

STATISTICAL ANALYSIS PLAN

What is the impact of a modified Roux-en-Y-gastric bypass operation on people with type 2 diabetes mellitus?

The LONG LIMB-2 double-blinded randomised controlled clinical trial

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01/09/2025

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1. ABBREVIATIONS

Abbreviation	Definition
RCT	Randomised controlled trial
T2DM	Type 2 diabetes mellitus
RYGB	Roux-en-Y-gastric bypass
BMI	Body Mass Index
HbA1c	Glycated haemoglobin
ITT	Intent-To-Treat Population
PP	Per-Protocol Population
GLM	Generalised linear model
CSR	Clinical study report
SAE	Serious Adverse Event
TEAE	Treatment emergent adverse event
AE	Adverse Event
SOC	System organs class
PT	Preferred Term
CRF	Case Report Form

2. STUDY DESIGN

In the LONG LIMB-2 prospective double-blinded RCT we propose to recruit 80 patients with T2DM and obesity who are eligible for metabolic surgery based on NICE guidance 189 who are on the waiting list for bariatric surgery at Imperial College Healthcare NHS Trust, King's College Hospital NHS Foundation Trust, Whittington Health NHS Trust and North Bristol NHS Trust obesity services. Randomisation will take place intra-operatively. The surgeon will measure total intestinal length and decide if the patient can be randomised intraoperatively (only patients with a total intestinal length greater than 5.5 metres will be randomised) and if so will contact the randomiser who will make the allocation at the time to either:

- A "standard" RYGB or
- A "modified" RYGB

The randomisation ratio will be 1:2 i.e. 24 participants for standard RYGB and 48 for modified RYGB.

Key Inclusion criteria

- Diagnosis of type 2 diabetes mellitus (T2DM)
- BMI > 30 kg/m²
- Age 18-65 years
- Eligible for metabolic/bariatric surgery as per NICE CG189

Key Exclusion criteria

- Current use or need for insulin
- Unacceptably high risk for anesthesia or surgery
- Pregnancy/breastfeeding
- Total intestinal length <5.5 meters

3. STUDY OUTCOME MEASURES

Primary outcome

- Change in HbA1C from baseline to 12 months

Secondary outcomes

Change from baseline to 12 months for:

- Rate of remission of T2DM
- Number of glucose-lowering medications
- Body weight
- Arterial blood pressure
- Lipid profile
- Adverse events (including surgical complications, hypoglycaemia and micronutrient deficiencies)
-

2.1 INTERIM ANALYSIS

There will be no interim analysis for this study.

2.2 FINAL ANALYSES AND REPORTING

2.2.1 FINAL ANALYSIS

Our study will involve collecting data from all participants for 12 months. Once this follow-up period is complete, the database will be locked, and no further modifications will be made to it. The final analysis of the primary and secondary outcomes, as outlined in the protocol and this statistical analysis plan (SAP), will only be performed using the locked database.

3 STUDY OBJECTIVES AND ENDPOINTS

3.1 STUDY OBJECTIVE

To perform a double-blind RCT to compare the safety and efficacy of “modified” RYGB vs. “standard” RYGB in improving glycaemic control in patients with T2DM and obesity.

3.2 STUDY ENDPOINTS

3.2.1 PRIMARY EFFICACY ENDPOINT

Primary efficacy outcome:

Change in HbA1C from baseline to 12 months.

Secondary Efficacy Endpoints

For each secondary endpoint, change from baseline to 12 months will be analysed:

- Rate of remission of T2DM
- Number of glucose-lowering medications
- Body weight
- Arterial blood pressure
- Lipid profile
- Adverse events (including surgical complications, hypoglycaemia and micronutrient deficiencies)

4 SAMPLE SIZE

Based on our own data from the LONG LIMB-1 trial and the most relevant RCT in the field¹ we estimated that the absolute HbA1c reduction in the standard RYGB group will be 3.0% and in the modified RYGB group 4.0%. With a standard deviation of 1.2% around both means and using a 1:2 randomisation, we will need 24 participants in the standard RYGB and 48 in the modified RYGB to have a 90% power to detect statistically significant differences between the groups at α of 0.05. We will recruit 80 patients in total to account for an approximate 10% drop-out rate based on rates in similar trials we have conducted in this field (e.g. LONG LIMB-1 trial ISRCTN15283219).

5 ANALYSIS POPULATIONS

5.1 INTENTION TO TREAT POPULATION (ITT)

We will recruit 80 participants in total and use a 1:2 randomisation. The intention-to-treat (ITT) population will include all patients who were initially assigned to either medical care or obesity

surgery, regardless of any deviations from the trial protocol or the actual surgical procedure they actually underwent. The analysis of this population will be based on their original assignment group.

5.2 PER-PROTOCOL POPULATION (PP)

The Per-Protocol (PP) analysis population includes the patients who completed their trial follow up at 12 months, with no major deviations to the treatment protocol, but grouped by the surgical procedure they actually underwent.

6 GENERAL ISSUES FOR STATISTICAL ANALYSIS

6.1 ANALYSIS SOFTWARE AND GENERAL METHODS OF ANALYSIS

We will use Stata or all analyses. Two-sided P-values < 0.05 will be considered statistically significant.

6.2 DISPOSITION OF SUBJECTS AND WITHDRAWALS

All subjects who provide written informed consent will be accounted for. The number and percentage of ITT subjects who discontinued the study prior to 12 months will be presented by treatment group, overall and by reason of discontinuation (adverse event, discontinued by investigator, withdrawn consent/request to terminate, lost-to-follow-up, death, other). Percentages will be based on the number of ITT subjects.

6.3 METHODS FOR MISSING DATA

All efforts will be made to prevent the occurrence of missing data. Nevertheless, it is anticipated that withdrawals will occur and hence there will be missing data on primary and secondary efficacy endpoints. We will assume the data are missing at random. The number of participants with missing data per variable and reasons will be reported as recommended.

6.4 PROTOCOL VIOLATIONS

Protocol violations will be summarised in the clinical study report (CSR). This summary will include the number and percent of subjects with each violation type. Major violations in this study may be those that are related to:

- Informed consent deviation
- Inclusion/Exclusion criteria, if such protocol violation is likely to impact one of the two primary endpoints
- Participant not complying with trial protocol

The main reason for assessing the incidence of major violations during the study is to determine which patients are in the per-protocol population (the per-protocol population excludes “major” violations). Prior to database lock, all protocol violations will be reviewed and patients who have had major violations will be noted and excluded from the per-protocol population.

6.5 MULTIPLE ENDPOINT ADJUSTMENT

There will be no adjustment for the multiple secondary endpoints. All secondary outcomes will be considered exploratory.

7 DEMOGRAPHICS AND OTHER BASELINE CHARACTERISTICS

7.1 DEMOGRAPHICS

Demographics will be summarised by randomised treatment groups. There will be no formal statistical comparisons between treatment groups on demographic variables. We will present variables with a normal distribution as mean (standard deviation). Variables with a skewed distribution will be summarised as median (interquartile range). Categorical variables will be presented as numbers (percentages).

7.2 BASELINE MEDICAL HISTORY

The medical history of all ITT and the safety analysis population subjects will be summarised in a table by treatment group. Specifically, the number and per cent of subjects who currently have the condition will be presented for each condition.

7.3 BASELINE LABORATORY MEASUREMENTS

A table presenting descriptive statistics (sample size, mean, standard deviation, median, min and max) of laboratory variables by treatment group at baseline will be provided for the ITT analysis set. If the baseline value is missing for a given variable and patient, the screening value will be used before calculating the descriptive statistics.

8 EFFICACY ANALYSES

8.1 PRIMARY OUTCOME

- Change in HbA1C from baseline to 12 months Change in HbA1C (continuous) from baseline to 12 months: ANCOVA with 12-month HbA1C as response to treatment; fixed effects for treatment group, insulin use, and baseline HbA1C as a covariate.

8.2 SECONDARY EFFICACY OUTCOMES

- For T2DM remission (binary) we will use general linear model (GLM) assuming a binomial distribution and log link function. This will give group differences in the form of an adjusted risk ratio (RR), which will be reported with a corresponding 95% confidence interval. If there are convergence issues with this statistical approach, logistic regression will be used for the data analysis.
- For body weight (continuous) we will use ANCOVA (12-month weight with baseline weight as covariate) and report adjusted mean difference. Arterial blood pressure and lipid profile will be analysed using ANCOVA.
- *Secondary safety outcomes:* surgical complications, hypoglycaemia, micronutrient deficiencies will be analyzed using Fisher's exact test.

9 SAFETY ANALYSES

9.1 PRIMARY SAFETY OUTCOME

The primary safety outcome will be the incidence risk of treatment-emergent serious adverse events (SAEs) through the 12-month follow-up period. We will evaluate this outcome via a Fisher's exact test. Results will be presented as numbers of patients with the event (percentage).

9.2 SECONDARY SAFETY OUTCOMES

We will use Fisher's exact tests to analyse all secondary safety outcomes (described in more detail below). The results will be reported numbers of patients with the event and percentages by treatment group.

9.3 ADVERSE EVENTS

We will assess the number of participants with any treatment-emergent SAEs, any treatment-emergent adverse events (TEAEs), any-cause withdrawals/dropouts and the number of participants per severity grade. The Clavien-Dindo classification for grading the severity of complications will be used. Pregnancy rates will also be assessed and considered an adverse event.

9.3.1 *ALL ADVERSE EVENTS*

The total number of TEAEs and the number and percentage of subjects with at least one TEAE will be presented by treatment groups. A TEAE is an event starting or worsening in severity at or after initiation of the index procedure for the randomised treatment. For subject counts, subjects experiencing a given event more than once will be counted only once for that event.

A listing of all adverse events will include the subject number, adverse event (AE) number, the investigator description of the AE, the AE system organs class (SOC) and preferred term (PT), the severity of AE, whether or not the AE is classified as serious (SAE), the relationship of the AE to the procedure, the action taken, the outcome, and the adjudication status.

9.3.2 *ADVERSE EVENTS LEADING TO WITHDRAWAL*

A summary of number of TEAEs and of the incidence risk rates (number and percentage of subjects) of TEAEs leading to study withdrawal, by SOC and PT will be presented in a similar manner as discussed above (with the exception of the plot). A data listing of TEAEs leading to withdrawal will also be provided, displaying details of the event(s) captured on the case report form (CRF).

9.3.3 *SERIOUS ADVERSE EVENTS*

Summaries of serious TEAEs will be conducted in the same manner as for all TEAEs discussed above.

9.3.4 Deaths

If a death occurs during the trial, relevant information (including study day of death relative to index initiation, cause of death, and adverse event leading to death) will be supplied in a data listing.

10 OTHER PLANNED ANALYSES

10.1 PLANNED SUBGROUP ANALYSES

The planned subgroup analyses aim to explore the differential treatment effect of “modified” vs. “standard” RYGB surgery on the primary outcome:

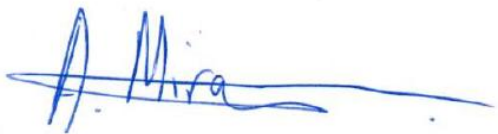
1. Baseline body mass index (BMI). This analysis will evaluate the treatment effect in patients with different baseline BMI values. Patients will be divided into two groups based on their baseline BMI, with one group having BMI $<45 \text{ kg/m}^2$ and the other having BMI $\geq 45 \text{ kg/m}^2$.
2. Age at randomization. This analysis will evaluate the treatment effect on patients of different age groups. Patients will be divided into two groups based on age: one group aged <40 years and the other aged ≥ 40 years.

For the above-mentioned analyses, we will use logistic regression or ANCOVA and include the subgroup variable (either baseline BMI or age at randomization) and group (“modified” vs. “standard”) and analyse the interaction between the two.

11 REFERENCES

1. Risstad, H., et al. (2016). "Standard vs Distal Roux-en-Y Gastric Bypass in Patients With Body Mass Index 50 to 60: A Double-blind, Randomized Clinical Trial." JAMA surgery 151(12): 1146–1155.

Approved and signed on 01 September 2025



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