2 SYNOPSIS

Name of Sponsor/Company: Bracco S.p.A., Via E. Folli 50, I-20134 Milan, Italy

Name of Active Ingredient: T3 Sulfate (T3S) – Sodium Salt

Name of Finished Product: Not applicable

Title of the study: Substitutive therapy of hypothyroid patients with L-thyroxine (T4) plus T3 sulfate (T3S). A phase II, open-label, single centre, parallel groups study on therapeutic efficacy and tolerability

Investigators: one principal investigator in Italy

Study centres: one investigational study site in Italy

Publication (reference): None

Study period: First subject enrolled: 01 September 2010; Last subject completed: 17 August 2011

Phase of development: II

Objectives: The primary objective of this study was to investigate the possibility of maintaining the metabolic control (evaluated on the basis of the TT3, FT3, FT4 and TSH circulating levels) in hypothyroid patients by partially substituting the T4 with T3S administration.

The secondary objectives of the study were: to assess the safety of T3S prolonged administration; to identify the optimal T4/T3S substitutive ratio; to compare the judgment of the patients on T4 vs T4+T3S therapy; to evaluate the effects of the combined therapy on serum lipids profile.

Methodology:
The study was performed according to a phase II, open-label, uncontrolled, single centre, parallel group study design.
The patients suitable for the study were thyroidectomized patients without endogenous hormonal production (Tg<5) in stable (almost from 3 months) substitutive therapy with levothyroxine (T4). Three group of patients (n=12 for each group) were enrolled according to the ongoing T4 dosage: 100, 125, 150 µg daily. The T4 dose was partially replaced by T3S, according to the ratio T4 25 µg → T3S 40 µg, i.e.

→ T4 100 µg group >75 µg of T4+40 µg of T3S
→ T4 125 µg group >100 µg of T4+40 µg of T3S
→ T4 150 µg group >125 µg of T4+40 µg of T3S

During the study the T4 dose remained unchanged, whereas the T3S dose was suitable for decrease or increase (until 100 µg maximum dose) according to the hormonal status (FT3, FT4, TSH), the clinical findings and the Investigator opinion. The patients were checked every 15 days (for a maximum of 45 days) until the initial metabolic control was achieved (or maintained): thereafter the control visits were performed monthly for 2 months

Number of subjects (total and in each arm):

	Evaluable for safety	**Evaluable for absorption**	**Completers**
Total	36	36	36
75+40 µg group	12	12	12
100+40 µg group	12	12	12
125+40 µg group	12	12	12

Diagnosis and main criteria for inclusion:

Subjects were enrolled if they met all the following criteria: written informed consent obtained; both gender; age between 18 and 70 years; hypothyroidism for any reason, in T4 substitutive therapy (daily dose: 100/125/150 µg);

undetectable Tg (<5); acceptable metabolic control (FT4, FT3 and TSH values inside the normal ranges for hypothyroid patients in substitutive therapy; patient's co-operative attitude and able to understand and adhere to study protocol procedures and timelines.

Test product, dose and mode of administration, batch no:

During the study T4 and T3S tablets were used. The T4 dose was 75 μg in 12 patients; 100 μg in 12; 125 μg in 12. The T3S was administerd at the dose of 40 μg in 34 patients; at the doses of 40 and 60 μg in one patient and of 40-60-80 μg in one patient. The IMPs were administered in single morning dose, in fasting conditions. Below the batch number of the IMPs provided (the T3S 20 and 100 μg strenght were not used) :

IMPs	*Strenght (/μg)*	*Batch n.*	*expiry date*
T4 tablets	75	9357P	02/2012
	100	9466P	02/2012
	125	9522P	02/2012
T3S tablets	20	TFR10134	08/2011 (extended date)
	40	TFR10135	08/2011 (extended date)
	60	TFR10136	08/2011 (extended date)
	80	TFR10137	08/2011 (extended date)
	100	TFR10138	08/2011 (extended date)

Duration of treatment: The treatment time-frame in the patients at stable T3S dose was 72,6±6 days (mean±SD) (min 60-max 85); in the patient 04/100-01 T4 (dose increased until 80 mg daily) was 103 days and in the patient 20/75-09 (T3S dose increased until 60 mg daily) 96 days.

Reference therapy, dose and mode of administration, batch no: Not applicable.

Criteria for evaluation:

Efficacy variables :
The primary objective of this study was to investigate the possibility of maintaining the euthyroid state -based on TT3, FT3, FT4 and TSH circulating levels- in hypothyroid patients, by partially substituting the actual dose of T4 with T3S.
The secondary objectives of the study were:
to assess the safety of T3S prolonged administration;
to identify the optimal T4/T3S substitutive ratio;
to compare the judgment of the patients on T4 vs T4+T3S therapy

Safety variables:
The safety variables were: incidence of adverse events, safety laboratory tests (haematology and blood chemistry), physical examination and vital signs (heart rate and blood pressure).

Statistical methods:

Data management and statistical analysis were carried out by the Studio Associato Airoldi, Cicogna e Ghirri (Via Manzoni 43, I-20121 Milan, Italy). Statistical analysis was performed using SAS® system, PC release 9.2 (SAS Institute, Cary, US). TT3 and TSH levels were markedly right-skewed and were therefore log-transformed to obtain approximately normal distributions as required to use parametric statistics. These variables were therefore expressed using geometric rather than arithmetic means and ratios rather than differences. As summary statistics of transformed variables are less easily interpreted, variables with a moderate departure from a Gaussian distribution were not log-transformed even if this would further improve approximation to normality. Categorical data were summarised by reporting frequencies and proportions, i.e. number of patients with a given characteristic out of the total number of patients in the relevant population. Continuous variables were summarised using minimum and maximum values and quartiles including median; arithmetic mean and SD have been reported if the variable distribution was not exceedingly skew; geometric mean was used instead if the variable distribution was approximately log-normal. The summary statistics of log-transformed variables were converted back to the

original untransformed scale for reporting. Conventionally, $p \leq 0.05$ was considered statistically significant, therefore 95% CIs (i.e. CIs with $\alpha = 0.05$) were reported. All reported p-values and CIs are two-sided. No formal adjustment of p-values and CIs was calculated for performing multiple analyses. Exact values were reported for the CIs of proportions. Summary statistics of thyroid hormone levels at each visit from V1 through V5 were reported overall and by T4 dose group. Means of differences from V1 for FT3 and FT4 levels (normally-distributed) and geometric means of ratios vs V1 for T3S, TT3 and TSH levels (log-normally-distributed) were calculated. For each hormone was first analysed the change from V1 to V3a, when the effect of the initial T3S dosage was measured in all 36 patients and there was no bias in comparing T4+T3S doses due to T3S dose adjustment in poor responders to the initial dosage. Changes (differences or ratios) from V1 to V3a were tested using Student's paired t test both overall and within dose groups, and their relationship with T4 dose was examined by one-way ANOVA with T4 dose as an interval variable. The subsequent analysis from V3a to V5 was restricted to the 34 patients who did no require T3S dose adjustment, thereby excluding the two subjects whose thyroid hormone levels after V3a were clearly affected the T3S dose increase. Mixed-model linear regression analysis of changes (differences or ratios) vs V1 was used, with visit and dose as fixed effects and subjects as random effects. Visit (3,4,5) was always modeled as an interval variable, a one-unit difference representing a one-month interval between scheduled time of visits. T4 dosage was modeled as an interval variable, except in the models estimating the effect of visit within dosage where it had to be nominal. The covariance structure was chosen according to the Akaike criterion corrected for small samples (AICC) as compound symmetry for FT3 and FT4 and heterogeneous compound symmetry for T3S, TT3 and TSH. Individual lipid plasma values flagged as exceeding normal reference ranges provided by the laboratory have been described. Summary statistics of lipid hormone plasma levels at V1 and V5 have been reported overall and by T4 dose group. Means of V5−V1 differences for cholesterol levels (normally-distributed) and geometric means of V5/V1 ratios for triglycerides levels (log-normally-distributed) were calculated with their 95% CIs and p-values by Student's paired t test both overall and within dose groups. The relationship of changes (differences or ratios) with T4 dose was examined by one-way ANOVA with T4 dose as an interval variable. Lipid level analyses were repeated both including and excluding the two patients who increased the T3S dose during the study. Individual hematology and blood chemistry values flagged as exceeding normal reference ranges provided by the laboratory have been described. Summary statistics of values at V1 and V5 and of changes from V1 to V5 have been reported overall. Means of V5−V1 differences for normally-distributed variables and geometric means of V5/V1 ratios for log-normally-distributed variables were calculated with their 95% CIs and p-values by Student's paired t test. Summary statistics of SBP, DBP and HR values at each visit from V1 through V6 were reported overall and by T4 dose group. Inferential analysis was conducted on differences from V2 (start of T4+T3S treatment) to V3a, V4 and V5 (end of T4+T3S treatment). The same methods reported above for the analysis of thyroid hormone levels were used. The covariance structure in mixed models was chosen as compound symmetry for HR and unstructured for SBP and DBP. Additionally, mean changes from V1 to V2 (when not performed on the same day) and p-values by Student's one-sample t test were calculated. AEs reported during the study have been summarised descriptively. Incidence rates with 95% CIs were calculated. Patients' judgment on drug regimens was reported descriptively along with the reason for preference if any. An exact test based on the binomial distribution was conducted comparing preferences for T4+T3S with preferences for T4 alone in the overall population. The safety population was defined as all randomised patients who received at least one dose of the experimental drug. The efficacy population was defined as all patients of the safety population whose blood samples were collected at each of the planned visits. Subset analyses by initial T4 dosage group were performed as planned. In a few analyses only the subjects who maintained the initial T3S dosage throughout the study were considered.

Study population: The study population included 36 patients in total, who were enrolled in sequential order. The 36 patients were distributed in the 3 treatment-dose groups as follows: 12 patients received the T4 75 µg +T3S 40 µg dose, 12 received the T4 100 µg +T3S 40 µg dose and 12 received the T4 125 µg +T3S 40 µg dose. All the 36 enrolled patients regularly completed the entire study procedures, as defined in the study protocol and, therefore, all of them were included in both the efficacy analysis population and in the safety analysis population. During the study the T3S dose was increased in 2 patients on the basis oft FT3, FT4 and TSH results: pt n°20 (screening number), 75 µg group: the T3S dose was increased to 60 µg from V3a to V5; pt n° 04, 100 µg group: the T3S dose was increased to 60 µg from V3a to V3b, to 80 µg from V3b to V5.

Extent of exposure and compliance: The treatment time-frame in the patients at stable T3S dose was 72,6±6 days (mean±SD) (min 60-max 85); in the patient 04/100-01 T4 (dose increased until 80 mg daily) was 103 days and in the patient 20/75-09 (T3S dose increased until 60 mg daily) 96 days. Compliance was assessed on the basis of the tablets consumption, according to the prescribed dose and the treatment duration. All patients followed the scheduled drug regimen, with difference greater than one unit between expected and actual number of tablets returned observed only in three patients. One patient assumed two T3S tablets and no T4 tablet one morning between V2 and V3a.

Efficacy results:

A the end of the study all patients were under controlled metabolic state.

The T3S serum levels changed erratically during the study, but at the end of the study were near the baseline values in all groups.

At the time 15 the TT3 serum levels increased slightly in the 75 and 100µg groups (+10% about) and decreased slightly in the 125 µg group (-10% about); at following times returned to the baseline values.

The FT3 serum levels remained almost unchanged in the 100 and 125 µg groups, decreasing slightly in the 75 µg group.

The FT4 serum levels decreased in all groups, being the greatest change observed in the 75 µg group (-25% at last control) and the smallest in the 125 µg group (-15%) [according to the reduction of T4 dose at the start of the study:-25% in the 75 µg group, -20% in the 100 µg group and -17% in the 125 µg group].

The TSH increased from the baseline value of 0,382 (geometric mean) µUI/mL to the 1,954 value at last visit in the 75 µg group, from 0,362 to 1,526 in the 100 µg group and from 0,402 to 0,742 in the 125 µg group. At the baseline 1/11patient in the 75 µg group and 1/11 in the 100 µg group showed a TSH value over 1,500 µUI/mL; at the end of the study the patients with a TSH value over this limit were 7/11 (63.6%) in the 75 µg group, 5/11(45,4%) in the 100 µg group and 4/12 (33,3%) in the 125 µg group.

At the start of the study the 2 patients with T4/T3S dose ratio 1,25 were inside the reference range of the circulating FT4/FT3 ratio; 4/11 patients of the 1,88 group, 5/11 in the 2,50 group and 7/12 in the 3,13 group were over the upper limit of reference range (45,4% of the total population). At last control the two patients with T4/T3S ratio 1,25 stayed inside the reference FT4/FT3 ratio range; in the others groups the patients over the upper limit of reference range were respectively 0/11, 1/11 and 3/12. Therefore, after the combined therapy the FT4/FT3 ratio was inside the normal range in 32/36 patients (88,9%).

Both in the 75 µg and 100 µg groups 5 patients judged the T4+T3S treatment better than T4 alone and 7 as the same; in the 125 µg group group the respective numbers were 2 and 10. The differences inside the groups were not statistically significant, but achieved statisticall significance in the whole (T4+T3S better for 12/36 patients, $p < 0,001$).

The total cholesterol serum levels (mean values) were almost unchanged in 75 and 125 µg groups, while slightly increased (+10,6%) in the 100 µg group. The LDL cholesterol serum levels increased of 5,8% in the 75 µg group and of 8,2% in the 100 µg group, decreased of 4,5% in the 125 µg group. The HDL cholesterol and triglycerides showed no clinically significant changes throught the study in all groups.

Safety results:

Adverse events:

Four patients complained of an AE: upper airways inflammation, lumbar pain, chest pain and road accident. The patients with chest pain and road accident required hospitalization. All patients recovered. No AE was judged as experimental treatment-related. No patient discontinued the IMPs treatment.

Safety laboratory parameters:

Safety laboratory values were checked at the screening visit and at the final visit (V5). No clinically significant changes of mean values were recorded.

Vital signs:

The results of vital signs (blood pressure and heart rate) did not show changes from baseline with all treatment doses.

Conclusions:

Three groups of outpatients at stable T4 dose (100-125-150 µg daily) were selected; in all patients 25 µg of T4 were substituted with 40 µg of T3S (>75-100-125 groups) on empirical basis (animals studies; pharmacokinetic study; current knowledge on physiology of thyroid hormones in human being). Throughouth the study a T3S dose change was allowed, but in two patients only the dose was increased (until 60 and 80 µg respectively). The results of the study confirmed that the T3S administered orally is absorbed and converted to FT3, acting a physiologic role. As a matter of fact, all patients were in a controlled metabolic state at the end of the study.

The serum levels of FT4 decreased in all groups, in proportional fashion to the l-thyroxine dose reduction, being the greatest decrease observed in the 75 µg group (25% of T4 dose replaced), the intermediate in the 100 µg group (20%) and the lowest in the 125 µg group (16,7%). Despite FT4 reduction, serum FT3 did not show significant changes, while TSH serum levels tended to increase. The T4 and T3S dose adjusted for the body weight did not correlate with the FT3 serum levels: this may indicate that serum T3 derives from both precursors. The reduction of FT4 and the minor changes in the FT3 produced a reduction of FT4/FT3 ratio, bringing the ratio back into the normality in most of the patients. The T4/T3S 2,5 ratio was the one that best approached the FT4/FT3 ratio and the TSH levels observed in normal controls. On the basis of the variable and personalized doses of exogenous thyroid hormones required by hypothyroid patients the useful combinations of the associated T4+T3S available in clinical practice could be (µg) 50+20 - 62,5+25 - 75+30-87,5+35 - 100+40 - 112,5+45 - 125+50 - 137,5+55 - 150+60 µg.

No concern raised from the safety viewpoint. No significant variations of the laboratory tests were detected and the clinical adverse events (upper airways inflammation, lumbar pain, shoulder fratture due to high speed motorcycle accident and chest pain not due to coronary disease) showed no reasonable correlation with the experimental treatment.

In conclusion, the results of this study show that the combination of l-thyroxine+triiodothyronine sulfate might get the golden treatment for hypothyroid patients.