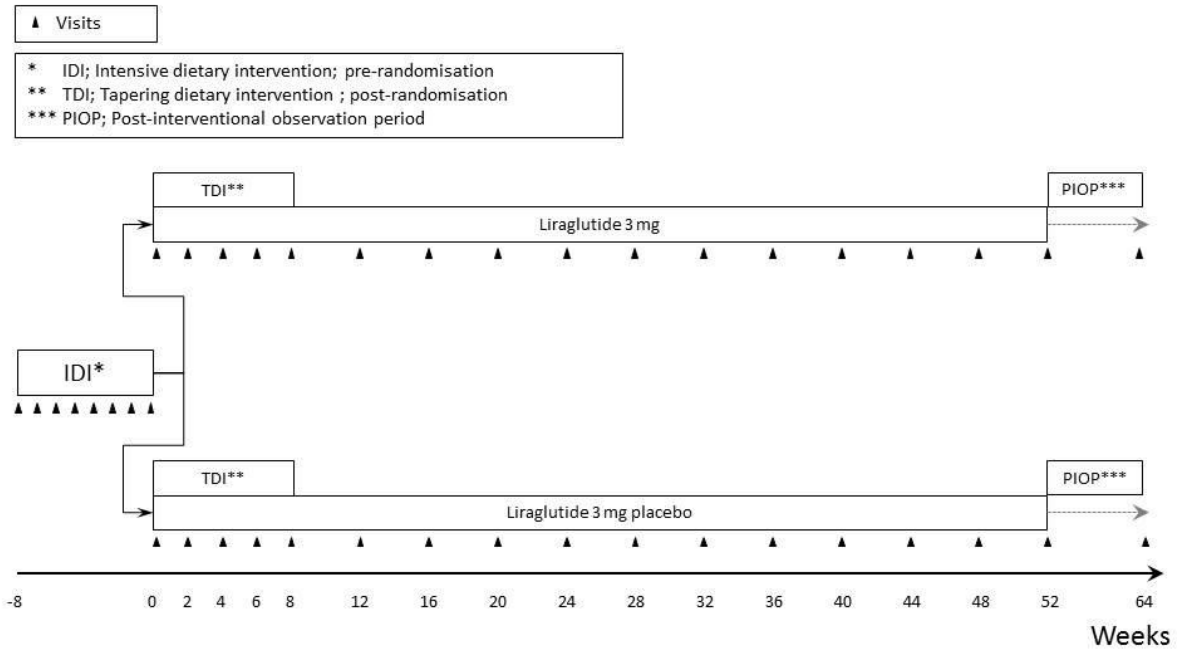
SECTION 3: STUDY METHODS

TRIAL DESIGN

The trial was designed as a single centre, randomized, parallel-group, placebo-controlled trial. Treatment allocation was done using a 1:1 ratio. The trial contained three periods (please see the FIGURE: TRIAL DESIGN below).

Participants were enrolled in an 8-week intensive dietary intervention (IDI) period (week -8 to 0). Upon successful achievement of a weight loss of minimum 5 % during this period participants continued with a tapering dietary intervention (TDI) for 8 weeks (week 0 to 8) and were randomized to receive either liraglutide 3 mg/day or identically appearing placebo throughout the 52-week main trial period (week 0 to 52).

FIGURE: TRIAL DESIGN



RANDOMIZATION

Patients were randomized in a 1:1 manner to receive either 3 mg/day liraglutide or identically appearing placebo at week 0, i.e., after the initial 8-week IDI period. Stratified randomization was done based on sex (male vs. female), age (<60 years vs. ≥60 years), and obesity class (BMI; < 40

kg/m^2 vs. ≥ 40 kg/m^2) status at trial enrolment (week -8). A computer-generated randomization sequence was produced using SAS PROC PLAN to generate eight separate randomization schedules before any participant was enrolled, allocating participants in permuted blocks of 2 to 6 to the daily liraglutide 3 mg/day or placebo group. The randomization sequence was concealed and entered directly into the eCRF by the data manager.

Allocation concealment was secured by utilizing the computer-generated allocation process (above) where the patient identifier was coupled to one of the experimental groups only once the physician clicked on the 'randomization button' appearing at the baseline visit (week 0, visit T0) in the eCRF system. Upon allocation to one of the two experimental groups the patient identifier was automatically coupled to specific pens, each of them labelled by a single and unique Dispensing Unit Number (DUN). The entire process was blinded for all investigators, clinical, academic, and administrative trial personnel.

TIMING OF FINAL ANALYSIS

The analyses of study-related data were initiated after the last patient's last visit (LPLV) (Visit T15) and timed to ensure transmission of data from the listed assessments and examinations.

TIMING OF OUTCOME ASSESSMENTS

The timing of outcome assessments is visualized in the visit schedule displayed in the trial protocol (22).

RADIOGRAPHY

Radiographic examinations included standard clinical fixed-semi-flexed weight-bearing posterior-anterior (PA) radiographs of both knees [7]. During the examination, patients were facing the plate of the radiography equipment with their knees touching the cassette holder. The radiography plate or cassette holder was placed so that the centre of the film was at the level of the patient's tibiofemoral joint line. The radiography beam was centered between the knees with a 10 degree cranio-caudal angle and the body weight was distributed equally between the two legs.

EXTRACTION OF RADIOGRAPHIC STUDIES

Radiographic studies are stored in the Picture Archiving and Communications System (PACS) in the Capital Region of Denmark. For this study, they must be extracted and pseudonymised. Extraction and pseudonymisation of Digital Imaging and Communications in Medicine (DICOM) headers will be done using the Teal Engine (Teal Medical ApS). Several images have burned in patient information. These will be removed with a custom script which blacks out the first and last 40 pixel-rows of images with burned in data. Afterwards, pixel data is recompressed using lossless JPEG2000 encoding ensuring lossless 16-bit compression.
If patients have more than one image for each laterality, the best projection will be selected by a trained technologist reviewer who will also verify that correct laterality is assigned to the respective image.

MACHINE LEARNING ANALYSIS TOOL

The weight-bearing, PA radiographs of both knees will be analysed in a standardised fashion by an imaging analysis software, RBknee (referred to as the ML tool). RBknee is a FDA-cleared and CE-marked tool which uses machine learning and computer vision, particularly deep learning, i.e. deep CNNs combined with advanced image processing, to analyse PA of patients with KOA, outputting Kellgren-Lawrence grades, OARSI grades, and Joint Space Width measurements.

SECTION 4 – STATISTICAL PRINCIPLES

CONFIDENCE INTERVALS AND P-VALUES

Level of statistical significance and use of confidence intervals: All applicable statistical tests will be two-sided and will be performed using a 5% significance level.

ANALYSIS POPULATIONS

The Available Case (AC) Population will include all enrolled individuals for whom the outcome is available on week -8 as well as week 52.

SECTION 5 – TRIAL POPULATION

ELIGIBILITY

Inclusion criteria

- Enrolment in the NCT02905864 trial

Exclusion criteria

- Insufficient image quality for evaluation of medial minimal joint space width

BASELINE PATIENT CHARACTERISTICS

Height (cm), age (years), sex (male/female), body weight (kg), baseline BMI (kg/m2), hip and waist circumference (cm), and KOOS score (0-100) will be reported as patient characteristics in table 1.

SECTION 6 – ANALYSIS

OUTCOMES

Primary outcome

- Change in medial mJSW from enrolment (week -8) to the end of the main trial period (week 52) in liraglutide + weight loss vs placebo + weight loss.

- Increase in per OARSI grade from enrolment (week -8) to the end of the main trial period (week 52) in liraglutide + weight loss vs placebo + weight loss.
- Increase in KL grade from enrolment (week -8) to the end of the main trial period (week 52) in liraglutide + weight loss vs placebo + weight loss.

Confirmatory secondary outcome

- Weight loss in kg (absolute). (Adjusted for baseline BMI).
- Increase in per OARSI grade from enrolment (week -8) to the end of the main trial period (week 52) adjusted for weight loss in kilograms and baseline BMI.
- Change in medial median JSW from enrolment (week -8) to the end of the main trial period (week 52) adjusted for weight loss in kilograms and baseline BMI.
- Increase in KL grade from enrolment (week -8) to the end of the main trial period (week 52) adjusted for weight loss in kilograms and baseline BMI.

COVARIATES

Basics

Body weight (kilograms), height (centimeters) and BMI (kg/m2) at week -8.

Kellgren-Lawrence grades

The Kellgren & Lawrence (KL) grading system applies a categorical grading scale of knee OA from standing semi-flexed radiographs going from 0 to 4 and incorporates the evaluation of osteophytes, joint space narrowing, sclerosis, and altered bone shapes. The original Kellgren & Lawrence description is depicted below.

- KL grade 0; no knee OA
- KL grade 1; doubtful narrowing & possible osteophyte
- KL grade 2; possible narrowing & definite osteophyte
- KL grade 3; definite narrowing & multiple osteophytes & possible bone deformity / some sclerosis
- KL grade 4; marked narrowing & large osteophyte & definite bone deformity / severe sclerosis

Joint space measurements

Radiographs will also be analysed with the ML tool, outputting minimal and median joint space width, Kellgren-Lawrence grade, and Osteoarthritis Research Society International (OARSI) atlas criteria gradings. Please see the details below:

- Joint Space Width (JSW)
    - The joint space width (JSW) is calculated by finding the minimum vertical distance between the contours of the femur and tibia at five distinct locations in both the medial and lateral compartment of each knee.
    - The raw output of the JSW measurement is five measurements for each compartment.

- Minimum Joint Space Width (minimum JSW)
    - The minimum JSW (mJSW) is defined as the minimum of the five JSW measures for each compartment.
    - mJSW is one number for each compartment.
- Median Joint Space Width (median JSW)
    - The median JSW is defined as the median of the five JSW measurements for each compartment.
    - median JSW is one number for each compartment.
- The JSW, minimal JSW and median JSW is calculated for each of the tibiofemoral compartments and will be named the medial and lateral JSWs, minimal JSWs and median JSWs, respectively.

Osteoarthritis Research Society International (OARSI) grades

The OARSI atlas criteria grading system will be used to grade all knee radiographs [8]. The OARSI atlas criteria classify KOA from 0 to 3 (none, mild, moderate, severe) based on osteophytes and JSN. The system classifies medial tibial sclerosis and lateral femoral sclerosis as being present (1) or absent (0). Medial tibial attrition is not evaluated.

ANALYSIS METHODS

Analyses of medial mJSW and medial median JSW will be performed to estimate change over 60 weeks (week –8 to 52).
Primary outcomes will be evaluated as follows:

- Change in medial minimal JSW (continuous outcome): linear regression will be used with treatment group and absolute weight change as dependent variables.
- Increase in per OARSI grade (binary outcome): logistic regression will be used with treatment group and absolute weight change as dependent variables.
- Increase in KL grade (binary outcome): logistic regression will be used with treatment group and absolute weight change as dependent variables.

Secondary confirmatory outcomes will be evaluated as follows:

- Change in medial minimal JSW (continuous outcome): linear regression will be used with treatment group as well as absolute weight change, age, sex, and baseline BMI as dependent variables.
- Increase in per OARSI grade (binary outcome): logistic regression will be used with treatment group as well as absolute weight change, age, sex, and baseline BMI as dependent variables.
- Increase in KL grade (binary outcome): logistic regression will be used with treatment group as well as absolute weight change, age, sex, and baseline BMI as dependent variables.

STATISTICAL SOFTWARE

The level of significance will be set at P = 0.05. All analyses will be carried out using R version 4 or newer (R Foundation, Vienna, Austria), or Python version 3.6 or newer (Python Software Foundation)

HARMS

Use of radiographic studies in this project will have no impact on patients. Studies have already been read by a radiologist. Thus, no new actionable findings are expected.

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MANUSCRIPT OUTLINE

Table 1: Patient Characteristics

Characteristic	**Total** (N=)	**Placebo** (N=)	**Liraglutide** (N=)
Female sex — no. (%)			
Age — years (range)			
Height — m (+/- sd)			
Weight — kg (+/- sd)			
BMI – kg/m2 (+/- sd)			

Waist circumference			
Hip circumference			
KOOS pain			
Pain			
Symptoms			
Function in activities of daily living			
Knee-related quality of life			
Function in sports and recreation			

Table 2. Characteristics and analysis output

Characteristic	**Baseline**			**Follow-up**		
	Total (N=)	**Liraglutide** (N=)	**Placebo** (N=)	**Total** (N=)	**Liraglutide** (N=)	**Placebo** (N=)
Weight — kg (+/- sd)						
BMI – kg/m2 (+/- sd)						
Medial Minimal JSW - mm (+/- sd)						
Medial Median JSW - mm (+/- sd)						
Lateral Minimal JSW - mm (+/- sd)						
Lateral Median JSW - mm (+/- sd)						

Median JSWs						
Measurement 1 - mm						
Measurement 2 - mm						
Measurement 3 - mm						
Measurement 4 - mm						
Measurement 5 - mm						
Lateral JSWs						
Measurement 1 - mm						
Measurement 2 - mm						
Measurement 3 - mm						
Measurement 4 - mm						
Measurement 5 - mm						
Kellgren-Lawrence grades						
KL0 - no.						
KL1 - no.						
KL2 - no.						
KL3 - no.						
KL4 - no.						
OARSI atlas criteria grading						
Medial femoral condyle						

Medial tibial plateau						
Lateral femoral condyle						
Lateral tibial plateau						
JSN Medial compartment						
JSN Lateral compartment						
Medial tibial sclerosis						
Lateral femoral sclerosis						

Supplementary file 1: Latest updated study protocol version 6 dated the 30th of January 2017, 15:30 {Gudbergsen, H, January 2017}