CLINICAL TRIAL PROTOCOL

STUDY DRUG:	Tirosint®-SOL (levothyroxine sodium) oral solution
PROTOCOL ID:	20US-T414
PHASE:	IV
REGISTRY NAME:	Clinicaltrials.gov (NCT05228184)
TITLE:	A Randomized Comparative Study Between Liquid (Tirosint®-SOL) and Tablet Formulations of Levothyroxine in Neonates and Infants with Congenital Hypothyroidism (CH)
SPONSOR:	IBSA, Institut Biochimique S.A. [redacted]
SPONSOR STUDY MANAGER:	[redacted]
DOCUMENT VERSION:	V.2.0 FINAL
DOCUMENT DATE:	07 September 2023
This study will be performed in compliance with Good Clinical Practices, including the archiving of essential documents	
This protocol is confidential property of IBSA, not to be copied or disclosed to third parties without written authorization from IBSA Institut Biochimique SA.	

1 DOCUMENT HISTORY

Version	Issue Date	Protocol Amendment Number and Description of Change(s)
1.0	22 June 2021	NA
2.0	07 September 2023	Amendment N. 1 Major/Substantial changes: • Sections 7 Protocol Synopsis and 12.1 Inclusion Criteria: Inclusion criterion #2 has been changed from "Primary CH diagnosis with elevated TSH and low T4…" to "Primary CH diagnosis with elevated TSH and low or normal FT4…", in order to include not only subjects with severe disease, but also those with mild to moderate disease, and to account for the new AAP guidelines on screening and management of congenital hypothyroidism recommending diagnosis based on TSH and FT4 (Rose 2023). Inclusion criterion #2 has moreover been changed from "…Infants (aged 29 days to 277 days) previously diagnosed with primary CH and who are already on LT4 therapy for at least 4 weeks" to "…Infants (aged 29 days to 277 days) previously diagnosed with primary CH and who are already on LT4 therapy for at least 3 weeks", to account for the recommended periodicity of therapy monitoring with one scheduled control usually falling at 3 to 4 weeks after the initiation of LT4 treatment (Rose 2023). Rationale on the choice of the inclusion criterion has been added in Section 11.2. • Sections 7 Protocol Synopsis and 12.2 Exclusion Criteria: Exclusion criterion #2 has been changed from "Low birth weight (LBW) and very low birth weight (VLBW) neonates (weight < 2.5 kg)" to " Low birth weight (LBW) or very low birth weight (VLBW) neonates (weight < 2.5 kg) or VLBW infants (weight < 1.5 kg)" in order to exclude from the study infants who were born VLBW. This population is indeed considered particularly fragile and at risk of death for the first year of life and this may jeopardize the participation in the study. Rationale on the choice of exclusion of VLBW subjects has been added in Section 11.2.

		• Sections 7 Protocol Synopsis and 12.2 Exclusion Criteria: Exclusion criterion #3 has been changed from "Neonates in neonatal intensive care units (NICU) or requiring admission to NICU or neonates/infants hospitalized or requiring hospitalization" to "Neonates in neonatal intensive care units (NICU) or requiring admission to NICU or neonates/infants hospitalized or requiring hospitalization or in fragile health conditions (e.g. with serious health problems or complications)", fragile health conditions may jeopardize the participation in the study. Rationale on the choice of this exclusion criterion has been added in Section 11.2. • Sections 12.4 Screen Failures and 16.1 Screening/Inclusion (Randomization) – Visit 1 (Day -14 To 1): the re-screening of subjects will be allowed, if eligibility has changed, but re-screening may happen only once, upon re-consenting and upon assignment of a new Subject Number. • Sections 7 Protocol Synopsis, 15.1.1 Laboratory Parameters, 15.3.1 Dosing Evaluation and 16 Overall Study Schedule: the analysis of TT4 has been made optional, at the discretion of the Investigator, because the most recent AAP guidelines on medical management of congenital hypothyroidism (Rose 2023) recommend TSH and FT4 as the main parameters for laboratory monitoring of the LT4 treatment. Rationale has been included under section 11.2. • Sections 15.1.1 Laboratory Parameters, 16.2 Treatment Phase and Table 1 Schedule of Evaluations: the window for blood samples collection for serum FT4, TT4 and TSH monitoring, as well as for the conduct of all visit procedures, has been extended from 0-4 days to 0-7 days within the allowed visit time frame, to allow more flexibility to subjects and sites. • Sections 15.1.1 Laboratory Parameters and 22.1 Biological Samples have been updated in order to allow the possibility to use more than one single laboratory at each site, as well as to have blood drawn as per SOC at a laboratory patient service center, so to decrease the discomfort for the subjects related to having all blood draws at the clinical site, particularly when living distant from the site. In order to reduce variability, the request to use the same lab for the same subject has been added. Also, the timing for samples analysis has been extended from 24 to 24-72 hours.

		• Section 16.2.3 Visit 2 (Study Day 8-15, i.e. 7-14 Days After Treatment Start) and Table 1 Schedule of Evaluations: blood draw at Visit 2 (7-14 days after treatment start) has been changed from mandatory to optional, at the discretion of the Investigator, for infants already on LT4, because this early control is not SOC for infants who are stable on LT4 and it would cause unnecessary discomfort to them. • Section 16.2.16 Unscheduled Visits: since unscheduled visits are frequently done to the sole purpose of drug dispensing, AEs and concomitant medications recording will not be mandatory in that case. • Section 16.1 Screening/Inclusion (Randomization) – Visit 1 (Day -14 to 1) and Table 1 Schedule of Evaluations: for infants presenting at Visit 1 with routine laboratory results already available, the acceptability of these results has been extended from 2 weeks to <28 days, in order to reduce the discomfort of additional blood sampling in a short period of time. Though, the possibility to repeat blood tests at V1, if needed as per Investigator's judgement, has been maintained. In addition, it has been clarified that, where acceptable routine lab results are available, these will have to be recorded in the CRF. Minor changes/Clarifications: • Table 1 Schedule of Evaluations and Section 16 Overall Study Schedule have been updated to correct a few discrepancies in Study Day numbers between Table 1 and Section 16, and to provide additional clarifications, without substantial changes in the procedures other than those described above. • Table 1 Schedule of Evaluations has been updated for release of CareCAT during the third week of treatment on days 15-21 instead of days 15-22. • Table 2 Outline of Visit Schedule Based on Age has been updated in order to correct few discrepancies in Study Day numbers between Table 2 and the text in Section 16 Overall Study Schedule, without substantial changes in the procedures other than those described above. • Background information provided in Sections 8 Introduction, 11 Trial Design and 13 Treatments has been reviewed in light of the new guidelines on the screening and management of

		congenital hypothyroidism by the AAP (Rose 2023), with no substantial impact on the study plan and procedures. • The literature overview in Section 8.1 Background has been updated with the most recent papers published on the use of LT4 oral solutions in congenital hypothyroidism, with no impact on the risk/benefit profile of the study. • Sections 11.2 Scientific Rationale for Study Design has been updated to clarify reliance on SOC for CH diagnosis, the definition of "elevated", "low" and "normal" TSH and FT4 results and the fact that local laboratory normal ranges are not the only reference ranges that the Investigator can take into account in their evaluation of hormonal results, but medical guidances, as well as relevant literature and manuals may also be taken in consideration. This is related to the fact that local laboratories normal ranges are highly variable and not always age-specific in terms of days from birth. This change is not intended to modify the study procedures, but to provide more clarity on the topic. In addition, the rationale for the changes in inclusion/exclusion criteria and assessments has been added. • In Sections 7 Protocol Synopsis and 12.2 Exclusion Criteria, Exclusion criterion #4 has been re-worded for uniformity of wording with other criteria. In addition, the clarification has been provided that the wording "neonates" and "infants" are to be intended as defined in inclusion criterion #2. These modifications don't change the meaning of the criteria, but are intended to clarify it. • In Sections 7 Protocol Synopsis and 13.1 Study Drug (IP), the strength of 13 mcg has been added among the strengths supplied in order to cover potential needs of fine dose adaptations. Supply of additional strengths among those authorized for Tirosint-SOL was already foreseen by the protocol. • Section 12.5 Strategies for Recruitment and Retention: the aim of external recruitment efforts has been better specified and the possibility of dissemination of information to the public has been added. • Section 13.2.1 Packaging and Labelling has been corrected to reflect actual labelling information.

		• In Section 15 Study Assessments and Procedures it has been clarified when clinically significant abnormalities detected at study assessments are to be recorded as medical history or AEs and when not. • Section 15.1.2 Parents' Satisfaction Questionnaire has been updated to ask Investigators to verify completion of the questionnaire and, if not completed, ask parents to complete it on site during the visits, in order to improve compliance with this assessment. • Section 16.1 Screening/Inclusion (Randomization) – Visit 1 (Day -14 to 1) and Table 1 Schedule of Evaluations: the definition of "first day of study drug administration" for subjects who were already on tablets and remain on tablets during the study has been clarified. • Sections 16.2.1 Normalization Phase, 16.2.2 Long-Term Follow-Up Phase and Table 1 Schedule of Evaluations: it has been clarified that if a Follow-Up Visit (Type 1 or 2) overlaps with a planned visit, it is possible to perform only the planned visit, consistently with SOC. • Section 16.2.2 and Table 1 Schedule of Evaluations: Follow-up Type 2 visits have been made optional, at Investigator's judgement, in case an abnormal T4 or TSH are found, for consistency with the SOC (they remain mandatory in case of dose adjustment). • Sections 7.2 Organizational and Administrative Structure and 17.5 Reporting and 17.7 Follow-up: a new email address of Drug Safety Unit for SAEs notification has been added, the list of persons to be informed has been updated, and some instructions regarding the information to be provided by the Investigator have been added. • Section 18.1 Data Collection: the latest definition of ALCOA has been added. • Sections 7 Protocol Synopsis and 19.3.2 Efficacy Outcomes: statistical analysis of TT4 will be conducted only if an adequate number of observations will be available, since the evaluation of this laboratory parameter at each visit will be optional. Moreover, the statistical analysis for events of TSH values above 4.5 mIU/L and FT4 values below the middle of the laboratory normal range has been modified from analysis

		of variances into logistic models because it is deemed more appropriate for the data type. • Section 20.2 Quality Control And Quality Assurance: the monitoring of Quality Tolerance Limits during the course of the study has been added. • Minor inconsistencies/discrepancies have been corrected throughout the text. Administrative changes: • Cover page: Clinicaltrial.gov number has been added • Section 3 List of Abbreviations: the list has been updated • Cover page and Section 7.2 Organizational and Administrative Structure: Sponsor's Legal Address has been updated. • Cover page and Section 7.2 Organizational and Administrative Structure: Sponsor's Study Manager's information have been updated. • Section 7.2 Organizational and Administrative Structure: Sponsor's United States Representative has been deleted. • Section 7 Protocol Synopsis: Due to delays in recruitment, overall study duration has been extended from 22 to 39 months (enrolment time from 10 to 26 months) and the estimated date for last patient completed from July 2023 to December 2024. • Section 19.3.1 General Statistical Methods: SAS version in use for the statistical analysis has been updated.

2 STATEMENT OF COMPLIANCE

The trial will be carried out in accordance with the International Conference on Harmonization Good Clinical Practice (ICH GCP) and the United States (US) Code of Federal Regulations (CFR) 45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56, 21 CFR Part 312, 21 CFR Part 11, and/or 21 CFR Part 812.

The protocol, informed consent form(s), recruitment materials, and all subject materials will be submitted to the Institutional Review Board (IRB) for review and approval. Approval of both the protocol and the consent form(s) will be obtained before any subject is enrolled. Any major amendment to the protocol will require review and approval by the IRB before the changes are implemented. In addition, all changes to the consent form will be IRB-approved; a determination will be made regarding whether a new consent needs to be obtained from subject who already provided consent using a previously approved consent form.

3 LIST OF ABBREVIATIONS

AAP	American Academy of Pediatrics
ADR	Adverse Drug Reaction
AE	Adverse Event
ALCOA	Attributable, Legible, Contemporaneous, Original and Accurate
ATC	Anatomical Therapeutic Chemical
BW	Body Weight
CareCAT	Caregiver Administered Children's Acceptance Tool
CFR	Code of Federal Regulations
CH	Congenital Hypothyroidism
CMP	Clinical Monitoring Plan
CRO	Clinical Research Organization
CSR	Clinical Study Report
CTA	Clinical Trial Agreement
DMC	Data Monitoring Committee
DMP	Data Management Plan
DSU	Drug Safety Unit
eCRF	Electronic Case Report Forms
EDC	Electonic Data Capture
EOS	End of Study
EU	European Union
FAS	Full Analysis Set
FDA	Food and Drug Administration
FPFV	First Patient First Visit
FT4	Free Thyroxine
FU	Follow-Up
GCP	Good Clinical Practice
GI	Gastrointestinal
HR	Heart Rate
ICF	Informed Consent Form
ICH	International Conference of Harmonization
ID	Subject Identification
IND	Investigational New Drug
IP	Investigational Product (Study Drug)
IQ	Intellectual Quotient
IRB	Institutional Review Board
ISF	Investigator Site File
IWRS	Integrated Web Response System

LBW	Low Birth Weight
LPLV	Last Patient Last Visit
LT4	Levothyroxine
LOCF	Last Observation Carried Forward
MedDRA	Medical Dictionary for Regulatory Activities
NICU	Neonatal Intensive Care Unit
PI	Principle Investigator
PPI	Proton Pump Inhibitors
PPS	Per Protocol Set
PT	Preferred Term
PTAE	Pre-Treatment Adverse Event
SAE	Serious Adverse Event
SAF	Safety Analysis Set
SAP	Statistical Analysis Plan
SAR	Serious Adverse Reaction
SOC	Standard of Care
SOC (MedDRA)	System Organ Class
SOE	Schedule of Evaluations
SOP	Standard Operating Procedure
SUSAR	Suspected Unexpected Serious Adverse Reaction
T3	Triiodothyronine
T4	Thyroxine
TT4	Total Thyroxine
TFT	Thyroid Function Test
TH	Thyroid Hormone
TM	Telemedicine
TSH	Thyroid Stimulating Hormone
US	United States
USPI	United States Prescribing Information
VLBW	Very Low Birth Weight
WHO-DRL	World Health Organization – Drug Reference List

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7 PROTOCOL SYNOPSIS

Title:	A Randomized Comparative Study Between Liquid (Tirosint®-SOL) and Tablet Formulations of Levothyroxine in Neonates and Infants with Congenital Hypothyroidism (CH)
Protocol:	20US-T414
Phase:	IV
Study Site(s):	Up to 20 sites in the United States
Study Objective:	The objective of this study is to gather information on the use of Tirosint®-SOL oral solution as thyroid hormone (TH) replacement therapy in neonates and infants with CH compared to the conventional treatment with crushed levothyroxine sodium (LT4) tablets. Specifically of interest is comparing the ease of administration and acceptability, the thyroid function tests (TFTs) profile, the LT4 dose required to maintain thyroid stimulating hormone (TSH) in the target range, as well as the growth pattern between the treatment groups.
Study Design:	This is a multi-center, prospective, parallel-group, open-label, randomized clinical study in one hundred and twenty-six (126) subjects (neonates and infants diagnosed with CH). Subjects will be randomized in a 2:1 ratio to Treatment (Tirosint®-SOL) or Control (levothyroxine sodium crushed tablets). Newly diagnosed neonates will be randomly assigned to start therapy with LT4 at the initial dose recommended by the Standard of Care (SOC). Infants already on LT4 therapy will continue at the same daily dose within the randomly assigned treatment group (dose adjustments are allowed, if needed based on laboratory parameters and clinical response). Once enrolled, subjects will be treated and followed for 12 months (±1.5 months), participating in 7-8 study visits, consisting of 6-7 in-clinic and 1-2 (or more if follow-up visits are required) telemedicine (TM) visits. The total number of visits depends on the age at inclusion as reported in **Table *2***. In-clinic visits are performed between 3-4 weeks after randomization, when the subject is 4, 6, and 9 months of age, and then every 3 months as required depending on the age at the inclusion. TM visits will be performed between 1-2 weeks after randomization, when the subject is 2 months of age, if

	applicable, and any time an additional follow-up is required for TSH and thyroxine (T4) monitoring.
Study Duration:	Planned treatment duration per subject: ~12 months Overall study duration: ~39 months (26 months to enroll all subjects).
Treatment:	**Study Drug:** Tirosint®-SOL (levothyroxine sodium) oral solution (IBSA Pharma Inc.) at the following strengths: 13, 25, 37.5, 44, 50, 62.5, 75, 88, 100 mcg. **Control**: Currently prescribed crushed LT4 tablets or, in case of newly diagnosed neonates, crushed LT4 tablets as determined by SOC. Dose: The dose will be based on body weight (BW) and age of each subject, and adjusted based on clinical response and laboratory parameters according to SOC and Investigator's judgement. The general aim of therapy should be to normalize the serum TSH level (it is suggested to target the mid- to lower half of the normal range whenever appropriate), and to increase the serum T4 into the upper half of the normal range. Mode of administration: Both products will be used according to the United States Prescribing Information (USPI) and SOC.
Study Population:	126 subjects meeting the following criteria will be enrolled. **Inclusion Criteria:** 1. Male and female subjects aged 0 to 9 months (277 days) at Inclusion; 2. Primary CH diagnosis with elevated TSH and low or normal FT4, requiring treatment with LT4, under either of the following conditions: • Neonates (aged 0 to 28 days) newly diagnosed with primary CH and needing to initiate LT4 therapy, or • Infants (aged 29 days to 277 days) previously diagnosed with primary CH and who are already on LT4 therapy for at least 3 weeks; 3. Parent or legal guardian of the subject provides voluntary written and informed consent prior to initiation of any study procedures; and 4. Parent or legal guardian of the subject is willing and able to comply with scheduled visits and all study activities. **Exclusion Criteria:**

	1. Preterm neonates with a gestational age < 37 weeks; 2. Low birth weight (LBW) or very low birth weight (VLBW) neonates (weight < 2.5 kg) or VLBW infants (weight < 1.5 kg); 3. Neonates in neonatal intensive care units (NICU) or requiring admission to NICU or neonates/infants hospitalized or requiring hospitalization or in fragile health conditions (e.g. with serious health problems or complications); 4. Neonates with CH diagnosis > 4 weeks after delivery; 5. Diagnosis of primary gastrointestinal (GI) disease: a. Gastroesophageal reflux requiring medical therapy (beyond thickening of formula or position); b. Anatomic defects (e.g. intestinal atresia, malrotation, tracheoesophageal fistula, pyloric stenosis, Hirschsprung's disease, gastroschisis); c. Dietary allergy (e.g. cow's milk protein allergy); d. Malabsorption related to cystic fibrosis, celiac disease and others; e. Necrotizing enterocolitis requiring surgical resection; 6. Known or suspected adrenal insufficiency (e.g. congenital adrenal hyperplasia, hypopituitarism); 7. Diagnosis of congenital cardiac disease, cardiac insufficiency or risk for cardiac failure; 8. Diagnosis of chromosomopathy; 9. Diagnosis of central hypothyroidism; 10. Hypersensitivity to glycerol; 11. Concomitant anticonvulsant medications, liothyronine, combination of LT4 and liothyronine, thyroid extracts and/or chronic or long-term use of systemic glucocorticoids (see **Section 13.2.5** for details); 12. History of nonadherence with medication or medical visit schedule; or 13. Any condition for which, participation would not be in the best interest of the subject or that could limit protocol specified assessments, according to the Investigator.
Study Assessments:	Efficacy Assessments: • Laboratory parameters (TSH, free thyroxine [FT4], and optional total thyroxine [TT4]); • Parents' Satisfaction Questionnaire; and

	• Caregiver Administered Children's Acceptance Tool (CareCAT). Safety Assessments: • Physical examination and vital signs; • Growth assessments (length, BW, head circumference); • Hypo- and hyper- thyroidism signs and symptoms; and • Adverse events (AEs). Other Assessments: • Dosing evaluation; • Treatment compliance; and • Nutritional intake.

Main Outcomes:	• Daily LT4 dose/kg required to maintain TSH in target range;
	• Frequency of dose adjustments;
	• Overall TSH, FT4 and, where available, TT4 values;
	• The number of events (TSH values) above 4.5 mU/L for TSH;
	• The number of events (FT4 values) below the middle of the laboratory normal range for FT4 for age;
	• Presence of signs and symptoms of hypo- and hyper-thyroidism;
	• Acceptance of medications evaluated by means of the CareCAT;
	• Ease of administration evaluated by means of Parents' Satisfaction Questionnaire; and
	• Growth patterns:
	  ○ Length;
	  ○ BW;
	  ○ Head circumference.

Exploratory Outcomes:	For newly diagnosed neonates: • Time to normalize TSH into reference range; and • Time to normalize FT4 into the upper half of the laboratory normal FT4 range for age.
Statistical Methods:	**Sample size:** Subjects will be randomized in a 2:1 ratio to Treatment (Tirosint®-SOL) or Control (conventional therapy with levothyroxine sodium crushed tablets). Considering a mean value of 6 mcg/kg/day of LT4 dose at 12 months of age and a standard deviation of 2 mcg/kg, a sample size of 102 neonates and infants (68 Treatment and 34 Control)

	will have 80% power to detect a difference of 20% in the LT4 dose between the groups using an independent t-test with a 0.050 two-tailed significance level. To account for a drop-out rate of maximum 20%, 126 neonates and infants will be enrolled in the study (84 Treatment and 42 Control). **General Methods:** Continuous variables will be summarized using the following descriptive statistics: n (non-missing sample size), mean, standard deviation, median, maximum and minimum. The frequency and percentages (based on the non-missing sample size) of observed levels will be reported for all categorical measures. Standard statistical tests (chi-square, Cochran-Mantel-Haenszel chi-square, Student's t) will be used to compare Treatment vs. Control. If distributions are severely non-normal, the analyses of continuous variables will be performed on the log-transformed values. If the log transformation does not produce normally distributed data, the Wilcoxon test will be used for comparisons. All analyses will be conducted using a two-tailed significance level of 0.05 unless otherwise stated. P-values less than or equal to 0.05 will be considered statistically significant. No adjustments for multiple comparisons are planned for exploratory outcomes.
Expected start date:	Estimated date first patient enrolled: September 2021
Expected end date:	Estimated date last patient completed: December 2024

7.1 SCHEDULE OF EVALUATIONS (SOE)

Table 1 Schedule of Evaluations

Visit	V1	Treatment start	V2	V3	FU1[a]	V4	V5	V6	V7	V8	V9	V10	V11	FU2[b]
Age at Visit	Varies		Varies	Varies		2 months (60 days ±7)	4 months (120 days ±14)	6 months (180 days ±14)	9 months (270 days ±14)	12 months (365 days ±21)	15 months (455 days ±21)	18 months (545 days ±21)	21 months (635 days ±21)	
Study Day	-14/1[c]	1	8-15[d]	22-29[d]		Varies[d]	Varies[d]	Varies[d]	Varies[d]	Varies[d]	Varies[d]	Varies[d]	Varies[d]	
Phase			TFTs normalization phase			Long-term follow-up phase								
Visit Type	Clinic		TM	Clinic	TM	TM	Clinic	Clinic	Clinic	Clinic	Clinic	Clinic	Clinic	TM
Informed Consent	X													
Demographics	X													
Medical History	X													
Concomitant Medications	X		X	X	X	X	X	X	X	X	X	X	X	X
Eligibility	X													
Adverse Events	X		X	X	X	X	X	X	X	X	X	X	X	X
Randomization	X[c]													
Physical Exam[e]	X			X			X	X	X	X	X	X	X	
Signs and symptoms of hypo- and hyper-thyroidism	X		X	X	X	X	X	X	X	X	X	X	X	X
Body Measurements[f]	X			X			X	X	X	X	X	X	X	
Nutritional Intake	X		X	X	X	X	X	X	X	X	X	X	X	X

[a] Subjects who at V3 have not yet achieved TFTs normalization will undergo one or more additional follow-up visits at appropriate time intervals (usually every 2 weeks) until TFTs are normalized (e.g. Follow-Up Type 1, FU1). If the timing of FU1 visit overlaps with the next planned visit (V4-V8), it is possible to perform only the planned visit.
[b] After V4, if a dose adjustment is needed or, at Investigator's judgement, if an abnormal T4 or TSH are found, additional follow-up visit(s) (2-6 week intervals) should be performed until normalization occurs (e.g. Follow-Up Type 2, FU2). If the timing of FU2 visit overlaps with the next planned visit (V5-V11), it is possible to perform only the planned visit.
[c] The procedures of screening and inclusion at V1 may be spread over multiple days (particularly when laboratory results need to be collected), but should be completed within 2 weeks with Randomization on Day 0 or 1. Day 1 is the first day of study drug administration (for subjects continuing on tablets, the first day of drug administration within the study).
[d] The procedures conducted at each visit may take place on multiple days (0-7 days) all within the allowed time frame.
[e] Including standard health maintenance exam for age and vital signs (Heart Rate and Temperature).
[f] Including length, body weight (BW) and head circumference.

Visit	V1	Treatment start	V2	V3	FU1[a]	V4	V5	V6	V7	V8	V9	V10	V11	FU2[b]
Age at Visit	Varies		Varies	Varies		2 months (60 days ±7)	4 months (120 days ±14)	6 months (180 days ±14)	9 months (270 days ±14)	12 months (365 days ±21)	15 months (455 days ±21)	18 months (545 days ±21)	21 months (635 days ±21)	
Blood Sampling for TFTs[g]	X[h]		X[m]	X	X	X	X	X	X	X	X	X	X	X
Dosing Evaluation	X		X	X	X	X	X	X	X	X	X	X	X	X
eDiary dispensing and instruction	X													
Treatment		←	—	—	—	—	—	—	—	—	—	—	—	→
Daily eDiary		←	—	—	—	—	—	—	—	—	—	—	—	→
CareCAT[i]			X	X		X	X	X	X	X				
Parents' Satisfaction Questionnaire[j]	X			X			X	X	X	X	X	X	X	
Subject Visit Reminder Card	X			X			X	X	X	X	X	X		
Drug dispensing and instruction[k]	X			X			X	X	X	X	X	X		
Drug return				X			X	X	X	X	X	X	X	
Treatment compliance			X	X	X	X	X	X	X	X	X	X	X	X
End of Study[l]										X	X	X	X	

[g] May be collected at the study visit or prior (0-7 days) to the study visit, within the visit allowed time frame.
[h] Subject's own lab results are acceptable. For newly diagnosed neonates, the lab results obtained from routine diagnostic workup will be recorded at V1; labs do not need to be repeated at Screening Visit for the purpose of the study. For infants who are already on TH therapy, the lab results obtained during routine follow-up will be recorded at V1, if not older than 4 weeks (<28 days); blood will be collected and analyzed if a lab report dated less than 28 days is not available, or if necessary as per Investigator's medical judgement.
[i] CareCAT will be completed after the daily drug administration in the eDiary during the first week of treatment (on days 1-7), during the third week of treatment (on days 15-21), and during the eighth week of treatment (on days 50-56).
[j] Parents' Satisfaction Questionnaire will be completed in the eDiary at V1 only for infants who are already on TH therapy and 0-3 days prior to all other in-clinic visits for all subjects.
[k] Further drug may be dispensed at FU visits, TM visits and/or unscheduled visits. Drug will not be dispensed at Visits 8, 9 or 10, if it is the final visit (End of Study).
[l] End of Study may be at any of these visits, depending on the age of the subject at inclusion.
[m] Optional, at the discretion of the Investigator, for infants already on LT4.

Table 2: Outline of Visit Schedule Based on Age

Visit	V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	V11
Age at Randomization	Varies	Varies	Varies	2 months (60 days ±7)	4 months (120 days ±14)	6 months (180 days ±14)	9 months (270 days ±14)	12 months (365 days ±21)	15 months (455 days ±21)	18 months (545 days ±21)	21 months (635 days ±21)
Study Day	-14/1	8-15	22-29	Varies	Varies	Varies	Varies	Varies	Varies	Varies	Varies
≤ 15 days	X	X	X	X	X	X	X	X			
> 15 and ≤ 40 days	X	X	X		X	X	X	X			
> 40 days and ≤ 78 days	X	X	X		X	X	X	X	X		
> 78 days and ≤ 138 days	X	X	X			X	X	X	X		
> 138 days and ≤ 228 days	X	X	X				X	X	X	X	
> 228 days and ≤ 277 days	X	X	X					X	X	X	X

7.2 ORGANIZATIONAL AND ADMINISTRATIVE STRUCTURE

OVERALL STUDY MANAGEMENT

[redacted]

STUDY MONITORING

[redacted]

DATA MANAGEMENT & STATISTICAL ANALYSIS

[redacted]

DRUG SAFETY

[redacted]

CLINICAL QUALITY ASSURANCE

[redacted]

SPONSOR'S REPRESENTATIVE

[redacted]

SPONSOR'S MEDICAL ADVISORS

[redacted]

8 INTRODUCTION

8.1 BACKGROUND

Primary hypothyroidism or thyroid hormone (TH) deficiency due to abnormality in the thyroid gland is the most common endocrine disease. The prevalence of hypothyroidism in the general population ranges from 3.8% to 4.6% (Chakera 2012)

Congenital hypothyroidism (CH) is one of the most common endocrine disorders in infancy, with an observed incidence in the United States of approximately 1:2000 to 1:4000 newborns. The clinical manifestations are often subtle or not present at birth. Common symptoms include decreased activity and increased sleep, feeding difficulty, constipation and prolonged jaundice. Common signs include myxedematous facies, large fontanels, macroglossia, a distended abdomen with umbilical hernia and hypotonia. CH is classified into permanent and transient forms, which in turn are divided into primary, secondary, or peripheral etiologies. The most prevalent form of permanent, primary CH is an abnormality in thyroid gland development (thyroid dysgenesis or agenesis) which accounts for 85% of cases, while inborn errors of TH biosynthesis (dyshormonogeneses) account for 10-15% of cases. Less commonly, the altered neonatal thyroid function is transient, attributable to the transplacental passage of maternal medication, maternal blocking antibodies or iodine deficiency or excess. Transient CH most commonly occurs in preterm infants born in areas of endemic iodine deficiency. In rare cases, CH may result from a pituitary or hypothalamic abnormality (central or secondary/tertiary hypothyroidism)(AAP 2006).

Unrecognized/untreated CH can result in cognitive impairment, mental retardation and growth complications (decreased height/length). Newborn screening and thyroid therapy started within two weeks of age can normalize cognitive development. In the United States (US), newborn screening for primary CH is part of the Recommended Uniform Screening Panel and is typically performed 24–72 hours after birth (Jones 2018, Rose 2023).

Two screening strategies for the detection of CH are generally used: (1) a primary TSH/backup T4 method and (2) a primary T4/backup TSH method. In addition, an increasing number of programs use a third, combined primary TSH plus T4 approach. Since methods for the simultaneous measurement of T4 and TSH are available, this represents the ideal screening approach (AAP 2006).

In addition, to avoid missing cases among neonates at risk of delayed increase in TSH, some states and countries adopt a two-screen approach, with repeat sampling at two weeks of life or two weeks after the first screening test was carried out, particularly in the following situations: preterm birth (< 37 weeks gestation), LBW and VLBW neonates, ill and preterm newborns admitted to neonatal intensive care units (NICUs), specimen collection within the first 24 hours of life, and multiple births (AAP 2006, Leger 2014, Rose 2023).

In the US, screening for CH is usually based on the measurement of whole blood TSH and T4. Heel-prick blood samples are collected on specialized filter paper (dried blood spot) from all

newborn babies in the first days of life (ideally at 2-4 days of age or at time of discharge) and immediately sent for testing. Positive screening results trigger immediate measurement of serum TSH and T4 concentrations (ATA 2017).

Some laboratories report screening results per unit of blood, a value that is approximately half the concentration in serum. The AAP recommends that all laboratories report results per unit of serum, because TSH and T4 are preferentially distributed into the serum.

There is wide variability in newborn screening TSH cutoffs, which depend on the baby's age at sample collection and distance from the postnatal surge in TSH. After delivery, serum TSH concentrations in term infants increase to a mean of approximately 80 mIU/L at 30 minutes of life and then decrease rapidly over the next two days. The serum TSH concentration is usually less than 10.0 mIU/L by the end of the first week, and is usually within the normal range for children and adults by the second week (Caiulo 2021). The assay used and whether TSH levels are expressed directly as blood levels or converted to serum (typically using a conversion factor of 2.2) are important factors to keep in mind when considering cutoffs.

Since the introduction of newborn screening in the mid-1970s, TSH cutoff values have been progressively lowered in many screening programs, with some programs now recalling babies with screening TSH levels as low as 6 to 10 mIU/L. Programs that have recently reduced cutoffs report an increase in the incidence of CH. The benefit of reducing screening thresholds for TSH is yet uncertain. Detractors say lower TSH cutoffs lead to more false positive results, can lead to overdiagnosis and medicalization of children with clinically inconsequential biochemical abnormalities, and increases the burden on families, while it is not clear whether newborns with mild TSH elevation are at risk of neurocognitive impairment without treatment (West 2020).

A recent study evidenced that there is variation in practice amongst newborn screening programs across the USA in the approach to screening for CH (Kilberg 2018).

According to the American Academy of Pediatrics (Rose 2023) current guidelines on newborn screening, in infant with TSH concentration greater than 40 mIU/L (serum equivalents) treatment with replacement levothyroxine should be initiated as soon as confirmatory serum has been drawn and before the results of the confirmatory tests are available. For cases in which the screening TSH concentration is ≤40 mIU/L, the results of the confirmatory serum sample should be awaited (preferably with a 24-hour turnaround time) before starting levothyroxine treatment andsubsequent actions are based on the results of TSH and FT4 obtained from the confirmatory serum sample.

Further (optional) diagnostic workup may include thyroid ultrasonography or ^{123}I or ^{99m}Tc thyroid uptake and/or scan to identify functional thyroid tissue (AAP 2006, Rose 2023).

All infants with CH should be rendered euthyroid as promptly as possible by replacement therapy with TH. An optimal cognitive outcome depends on both the adequacy and timing of postnatal therapy. Levothyroxine sodium (LT4) alone is the treatment of choice. LT4, although synthetic, is identical to the thyroxine (T4) produced by the body and mimics its effects. An initial dosage of 10 to 15 mcg/kg/day of LT4 is recommended, depending on the severity of the initial

hypothyroidism. Upon treatment start, the goal of therapy is to rapidly normalize serum FT4 and TSH levels (optimally within 2 to 4 weeks of treatment initiation), while after initial normalization therapeutic targets are to maintain TSH within the age-specific reference range and FT4 (or total T4) in the upper half of the age-specific reference range unless achieving a serum FT4 level in this range would result in a TSH level less than the reference range. Frequent laboratory monitoring in infancy is essential to ensure optimal neurocognitive outcomes. The LT4 dose should be adjusted according to the infant's clinical response and serum T4 and TSH concentrations (Rose 2023).

In general, the prognosis of infants detected by screening and started on treatment early is excellent, with intellectual quotient (IQ) similar to sibling or classmate controls. Studies show that a lower neurocognitive outcome may occur in those infants started at a later age (> 30 days of age), on lower LT4 doses than currently recommended, and in those infants with more severe hypothyroidism (Rastogi 2014).

LT4 in tablet form is considered the SOC for treatment of hypothyroidism by the AAP Guidelines. For neonates and infants, the tablet should be crushed and suspended in a few milliliters of formula, breast milk, or water. LT4 suspensions that may be prepared by individual pharmacists may lead to unreliable dosage (Meyer 2020, AAP 2006, Rose 2023).

Recently a liquid LT4 formulation was approved by the US FDA, Tirosint®-SOL (levothyroxine sodium) oral solution. This formulation can be administered without any manipulation and offers a therapeutic alternative to crushing tablets. Tirosint®-SOL is approved for the following indications:

- Hypothyroidism – As replacement therapy in primary (thyroidal), secondary (pituitary), and tertiary (hypothalamic) congenital or acquired hypothyroidism, and
- Pituitary Thyrotropin (TSH) Suppression – As an adjunct to surgery and radioiodine therapy in the management of thyrotropin-dependent well-differentiated thyroid cancer.

The product is provided in unit-dose ampules, it is appropriately labeled for use in all relevant pediatric populations and is commercially available at the following strengths: 13, 25, 37.5, 44, 50, 62.5, 75, 88, 100, 112, 125, 137, 150, 175, 200 mcg.

Tirosint®-SOL is bioequivalent to Tirosint (levothyroxine sodium) capsules when administered in healthy adult volunteers. In addition, administration of Tirosint®-SOL upon dilution in water is bioequivalent to administration of Tirosint®-SOL directly into the mouth in healthy adult volunteers (Tanguay 2019).

Although use of LT4 oral solutions in the US is limited, use in other countries indicates that they are safe and effective in the treatment of CH and may present some advantages in terms of ease of administration. The relevant clinical experience is detailed below.

In a retrospective study of 78 newborns with CH taking liquid LT4, it was demonstrated that normalization of serum TH concentration was obtained within 7-10 days of administration in 87% of group A (liquid) subjects and 82% of group B (tablet) subjects, at doses ranging between 10

and 15 µg/kg/day. Group A subjects had significantly lower TSH values compared with those of group B at 7-10 days (p = 0.05) and 6-8 months (p = 0.043) of treatment, despite similar L-T4 dose and FT4 concentration. Mean Developmental Quotient scores were within normal range in all subjects. When comparing the effects of liquid and tablet formulation in the treatment of infants with CH, the study confirmed the efficacy and safety of both formulations using dosages recommended by CH guidelines (Peroni 2013).

In a prospective study in 42 newborns with CH, there were significantly more subjects with suppressed TSH concentrations in group 1 (liquid) than in group 2 (tablet). In the moderate/mild form of CH, the subjects of group 1 and group 2 showed median values of TSH, FT3, and FT4 that were not significantly different. No clinical or electrocardiographic signs of heart disease were found. There were no significant differences in the developmental quotient between the groups. The authors concluded that data indicated non-complete bioequivalence between the liquid and tablet formulations, especially in infants with severe CH (Cassio 2013).

Von Heppe reported that in 28 newborns with primary CH, the initial dose necessary to normalize TSH is not lower when a liquid solution is used. The higher dose used in tablets is not due to inefficient absorption, but rather reflects the increased demand for TH in the first weeks of life. The liquid formula was easier to handle for the parents, and enabled more individualized dosage of newborns, especially those with a LBW. In comparison with parents whose children received LT4 in tablet form, the parents of newborns who received the liquid formula were more content with the treatment (von Heppe 2004).

Further knowledge on the use of LT4 oral solutions in the pediatric population has been gained recently.

A multicenter study compared the effectiveness of an LT4 oral solution (group D, 117 subjects) with that of a tablet formulation (group T, 137 subjects) in children with CH up to 3 years of age. Auxological parameters, LT4 dose and thyroid function values (TSH and FT4) at diagnosis, 3, 6, 12, 24, 36 months were not significantly different. TSH at 15 days (p = 0.002) and 1 month (p = 0.009) was significantly reduced in group D. At 2-year follow-up, median TSH was significantly lower in group T (p = 0.03). Both therapeutic strategies were considered effective in the treatment of CH, with a higher risk of overtreatment in the first months of therapy associated with oral solution. No negative effects on cognitive development were observed (Vigone 2021).

A prospective randomized control study compared a liquid LT4 formulation available in Greece (Group B, 19 subjects) with tablets (Group A, 17 subjects) in the substitutive treatment of 3-12 years old children with CH, who were evaluated at 0, 2, 4 and 6 months. TSH values showed a statistically significant difference (p=0.017) between groups only at 6 months (Group A having higher TSH levels than Group B), while FT4 levels had no statistical difference throughout the 6-month study period and were always within the normal range. Dose adjustments were more frequent in Group A (p=0.038). Liquid LT4 substitutive treatment exhibited no statistically significant adverse effects in comparison to tablets (Tzifi 2021).

A recent study comparing two types of liquid LT4 formulations available in Italy (TF and TS) in 21 newborns newly diagnosed with primary CH and followed-up for 1 month highlighted differences in serum TSH levels 15 days after treatment start (TSH in the TF Group was 0.08 ± 0.02 mcUI/mL, while in the TS Group it was 36.7 ± 14.7 mcUI/mL p=0.04), with no differences at 30 days, as well as at FT4 and at LT4 requirement. The study confirmed the efficacy of the two liquid formulations in normalizing the thyroid hormonal profile in newborns with CH, although the response seemed to be different in timing, therefore the authors recommended an individual approach considering the type of formulation used, the diagnostic category of CH and the clinical features (Tuli 2022).

The differences in absorption between oral solutions and tablet formulations are not unexpected considering that differences in the bioavailability have been reported even among generic tablet formulations in subjects with CH (Carswell 2013, Lomenick 2013). Moreover, the oral solution is ready to use and therefore the full dose is administered, while the tablet requires crushing and dispersing, which may lead to some loss of the active ingredient.

Successful use of Tirosint®-SOL has been reported in a case of delayed diagnosis of CH in a child with trisomy 21 and biotinidase deficiency. At 8 weeks of life, CH was diagnosed and the child was started on the LT4 tablet. The subject's parents reported difficulty in administering the dose via a crushed tablet in a bottle of formula. At 16 weeks of life, the parents still reported difficulty with cutting and crushing the LT4 tablet, resulting in inconsistent dosing. The patient was switched to Tirosint®-SOL and the parents reported that administration was much easier. At 24 weeks of life, both TSH and FT4 were normal and the family reported being very happy with the LT4 oral solution (Feldt 2020).

The aim of this study is to gather information on the use of Tirosint®-SOL oral solution as TH replacement therapy in neonates and infants with CH compared to the conventional treatment with crushed LT4 tablets in a real-life setting. Specifically of interest is comparing the ease of administration for parents and the acceptability by the subject, the TFTs profile, and the LT4 dose required to maintain TSH in the target range.

9 RISK-BENEFIT ASSESSMENT

9.1 KNOWN POTENTIAL RISKS

It is known that LT4 is well tolerated for the treatment of CH. Potential risks related to its use in the pediatric population are as follows:

- Infants with CH appear to be at increased risk of other congenital anomalies. Cardiovascular anomalies, including pulmonary stenosis, atrial septal defect, and ventricular septal defect are the most common (AAP 2006).

- Subjects should be closely monitored during the first two weeks of LT4 therapy for cardiac overload, arrhythmias, and aspiration from suckling (Synthroid USPI, Tirosint®-SOL USPI).
- Undertreatment may have deleterious effects on intellectual development and linear growth (Synthroid USPI, Tirosint®-SOL USPI).
- Overtreatment has been associated with craniosynostosis in infants, and may adversely affect the tempo of brain maturation and accelerate bone age with resultant premature closure of the epiphyses and compromised adult stature (Synthroid USPI, Tirosint®-SOL USPI).
- Potential risks due to switching from tablets to liquid solution in infants, are considered minimal in the conduct of this study due to titration method implemented while switching and will be futher mitigated by the frequency of study visits. Switching from tablets to liquid solution does not apply to neonates.

9.2 KNOWN POTENTIAL BENEFITS

Tirosint®-SOL addresses the issues of crushing and dispersing the tablets and is also aligned with guidelines for the treatment of hypothyroidism from the American Thyroid Association (ATA) that discourage the use of compounded solutions (Meyer 2020, Jonklaas 2014).

With respect to solutions in multi-dose bottles, it is expected that Tirosint®-SOL will provide an improved consistency of dosing since each unit-dose ampule contains the exact dose to be administered. This is particularly important in the studied population and in the administration of drugs like LT4 that are narrow therapeutic index drugs.

Tirosint®-SOL's most significant clinical benefit is its ease of admisinistration as a pleasant-tasting liquid. Tirosint®-SOL can be taken either directly in the mouth or upon dilution in water. Having a more user-friendly medication may help improve compliance and therefore reduce sequelae of inadequately treated hypothyroidism.

The chemical composition of Tirosint®-SOL contains only three ingredients: levothyroxine sodium, glycerol, and water [free of sugars, dyes, alcohol, wheat starch (gluten), lactose, or any other excipients (inactive ingredients) used to make traditional LT4 tablets]. This property of Tirosint®-SOL makes it particularly suitable in subjects with food or ingredient sensitivities.

9.3 ASSESSMENT OF POTENTIAL RISKS AND BENEFITS

The treatments used in this study are FDA-approved products. The products are used according to the labeling and the SOC. Therefore, the potential risks and benefits for the subjects participating in this study are the same or similar to the risks and benefits for subjects being treated with these commercially available FDA-approved drugs.

Treatment-emergent AEs, clinical laboratory results, vital signs and electrocardiogram findings collected during the clinical development of Tirosint®-SOL did not identify any new safety concerns with respect to the known safety profile of LT4.

10 TRIAL OBJECTIVES

10.1 PRIMARY OBJECTIVES

The main objectives of this study are to compare the use of Tirosint®-SOL and conventional treatment with crushed LT4 tablets in terms of:

- LT4 dose required to maintain TSH in target range;
- hormonal (TFTs) profile;
- parent/caregiver reports of satisfaction and ease of administration; and
- subject's acceptance of the treatment.

10.2 SECONDARY OBJECTIVES

The secondary objective is to compare the study drug and the control treatment by assessing frequency of dose adjustments, and growth patterns (length, BW, head circumference).

10.3 EXPLORATORY OBJECTIVES

An exploratory objective will be to compare the study drug and the control treatment in neonates only for the time required to normalize TSH and FT4 into their target ranges.

11 TRIAL DESIGN

11.1 OVERALL DESIGN

This is a phase IV, multi-center, prospective, parallel-group, open-label, randomized clinical study in one hundred and twenty-six (126) subjects [neonates and infants (0-9 months)] diagnosed with CH. Subjects will be randomized in a 2:1 ratio to Treatment (Tirosint®-SOL) or Control (conventional therapy with crushed LT4 tablets).

Newly diagnosed neonates will be randomly assigned to start therapy with LT4 at the initial dose recommended by the SOC. Infants already on LT4 therapy will continue at the same daily dose within the randomly assigned treatment group (dose adjustments are allowed, if needed based on laboratory parameters and clinical response).

Once enrolled, subjects will be treated and followed for 12 months (±1.5 months), participating in 7-8 study visits, consisting of 6-7 in-clinic and 1-2 TM visits (or more if follow-up visits are required). The total number of visits depends on the age at inclusion as reported in **Table *2***. In-clinic visits are performed 3-4 weeks after randomization, when the subject is 4, 6, and 9 months of age, then every 3 months as required depending on the age at the inclusion. TM visits will be performed 1-2 weeks after randomization, when the subject is 2 months of age, if applicable, and any time an additional follow-up is required for TSH and T4 monitoring.

11.2 SCIENTIFIC RATIONALE FOR STUDY DESIGN

The current clinical experience in the pediatric population with liquid LT4 is limited. This study is designed to understand the clinical experience with Tirosint®-SOL in the US pediatric population with CH in a real-life setting. Further elucidation of the potential benefits to this population will allow for clinicians and parents to make educated decisions regarding the best treatment for their subjects and children.

The study will be conducted in subjects up to 9 months (277 days) of age with CH, as this is the population that may benefit more from the availability of an oral solution, due to the challenge in administering solid dosage forms.

Subjects with diagnosis of primary CH based on elevated TSH and low or normal FT4 will be included. No cut-off values are set in the protocol to define elevated TSH and low or normal FT4, because this phase IV study is not designed to explore the diagnosis of CH, but to evaluate the ease of administration of the solution versus the tablet. The diagnosis of primary CH is done by the clinician based on established guidelines (Rose 2023). The terms “elevated”, “low” and “normal" are not strictly and simply referred to the local laboratory normal ranges, which are highly variable from one laboratory to another in this patient population and do not always take into appropriate consideration the age of the subject. Further to local lab normal ranges, other age-specific reference ranges, provided by medical guidances, relevant literature and manuals may be taken in consideration by the Investigators in their evaluation of hormonal results (e.g. Rose 2023, Elmlinger 2001)

The following subjects will be excluded secondary to confounding variables:

A. Subjects with hypothyroxinemia, characterized by low T4 and normal TSH values, will not be included in the study for several reasons: (1) there is controversy regarding the need for TH therapy in these settings and there is no clear consensus regarding optimal follow-up, (2) monitoring of TFTs may extend beyond 1 month of age to evaluate persistence of the abnormality, and (3) it would not be possible to assess the time to reaching target TSH and T4 values, that is one of the outcomes of the study (AAP 2006).
B. Premature and LBW and VLBW newborns have multiple clinical and developmental variables that increase the difficulty to diagnose and treat CH, including: (1) non-thyroidal illness (sick-euthyroid syndrome), (2) medications that decrease TSH secretion (steroids

and others), and (3) physiologic, delayed TSH elevation. For these reasons these subjects will be excluded from the study (AAP 2006). In addition, infants who were born VLBW will be excluded from the study since they are considered at significant risk of death during the first year of life. For the same reason, subjects who are in fragile health conditions will also be excluded.

C. Children with chromosomopathies will also be excluded as these conditions may be associated with altered tone in the hypothalamic-pituitary-thyroid axis (i.e. Trisomy 21), feeding issues secondary to an increased risk of congenital cardiac and gastrointestinal disorders, and impaired physical and intellectual development independent of thyroid insufficiency.

The study will be conducted in parallel groups, since the long follow-up does not allow a cross-over and in order to avoid further switches in the formulation. The two groups will be unbalanced as the response to treatment with LT4 tablets is very well known and adequately described in the literature. Unbalanced randomization will allow for the maximum amount of data regarding the treatment with Tirosint®-SOL to be gathered.

Subjects will be followed for 12 months in order to collect sufficient information on the TFTs profile at different ages, on growth, and to assess changes in the parents' satisfaction during the growth of the subject.

Because routine screening programs for CH vary among states and there is no common consensus on TH theresholds to be used for the diagnosis of CH (Kilberg 2018), each site will follow the SOC for routine screening in newborns for establishing the diagnosis of CH. All the routine screening will be conducted prior to the study Screening Visit and is outside the scope of this protocol.

The assessments to evaluate therapy (serum TSH and FT4, clinical examination, and growth), have been selected based on recommendations by the AAP and USPI (AAP 2006, Rose 2023, Tirosint®-SOL USPI, Synthroid USPI) and to specifically address the study objectives. Because the most recent guidelines on medical management of congenital hypothyroidism recommend TSH and FT4 as the main parameters for laboratory monitoring of the LT4 treatment, the analysis of TT4 will be left optional to the Investigator's judgement. **The assessments included in this study are not intended to be an exhaustive list of the assessments that should be performed during routine therapeutic monitoring of these subjects. Investigators should still provide routine monitoring based on the SOC and that is outside the scope of this protocol.**

The timing for the visits has been selected based on the current AAP recommendations (AAP 2006, Rose 2023) and the USPI (Tirosint®-SOL USPI, Synthroid USPI).

An overview of the recommendations provided by the most relevant current guidelines in terms of timing of follow-up is provided below.

According to the AAP Guidelines (Rose 2023), clinical examination, including assessment of growth and development and laboratory TFTs, should be performed every few months during the first 3 years of life, to ensure optimal LT4 dosage and adherence to therapy.

Serum FT4 and TSH measurements should be performed:

- At 1 to 2 weeks after the initiation of LT4 treatment and every 2 weeks until serum TSH level is normal;
- Every 1 to 2 months during the first 6 months of life (monthly in infants with severe CH);
- Every 2 to 3 months during the second 6 months of life;
- Every 3 to 4 months between 1 and 3 years of age;
- At more frequent intervals in children with severe CH, when compliance is questioned, abnormal values are obtained, or dose or source of medication has been changed;
- FT4 and TSH measurements should be repeated 4 to 6 weeks after any change in LT4 dosage or formulation (Rose 2023).

According to the ATA, once the proper dose is identified, surveillance testing with a serum thyrotropin and T4 should be performed every 1 to 2 months during the first year of life with decreasing frequency as the child ages (Jonklaas 2014)

The most recent ENDO-European Reference Network guidelines outline:

- The first clinical and biochemical follow-up evaluation should take place 1 to 2 weeks after the start of LT4 treatment (1 week at the latest in case of a starting dose of 50 mcg per day or an even higher dose);
- Subsequent (clinical and biochemical) evaluation should take place every 2 weeks until complete normalization of serum TSH is achieved; therafter, the evaluation frequency can be lowered to once every 1 to 3 months until the age of 12 months;
- Between the ages of 12 months and 3 years, the evaluation frequency can be lowered to every 2 to 4 months; thereafter, evaluations should be carried out every 3 to 6 months until growth is completed;
- If abnormal FT4 or TSH values are found, or if compliance is questioned, the evaluation frequency should be increased; and
- After a change of LT4 dose or formulation, an extra evaluation should be carried out after 4 to 6 weeks (Van Trotsenberg 2021).

This is also in line with the USPI of Tirosint®-SOL and LT4 tablets (Synthroid USPI, Tirosint®-SOL USPI) stating that in subjects with CH, the adequacy of replacement therapy should be assessed by measuring both serum TSH and TT4 or FT4 as follows: 2 and 4 weeks after the initiation of treatment, 2 weeks after any change in dosage, and then every 3 to 12 months thereafter following dose stabilization until growth is completed. Poor compliance or abnormal values may necessitate more frequent monitoring.

The schedule of visits in the present study is in line with the recommendations. Importantly, **the Principle Investigator (PI) is left free to perform additional unscheduled visits if needed in the best interest of the subject**.

11.3 JUSTIFICATION OF DOSE

The recommended daily dose of LT4 solution and/or tablets in pediatric subjects with hypothyroidism is based on an individual subject's body weight and age (Tirosint®-SOL USPI, Synthroid USPI).

Table 3: Tirosint®-SOL Dosing Guidelines for Pediatric Hypothyroidism (Infants and Neonates)

Age	Daily Dose Per Kg Body Weight*
0-3 months	10-15 mcg/kg/day
3-6 months	8-10 mcg/kg/day
6-12 months	6-8 mcg/kg/day
1-5 years	5-6 mcg/kg/day

***The dose should be adjusted based on clinical response and laboratory parameters.**

While the general aim of therapy is to normalize the serum TSH level, TSH may not normalize in some subjects due to in utero hypothyroidism causing a resetting of pituitary-thyroid feedback. Failure of the serum T4 to increase into the upper half of the normal range within 2 weeks of initiation of therapy and/or of the serum TSH to decrease below 20 mIU/L within 4 weeks may indicate the child is not receiving adequate therapy. Assess compliance, dose of medication administered, and method of administration prior to increasing the dose (Tirosint®-SOL USPI, Synthroid USPI).

An overview of the recommendations provided by the most relevant current guidelines in terms of dose selection and adjustment is provided below.

According to the AAP, the goal of therapy is to normalize FT4 and TSH levels, optimally within 2 to 4 weeks of treatment initiation. An initial dosage of 10 to 15 mcg/kg of LT4 is recommended. After initial normalization, serum TSH should be maintained in the age-specific reference range; serum FT4 levels should be maintained in the upper half of the age-specific reference range unless achieving a serum FT4 level in this range would result in a TSH level less than the reference range (Rose 2023).

According to the American Thyroid Association , LT4 replacement at a dose of 10–15 mcg/kg/day should be initiated once newborn screening is positive, pending the results of confirmatory testing. Higher doses may be required for infants with severe CH. The aim of therapy is to maintain the serum T4 in the mid- to upper half of the pediatric reference range and the serum TSH in the mid-

to lower half of the pediatric reference range. The target should be to normalize serum T4 approximately 2–4 weeks after initiation of therapy (Jonklaas 2014).

The most recent ENDO-European Reference Network provides further details:

- LT4 treatment should be started as soon as possible, not later than two weeks after birth or immediately after confirmatory (serum) thyroid function testing in neonates in whom CH is detected by a second routine screening test.
- The LT4 starting dose should be up to 15 mcg/kg per day, taking into account the whole spectrum of CH, ranging from mild to severe.
- Infants with severe CH, defined by a very low pretreatment serum FT4 (<5 pmol/L) or TT4 concentration in combination with elevated TSH (above the normal range based on time since birth and gestational age), should be treated with the highest starting dose (10–15 mcg/kg per day).
- Infants with mild CH (FT4 > 10 pmol/L in combination with elevated TSH) should be treated with the lowest initial dose (~10 mcg/kg per day).
- The first treatment goal in neonates with primary CH is to rapidly increase the circulating amount of TH, reflected by normalization of serum TSH; therafter, TSH should be kept within the reference interval.
- If TSH is in the age-specific reference interval, FT4 concentrations above the upper limit of the reference interval can be accepted and recommends maintaining the same LT4 dose.
- Any reduction of the LT4 dose should not be based on a single higher than normal FT4 concentration, unless TSH is suppressed (i.e., below the lower limit of the reference interval) or there are signs of overtreatment (e.g., jitteriness or tachycardia) (Van Trotsenberg 2021).

11.3.1 REFERENCE RANGES AND TARGET RANGES FOR DOSE ADJUSTMENT

It is not possible to define a globally accepted reference range for TSH, TT4 or FT4. TH levels change markedly during childhood and therefore the reference range should be adapted based on age (Kapelari 2008). In the present study, age-specific reference ranges provided by the sites' laboratories will be adopted.

For TSH the AAP Guidelines proposed a target range of 0.5 and 2.0 mU/L during the first 3 years of life (AAP 2006), but the recent revision proposes to target TSH within the age-specific reference range (Rose 2023). LaFranchi indicates target ranges for TSH on treatment should be <5 mU/L, optimally 0.5–2.0 mU/L (LaFranchi 2011). Most commonly therapy is aimed at maintaining the serum TSH in the reference range (Van Trotsenberg 2021) or in the mid- to lower half of the pediatric reference range (Jonklaas 2014). No target range will be defined for TSH in the frame of the present study, but replacement therapy should target to maintain TSH levels within the age-

specific reference range (Rose 2023) (it is suggested within the mid- to lower half of the reference range whenever appropriate).

For FT4 and TT4, AAP Guidelines reported target values for FT4 of 1.4 – 2.3 ng/dL (or 18-30 pmol/L), and for TT4 of 10 – 16 µg/dL (or 130 – 206 nmol/L) during the first 3 years of life (AAP 2006), but the recent revision does not provide any target range (Rose 2023). LaFranchi also provides an example of FT4 reference range of 0.8 – 2.3 ng/dL, with a target range of 1.4 – 2.3 ng/dL (LaFranchi 2011). Therapy is most commonly aimed at maintaining the serum T4 in the mid- to upper half of the pediatric reference range (Jonklaas 2014). Reference ranges may vary greatly from one laboratory to another depending on the analytical method used (Spencer 2017). Therefore, no target ranges will be defined for T4 in the frame of the present study, but replacement therapy should target to maintain FT4 levels in the upper half of the age-specific reference range (Rose 2023).

11.4 END OF STUDY DEFINITION

A subject is considered to have completed the study if they have completed all phases including the End Of Study (EOS) Visit or the last scheduled procedure shown in the Schedule of Evaluations (**Table *1***). The EOS is defined as the completion of the last subject's Visit or last scheduled procedure.

12 SELECTION OF POPULATION

A total of 126 subjects will be enrolled in this study.

The PI is to give his/her approval to the participation of each subject in the study based on the inclusion/exclusion criteria below. Laboratory assessments for CH diagnostic purposes (routine screening) are not part of this protocol and Institution's should follow their own diagnostic procedures and complete them before the study Screening Visit.

Subjects will be included in the study consecutively, by site, from the inclusion of the first eligible subject according to the screening criteria. The Subject Number will be assigned automatically through the electronic data capture (EDC) system during the screening visit (V1) and be consecutive to the previously assigned code. In case the subject is not included in the study, it will be considered a screening failure and the Subject Number will not be used again.

Example of a Subject Number: XX–XXX (2 digit Site Code – Subject Code starting with 001, 002, 003 etc.)

12.1 INCLUSION CRITERIA

Subjects must satisfy all of the following criteria to be enrolled in the study:

1. Male and female subjects aged 0 to 9 months (277 days) at Inclusion;
2. Primary CH diagnosis with elevated TSH and low or normal FT4, requiring treatment with LT4, under either of the following conditions:
    - Neonates (aged 0 to 28 days) newly diagnosed with primary CH and needing to initiate LT4 therapy, or
    - Infants (aged 29 days to 277 days) previously diagnosed with primary CH and who are already on LT4 therapy for at least 3 weeks;
3. Parent or legal guardian of the subject provides voluntary written and informed consent prior to initiation of any study procedures; and
4. Parent or legal guardian of the subject is willing and able to comply with scheduled visits and all study activities.

12.2 EXCLUSION CRITERIA

Subjects who meet any of the following criteria must be excluded *(the wording "neonates" and "infants" refer to the populations defined in inclusion criterion #2)*:

1. Preterm neonates with a gestational age < 37 weeks;
2. Low birth weight (LBW) or very low birth weight (VLBW) neonates (weight < 2.5 kg) or VLBW infants (weight < 1.5 kg);
3. Neonates in neonatal intensive care units (NICU) or requiring admission to NICU or neonates/infants hospitalized or requiring hospitalization or in fragile health conditions (e.g. with serious health problems or complications);
4. Neonates with CH diagnosis > 4 weeks after delivery;
5. Diagnosis of primary gastrointestinal (GI) disease:
    a. Gastroesophageal reflux requiring medical therapy (beyond thickening of formula or position);
    b. Anatomic defects (e.g. intestinal atresia, malrotation, tracheoesophageal fistula, pyloric stenosis, Hirschsprung's disease, gastroschisis);
    c. Dietary allergy (e.g. cow's milk protein allergy);
    d. Malabsorption related to cystic fibrosis, celiac disease and others;
    e. Necrotizing enterocolitis requiring surgical resection;
6. Known or suspected adrenal insufficiency (e.g. congenital adrenal hyperplasia, hypopituitarism);
7. Diagnosis of congenital cardiac disease, cardiac insufficiency or risk for cardiac failure;
8. Diagnosis of chromosomopathy;
9. Diagnosis of central hypothyroidism;
10. Hypersensitivity to glycerol;
11. Concomitant anticonvulsant medications, liothyronine, combination of LT4 and liothyronine, thyroid extracts and/or chronic or long-term use of systemic glucocorticoids (see **Section 13.2.5** for details);

12. History of nonadherence with medication or medical visit schedule; or
13. Any condition for which, participation would not be in the best interest of the subject or that could limit protocol specified assessments, according to the Investigator.

12.3 LIFESTYLE CONSIDERATIONS

Not applicable

12.4 SCREEN FAILURES

Screen failures are defined as subjects for whom written informed consent has been obtained from parent or legal guardian but who are not subsequently randomized. A minimal set of screen failure information is required to ensure transparent reporting and respond to potential queries from regulatory authorities; this information includes: informed consent signature, demographics, eligibility criteria, screen failure details including reason for exclusion and study termination, adverse events (AEs) and serious adverse events (SAEs).

Subjects who fail the screening may be re-screened, if the subject's eligibility has changed. Re-screening may only happen once, upon re-consenting and upon assignment of a new Subject Number.

12.5 STRATEGIES FOR RECRUITMENT AND RETENTION

It is anticipated that individuals with CH will present to participating study sites. External recruitment efforts may be needed to ensure awareness is given to all populations including but not limited to local, diverse, and all income levels. If efforts are needed, they may include dissemination of information about this study to other medical professionals/hospitals and to the public (such as within surrounding communities to each opened investigational site).

13 TREATMENTS

13.1 STUDY DRUG (IP)

13.1.1 DESCRIPTION OF STUDY DRUG

Study Drug: Tirosint®-SOL (levothyroxine sodium) oral solution (IBSA Pharma Inc.). Tirosint-SOL is a ready-to-use solution containing LT4. It is a clear, colorless to slightly yellow solution supplied in a 1 mL white, nontransparent, unit-dose ampule and is available in the following strengths (mcg/mL): 13, 25, 37.5, 44, 50, 62.5, 75, 88, 100, 112, 125, 137, 150, 175, 200. The inactive ingredients contained in the product are glycerol and water.

Tirosint®-SOL oral solution will be provided by the Sponsor at the following strengths: 13, 25, 37.5, 44, 50, 62.5, 75, 88, 100 mcg. This strengths range is considered sufficient based on the potential weight of the subjects enrolled in the study. However, additional strengths among those authorized for Tirosint®-SOL may be provided upon request in particular cases (e.g. obese subjects).

Control: Crushed levothyroxine sodium tablets. LT4 tablets will vary based on the Subject and SOC. Newly diagnosed neonates will start treatment with one of the FDA-approved LT4 tablets available on the market, as determined by SOC. Infants already on therapy will continue taking the same LT4 tablets that they are on as a prescription before inclusion in the study. Both branded and generic products will be allowed in the study, the choice being left to the PI.

Any change in source of the LT4, especially if not a standard brand, requires repeat TFTs (serum T4 and TSH) two to six weeks after the change to assess for the potential retitration of the dose (AAP 2006, Van Trotsenberg 2021, Rose 2023). This situation should be avoided when possible.

LT4 tablets are commercially available at the following strengths: 25, 50, 75, 88, 100, 112, 125, 137, 150, 175, 200, and 300 mcg. Inactive ingredients vary between formulations.

LT4 tablets will **NOT** be supplied by the Sponsor. Tablets will either be provided by the site hospital pharmacy or purchased by the parent/guardian of the subject at a local pharmacy and reimbursed. Any strength, among the commercially available ones, can be used in the study, though it is expected that the following strengths will most commonly be utilized: 25, 50, 75, 88, 100 mcg. If needed, tablets can be cut in halves to obtain the missing intermediate strengths, for example:

- 37.5 mcg daily dose will be obtained as half 75 mcg tablet;
- 44 mcg daily dose will be obtained as half 88 mcg tablet; or
- 62.5 mcg daily dose will be obtained as half 125 mcg tablet.

13.1.2 DOSAGE AND ADMINISTRATION

Both products will be used and dosed according to the USPI and SOC.

The dose administered will be based on the subject's BW and age, and adjusted based on clinical response and laboratory parameters according to SOC and PI's judgement.

These procedures are informed by the main treatment guidelines, as well as in the USPI of Tirosint®-SOL and LT4 tablets (Synthroid USPI, Tirosint®-SOL USPI), as described in **Section 11.3**.

Further to these general principles, Investigators are required to follow the SOC in order to define the correct dose and treatment goal for each subject.

Initial Dosing in newly diagnosed neonates:

- Replacement therapy with LT4 should be started as promptly as possible upon diagnosis of CH.
- **Inclusion in the study must not delay the start of LT4 treatment.**
- Initial neonate LT4 dose should be between 10-15 mcg/kg;
- The initial dose should take into account the severity of the disease; and
- The goal of therapy should be to normalize FT4 into the upper half of the reference range and TSH within the reference range (it is suggested, within the mid- to lower half of the reference range whenever appropriate).

Initial Dosing in infants already under LT4 therapy:

- Subjects will be switched directly from crushed LT4 tablets to Tirosint®-Sol or continue on crushed LT4 tablets, with **no treatment interruption**.
- Same dose used before screening should be maintained, if replacement is adequate at screening/inclusion; or
- Dose should be adjusted at inclusion, if needed based on laboratory parameters and clinical response.

Dosing throughout study in neonates and infants:

- Dose adjustments can be done whenever required throughout the study and will be defined based on the evaluation of T4 and TSH values and clinical response according to SOC; and
- Therapy should aim to maintain serum TSH concentration in the age specific laboratory reference range (it is suggested, to target the mid- to lower half of the reference range whenever appropriate) and serum T4 concentration in the upper half of the age specific laboratory reference range.

If dose fine tuning is required, which cannot be obtained with the available strengths, alternating two strengths over the course of the week may be allowed. All dosing schemes must be documented in the EDC.

Parents should be adequately trained on dosing procedure prior to administering the first dose.

LT4 will be administered as a single daily oral dose.

LT4 should be administered on an empty stomach, one-half to one hour before breakfast and at least 4 hours before or after drugs known to interfere with LT4 absorption. The need for dose adjustments should be evaluated when regularly administering within an hour of certain foods that may affect LT4 absorption [soybean flour (infant formula), cottonseed meal, walnuts, dietary fiber] (Tirosint® SOL USPI, Synthroid USPI).

Tirosint®-SOL may be administered in water or directly into the mouth (Tirosint® SOL USPI).

- To administer Tirosint®-SOL in water, the contents of one single unit-dose ampule should be squeezed into a glass or cup containing water. The diluted Tirosint®-SOL should be stirred and all of it drunk immediately. The glass or cup should be rinsed with additional water and the contents drunk to ensure that the total dose is taken. Tirosint®-SOL should not be diluted in a medium other than water. The ampule should be opened and the solution prepared immediately before intake; or
- To administer Tirosint®-SOL directly (without water), it can either be squeezed into the mouth or onto a spoon and immediately consumed.

LT4 tablets should be administered to infants and children who cannot swallow intact tablets by crushing the tablet, suspending the freshly crushed tablet in a small amount (5 to 10 mL or 1 to 2 teaspoons) of water and immediately administering the suspension by spoon or dropper. The suspension should not be stored (Synthroid USPI).

13.1.3 ADDITIONAL DOSING INSTRUCTIONS

It is recommended that parents are educated, by trained personnel, on appropriate TH administration, the substances (e.g. iron, calcium, soy, fiber) that can interefere with TH absorption, the importance of adherence to the treatment plan, and the importance of periodic follow-up care (AAP 2006, Rose 2023, Synthroid USPI, Tirosint® SOL USPI). Education will be provided as per SOC.

While LT4 intake is recommended on an empty stomach, one-half to one hour before breakfast, literature is quite consistent in stating that such a recommendation may be difficult to adhere to in infants (LaFranchi 2011). The most recent guidelines on CH, issued by ENDO-European Reference Network, suggest that, in contrast to adults, neonates, infants and children can be administered LT4 together with food (but with avoidance of soy protein and vegetable fiber). Importantly, LT4 should be administered at the same time every day, also in relation to food intake; while this approach can improve compliance, it ensures as constant as possible LT4 absorption and, with that, as good as possible LT4 dose titration (Van Trotsenberg 2021). The most recent AAP guidelines also suggest that LT4 tablets can be crushed and suspended in 2 to 5 mL (about 1 teaspoon) of human milk, nonsoy-containing formula, or water and also that LT4 can be administered at any time of day in infants and toddlers (morning or evening, with or without feeds), as long as the timing and manner of administration are consistent (Rose 2023).

For the present study, in order to standardize the mode of administration, parents of newly diagnosed neonates will be instructed to administer LT4 first thing in the morning, possibly before the first bottle or feeding.

Parents of infants already on LT4 treatment who will remain on LT4 tablets will not be asked to change their habits (in terms of administration time and interval to food intake) upon inclusion in the study if these are providing adequate replacement, in order to ensure constant LT4 absorption and not compromise compliance.

Parents of infants already on LT4 treatment assigned to Tirosint®-SOL will be advised at inclusion to administer Tirosint-SOL as first thing in the morning, possibly before the first bottle or feeding. If this is in contrast with their previous habits and those were providing adequate replacement, upon discussion with the Investigator, they may be allowed to choose between the new habit and the old one (in terms of administration time and interval to food intake), in order not to compromise compliance with the treatment.

The intake of simethicone, iron, calcium, soy and fiber at the time of L-T4 administration should be avoided as they can interfere with TH absorption. LT4 should not be mixed with these substances and their intake should be delayed by about 4 hours from LT4 (Van Trotsenberg 2021, AAP 2006, LaFranchi 2011, Rose 2023) (see also **Section 13.2.5**).

Typically, the daily LT4 tablet is crushed and mixed with water, expressed breast milk, or formula. The resulting suspension is drawn up in a syringe and squirted into the cheek pad or put in an open nipple for the infant to suck (LaFranchi 2011). SOC instructions about how to administer LT4 tablets will be provided to parents of neonates newly diagnosed with CH.

For Tirosint-SOL, reference is made to the instructions provided in the USPI.

13.2 PREPARATION, HANDLING, STORAGE AND ACCOUNTABILITY

13.2.1 PACKAGING AND LABELLING

The Sponsor will supply Tirosint®-SOL in the FDA-approved commercial packaging with study-specific labelling. Tirosint®-SOL is commercially available in 30-count boxes. Each box contains 6 pouches with 5 ampules of Tirosint®-SOL in each pouch (30 total doses). Each box and ampule are color coded to help clearly identify the dosage strength of Tirosint®-SOL.

Study specific label on the pouch will not obscure the original commercial labelling (lot, expiry date, and storage conditions) and will report the following information:

- Name and address of the sponsor;
- Study number;
- Box number;
- Clinical Batch number; and
- For clinical trial use only.

Study specific label to be added to the box will not obscure the original labelling and will report the following information:

- Name and address of the sponsor;
- Study number;
- Box number;
- Subject Number;
- Clinical Batch number; and

- For clinical trial use only.

The box number is a unique identifier (X-NN-NNNN) on each box and pouch that includes a Letter (identifying the strength), 2-digit Number (identifying the packaging campaign), and 4-digit Number (identifying the box number).

Table 4: Dose Identifier Key

Letter Identifier	Color Identifier	Dose Strength
I	GREEN	13 mcg
A	ORANGE	25 mcg
B	DARK BLUE	37.5mcg
C	RED	44 mcg
D	WHITE	50 mcg
E	GREY	62.5 mcg
F	PURPLE	75 mcg
G	OLIVE	88 mcg
H	YELLOW	100 mcg

For example, A-01-0001 indicates that this contains the 25 mcg dose and is the first box of the first packaging campaign. The Subject Number on the carton will be left blank. The Subject Number must be written by the PI or designee.

13.2.2 PREPARATION ONSITE

Not applicable.

13.2.3 RECEIPT, STORAGE AND ACCOUNTABILITY

The PI or designee should inventory each shipment of Tirosint®-SOL, which should then be transferred to a secure, temperature-controlled storage area that is accessible only to delegated site personnel. A temperature-recording device will be used to monitor the temperature in the storage area for the duration of the study.

Tirosint®-SOL must be stored in the original container (closed pouch) at 20°C to 25°C (68°F to 77°F); excursions permitted to 15° - 30°C (59° - 86°F). Any temperature deviations should be recorded in the IP log and reported to the Sponsor to determine if the product can still be used.

TIROSINT®-SOL oral solution must be used within three (3) months after opening the pouch. The ampules must be kept in the pouch until ready to use as important information may be lost (i.e., manufacturer/distributor names and distributor contact phone number).

Unused IP will be returned to the Sponsor or destroyed on-site according to clinical site standard operating procedures (SOPs). The clinical monitor or Sponsor designee will determine when these activities should occur.

IP accountability will be supported through documentation of receipt, dispensing, return and destruction, and overall reconciliation of these activities. All observed discrepancies in IP usage should be documented and explained.

For subjects assigned to Tirosint®-SOL, at each in-clinic visit , each subject will receive a sufficient number of cartons to ensure the daily adminstration of the dose until the following planned in-clinic visit. During the following in-clinic visit, the subject's parent/guardian will bring back to the study site all cartons, pouches, ampules previously received (partially used pouches including unused ampules and unused cartons, pouches and ampules) and receive a new supply of the IP. Returned supplies will not be redispensed.

For subjects assigned to LT4 tablets, each subject will either receive a sufficient number of tablets to ensure the daily adminsitration of the dose until the following planned in-clinic visit or will purchase it from a local pharmacy upon prescription. The study site personnel are responsible for recording the product name and strength into the electronic case report form (eCRF).

In special circumstances (e.g. TM visits, pandemic restrictions) the study drugs might be shipped directly to patient.

13.2.4 PRECAUTIONS AND OVERDOSAGE

Additional information concerning relevant precautions and the procedures to be undertaken in the event of overdosage can be found in the Tirosint® SOL USPI.

13.2.5 CONCOMITANT MEDICATIONS

Subjects will be allowed to receive any concomitant medication necessary for the treatment of pre-existing concomitant pathologies or for intercurrent diseases that are not exclusionary.

Exclusionary medications are:

- liothyronine, from two weeks prior to start of screening;
- combination of LT4 and liothyronine, and thyroid extracts, from four weeks prior to start of screening;
- anticonvulsants, as they increase degradation of LT4;
- chronic or long-term systemic glucocorticoids, as they may affect interpretation of TFTs (short term use of systemic glucocorticoids, as well as inhalatory or topical corticosteroids are allowed).

All concomitant medications will be recorded in the eCRF.

All medications used by a particular subject should be listed, reviewed, and approved by the PI or designee.

Drugs known to interfere with LT4 absorption (such as calcium carbonate, ferrous sulfate, bile acids sequestrants and ion exchange resins), as well as simethicone, should be administered at least 4 hours apart from Tirosint®-SOL or LT4 tablets.

Biotin has been reported to interfere with some immunoassays using a streptavidin-biotin complex, including TSH and FT4 and clearance from the blood is yet unknown. For this reason, information on the use of biotin should be collected in the eCRF and parents/guardian should be advised to withhold biotin supplementation for four days before each blood draw (Charles 2018).

Consumption of certain foods may affect LT4 absorption thereby necessitate adjustments in dosing. Soybean flour (infant formula), cottonseed meal, walnuts, and dietary fiber may bind and decrease the absorption of LT4 from the gastrointestinal tract. Grapefruit juice may delay the absorption of LT4 and reduce its bioavailability.

Care should be taken to avoid concomitant administration of soy and fiber.

13.2.6 CONTRAINDICATIONS

TIROSINT®-SOL is contraindicated in subjects with:

- Hypersensitivity to glycerol, the inactive ingredient in TIROSINT®-SOL; and/or
- Uncorrected adrenal insufficiency.

13.2.7 RESCUE MEDICATION

No rescue medications will be used in this study.

13.3 BLINDING, TREATMENT ALLOCATION AND RANDOMIZATION PROCEDURES

This study will not be blinded, all study staff will know what treatment is assigned.

All eligible subjects will be randomly assigned at Visit 1. Subject randomization will be 2:1 (Treatment:Control) and stratified by site through an Integrated Web Response System (IWRS). Randomization will be stratified based on age:

- neonates newly diagnosed ≤ 28 days; and
- infants > 28 days already on therapy with LT4.

13.4 EMERGENCY CODE AND UNBLINDING PROCEDURES

This is an open study therefore unblinding procedures are not needed.

14 DISCONTINUATION/WITHDRAWAL

14.1 DISCONTINUATION/WITHDRAWAL

Participation will be discontinued for any of the following reasons:

- Voluntary subject/legally authorized representative withdrawal for any reason;
- PI's discretion;
- An AE, including onset or worsening of a concomitant illness, that is considered by the PI to be incompatible with continuation in the study;
- Administration of a drug that is not permitted according to the exclusion criteria;
- Failure to comply with the requirements of the protocol or major protocol deviation (e.g., inclusion error, evidence of noncompliance with exclusion/inclusion criteria during the study, subject misses study visits, failure to take assigned medication, or provide blood sample as required, etc.);
- Lost to follow-up; or
- Subject death.

Subjects whose parents or legal guardians provide informed consent, receive the IP and subsequently withdraw, or are withdrawn or discontinued from the study, will not be replaced.

14.2 PREMATURE TERMINATION

The trial may be suspended temporarily or terminated prematurely if there are any relevant medical or ethical concerns, or if completing the trial is no longer practicable. If such action is taken, the reasons for terminating the trial must be documented in detail and submitted to the overseeing IRB. All subjects still under treatment at the time of termination will undergo a final examination and the results will be documented in the eCRF.

14.3 LOST TO FOLLOW-UP

The PI or designee must take the following actions if a parent/guardian of a subject fails to return to the site for a required study visit:

- Attempt to contact the parent/ legal guardian and reschedule the missed visit as soon as possible; counsel parent/ legal guardian on the importance of maintaining the assigned visit schedule and ascertain if the subject wishes to and/or should continue in the study;
- Make every effort to regain contact with the parent/ legal guardian. Where possible, three (3) telephone calls should be made and, if necessary, a certified letter sent to the parent/legal guardian's last known mailing address. These contact attempts should be documented in the source documents and eCRF;

- Should the parent/legal guardian continue to be unreachable, they will be considered to have withdrawn from the study with a primary reason of lost to follow-up.

15 STUDY ASSESSMENTS AND PROCEDURES

15.1 EFFICACY ASSESSMENTS

15.1.1 LABORATORY PARAMETERS

Laboratory parameters evaluated in this study include:

- Thyroid Stimulating Hormone (TSH);
- Free Thyroxine (FT4); and
- Total Thyroxine (TT4), optional, at the discretion of the Investigator.

The analyses will be conducted on serum samples.

Blood samples for serum FT4, TT4 and TSH monitoring will be collected 0 (during the visit) to -7 days before each visit (within the allowed visit time frame) to allow for adequate time to evaluate the measurements.

Blood samples should be collected at least 4 hours after the last LT4 administration or before taking the LT4 daily dose (VanTrotsenberg 2021, Rose 2023). Whenever possible perform the collection in the same way and at the same time at each visit.

At each visit, 1-4 mL of blood will be collected; 7-32 mL of blood will be collected per subject for the entire study (7-8 time points). If considering a maximum of two additional unscheduled visits during the course of the study, a maximum of 40 mL of blood will be collected over one year.

A venipuncture sample should be collected by accessing the veins of the antecubital fossa (inside the elbow), in order to obtain serum. If unable to obtain from antecubital fossa, a heel or finger prick is preferable to no sample. This must be clearly specified on the request to the laboratory and results will have to be converted by the laboratory to serum equivalents. In exceptional cases, a subject's blood draw may be performed at a different location (e.g. laboratory patient service center), as per SOC.

- Each site will attempt to use the same laboratory throughout the study (main laboratory). Additional laboratories may be used, but, in order to reduce variability, these should be limited to not more than 3 analytical lab facilities per site (e.g. hospital lab and other providers of diagnostic services such as Quest and LabCorp).
- Each site will attempt to use the same laboratory for the same subject throughout the study.
- Laboratory sampling and analytical procedures should be followed.

- Local lab reference ranges will be collected and any change in reference ranges should be recorded in the EDC.
- The laboratory assessments done for CH diagnostic purposes (routine newborns screening) are not part of this protocol (they precede screening/inclusion for the study). Results from the site's laboratory or other laboratories following different procedures are both acceptable.

When abnormalities in the laboratory parameters are detected, they will be assessed by the Investigator as clinically significant or not clinically significant. Because laboratory parameters are the main parameter of therapy monitoring and abnormalities are expected in relation to growth and the development of the disease and therapy, abnormalities will not be recorded in the subject's eCRF as medical history, nor as AEs, even when clinically significant.

15.1.2 PARENTS' SATISFACTION QUESTIONNAIRE

The Parents' Satisfaction Questionnaire will be completed by the parents/guardian in the eDiary: at V1 only for infants who are already on TH therapy, and 0-3 days before in-clinic visits for all subjects. Parents will answer questions about the following topics:

- Coping and mental well-being;
- Drug preparation & administration;
- Treatment satisfaction; and
- Medication preference.

During the visits the Investigator should verify completion of the Questionnaire and, if not completed, should ask the parent(s) to complete it on site.

15.1.3 CAREGIVER ADMINISTERED CHILDREN'S ACCEPTANCE TOOL (CareCAT)

CareCAT is a 5-point nominal scale used to assess the acceptance of oral medicines in infants and toddlers who are unable to verbally give their opinion about a medicine (Blume 2018).

The CareCAT is designed to be administered by parents/caregivers in their home environment. This questionnaire will be completed within the eDiary during the first, the third and the eighth week of treatment.

Figure 1: Caregiver Administered Children's Acceptance Tool (CareCAT)

Descriptors:

Swallows well – Medicine is swallowed down

- Observing the act of swallowing the medicine – '[I see that she] drinks the medicine and swallows'
- Ingesting the medicine in absence of negative behaviors – 'He swallows and does not give me a hard time'

Refusal – Resistance of the child (e.g. fighting, crying)

- Defensive behavior preventing the intake of medicine – 'By pushing the spoon' or 'fights with her hands' or 'turns away her head'

Spitting up – Some medicine is coming out of the mouth immediately

- Forcing the medicine out actively – 'He spits or maybe blows the medicine out'
- Medicine passively leaving the mouth ('overflow' or 'spilling') – 'When the medicines runs down the mouth

Vomiting – Within 30 minutes after swallowing the medicine the child brings up something [vomits]

- 'She takes out the medicine after feeling nauseous, then vomits it with food'

Medication not taken – No medicine went into the mouth of the child

- No oral intake of medicine – 'She does not want [to take the medicine] until the medicine did not get in [the mouth]'
- Intake without ingesting – 'I have tried to give her but [the medicine] was still not swallowed

15.2 SAFETY ASSESSMENTS

15.2.1 PHYSICAL EXAMINATION AND VITAL SIGNS

A full physical examination (standard health maintenance exam for age) will be performed, and abnormalities found during the examination will be recorded specifying the body system in the EDC.

Vital signs that will be collected and recorded in the EDC are: heart rate and temperature.

Clinically significant abnormalities in physical examination or vital signs will be recorded as medical history (if starting before ICF signature) or as AEs (if occurring after ICF signature).

15.2.2 GROWTH ASSESSMENTS

Growth assessments include the following:

- Length (cm);
- Body weight (kg); and
- Head circumference (cm).

Clinically significant abnormalities in growth assessments will be recorded as medical history (if starting before ICF signature) or as AEs (if occurring after ICF signature).

15.2.3 HYPO- AND HYPER- THYROIDISM SIGNS AND SYMPTOMS

Signs and symptoms of hypo- and hyper- thyrodism will be evaluated by the PI based on clinical assessment and interview of the subject's parent or legal guardian.

Signs and symptoms of hypo-/hyper-thyroidism will not be assessed as AEs, unless they are judged clinically significant. Clinically significant signs and symptoms of hypo-/hyper-thyroidism will be recorded in the medical history (if starting before ICF signature) or as AEs (if occurring after ICF signature).

Examples of signs and symptoms of hypothyroidism that could be observed include, but are not limited to jaundice, hypotonia, puffy-looking face/myxedematous facies, cool and pale skin, large fontanel on the head, large tongue (macroglossia), distended abdomen with umbilical hernia, hoarse crying, decreased activity, sluggishness, increased sleeping, tiring easily, constipation, decreased appetite, slow feeding/difficulty swallowing, or slow growth including delayed neuropsychomotor development (Rastogi 2010; Saoud 2019; Rose 2023).

Examples of signs and symptoms of hyperthyroidism that could be observed include, but are not limited to jitteriness, hyperactivity, difficulty sleeping, increased appetite without weight gain, frequent stooling or diarrhea.

15.2.4 ADVERSE EVENTS (AEs)

The occurrence, frequency, nature and severity of all AEs that occur after signature of the written informed consent will be documented for each subject. At each visit the PI will ask the subject's parent/guardian about any untoward experiences since the previous visit in accordance with **Section 17**.

15.3 OTHER ASSESSMENTS

15.3.1 DOSING EVALUATION

At each visit the PI will assess the TSH, FT4 and, optionally, TT4 values as well as the clinical response to establish if the daily LT4 dose is appropriate or if adjustments are needed.

15.3.2 TREATMENT COMPLIANCE

Treatment compliance will be evaluated through the eDiary. Parents/guardians will indicate daily if the dose was effectively administered to the subject. The PI will review treatment compliance during the visits and in case of poor compliance will investigate reasons with the parents/guardians and instruct parents/ guardians about the importance of treatment adherence.

In addition, for Tirosint®-SOL the PI will count the returned unused IP. Significant discrepancies between IP count and eDiary records will need to be clarified with the parents/guardian during the visit and appropriately recorded.

15.3.3 NUTRITIONAL INTAKE

Nutritional intake will be recorded by the PI in the eCRF. After interviewing the parent/legal guardian of the subject the PI will report if the subject takes:

- breast milk, formula milk and type (cow's milk based formula, soybean based formula, hydrolysed protein formula, other), cow's milk, baby food (e.g. infant cereals, mashed/strained fruits and vegetables, pureed meat), adult-like food.

16 OVERALL STUDY SCHEDULE

The study schedule is summarized in **Section 7.1** and described below. Study visits are conducted as either in-clinic or telemedicine (TM) visits. The PI is allowed to perform in-clinic visits instead of TM if necessary. In exceptional circumstances (e.g. pandemic restrictions) in-clinic visits could be replaced by TM visits.

16.1 SCREENING/INCLUSION (RANDOMIZATION) – VISIT 1 (DAY -14 to 1)

The procedures of screening and inclusion may be spread over multiple days (particularly when laboratory results need to be collected), but should be completed within two weeks.

If a subject is found to be potentially eligible for the trial, parents/legal guardians will be informed both verbally and in writing about the study objectives and procedures. The Informed Consent Form (ICF) will be signed and dated by the parents/legal guardian and the PI. The informed consent process must be documented in the medical record of the subject.

AE collection and reporting should begin after informed consent is given.

After informed consent to participate in the study has been obtained, the neonate/infant will be assigned a Subject Number and the PI or designee will do the following to confirm eligibility:

- Record demographic data and medical history; and
- Record prior and concomitant medications;
- Verify compliance with the inclusion/exclusion criteria.

If eligibility for the trial is confirmed, the PI or designee will do the following:

- Perform a physical examination including body measurements and vital signs;
- Assess the presence of signs and symptoms of hypo- or hyper- thyroidism;
- Record nutritional intake;
- Record results of TSH, FT4 and/or TT4 from original diagnosis from medical records (both results from dried spot whole blood, if available, and from confirmatory serum tests, specifying if results are expressed per unit of blood or per unit of serum);
- Take blood samples for serum TSH, FT4 and, optional, TT4 (for infants who are already on TH therapy and for whom a lab report dated < 28 days is not available) or record results of TSH, FT4 and TT4 (for infants who are already on TH therapy and for whom a lab report dated < 28 days is available; in this latter case a blood draw and re-assessment of hormonal values can be done, if necessary as per Investigator's medical judgement);
- Randomize the subject using the IWRS (Day 0 or Day 1 of the study);
- Perform dosing evaluation;
- Dispense the assigned drug product;
- Instruct parents/guardians about drug product administration and indicate the date of the first dose to be administered;
- Verify with parents/guardians that they can access and how to use the eDiary including instruction on how to perform and timelines of the:
    - Drug administration;
    - CareCAT;
    - Parents' Satisfaction Questionnaires;

- Ask parents/guardians to complete the Parents' Satisfaction Questionnaire in the eDiary (for infants already on LT4 therapy); and
- Complete and provide parents/ guardians with the Subject Visit Reminder Card.

The **first day of study drug administration** (**Day 1**) (for subjects continuing on tablets, the first day of drug administration within the study) should be either the same day of randomization (preferred) or the day after.

16.2 TREATMENT PHASE

The treatment phase is divided into two phases: the TSH normalization phase and the long-term follow-up phase.

The procedures conducted at each visit may take place on multiple days (0-7 days) all within the allowed time frame.

16.2.1 NORMALIZATION PHASE

The normalization phase encompasses from Screening/Inclusion until complete normalization of serum TSH and T4 within the reference range for age is achieved and includes Visits 2 and 3. Subjects who at Visit 3 have not yet achieved TFTs normalization, based on the Investigator's judgement, will undergo one or more additional follow-up visits at appropriate time intervals (usually every two weeks) and dose adjustments until TFTs are normalized (e.g. Follow-Up Type 1, FU1). If the timing of FU1 visit overlaps with the next planned visit (V4-V8), it is possible to perform only the planned visit.

16.2.2 LONG-TERM FOLLOW-UP PHASE

The long-term follow-up phase begins once TFTs normalization has been achieved and includes Visits 4 to 11.

If a change in LT4 dose is needed or, at the discretion of the Investigator, if an abnormal T4 or TSH are found, an additional follow-up visit should be performed 2-6 weeks after the dose change or abnormal finding (e.g. Follow-Up Type 2, FU2). If the timing of FU2 visit overlaps with the next planned visit (V5-V11), it is possible to perform only the planned visit.

16.2.3 VISIT 2 (STUDY DAY 8-15, i.e. 7-14 DAYS AFTER TREATMENT START)

During Visit 2 the PI or designee will complete the following via a TM visit, except for blood sampling:

- Verify concomitant medications;
- Record AEs;

- Assess the presence of signs and symptoms of hypo- or hyper- thyroidism (by parents' interview);
- Take blood samples for serum TSH, FT4 and, optional, TT4 (blood sampling is optional, at the discretion of the Investigator, for infants already on LT4);
- Perform dosing evaluation;
    - A different LT4 dose may be indicated and drug dispensed or shipped to parents/guardians if the dose must be adjusted;
- Record nutritional intake;
- Review eDiary completion (Treatment Compliance and CareCAT); and
    - If needed, further instructions will be provided to the parent/guardian on how to complete the eDiary and how to comply with the study requirements.

16.2.4 VISIT 3 (STUDY DAY 22-29, i.e. 21-28 DAYS AFTER TREATMENT START)

During Visit 3 the PI or designee will complete the following via an in-clinic visit:

- Verify concomitant medications;
- Record AEs;
- Perform a physical examination including body measurements and vital signs;
- Assess the presence of signs and symptoms of hypo- or hyper- thyroidism;
- Take blood samples for serum TSH, FT4 and, optional, TT4;
- Perform dosing evaluation;
    - A different LT4 dose may be indicated and drug dispensed or shipped to parents/guardians if the dose must be adjusted;
- Record nutritional intake;
- Review eDiary completion (Treatment Compliance, Parents' Satisfaction Questionnaire, CareCAT);
    - If needed, further instructions will be provided to the parent/guardian on how to complete the eDiary and how to comply with the study requirements;
    - Report and resolve discrepancies that are noted in treatment compliance;
- Return and dispense drug, if applicable; and
- Update the Subject Visit Reminder Card.

16.2.5 FOLLOW UP TYPE 1 (FU1):

During FU1 the PI or designee will complete the following via a TM visit, except for blood sampling:

- Verify concomitant medications;
- Record AEs;

- Assess the presence of signs and symptoms of hypo- or hyper- thyroidism (by parents' interview);
- Take blood samples for serum TSH, FT4 and, optional, TT4;
- Perform dosing evaluation;
    - A different LT4 dose may be indicated and drug dispensed or shipped to parents/guardians if the dose must be adjusted;
- Record nutritional intake;
- Review eDiary completion (Treatment Compliance);
    - If needed, further instructions will be provided to the parent/guardian on how to complete the eDiary and how to comply with the study requirements.

16.2.6 VISIT 4 (2 MONTHS, 60 DAYS ±7 OF AGE):

During Visit 4 the PI or designee will complete the following via a TM visit, except for blood sampling:

- Verify concomitant medications;
- Record AEs;
- Assess the presence of signs and symptoms of hypo- or hyper- thyroidism (by parents' interview);
- Take blood samples for TSH, FT4 and, optional, TT4;
- Perform dosing evaluation;
    - A different LT4 dose may be indicated and drug dispensed or shipped to parents/guardians if the dose must be adjusted;
- Record nutritional intake;
- Review eDiary completion (Treatment Compliance and CareCAT);
    - If needed, further instructions will be provided to the parent/guardian on how to complete the eDiary and how to comply with the study requirements.

16.2.7 VISIT 5 (4 MONTHS, 120 DAYS ±14 OF AGE):

During Visit 5 the PI or designee will complete the following via an in-clinic visit:

- Verify concomitant medications;
- Record AEs;
- Perform a physical examination including body measurements and vital signs;
- Assess the presence of signs and symptoms of hypo- or hyper- thyroidism;
- Take blood samples for serum TSH, FT4 and, optional, TT4;
- Perform dosing evaluation;
    - A different LT4 dose may be indicated and drug dispensed or shipped to parents/guardians if the dose must be adjusted;

- Record nutritional intake;
- Review eDiary completion (Treatment Compliance, Parents' Satisfaction Questionnaire, CareCAT); and
    - If needed, further instructions will be provided to the parent/guardian on how to complete the eDiary and how to comply with the study requirements;
    - Report and resolve discrepancies that are noted in treatment compliance;
- Return and dispense drug, if applicable; and
- Update the Subject Visit Reminder Card.

16.2.8 VISIT 6 (6 MONTHS, 180 DAYS ±14 OF AGE):

During Visit 6 the PI or designee will complete the following via an in-clinic visit:

- Verify concomitant medications;
- Record AEs;
- Perform a physical examination including body measurements and vital signs;
- Assess the presence of signs and symptoms of hypo- or hyper- thyroidism;
- Take blood samples for serum TSH, FT4 and, optional, TT4;
- Perform dosing evaluation;
    - A different LT4 dose may be indicated and drug dispensed or shipped to parents/guardians if the dose must be adjusted;
- Record nutritional intake;
- Review eDiary completion (Treatment Compliance, Parents' Satisfaction Questionnaire, CareCAT);
    - If needed, further instructions will be provided to the parent/guardian on how to complete the eDiary and how to comply with the study requirements;
    - Report and resolve discrepancies that are noted in treatment compliance;
- Return and dispense drug, if applicable; and
- Update the Subject Visit Reminder Card.

16.2.9 VISIT 7 (9 MONTHS, 270 DAYS ±14 OF AGE):

During Visit 7 the PI or designee will complete the following via an in-clinic visit:

- Verify concomitant medications;
- Record AEs;
- Perform a physical examination including body measurements and vital signs;
- Assess the presence of signs and symptoms of hypo- or hyper- thyroidism;
- Take blood samples for serum TSH, FT4 and, optional, TT4;
- Perform dosing evaluation;

    - A different LT4 dose may be indicated and drug dispensed or shipped to parents/guardians if the dose must be adjusted;
- Record nutritional intake;
- Review eDiary completion (Treatment Compliance, Parents' Satisfaction Questionnaire, CareCAT);
    - If needed, further instructions will be provided to the parent/guardian on how to complete the eDiary and how to comply with the study requirements;
    - Report and resolve discrepancies that are noted in treatment compliance;
- Return and dispense drug, if applicable; and
- Update the Subject Visit Reminder Card.

16.2.10 VISIT 8 (12 MONTHS, 365 DAYS ±21 OF AGE):

During Visit 8 the PI or designee will complete the following via an in-clinic visit:

- Verify concomitant medications;
- Record AEs;
- Perform a physical examination including body measurements and vital signs;
- Assess the presence of signs and symptoms of hypo- or hyper- thyroidism;
- Take blood samples for TSH, FT4 and, optional, TT4;
- Perform dosing evaluation;
    - A different LT4 dose may be indicated and drug dispensed or shipped to parents/guardians if the dose must be adjusted;
- Record nutritional intake;
- Review eDiary completion (Treatment Compliance, Parents' Satisfaction Questionnaire, CareCAT);
    - If needed, further instructions will be provided to the parent/guardian on how to complete the eDiary and how to comply with the study requirements:
    - Report and resolve discrepancies that are noted in treatment compliance;
- Return and dispense drug, if applicable;
    - Drug will not be dispensed at Visit 8 if it is the EOS; and
- Update the Subject Visit Reminder Card (not if it is EOS).

16.2.11 VISIT 9 (15 MONTHS, 455 DAYS ±21 OF AGE):

During Visit 9 the PI or designee will complete the following via an in-clinic visit:

- Verify concomitant medications;
- Record AEs;
- Perform a physical examination including body measurements and vital signs;
- Assess the presence of signs and symptoms of hypo- or hyper- thyroidism;

- Take blood samples for TSH, FT4 and, optional, TT4;
- Perform dosing evaluation;
    - A different LT4 dose may be indicated and drug dispensed or shipped to parents/guardians if the dose must be adjusted;
- Record nutritional intake;
- Review eDiary completion (Treatment Compliance and Parents' Satisfaction Questionnaire);
    - If needed, further instructions will be provided to the parent/guardian on how to complete the eDiary and how to comply with the study requirements;
    - Report and resolve discrepancies that are noted in treatment compliance;
- Return and dispense drug, if applicable;
    - Drug will not be dispensed at Visit 9 if it is the EOS; and
- Update the Subject Visit Reminder Card (not if it is EOS).

16.2.12 VISIT 10 (18 MONTHS, 545 DAYS ±21 OF AGE):

During Visit 10 the PI or designee will complete the following via an in-clinic visit:

- Verify concomitant medications;
- Record AEs;
- Perform a physical examination including body measurements and vital signs;
- Assess the presence of signs and symptoms of hypo- or hyper- thyroidism;
- Take blood samples for TSH, FT4 and, optional, TT4;
- Perform dosing evaluation;
    - A different LT4 dose may be indicated and drug dispensed or shipped to parents/guardians if the dose must be adjusted;
- Record nutritional intake;
- Review eDiary completion (Treatment Compliance and Parents' Satisfaction Questionnaire);
    - If needed, further instructions will be provided to the parent/guardian on how to complete the eDiary and how to comply with the study requirements;
    - Report and resolve discrepancies that are noted in treatment compliance;
- Return and dispense drug, if applicable;
    - Drug will not be dispensed at Visit 10 if it is the EOS; and
- Update the Subject Visit Reminder Card (not if it is EOS).

16.2.13 VISIT 11 (21 MONTHS, 635 DAYS ±21 OF AGE):

During Visit 11 the PI or designee will complete the following via an in-clinic visit:

- Verify concomitant medications;

- Record AEs;
- Perform a physical examination including body measurements and vital signs;
- Assess the presence of signs and symptoms of hypo- or hyper- thyroidism;
- Take blood samples for TSH, FT4 and, optional, TT4;
- Perform dosing evaluation;
    - A different LT4 dose may be indicated and drug dispensed or shipped to parents/guardians if the dose must be adjusted;
- Record nutritional intake;
- Review eDiary completion (Treatment Compliance and Parents' Satisfaction Questionnaire);
    - Report and resolve discrepancies that are noted in treatment compliance; and
- Return drug, if applicable.

16.2.14 FOLLOW-UP TYPE 2 (FU2):

During FU2 the PI or designee will complete the following via a TM visit, except for blood sampling:

- Verify concomitant medications;
- Record AEs;
- Assess the presence of signs and symptoms of hypo- or hyper- thyroidism (by parents' interview);
- Take blood samples for serum TSH, FT4 and, optional, TT4;
- Perform dosing evaluation;
    - A different LT4 dose may be indicated and drug dispensed or shipped to parents/guardians if the dose must be adjusted;
- Record nutritional intake;
- Review eDiary completion (Treatment Compliance);
    - Report and resolve discrepancies that are noted in treatment compliance; and
    - If needed, further instructions will be provided to the parent/guardian on how to complete the eDiary and how to comply with the study requirements.

16.2.15 END OF STUDY (EOS) VISIT:

The EOS vist should be performed at Visits 8 through 11 according to the age of inclusion outlined in **Table 2**.

At the EOS the PI will prescribe the appropriate LT4 product to allow continuation of TH therapy, upon presentation to parents of the possible alternatives and questioning about their preferential choice.

16.2.16 UNSCHEDULED VISITS

Unscheduled visits may be performed during the study or after the end of the study, by TM or in clinic, if judged necessary by the PI. During an unscheduled visit the following information will be recorded at a minimum:

- Reason for the visit;
- AEs (not mandatory if the visit is to the sole purpose of drug dispensing); and
- Concomitant medications (not mandatory if the visit is to the sole purpose of drug dispensing).

If blood samples for serum TSH, FT4 and TT4 are collected, results will be recorded.

If study drug is dispensed/returned, this will be recorded.

17 ADVERSE EVENT DESCRIPTION AND REPORTING

17.1 ADVERSE EVENTS (AEs)

An AE is any untoward medical occurrence in a clinical trial subject, whether or not there is a suspected causal relationship between the event and the Study Drug. AEs include exacerbation of a pre-existing medical condition, that is, the condition has increased in severity, frequency and/or duration. A pre-existing condition that did not deteriorate during the study, but was treated with a medical or surgical procedure, should not be recorded as an AE. However, the procedure itself should be in the eCRF.

An adverse drug reaction (ADR) is any AE with a suspected causal relationship to the Study Drug. This means that there is at least reasonable evidence that the event might have been elicited by administration of the Study Drug.

17.2 SERIOUS ADVERSE EVENTS (SAEs)

SAEs are a subset of AEs that resulted in one of the following outcomes:

- Death;
- Life-threatening illness or injury (i.e. the patient was at risk of death at the time of the event; it does not refer to an event that hypothetically might have caused death if it were more severe);
- Inpatient hospitalization or prolongation of existing hospitalization;
- Persistent or significant disability or incapacity;
- A congenital anomaly or birth defect; or

- An important medical event (i.e. an event that jeopardize the patient or may require intervention to prevent one of the other outcomes listed above).

A serious adverse reaction (SAR) is any serious adverse event with a suspected causal relationship to the Study Drug. This means that there is at least reasonable evidence that the event might have been elicited by administration of the Study Drug.

17.3 CAUSALITY ASSESSMENT AND CLASSIFICATION

The PI must make a causality assessment for each adverse event experienced by a study subject following at least one dose of Study Drug (IP) according to the following guidance. However, any adverse event that occurs between written informed consent and administration of the first dose of IP will be considered a pre-treatment adverse event (PTAE), and this option should be selected in the applicable form rather than making a causality assessment.

- Certain: There is clear evidence to suggest a causal relationship and other possible contributing factors can be ruled out. The clinical event, including laboratory test abnormality, occurs in a plausible time relationship to IP administration and cannot be explained by concurrent disease or other drugs or chemicals. The response to withdrawal of the drug (de-challenge) should be clinically plausible. The event must be pharmacologically or phenomenologically definitive, with use of a satisfactory re-challenge procedure if necessary.
- Probable: There is evidence to suggest a causal relationship and the influence of other factors is unlikely. The clinical event, including an abnormal laboratory test, occurs within a reasonable time after administration of the Study Drug, is unlikely to be attributed to concurrent disease or other drugs or chemicals, and follows a clinically reasonable response on withdrawal (de-challenge). Re-challenge information is not required to fulfil this definition.
- Possible: There is some evidence to suggest a causal relationship; however, other factors may have contributed to the event (e.g. the subject's clinical condition, other concomitant treatments). The clinical event, including an abnormal laboratory test, occurs within a reasonable time after administration of Study Drug, but could be explained by concurrent disease or other drugs or chemicals. Information on drug withdrawal may be lacking or unclear.
- Unlikely: There is another reasonable explanation for the event occurrence. A clinical event, including an abnormal laboratory test, whose temporal relationship to Study Drug administration makes a causal relationship improbable, and which other drugs, chemicals or underlying disease provide plausible explanations.
- Not related: There is no evidence of any causal relationship between the clinical event and Study Drug administration, and/or sufficient information exists to indicate that the event is related to another etiology.

17.4 SEVERITY CLASSIFICATION

Regardless of the classification of an adverse event as serious or non-serious, the severity of an adverse event will be rated according to the following definitions:

- Mild: Symptom barely noticeable to subject and does not interfere with subject's daily activities. Minimal or no treatment typically needed for relief of symptom.
- Moderate: Symptom may cause some discomfort and may interfere with subject's daily activities. The subject can continue the study, even if treatment of symptom may be needed.
- Severe: Symptom causes severe discomfort and interrupts the subject's daily activities. They may be of such severity that IP administration has to be stopped and the subject may be treated for symptoms and/or hospitalized.

It should be noted that a severe adverse event does not have to be serious in nature and vice versa. Contrary to the other two classifications (seriousness and causality), the classification of severity does not have any impact on reporting procedures.

17.5 REPORTING

All non-serious AEs experienced by a study subject from the time of written informed consent through EOS or discontinuation must be recorded in the source documents and eCRFs.

All SAEs experienced by a study subject from the time of consent signing through twenty-five (25) days (which is equivalent to 5 half-lives of LT4 in children (Colucci 2013)), post-discontinuation or intended follow-up period (EOS), must be recorded in the source documents and eCRF. Within 24 hours of discovery, the PI (or designee) should complete an initial form and submit to:

IBSA – Drug Safety Unit (DSU)
[redacted]

The initial form should contain at least the following information:

- Protocol number;
- Subject's study identifiers (screening and/or randomization number, age, sex);
- Relevant medical history and any concomitant medications taken during the study;
- SAE description and onset;
- Causality assessment;
- Study Drug and batch number;
- Date of initial dose and the last dose before onset of SAE;
- Detailed description of the circumstances leading to the SAE;

- Specific treatment of the SAE;
- Outcome;
- Principal investigator's name, address, phone number and email address; and
- The principal investigator's signature and date.

If applicable, the Investigator should also send to IBSA (upon anonymization):

- any examinations carried out to investigate the SAE;
- hospital discharge summary.

Missing information should not lead to a delay in reporting to IBSA. All effort should be made by the Investigator to obtain any useful information for the report of the SAE.

In case of death, the Investigator will be requested to forward the autopsy report, if available.

When additional information becomes available, the PI or designee will complete a follow-up form and submit it to the above entities within 48 hours.

Although a designee of the PI may collect information on the initial and follow-up form, the PI will review and sign and date the form(s) to attest to the accuracy and completeness of the information therein.

PIs are responsible for submitting any SAE that occurs to their local IRB, in accordance with the local IRB guidelines.

The Sponsor will review all SAEs to determine whether they meet the definition of a Suspected Unexpected Serious Adverse Reaction (SUSAR) using reference safety information in the IB and USPI, if applicable. In such instances, the Sponsor expects the PI or designee to expedite retrieval of necessary information on the subject and event. Sponsor will submit a safety report to FDA and participating PIs within 15 calendar days of the determining the event qualifies for expedited reporting. PIs will submit safety report to the IRB, as applicable within the appropriate timelines.

17.6 PREGNANCY

N/A

17.7 FOLLOW-UP

Following reporting of an AE, neonates and infants will be followed through the end of the study, or if the AE is treatment-related, thirty (30) days after the completion of the study or until resolution (whichever is sooner). SAEs, SARs and SUSARs should be followed until resolution or acceptable stabilization in the event of chronicity. Medical or surgical procedures that occur during the study that did not result from an AE or SAE will not require follow-up.

18 DATA MANAGEMENT AND STATISTICAL ANALYSIS

18.1 DATA COLLECTION

Source data are all information, original records of clinical findings, observations, or other activities necessary for reconstruction of a clinical trial. Source data can be either electronic or handwritten, and includes but is not limited to hospital records, clinical and office charts, progress notes, laboratory notes and records, pharmacy records, informed consent forms, subject diaries and notes, and study visit worksheets.

Designated clinical site personnel will be responsible for data collection under the supervision of the PI, who is ultimately responsible for ensuring data are attributable, legible, contemporaneous, original and accurate (ALCOA+), with the plus (+) including data to be available, enduring, complete and consistent. To the extent possible, all study data will be entered directly into the eCRF by the PI or designee and such data will be considered electronic source (eSource). Study data will also be collected in an electronic Participant Diary (eDiary) and such data will be transmitted directly to the eCRF through a native integration between these systems. Although designated clinical site personnel are not directly responsible for collection of diary data, they should nonetheless review the diaries to ensure collection of required data.

The PI will review and electronically sign the completed eCRF for each subject before data are archived, in order to maintain accurate case histories.

When it is not possible to enter data directly into the eCRF the PI or designee may use paper worksheets that will be considered source. All paper source documents should be completed in a neat, legible manner to ensure accurate interpretation and transcription into other media, as applicable. Designated clinical site personnel will be responsible for entry of all relevant paper source data into the eCRF under the supervision of the PI. Data entry should be completed contemporaneously with each visit; preferably within 24 hours.

All eCRF users will receive a User Manual and training on system use prior to enrollment of the first participant. Access to the eCRF will be controlled by the Sponsor (or designee) through provision of user-specific login credentials that will only be provided after documentation of eCRF training. All personnel authorized to make entries in the eCRF will be listed by name, title, signature, and initials. The list should be updated during the study duration, if needed.

The PI and/or designated clinical site personnel should immediately alert the clinical monitor and/or Sponsor designee about eCRF operational issues. In the unlikely event that the eCRF is unavailable for an extended period of time, the clinical monitor may direct designated clinical site personnel to utilize paper CRFs for data collection.

All data originators (i.e., PI, designee(s) and participants) at each clinical site will be documented and made available, if needed. Each data element in the eCRF will have an associated identifier

comprised of a data originator, date/time stamp, and clinical trial participant, and both the data element and identifier will be present in a human-readable audit trail.

18.2 DATA MANAGEMENT

A separate Data Management Plan (DMP) will be used to define required data management practices. Briefly, the computer system(s) used in this study will be designed and validated according to contemporary standards, including 21 CFR Part 11, prior to deployment. The eCRF will be designed with internal edit checks, including but not limited to automatic range checks, to promote data quality. In addition, manual queries can be created in the eCRF by clinical monitors or other Sponsor designee(s). All changes in the eCRF will be documented in an audit trail that cannot be manipulated by system users. Following data review and resolution of queries, as applicable, the PI will electronically sign each subject's study record, thereby locking the dataset and preventing additional changes. The electronic signature represents the PI's attestation to the accuracy and completeness of the data and has the same legal meaning as a hand-written signature.

After database cleaning has occurred and a data review has been performed, the trial database will be considered locked. Following finalization of the Statistical Analysis Plan (SAP), the statistical analyses will be performed. Any further modification of recorded data that requires the database to be unlocked and subsequently re-locked will be documented.

18.3 CODING DICTIONARIES

AEs and prior concomitant diseases will be coded using the Medical Dictionary for Regulatory Activities (MedDRA). All verbatim terms will be assigned to a Preferred Term and will be classified by primary System Organ Class according to the MedDRA thesaurus., version 23.0 or higher. Prior and concomitant medications will be coded using the World Health Organization – Drug Reference List (WHO-DRL) Dictionary version March 1, 2020 or higher and classified according to at least the 3rd level Anatomical Therapeutic Chemical (ATC) Classification System level subgroup.

19 STATISTICAL ANALYSIS

The following is a brief description of the planned statistical analyses. A detailed SAP will be written after finalizing the protocol. The SAP may be updated before the end of data management activities or after a discussion of protocol deviations by the clinical team during the data review.

19.1 SAMPLE SIZE

Subjects will be randomized in a 2:1 ratio to Treatment (Tirosint®-SOL) or Control (conventional therapy with LT4 crushed tablets). Considering a mean value of 6 mcg/kg/day of LT4 dose at 12

months of age (**Table *3***) and a standard deviation of 2 mcg/kg, a sample size of 102 neonates and infants (68 Treatment and 34 Control) will have 80% power to detect a difference of 20% in the LT4 dose between the groups using an independent t-test with a 0.050 two-tailed significance level. To account for a drop-out rate of maximum 20%, 126 neonates and infants will be enrolled in the study (84 Treatment and 42 Control) (Peroni 2013)

19.2 POPULATION FOR ANALYSIS

The following subject populations will be evaluated and used for presentation and analysis of the data:

- **Full Analysis Set (FAS):** all randomized subjects;
- **Per Protocol Set (PPS)**: all randomized subjects with no major protocol deviations; and
- **Safety Analysis Set (SAF)**: all randomized subjects who received at least 1 dose of study medication.

If there are treatment errors, the FAS will be analyzed according to randomized treatment while the SAF will be analyzed according to the actual treatment received. The SAF is the primary population for the analysis of safety endpoints.

19.3 STATISTICAL METHODS

19.3.1 GENERAL STATISTICAL METHODS

Statistical analysis will be performed by IBSA Data Management & Statistics, R&D Dept. IBSA, Switzerland, under the responsibility of Data Management and Statistics Unit Manager.

All statistical analyses and data processing will be performed using SAS® Version 9.4 on a Windows operating system.

All p-value(s) will be rounded to three decimal places. Statistical significance will be declared if the rounded p-value will be less than 0.050. Two-sided 95% confidence intervals (CI) will be calculated.

The last observation carried forward (LOCF) technique will be used to deal with missing data or in the case of early study discontinuations. The SAP will describe in details how missing data will be managed.

19.3.2 EFFICACY OUTCOMES

The LT4 dose/kg will be calculated based on the daily LT4 dose used in the time period preceding the visit and the body weight measured during the visit. When dosing schedules alternating strengths over the course of the week are used, the daily dose will be calculated as the average daily dose on a weekly basis. The LT4 dose/kg required to maintain TSH in target range will be

analyzed at 12 months of age visit (V8) and when the subject is 2, 4, 6, 9, 15, 18 and 21 months of age (V4, V5, V6, V7, V9, V10, V11) by means of analysis of covariance, including treatment group, newly diagnosed neonates vs infants already on LT4 therapy, age at inclusion and center as covariate.

Laboratory parameters (TSH and FT4) will be analysed at each time point by means of analysis of covariance, including basal value, treatment group, newly diagnosed neonates vs infants already on LT4 therapy, age at inclusion and center as covariate.If an adequate number of observations will be reached, the same analysis will be repeated for the TT4 parameter; otherwise the TT4 results will only be summarised by descriptive statistics.

The events of TSH values above 4.5 mU/L will be analyzed with a logistic model for each study visit with treatment group, newly diagnosed neonates vs infants already on LT4 therapy, age at inclusion and center as covariates. The laboratory result with a TSH value above 4.5 mU/L reported for the visit will be considered the analysis event.

The events of FT4 values below the middle of the laboratory normal range will be analyzed with a logistic model for each study visit with treatment group, newly diagnosed neonates vs infants already on LT4 therapy, age at inclusion and center as covariates. The laboratory result with a FT4 value below the middle of the laboratory normal range reported for the visit will be considered the analysis event.

Parent satisfaction assessed by “Parents’ Satisfaction Questionnaire” at each in-clinic visits (and during visit V1 only for infants that are already in therapy) will be analyzed by means of analysis of covariance, including treatment group, newly diagnosed neonates vs infants already on LT4 therapy, age at inclusion and center as covariate.

The subject medicine acceptance assessed by “Caregiver Administered Children’s Acceptance Tool (CareCAT)” will be analyzed during the first week, the third week, and the eighth week of administration comparing the treatment groups by means Cochran-Mantel-Haenszel chi² test.

19.3.3 EXPLORATORY OUTCOMES

For newly diagnosed neonates the time to normalize TSH into reference range and the time to normalize FT4 into the upper half of the laboratory normal FT4 range for age will be analysed by means of the Kaplan–Meier methods and log rank test.

19.3.4 SAFETY OUTCOMES

Vital signs at each in-person visit will be analyzed descriptively within treatment groups.

Length, weight and head circumference will be analysed at each in-person visit by means of basal value, treatment group, newly diagnosed neonates vs infants already on LT4 therapy, age at inclusion and center as coviarate.

The presence of signs and symptoms of hypo- and hyper-thyroidism at each planned visit will be compared between treatment groups by means of Chi² test.

Number of subjects experiencing AEs and SAEs during the study will be calculated. Number of AEs and SAEs will also be calculated. Difference between treatment groups on subjects who experienced AEs and SAEs will be evaluated using a Chi² test or a two-tailed Fisher's exact test as appropriate. Moreover adverse events will be described by MedDRA's System Organ Classes (SOCs) and Preferred Term (PT).

Similar summary tables will be provided for the number of AEs/SAEs at least possibly related to study treatment and for the number of AEs/SAEs leading to study discontinuation.

The number and percentage of subjects with AEs/SAEs will be summarized by their MedDRA preferred term within SOC.

Any subject reporting multiple episodes of the same AE/SAE (i.e. same preferred term) will be counted once.

Compliance will be calculated as the percentage of medication taken in relation to the recommended dose and the actual period of treatment (number of doses reported to be taken / number of doses envisaged x 100). Treatment Compliance will be described by means of descriptive statistics.

The number of subjects who need a dose re-adjustement during the long-term follow-up phase of the study will be compared between treatment groups by means of Chi² test.

19.3.5 ADJUSTMENT FOR MULTIPLE COMPARISONS

Not applicable

19.3.6 MISSING DATA

In general, there will be no substitutions made to accommodate missing data points. All data recorded on the CRF will be included in data listings that will accompany the clinical study.

For the efficacy and safety end points analysis, missing values will not be replaced.

If the start date of an AE is partially or completely missing, then the date will be compared as far as possible with the date of first study treatment dosing. The AE will be assumed to be treatment emergent (worst case approach) if it cannot be definitively shown that the AE did not occur or worsen during the safety period. The following general rules will be used:

- If the start day is missing, but the start month and year are complete, an AE will only be excluded as being treatment emergent if the start month/year is before the month/year of randomization or if the stop date is before randomization.

- If the start day and month are missing, but the start year is complete, an AE will only be excluded as being treatment emergent if start year is before the year of randomization or if the stop date is before randomization.
- If the start date is completely missing, an AE will be considered treatment emergent unless the stop date is before randomization.

19.3.7 INTERIM ANALYSIS

An interim analysis will be conducted on the TFTs normalization phase data, when 63 subjects (half of the recruitment target) complete the TFTs normalization phase of the study.

19.3.8 SENSITIVITY ANALYSES

Sensitivity analyses may be defined in the SAP in order to investigate the effects of missing values.

19.3.9 SUBGROUP AND COVARIANCE ANALYSES

Subgroup and additional covariance analyses may be performed and will be defined in the SAP in order to investigate the effect of baseline variables on efficacy and safety outcomes.

19.3.10 INDIVIDUAL PARTICIPANT DATA

All individual subject data recorded in the eCRF and all derived subject data used for statistical analyses will be provided in individual subject listings.

19.3.11 PROTOCOL DEVIATIONS

A protocol deviation is any non-compliance with the clinical trial protocol, ICH GCP, or ancillary study requirements. The non-compliance may be on the part of the subject, the PI, or the clinical site personnel. Deviation from the protocol is prohibited, except in medical emergencies or in unforeseen, isolated instances in which minor deviations that do not increase the subject's risk or affect study endpoints are carried out.

It is the responsibility of clinical site personnel to be vigilant in identifying and reporting to the clinical monitor each protocol deviation that occurs during the study. Any deviation that does fall into one (or more) of the following categories should be reported immediately to the clinical monitor: deviations that alter the benefit-risk profile, jeopardize subject safety or impact data integrity. Deviations that do not alter the benefit-risk profile, jeopardize subject safety or impact data integrity should be reported to the clinical monitor during the next monitoring visit. Following review of a deviation, the clinical monitor or Sponsor designee will discuss the deviation with the PI and, if applicable, suggest corrective action to prevent recurrence. Any corrective action should be implemented promptly by the clinical site. All protocol deviations will be listed in the Data

Review document, wherein they will be classified as major or minor according to the impact on subject safety, data integrity, and efficacy assessments.

20 MONITORING AND ACCESS TO DATA

20.1 MONITORING

Monitoring of the clinical site will be performed to ensure the rights and well-being of study subjects, that the reported study data are accurate, complete and verifiable, and that the study is being conducted in compliance with the protocol and any amendments, the most recent ICH GCP guidelines, FDA guidance and any applicable local regulations.

Clinical site monitoring procedures are described in detail in the Clinical Monitoring Plan (CMP). In general, monitoring of this study will be performed through on-site, remote and centralized activities. Risk based monitoring will be used by identifying, assessing, monitoring and mitigating the risks that could affect the quality of the study or subjects safety. The type, intensity and frequency of the monitoring activities will be revised periodically, if necessary, to ensure overall study quality. Any alteration in monitoring procedures will be reflected in the CMP. Audits are not mandated by the protocol but may be performed if needed.

Effective monitoring requires that the PI provide access to the clinical site facilities, applicable site personnel and source documents to the clinical monitor and/or Sponsor designee(s). In addition, the PI (or designee) must be prepared to allocate sufficient time to the clinical monitor and Sponsor designee(s) to facilitate completion of monitoring activities.

20.2 QUALITY CONTROL AND QUALITY ASSURANCE

The Sponsor (or designee) will deliver a study training session to each clinical site prior to allowing enrollment of neonates and infants. Clinical site personnel not in attendance at this session must be trained by the Sponsor, PI or respective designee(s), prior to participation in study-related activities.

Clinical monitors will follow written CRO's SOPs to verify that the study is being conducted in accordance with the protocol and any amendments, ICH GCP guidelines and applicable regulatory requirements.

The PI is responsible for ensuring the quality of overall study conduct at their clinical site, including but not limited to data collection and entry, control of Study Drug, biological sample collection, storage and shipment, and study document completion.

The PI will provide access to the clinical site facilities, applicable site personnel and source documents for monitoring and auditing by the Sponsor and/or designee, and inspection by local

and regulatory authorities. In addition, the PI or designee must allocate sufficient time to facilitate monitoring, auditing and/or inspectional activities.

Quality Tolerance Limits, as defined in a separate document, will be monitored during the course of the study.

20.3 SAFETY OVERSIGHT

The Sponsor will periodically review all AEs and SAEs individually and/or in aggregate to monitor the benefit-risk profile of the IP and thereby ensure the well-being of neonates and infants participating in the study. In addition, Medical Experts with appropriate expertise will review medical or safety issues upon Sponsor's request.

21 ETHICAL AND REGULATORY ASPECTS

21.1 DECLARATION OF HELSINKI

The study will be conducted in accordance with the Declaration of Helsinki (1996) and its amendments.

21.2 REGULATORY REQUIREMENTS

The sponsor or designated CRO will execute a Clinical Trial Agreement (CTA) with each clinical site that participates in the study.

This is a Phase 4 study and meets all the criteria for 21 CFR 312.2(b), and therefore is exempt from the IND requirements.

21.3 INSTITUTIONAL REVIEW BOARD (IRB) APPROVAL

The protocol and ancillary study documents will be submitted to the responsible IRB(s) and an unconditional approval document that references the protocol must be received before starting the study; consequently, IP will not be shipped to clinical sites and clinical sites will not be permitted to begin enrolling subjects until full approval has been obtained. Any subsequent protocol amendment and/or relevant information (e.g. serious adverse event) that becomes available after approval will be communicated to the IRB according to current effective guidance.

21.4 PROTOCOL AMENDMENTS

Neither the Sponsor nor PI will amend this protocol without first obtaining agreement from the other party. Each protocol amendment must be signed and dated by the Sponsor and PI. A major protocol amendment (e.g. informed consent form revision), except when it is necessary to

eliminate apparent immediate hazard to the subject, must be approved by the IRB before implementation. A minor protocol amendment (e.g. editorial change) does not need to be approved by the IRB before implementation. Minor amendments will be communicated to the IRB as part of the next major amendment, as applicable.

21.5 WRITTEN INFORMED CONSENT

IRB-approved documents, including the Subject Information Sheet and written or electronic ICF(s) describing the study intervention, procedures, and potential benefits and risks will be given to the parent or legally authorized representative prior to enrollment. These concepts will also be delivered verbally by the PI (or designee), in addition to insurance coverage and procedures in the event of a study-related injury. The PI (or designee) will also inform the parent or legally authorized representative that participation in the study is voluntary, that refusal to participate will not lead to loss of benefits or prejudice the relationship with the physician in any way, and that withdrawal from the study is possible at any time for any, or no reason.

The parent or legally authorized representative will have adequate time to read and consider the consent form(s) and clarify unanswered questions before being asked to sign. The informed consent process and the date performed will be recorded in the source documents and the relevant consent form(s) will be fully executed before the subject undergoes any study-specific procedures. The original signed consent form(s) will be kept with the subject's study documents, while the parent or legally authorized representative will be given a copy for future reference.

21.6 INSURANCE

Sponsor has undersigned an insurance policy covering neonates and infants who enter the study.

Sponsor will indemnify the PI and hold them harmless for claims or damages arising out of the investigation in excess of those covered by their own professional liability insurance provided that the IP was administered according to the protocol and any amendment, accepted medical practice, and under the supervision of the PI (or designee). This indemnification does not apply to claims for damages arising out of any act of omission or negligence by the PI or designee(s). The PI must notify Sponsor immediately upon notice of any claims or lawsuits.

21.7 CONFIDENTIALITY AND PRIVACY

Subject confidentiality and privacy is strictly held in trust by the PI, the Sponsor(s) and their respective representatives. This confidentiality is extended to cover testing of biological samples and genetic tests in addition to the clinical information relating to neonates and infants. Therefore, the study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study, or the resulting data will be released to any unauthorized third party without prior written approval of the Sponsor. The study will be registered

with clinicaltrials.gov, but subject-specific information will not be provided to or displayed on the website.

During and after the study, the subject's identity may become known to the Sponsor or designee as part of routine monitoring or auditing procedures, or regulatory authorities as part of study review. Disclosure of a subject's identity to any other party will require prior agreement of the subject or legally authorized representative, as applicable.

All research activities will be conducted in as private a setting as possible.

The Sponsor, clinical monitor or other authorized representative(s) of the Sponsor, representatives of the IRB or regulatory agencies may inspect all documents and records required to be maintained by the PI, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the neonates and infants in this study. The clinical site will permit access to such records. This information will be used by the Sponsor to support development of the Study Drug, and therefore may be disclosed to other investigators, development partners or regulatory authorities.

The study subject's contact information will be securely stored at each clinical site for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by the reviewing IRB, institutional policies, or Sponsor requirements.

Study subject research data, which is for purposes of statistical analysis and scientific reporting, will be transmitted to and stored by the statistician. This will not include the subject's contact or identifying information. Rather, individual neonates and infants and their research data will be identified by a unique study identification number. The study data entry and management systems used by clinical sites and data management will be secured, and password protected. At the end of the study, all study databases will be de-identified and archived by the Sponsor.

22 RECORD RETENTION

Study documents should be retained for a minimum of 2 years after the last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region, or until at least 2 years have elapsed since the formal discontinuation of clinical development of the Study Drug.

After completion of the study and analysis of the data, the essential documents (ICH GCP E6 R2, Chapter 8) will be stored in the Trial Master File in the Sponsor's archive for at least 25 years according to new European Union (EU) regulations. The essential documents will be stored in the Investigator Site File for the longest of the following periods: for 15 years or for the record retention period mandated by any federal, state, or local laws or regulations. The subjects' files (hospital charts, source documents, etc.) will be stored for the same period, or at least for the

maximum period allowed by institutional policies, if not otherwise agreed to by the Sponsor in writing.

Once the storage period has elapsed, the PI must contact the Sponsor (contacts are provided in the CTA). The PI is not allowed to destroy the study related documents without prior written authorization from the Sponsor. The Sponsor will inform the PI when such documents no longer need to be retained.

22.1 BIOLOGICAL SAMPLES

Biological samples will be managed, processed and analyzed at the sites selected laboratories. The samples will only be used for TH testing, and these tests should be performed within 24-72 hours of collection. Samples should not be stored for an extended period of time at the laboratory.

23 REPORTING

A clinical study report (CSR) will by prepared by the Sponsor (or designee) according to ICH guidelines.

24 PUBLICATION POLICY

The data collected during the study will be the property of the Sponsor. The Sponsor is entitled to publish and/or present any results of this study at scientific meetings, and to submit these clinical trial data to regulatory authorities. The PI agrees to treat any unpublished information supplied by the Sponsor or designee as confidential and ensure that confidentiality is maintained by all involved clinical site personnel.

The publication of any data derived from this study will be the result of a bilateral agreement between the PI and the Sponsor. The Sponsor has the right to review any manuscript about the study, and associated revisions will be delivered within 60 days of receipt. Revision of posters, abstracts or any other similar publications will be performed within 15 days. For multicenter studies, the Sponsor requires that the PI (or designee) does not publish any partial data before publication of the whole data set. The publication will neither disclose the identity of subjects nor any intellectual property information.

25 RESPONSIBILITIES

The PI is responsible for personally supervising study conduct at their clinical site to ensure compliance with the protocol, ancillary study processes, ICH GCP, the Declaration of Helsinki

and applicable regulatory requirements. By signing this protocol, the PI attests to their understanding of, and commitment to upholding this responsibility.

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27 SIGNATURES FOR APPROVAL

PRINCIPAL INVESTIGATOR

_____
[Name of the PI]

_____
Affiliation

Signature _____ *Date* _____/_____/_____
dd mmm yyyy

SPONSOR'S REPRESENTATIVE

[redacted]

IBSA

Signature _____ *Date* _____/_____/_____
dd mmm yyyy

OVERALL SPONSOR'S STUDY MANAGER

[redacted]

IBSA

Signature _____ *Date* _____/_____/_____
dd mmm yyyy

DATA MANANGER AND STATISTICIAN

[redacted]

IBSA

Signature _____ *Date* _____/_____/_____
dd mmm yyyy

DRUG SAFETY MANAGER

[redacted]

IBSA

Signature _____ *Date* _____/_____/_____
dd mmm yyyy