Name of the Sponsor: Azienda Ospedaliero Universitaria Meyer
Name of Finished Product: Eutirox/Tirosint
Name of Active Ingredient: Levothyroxine

Study title	Studio randomizzato crossover su pazienti con ipotiroidismo acquisito primario per valutare i possibili effetti a livello intestinale di due diverse formulazioni di L-Tiroxina.
Name of test drug/ investigational product	Eutirox e Tirosint
Name of the Sponsor	Azienda Ospedaliero Universitaria Meyer - Viale Pieraccini 24 50139 Firenze
Protocol Code	THYR69
EudraCT number	2015-001248-12
Development phase of study	Phase IV
Study initiation date (first patient enrolled, or any other verifiable definition)	31/03/2016
Study completion date (last patient completed)	14/03/2019
Date of report	04/03/2020

STATEMENT

This document has been prepared following the ICH guideline E3 on structure and content of clinical study reports.

The study was conducted in accordance with Good Clinical Practice, including the archiving of essential documents

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Publication (reference): None	
Study period (years): The trial had a 12-month treatment period and 12 months of follow-up period. The enrollment phase lasted 12 months. The total duration of the study, including the data analysis, were 36 months. The trial does not enrolled patients. The centre was opened on 08 June 2016 and was prematurely closed on 14 March 2019	**Phase of development**: IV

Objectives:

Primary objective:

Evaluate the effect of two different pharmaceutical forms of L-T4 in the gut in terms of inflammatory parameters and gut absorption.

Secondary objectives:

1) Evaluate the effect of two different pharmaceutical forms of L-T4 on the gut in terms of modification of gut microbiota;

2) Evaluate the effect of the disease (congenital hypothyroidism) on the gut in terms of modification of gut microbiota, inflammatory parameters, gut absorption;

3) Evaluate the incidence of celiac disease in hypothyroid patients.

Methodology:

The study is interventional, randomized, cross-over, open-label, monocentric and no profit.

Patients are randomized to:

- solid L-T4 arm (arm A): the subjects assigned to this arm receive the solid formulation of L-thyroxine (Eutirox tablets)

- liquid L-T4 arm (arm B): the subjects assigned to this arm receive the liquid formulation of L-thyroxine. (Tirosint oral solution)

The treatments are administered for 6 months, after which each patient are allocated to the alternate arm.

Number of patients (planned and analysed):

Planned:70

Enrolled: None

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Diagnosis and main criteria for inclusion: - Children and adolescents (<18 years) with primary acquired hypothyroidism needing replacement therapy with L-T4 - Informed consent obtained by subject/parents
Test product, dose and mode of administration, batch number: Arm A: Eutirox: 50 µg tablets for oral use. According to Eutirox Summary of Product Characteristics, the dosage are: 0-6 months: 10 µg/kg/die; 6-12 months: 8 µg/kg/die; 1- 5 years: 6 µg/kg/die; 5-10 years 4 µg/kg/die. Arm B: Tirosint: 50 µg/ml - 100 µg/ml, oral solution for oral use. According to Tirosint Summary of Product Characteristics, the dosage are: 0-6 months: 10 µg/kg/die; 6-12 months: 8 µg/kg/die; 1- 5 years: 6 µg/kg/die; 5-10 years 4 µg/kg/die;
Duration of treatment: Tirosint: 6 months Eutirox: 6 months At the end of the first 6 months, patients are allocated to the other treatment arm for the remaining 6 months (cross-over study)
Reference therapy, dose and mode of administration, batch number: N.A.

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Criteria for evaluation:

Efficacy:

Primary Endpoint: Evaluate the effect of two different pharmaceutical forms of L-T4 in the gut in terms of inflammatory parameters and gut absorption.

Secondary Endpoint:

- Differences between the two groups at time T6 - T0 and intra-group differences at time T12- T6 compared to T6-T0 (before-after analysis) in terms of percentage of microorganisms (Actinobacteria, Firmicutes, Bacteroidetes, proteobacteria). Differences will be calculated with the Shannon Index and Chao I. .
- Qualitative and quantitative (percentage) differences characterization of gut microbiota before the initiation of therapy (T0)
- Differences in gut microbiota among hypothyroid patients (T0) and healthy patients (data form Human Microbiome Project).
- Estimate parameters regarding the gut inflammation (calprotectin, osteoprotegerin, protein S100-A12) and gut absorption (Steatocrit)before the initiation of the therapy (T0)
- Estimate the incidence of positive patients to deaminated AGA at T6, T12 and T24 (follow-up).

Safety:

- Qualitative and quantitative description of adverse events

Statistical methods:

The results will be expressed as means and standard deviations. The statistical analysis will be carried out with the SPSS 15.0 program (SPSS Inc., Chicago, IL, USA). The primary endpoint regarding inflammatory parameters will be measured in the two groups by evaluating the average increases from baseline in the two arms (0-6 months) and before and after the cross-over (0-6 months vs 6-12months). The differences between populations will be analyzed with parametric and non parametric (ANOVA) statistical methods. The Chao I and Shannon index will be used to calculate microbial wealth and biodiversity.

The biodiversity index and the estimate of microbial wealth will be calculated analyzing the samples with QIIME, to eliminate bias due to sequencing. The values concerning the number of bacteria will be converted into logarithmic values before the statistical analysis. Given the low number of participants, the Mann-Whitney U test will be used to highlight changes in the number of bacteria and in the values of the biochemical variables between the 2 groups.

Nominal data will be evaluated with the chi-square test. Spearman's correlation coefficient will be calculated to estimate the correlations between variables. To control confounding factors a multiple regression analysis will be performed. Age, type of breastfeeding, age at weaning, age of first introduction of gluten, caesarean delivery will be used as confounding variables.

Statistical significance was set at a value of $p < 0.05$. The primary and safety analysis will be performed on all randomized subjects (ITT). For those lost to follow-up, the latest information collected at the scheduled

times (time 0, 6, 12, 24) will be considered. No intermediate analyzes are foreseen.
SUMMARY – CONCLUSIONS: **Efficacy results:** N.A. **Safety results:** N.A. **CONCLUSION: T**he study was opened for almost 3 years. Since no patients were enrolled in the study, due to organizational problems, the Principal Investigator decided to prematurely close the study at March 2019.
Date of the report: 04.03.2020