CLINICAL RESEARCH PROTOCOL

CONFIDENTIAL

Title

EFFICACY ASSESSMENT OF SYSTEMATIC FOLINIC ACID AND THYROID HORMONE TREATMENT ON THE PSYCHOMOTOR DEVELOPMENT OF YOUNG DOWN SYNDROME CHILDREN.
Phase 3 study - ACTHYF- IJL-AFHT-TH10

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List of the different amendments submitted and approved

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SIGNATURE PAGE OF A BIOMEDICAL RESEARCH PROTOCOL BY THE PRINCIPAL INVESTIGATOR AND THE REPRESENTATIVE OF THE SPONSOR

TITLE: EFFICACY ASSESSMENT OF SYSTEMATIC FOLINIC ACID AND THYROID HORMONE TREATMENT ON THE PSYCHOMOTOR DEVELOPMENT OF YOUNG DOWN SYNDROME CHILDREN

Phase 3 study - ACTHYF- IJL-AFHT-TH10

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I certify that I have read Version 19 of the Protocol, dated July 20, 2017, as well as the changes made by non-substantial amendment No. 9. I undertake to continue this study in accordance with this document, the national regulations in force and the principles of ICH and BPC.

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1. PROTOCOL SYNOPSIS

Sponsor	Institut Jérôme Lejeune
Title	**Evaluation of the efficacy of systematic folinic acid and thyroid hormone treatment on the psychomotor development of young Down syndrome children. ACTHYF**
Coordinator	Doctor Clotilde MIRCHER
Study site	Institut Jérôme Lejeune (IJL)
Study start date	02/04/2012
Phase and study duration	Phase 3, scheduled to last 60 months.
Primary objective	Evaluation of the following in newborn infants and very young children with Down syndrome: - the efficacy of systematic treatment with L-thyroxine at controlled doses (clinically and by ultrasensitive TSH assay) - the efficacy of systematic folinic acid treatment at a dose of 1 mg/kg/d - any interaction between these two treatments
Secondary objectives	Evaluation of the influence of genetic and biochemical factors (metabolism of monocarbons and redox) on the effect of the two treatments studied.
Diagnosis, selection criteria	Down syndrome, aged 6 to 18 months at inclusion.
Study treatments	L-thyroxine and folinic acid
Outcome measures	Griffiths mental development scale (GMDS) and Brunet-Lézine psychomotor development scale. Main Criteria: One-year evolution of the Global Quotient GQ, determined using the Griffiths mental development scale (GMDS).
Methodology	Double-blind, randomised, placebo-controlled trial for both treatments: thyroxine and folinic acid.
Number of cases	175
Statistical methods	The analysis of the primary endpoint, performed on the ITT population, will be an ANCOVA of the treatment effect (4 treatment groups: L-thyroxine alone, folinic acid alone, combination of the two treatments, placebo) with adjustment for the following covariates: sex, age class, value of primary endpoint at initial visit, and psychometric evaluator. This ANCOVA will make it possible to compare the three active treatments with the placebo by the Dunnett test.
Key words	Down syndrome thyroid hormone folinic acid clinical trial genetics paediatrics mental retardation psychomotor development

2. ABBREVIATIONS AND DEFINITIONS

2.1 ABBREVIATIONS

AIC	Akaike Information Criterion
ANSM	*Agence Nationale de Sécurité du Médicament* (French National Agency for Drug Safety)
GCP	Good Clinical Practices
BIC	Bayesian Information Criterion
BSC	Biological Sample Collection
C.P.P.	*Comité de Protection des Personnes* (Ethics Committee)
CNIL	*Commission Nationale Informatique et Libertés* (French data protection body)
DMC	Data Monitoring Committee
CRB	*Centre de Ressources Bologiques* (Biological resources centre)
CSP	*Code de la Santé Publique* (French Public Health Code)
INN	International Non-proprietary Name
SCH	subclinical hypothyroidism (mild thyroid failure)
NGS	Next Generation Sequencing
NSA	non-substantial amendment
SA	substantial modification
SAI	substantial modification for informational purposes
TH	thyroid hormones
IJL	Institut Jérôme Lejeune
D or d	day
kg	kilogram
µg	microgram
NA	not applicable
HC	head circumference
DQ	developmental quotient
IQ	intelligence quotient
SPC	summary of product characteristics
T	telephone contact
T21	trisomy 21 (Down syndrome)
V_1	initial visit
V_2	intermediate visit
V_3	final visit

2.2 KEY WORDS

Down syndrome, thyroid hormone, clinical trial, genetic, paediatrics, mental retardation, psychomotor development.

3. STUDY OUTLINE

The study outline, indicating the sequence for observation of each patient, in tabular format, is as follows:

Visit or telephone contact	***V_1***	***T***	***T***	***T***	***T***	***V_2***	***T***	***T***	***T***	***V_3***
Sequence	**D1**	**D15**	**D30**	**D60**	**D120**	**D180**	**D210**	**D240**	**D300**	**D360**
CLINICAL										
Selection criteria	X	X								
Functional signs	X		X	X	X	X	X	X	X	X
Clinical examination	X					X				X
Psychometric tests	X					X				X
LABORATORY										
FBC	X					X				X
T4,	X					X				X
TSH	X		X	X*		X	X*	X*		X
Ferritin	X					X				X
Monocarbon biochemistry	X					X				X
Redox biochemistry	X					X				X
Monocarbons by Next Generation Sequencing (NGS)	X									
BSC										X
Total blood volume taken	7 ml		2 ml			7 ml				9 ml
SAFETY		X	X	X	X	X	X	X	X	X
COMPLIANCE						X				X

* Optional assay

4. INTRODUCTION, STUDY RATIONALE

Down syndrome (trisomy 21 or T21) is the most common cause of mental retardation, affecting more than one baby in 1,000[1]. This proportion has remained relatively stable for the past 20 years[2], with the effect of neonatal screening being counter-balanced by the increase in maternal age. This means that there are over 50,000 people affected by T21 in France and 8 to 9 million worldwide. Their life expectancy has increased substantially, from approximately nine years in 1930 to over 50 years at present[3, 4].

The intelligence quotient of these patients varies considerably, ranging from 30 to 70, for an average IQ of approximately 50[5, 6], allowing some patients to live a relatively independent life, whereas others require substantial and permanent assistance throughout their lives.

Thyroid hormones and Down syndrome

Thyroid problems play a major role in the clinical characteristics of Down syndrome. The condition is frequently accompanied by hypothyroidism[7]. This link has long been known, even leading to confusion between the two entities in the early 20th century[8]. Indeed, several Down syndrome symptoms are also present in untreated congenital hypothyroidism: short stature, slowed development, dry skin, excess weight, bradycardia, and mental retardation. This high prevalence of hypothyroidism can be observed at all ages in the Down syndrome population[9]. In a series of 344 Down syndrome patients aged between 1 and 53 years, Rubello *et al.*[10] found a 35% rate of compensated subclinical hypothyroidism (SCH: normal T4 and elevated TSH). Among these cases, 18% had antithyroid antibodies. In the course of the longitudinal follow-up of 201 of these patients for a period of 24 months, progression towards patent hypothyroidism was demonstrated in 33% of cases. Hyperthyroidism, on the other hand, is much rarer[11]. In most cases, the dysthyroidism is of autoimmune origin. However, thyroid cancers, like many solid tumours, are exceptional in the Down syndrome population[12].

A study has estimated the proportion of congenital hypothyroidism to be 1% in newborn infants with Down syndrome[13], *i.e.* a risk that is 28 times higher than that of the general population. This observation of an abnormally high and, as yet, unexplained incidence of congenital hypothyroidism was recently examined in more detail, in an analysis of the results of screening for hypothyroidism in newborn babies[14]. Systematic assays of T4, conducted on all newborn infants in this study, demonstrated an original results profile in those affected by Down syndrome: the distribution of T4 is similar to that of the population, as a whole, but the mean is shifted downwards. After considering several factors (low birth weight, prematurity, or other medical problems as possible causes), the authors concluded that there is a disruption in thyroid metabolism specific to Down syndrome. This study also showed that, among all infants with low T4 levels, those affected by Down syndrome had an average TSH level that was twice that of the others. This elevation was recently confirmed[15]. It persists to at least the age of 26 months and does not appear to be of hypothalamic or autoimmune origin or to correspond to peripheral T3 resistance. This persistent elevation in TSH has clinical consequences. In a retrospective study in 94 Down syndrome children aged between 4 months and 4 years, Sharav *et al.* found an inverse correlation between TSH concentrations and weight in children aged between four months and two years[16].

Selikowitz[17] conducted a longitudinal study of the growth and intellectual development of 100 Down syndrome children, aged from two weeks to 11 years, over a period of five years. He did not find any significant difference for height and final IQ between the children with normal thyroid function (n = 88) and those with compensated subclinical hypothyroidism (n = 9). Conversely, when studying the intellectual and psychomotor repercussions in 35 adult Down syndrome patients, Bhaumik[18] found an inverse correlation between TSH levels and the score in two areas of the Vineland scale (adaptive behaviour scale).

Thyroid hormones and energy metabolism

Over 40 years after the introduction of systematic neonatal screening for hypothyroidism, the fundamental role of thyroid hormones in psychomotor development has been clearly established[19]. However, thyroid hormones do not only have a beneficial effect on brain development. The energy metabolism that they induce via the mitochondrial pathway can cause oxidative stress[20-22]. Thyroid hormone also plays an indirect role in oxidative stress, by inhibiting the expression of an enzyme - superoxide dismutase-1 - involved in the catabolism of reactive oxygen species (ROS)[23].

Treatment of hypothyroidism in Down syndrome

Although the need to treat patent hypothyroidism has not been questioned for a long time, the treatment of subclinical hypothyroidism is still the subject of debate. The issue is even more important for Down syndrome patients due to their mental retardation.

"Down syndrome treatment" using thyroid hormone has been proposed for many years, but studies conducted up until recently did not have a sufficiently rigorous methodology to allow confirmation of the relevance of this approach[8, 24].

A clinical study evaluating systematic thyroid hormone treatment of new-born babies and young children with Down syndrome was conducted in 2005 to assess the effect of thyroid hormone treatment (4 µg/kg/d) administered from birth until two years in 196 Down syndrome children[25]. The results demonstrate an excellent tolerance of thyroxine, with no major adverse effects recorded, and a significant benefit on the motor development and height and weight gain of these babies. However, psychomotor development was only shown to be significantly influenced by thyroid treatment in a secondary analysis, not in the principal intent-to-treat analysis. This ambiguous result may be partially explained by practical and methodological problems: by the authors' own admission, the primary outcome measure perhaps lacked the necessary sensitivity for medium and long-term evaluation. The exact conditions for these evaluations are also questionable.

Adverse effects of thyroid hormones

The excellent tolerance of thyroxine treatment observed in the study by van Trotsenburg *et al.* is consistent with the data in the literature as a whole. A review of the different possible side effects of thyroid hormones highlighted cardiovascular effects in subjects over the age of 60 years, whereas the data collected in children did not show any modification in cardiac function indices[26]. Thyroid hormone overdose has effects that can be easily detected clinically: increased heart rate, accelerated intestinal transit, rapid weight loss, sweating, nervousness, insomnia, and headaches. The results of the most recent therapeutic study[25] suggest that monitoring this clinical data is sufficient for optimal adjustment of treatment doses.

The development of osteoporosis or osteopenia secondary to long-term treatment with thyroid hormones has long been a subject of debate[27, 28, 29, 30, 31.] Recent data limits the possibility of this type of effect to cases in which TSH is completely blocked[32]. Other studies, conducted in children with

congenital hypothyroidism, are also reassuring in terms of bone mass integrity, even after several years of treatment[33-35].

In Down syndrome children aged from one to three years treated for several years, Koch *et al.* [36] did not find any major side effects, despite doses of up to 75 µg/day: the only adverse effects mentioned were diarrhoea, intolerance to heat, and irritability.

In view of these various studies, it can be concluded that regular clinical monitoring of treated children is all that is required, assisted by ultrasensitive assay of TSH, with the administration of thyroxine doses that do not lead to total suppression of TSH (optimal concentration: 1.5 ± 0.5 mIU/l, according to Williams[26].

Folates and Down syndrome

Folate deficiency is known to cause cognitive impairment and psychiatric disorders[37-39]. These problems can be effectively corrected by folic acid supplementation[40, 41].

For Down syndrome, a functional folate deficiency has been demonstrated *in vitro*, with an improvement in cell metabolism observed following the addition of folinic acid[42]. This may be explained by the fact that at least 7 genes involved in the metabolism of folates have been located on chromosome 21[43], including the reduced folate carrier gene (RFC); this carrier is used by methotrexate, which probably explains the significant toxicity of methotrexate in Down syndrome children treated for leukaemia[44]. Several studies have already attempted to evaluate the benefit of systematic folate treatment in Down syndrome children and adults, without obtaining conclusive results. Most studied only a very small number of patients and demonstrate methodological weaknesses. One was is restricted to a retrospective analysis of cases[45], whereas others studied mixtures of vitamins and minerals at non-controlled doses[46-48].

A more recent study was conducted on 156 Down syndrome infants to make a double-blind evaluation of the effect of folinic acid and several antioxidants[49]. The results do not demonstrate any significant effect of folinic acid on psychomotor development and cognitive performance. However, the folinic acid dose used in this study (0.1 mg/kg/d) appears to be too low to compensate for any real folate deficiency in these patients. In addition, the method for patient selection and recording of psychometric data may lead to a risk of a high dispersion of primary outcome measure results.

Another study was conducted on 113 Down syndrome infants who received double-blind treatment with folinic acid or placebo for a period of one year[50]. The dose used in the treated group was 10 times higher than the dose in the previous study (1 mg/kg/d). Tolerance was excellent, as no clinical or laboratory adverse effects related to the treatment being study were demonstrated. The efficacy of the treatment was evaluated on the basis of the psychomotor developmental age of the children using the Brunet-Lézine psychomotor test. The efficacy results did not permit unequivocal interpretation. Indeed, the intent-to-treat analysis did not reveal any significant difference in favour of the treatment, whereas the additional analysis, conducted on 87 infants and including psychometric assessments of each child performed by the same assessor, demonstrated a significant benefit of folinic acid treatment on developmental age.

Folates and the thyroid

In the most recent study published[50], contradictory results for the primary outcome measure were supplemented by a principal component analysis, suggesting that the potential benefit of folinic acid treatment might be particularly marked in infants treated with thyroid hormone at the same time. This positive interaction was not expected, and the study design did not enable more detailed analysis of this interaction. A hypothesis was been put forward to explain these results: as thyroid hormones stimulate mitochondrial energy metabolism, folinic acid may prevent oxidative stress linked to this stimulation.

Several studies conducted in patients without Down syndrome have demonstrated a correlation between thyroid status and blood homocysteine and folate levels[51; 52, 53; 54; 55]: homocysteine and TSH levels were positively correlated, whereas homocysteine and folate levels are inversely correlated. The mechanism of this relationship is still not fully understood and may involve functional polymorphisms of genes involved in folate metabolism[56]. A more recent study demonstrated a significant inverse correlation between IQ and homocysteine levels, and a significant direct correlation between IQ and folate levels in adults with Down syndrome[6]. No thyroid assay was conducted in this study.

Therefore, this possible positive interaction between folates and thyroid hormones still needs to be confirmed and examined in more detail.

5. CONCLUSION AND STUDY OBJECTIVES

It has been established that a good thyroid hormone balance is crucial for the correct development of a child's central nervous system. However, thyroid metabolism appears to be systematically impaired in newborn infants and young children with Down syndrome, with this chromosomal abnormality also consistently associated with mental retardation, to varying degrees, from a very young age.

In addition, folates act on brain function and their metabolism is disrupted in Down syndrome patients.

Despite the evidence, currently available data are not sufficient to predict whether the two potential treatments are actually beneficial for brain maturation and psychomotor development in Down syndrome patients, in the absence of demonstrated hypothyroidism and folate deficiency, nor whether they may have a synergistic effect when administered together.

The aim of this study is to evaluate the following aspects in very young children with Down syndrome, over a period of one year:

- the efficacy of systematic treatment with L-thyroxine at controlled doses clinically and by ultrasensitive TSH
- the efficacy of systematic folinic acid treatment at a 1 mg/kg daily dose
- any interaction between these two treatments

The psychometric assessment problems encountered in the various studied cited, including the most recent, led to the selection of an internationally validated psychomotor development scale, suitable for the population concerned by this study and recognised for its metrological qualities as the primary outcome measure: the Griffiths mental development scale (GMDS). This tool benefits from an established and standardised evaluation method[57-59].

It will be supplemented by a psychomotor development scale standardised in France (Revised Brunet-Lezine scale)[60].

6. SUBJECT SELECTION

6.1 INCLUSION CRITERIA

Patients corresponding to the following criteria will be selected:

1) Patients with a karyotype demonstrating homogeneous, free, or Robertsonian translocation of trisomy 21
2) Patients who have undergone a cardiac ultrasound demonstrating no severe heart disease or significant mitral valve leak
3) Patients aged 6 to 18 months at inclusion
4) Patients who can be reasonably expected to attend the visits scheduled as part of the study and capable of taking the study treatment and undergoing the scheduled tests, in particular the Griffiths test (GMDS), (in particular, no significant auditory or behavioural problem, as judged by the investigator)
5) Patients whose parents or legal representative can be contacted by telephone
6) Patients with at least one of the parents speaking to him in French
7) Patients whose parents or legal representative have received and understood the information document and signed the informed consent form

6.2 EXCLUSION CRITERIA

Patients corresponding to the following criteria will not be selected:

1) Gestational age < 231 days or 33 weeks (35 weeks of amenorrhea)
2) Apgar < 7 at 5 min after birth
3) If they have taken within the past three months or are currently taking folic or folinic acid
4) If they have taken or are currently taking L-thyroxine
5) Known allergy or hypersensitivity to calcium folinate (FOLINORAL) or any of its excipients
6) History of known allergy and / or hypersensitivity to LEVOTHYROX or any of its excipients
7) If they have presented with congenital hypothyroidism,
8) If they have hypothyroidism demonstrated by laboratory tests with TSH > 7 mIU/l
9) Presenting or having presented with hyperthyroidism
10) Having presented with a leukemoid reaction at birth
11) Presenting or having presented with leukaemia
12) Presenting or having presented with West syndrome or any other form of epilepsy or unstable neurological disease
13) Presenting or having presented with signs of central nervous system distress: CVA, postoperative hypoxia, meningitis
14) Presenting with severe heart disease or significant mitral valve leak on cardiac ultrasound, with haemodynamic effect
15) Presenting with non-controlled cardiac arrhythmia
16) Patients with a serious disease that could impact his participation to the study according to the investigator's opinion.

17) Patients participating or having participated in another interventional research protocol in the past two months
18) Parents that are not able to understand study's constraints

6.3 CRITERIA FOR PREMATURE WITHDRAWAL FROM THE STUDY

Parents have the option to stop their child's taking the experimental treatment at any time, for any reason.

The occurrence of an SAE does not necessarily lead to premature withdrawal from the trial.

Patients withdraw from the study in case of:

- any disease, which, in the investigator's opinion, could have a significant impact on the child's development and his/her assessment (West syndrome or other type of epilepsy, leukaemia, etc.)
- a request from the independent physician in charge of the follow-up of the TSH assays (see §8.1.1.3) in case of:
    - the occurrence of biological hyperthyroidism: that is, TSH < 0.5 mIU/L **and** if the independent physician deems it necessary
    - the occurrence of biological hypothyroidism:
        - for a randomized patient in one of the two groups treated with the thyroxine placebo: TSH > 7.0 mIU/L for two successive doses at 4 or 5-week intervals
        - for a randomized patient in one of the two groups treated with L-thyroxine: TSH > 7.0 mIU/L for two consecutive doses at 4 or 5-week intervals, despite a stable, adjusted daily dose of L-thyroxine for at least 4 weeks (maximum 8 µg/kg/day).
- a request of the trial sponsor or health authorities

In all cases, it is the responsibility of the independent physician to directly report to the investigator his decision to stop the study for the corresponding patient, as well as the reason and the measures to be taken.

6.4 PATIENT RECRUITMENT

Patients will be recruited among those consulting at the Investigational centre (Jérôme Lejeune Institute). The study is proposed by the investigating physicians to the parents of all patients meeting the inclusion criteria. For this purpose, a pre-inclusion test will be performed for all patients from 6 to 18 months of age in consultation.

Information on the purpose of this study and how to participate will be available on the website of the Institute and the Jérôme Lejeune Foundation so that families can contact us directly if they wish to obtain more information or to offer to participate. Progress of the study will also be communicated through letters from the Institute and the Jérôme Lejeune Foundation.

7. GENERAL STUDY OUTLINE

7.1 STUDY DESIGN

This is a single-centre, comparative, double-blind, placebo-controlled, therapeutic clinical trial conducted in parallel groups for the administration of each of the two treatments studied: L-thyroxine and folinic acid. Each patient will be treated and followed up for a period of one year.

Each patient will be randomised into one of the following four treatment groups:

- Folinic acid at a dose of 1 mg/kg/d and L-thyroxine at a controlled dose, initiated at 3 µg/kg/d,
- Folinic acid and L-thyroxine placebo,
- L-thyroxine and folinic acid placebo,
- Folinic acid placebo and L-thyroxine placebo.

Efficacy will be assessed after 6 and 12 months of treatment.

Where possible, patients who prematurely discontinue the study treatment will be assessed in the same way as the others during the final visit, to be scheduled as soon as possible after stopping treatment.

7.2 CHRONOLOGICAL DESCRIPTION

To avoid any recruitment bias, a form will be completed for all patients under the age of 18 months attending IJL for a consultation, specifying:

- Whether the patient has Down syndrome,
- The patient's exact age,
- Whether the legal representative can be contacted by telephone,

All patients consulting one of the investigating physicians and meeting the three criteria above will be invited to take part in the study.

The information and consent document will be given to the patient's parents. The document will be explained by the investigator, who will seek their consent.

Any reason for non-participation in the trial will be recorded: parents' refusal, selection criteria not met, etc.

The total observation period for a patient in the trial will be one year. Three visits will be necessary, corresponding to normal monitoring of a Down syndrome child of this age. Interim contact will be made by telephone, at least every two months.

With the parents' agreement, an information letter will be sent by IJL to the physician (paediatrician or general practitioner) responsible for routine follow-up of the patient on the day of his/her inclusion in the study.

Once the written consent of the patient's parents has been obtained, the detailed study sequence will be as follows:

7.2.1 Initial visit V_1

The initial assessment will include the following:

- Verification of selection criteria, including verifying the Down syndrome diagnosis, the presence of the karyotype, and the presence of a cardiac ultrasound in the medical records
- Assessment of the patient's psychomotor development, using the Griffiths test (GMDS) and the Brunet-Lézine test, by a qualified and trained assessor
- Recording of any medical and surgical history and concomitant medication and physical therapy during the study,
- Full clinical assessment: general examination, including biometrics (height, weight, head circumference), cardiac auscultation, and neurological assessment (systematic investigation for abnormal movements, tongue protrusion, tremor, examination of tone, standing, deep tendon reflexes),
- Heart rate and blood pressure (Dynamap®),
- Blood sample for initial biological assessment (FBC, free T4, TSH, plasma ferritin) and performance of the biochemical analyses scheduled:
    - Genomic analysis (by NGS sequencing) and biochemical assay of monocarbon metabolism. Biochemical analysis of oxidative stress markers.

In case of difficulties related to drawing of the blood sample, it may be performed outside the IJL in a biological analysis laboratory specialized in the drawing of paediatric blood samples. Patients will go there by taxi, paid for by the IJL.

- Prescription for biological assessment (TSH), to be performed 4 to 5 weeks later and to be repeated if necessary
- Randomisation and allocation of study treatments will then be performed, once all the tests and procedures scheduled in the protocol have been completed
- A follow-up diary will be given to the parents, along with all treatment required for the study until the next visit (V2), and the blood sampling kits for interim TSH controls.

7.2.2 *Telephone contacts, (T),*

After 4 to 5 weeks (D28-D35) for adjustment of L-thyroxine doses, if necessary, and at least every two months for assessment of treatment tolerance (behaviour, sleep, irritability, transit, weight gain curve, tremor) and recording of any adverse events.

7.2.3 Blood sample for assay of TSH between D28 and D35, repeated after the same time interval if necessary.

7.2.4 *Two months before visit V2:*

Reminder of the date for visit V2 by telephone.

7.2.5 Interim visit V_2, six months after the inclusion visit:

- Assessment of the patient's psychomotor development, using the Griffiths test (GMDS) and the Brunet-Lézine test, by a qualified and trained assessor, if possible the same assessor having performed the previous assessment
- Full clinical assessment: general examination, including biometrics (height, weight, head circumference), cardiac auscultation, and neurological assessment (tongue protrusion, systematic investigation for abnormal movements, tremor, examination of tone, standing, deep tendon reflexes)

- Overall assessment of treatment and tolerance by the practitioner (behaviour, sleep, irritability, transit, weight gain curve, tremor, etc.)
- Collection of the overall opinion of the IJL investigating physician concerning the child (CGI), using a semi-quantitative assessment grid
- Recording of modifications in concomitant medication or physical therapy and recording of any adverse events
- Blood sample for biological assessment (FBC, free T4, TSH, ferritin) and performance of the biochemical analyses scheduled:
    - biochemical assay of monocarbon metabolism, biochemical analysis of oxidative stress markers

In case of difficulties related to drawing of the blood sample, it may be performed outside the IJL in a biological analysis laboratory specialized in the drawing of paediatric blood samples. Patients will go there by taxi, paid for by the IJL.

- Recording of compliance with treatment by interview and collection of packaging for treatments supplied at the previous visit
- A follow-up diary will be given to the parents, along with all treatment required for the study until the final visit (V3), and the blood sampling kits for interim TSH controls.

7.2.6 *Telephone contacts, (T)*

After 4 to 5 weeks (D208-D215) for adjustment of L-thyroxine doses, if necessary, and at least every two months for assessment of treatment tolerance (behaviour, sleep, irritability, transit, weight gain curve, tremor) and recording of any adverse events.

7.2.7 Blood sample for assay of TSH between D208 and D215, repeated once if necessary.

7.2.8 Two months before visit V_3:

Reminder of the date for visit V3 by telephone.

7.2.9 Final visit V_3, 12 months after the inclusion visit:

- Assessment of the patient's psychomotor development, using the Griffiths test (GMDS) and the Brunet-Lézine test, by a qualified and trained assessor, if possible the same assessor having performed the two previous assessments
- Full clinical assessment: general examination, including biometrics (height, weight, head circumference), cardiac auscultation, and neurological assessment (systematic investigation for abnormal movements, tongue protrusion, tremor, examination of tone, standing, walking, deep tendon reflexes, sphincter control)
- Overall assessment of treatment and tolerance by the practitioner (behaviour, sleep, irritability, transit, weight gain curve, tremor, etc.)
- collection of the opinion of the IJL investigating physician concerning the child (CGI), using a semi-quantitative assessment grid
- Recording of modifications in concomitant medication or physical therapy
- Recording of any adverse events
- Recording of compliance with treatment by interview and collection of packaging for treatments supplied at the previous visit
- Blood sample for biological assessmennt (FBC, free T4, TSH, ferritin, plasma homocysteine) and performance of the analyses scheduled:
    - Biochemical assay of monocarbon metabolism
    - Biochemical analysis of oxidative stress markers

- Transformation of lymphocytes into lymphoblastoid cells for storage with DNA and plasma in a biological sample collection (BSC)

In case of difficulties related to drawing of the blood sample, it may be performed outside the IJL in a biological analysis laboratory specialized in the drawing of paediatric blood samples. Patients will go there by taxi, paid for by the IJL.

The last assessment will be considered to be the final assessment for efficacy and safety.

7.2.10 Follow-up after the end of the study

The study treatments will be stopped after the final visit (V3).

The independent physician may make special recommendations for monitoring the patient in view of the results of the V3 T4 and TSH.

Unless specifically recommended by the independent physician, two thyroid examinations (TSH, T4) will be systematically prescribed by the investigating physician to be carried out two and four months after stopping the study treatment.

Following the study, the patients will be monitored by their usual attending physician.

7.2.11 Exclusion period

Patients cannot be included in another study protocol during the two months following the last dose of the study treatment.

8. INVESTIGATIONAL DRUG

8.1 STUDY PRODUCTS

These are included in the products listed in article L 5311-1 of the French Public Health Code (CSP). They are not included in the products listed in article R. 1211-29 nor labile blood products.

8.1.1 Thyroxine

8.1.1.1 Form, packaging, and administration

The levothyroxine prescribed will be L-Thyroxine in the form of tablets containing 25 µg of levothyroxine sodium (INN). This form will be repackaged for the study and supplied to patients by each investigator. The French summary of product characteristics (SmPC) is attached in the appendix. The tablets will be designed to be divided to allow the administration of as accurate a dose as possible. Their contents will be crushed using a tablet crusher supplied with the treatments and then mixed with a small portion of food (fruit, jam, yoghurt, etc.) or cold liquid. The treatment must be taken immediately after preparation in the morning, before breakfast.

8.1.1.2 Initial dosage

This will be 3± 0.2 µg/kg/d, in accordance with the following rule:

WEIGHT (Kg)	Total dose / d (µg)	Number of tablets / d
≤ 5.2	12.5	½ (0.5)
5.2 – 7.28	18.75	¾ (0.75)
7.29 – 9.37	25	1 (1)
9.38 – 11.45	31.25	1 ¼ (1.25)
11.46 – 13.54	37.5	1½ 1.5
13.55 – 15.61	43.75	1¾ (1.75)
15.62 – 17.71	50	2 (2)

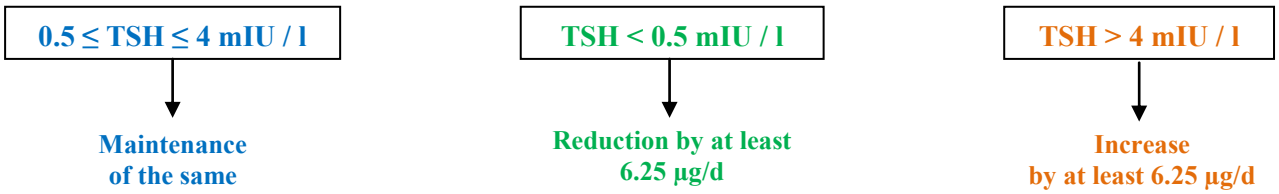
8.1.1.3 Dosage adjustment methods and compliance with double-blind conditions

A TSH Assay will be performed after 4 to 5 weeks of fixed-dose treatment for all infants taking part in the study.

A physician, independent of the sponsor and the investigators, who has no contact with them and remains geographically distant throughout the study, will be the only recipient of the TSH and T4 assay results for all the patients in the context of the study. Only this physician will know which treatment is given to each infant: L-thyroxine or placebo. Any dosage adjustment must be immediately communicated to the physician monitoring the child at the investigator centre (Institut Jerome Lejeune), following receipt of the laboratory results by the independent physician. It must then be

immediately communicated to the patient's parents by the investigator centre physician, by telephone. The independent physician will be able to contact the patient's parents directly by telephone should he deem it necessary.

For each infant treated with L-thyroxine, the dosage adjustment will be prescribed in accordance with the following three result scenarios:



The TSH assay will then be repeated, if the independent physician deems it necessary, after the same interval and according to the same conditions (after 4 to 5 weeks of fixed-dose treatment). This assay will be repeated at each visit scheduled by the study (6 and 12 months after the start of treatment) for all the infants included.

To maintain the double-blind conditions for all participants in the study, the independent physician will be the only recipient of the TSH results. He will prescribe the dosage adjustments for the group treated by placebo in a similar way to those prescribed in the group treated with L-thyroxine, in accordance with a decision rule reviewed by Effi-Stat and validated by the Data Monitoring Committee (CISD/DMC) for the study.

8.1.2 Thyroxine placebo

This will be supplied in an identical form and will consist of inactive excipients.

8.1.3 Folinic acid

8.1.3.1 Form and administration

Folinic acid will be administered in the form of calcium folinate capsules containing 5 mg. The product's characteristics are indicated in the SPC appended. The capsules must never be administered as they are, but must first be opened with scissors and the contents mixed with cold food (fruit purée, jam, yoghurt, etc.). The resulting preparation should be given to the patient immediately, as a single daily dose.

8.1.3.2 Dosage

The dosage adopted will be 1.0 ± 0.3 mg/kg/d.

The decision outline setting the daily dosage will be as follows:

WEIGHT (kg)	DOSE (mg/d)	Number of CAPSULES/d
≤ 7.3	5	1
7.4 – 10.9	10	2
11.0 – 15.9	15	3
16.0 – 28.0	20	4

.

8.1.4 Folinic acid placebo

This will be supplied in an identical form and will consist of inactive excipients.

8.1.5 Compliance and measures in the event of a missed dose

Treatment compliance will be assessed by interview and collection of treatment packaging. If a dose of product is missed, it will not be necessary to administer the missed dose at the same time as the next dose. Treatment compliance will be assessed by interview and collection of treatment packaging for both treatments.

8.1.6 Treatment duration

The treatment duration scheduled for each patient is one year.

8.2 RANDOM ALLOCATION METHOD (RANDOMISATION)

Randomisation will be performed by computer, at the end of each patient's inclusion visit, using a programme produced by ASCOPHARM, stratified based on age and gender. Randomisation will also include minimisation to ensure a balance in the treatment groups at the interim analysis scheduled.

8.3 CONCOMITANT TREATMENTS

All concomitant medication and physical therapy will be recorded in the case report form, with trade names and/or INN.

8.4 DIET, HEALTH AND DIETARY ADVICE, LIFESTYLE

Any major changes in the patient's routine habits will be recorded.

9. VIGILANCE, ADVERSE EVENTS

9.1 DEFINITIONS

The investigational products examined in the present trial are included in the products listed in article L 5311-1 of the French Public Health Code (CSP), with the exclusion of the products listed in article R. 1211-29 and labile blood products. The definitions are those of article R1123-39 in the CSP.

9.1.1 Adverse event

Any untoward medical occurrence in a person taking part in a biomedical research study, regardless of whether this event is related to the study or the product being investigated in the study.

9.1.2 Serious adverse event or reaction

Any reaction or event that meets at least one of the following conditions, regardless of the dose of the study product administered:

- results in death,
- is life-threatening to the patient taking part in the study,
- requires hospitalisation or prolongation of existing hospitalisation,
- causes incapacity or significant or persistent disability,
- leads to a congenital anomaly or malformation,
- is medically important.

Note:

The term "life-threatening" refers to an event in which the subject was at immediate risk of death at the time of the event. It does not refer to an event which hypothetically might have caused death if it were more severe.

Medical and scientific judgement should be exercised in deciding whether an event is serious if it does not lead to any of the conditions cited above. Any important event that can be life-threatening or might require intervention to prevent one of the other outcomes listed in the definition above should be considered serious.

9.1.3 Adverse reaction of a study drug

Any harmful or unintended reaction to a study product, regardless of the dose administered. All adverse events for which the investigator and sponsor consider that a causal relationship with the study drug may be reasonably envisaged must be considered to be suspected adverse drug reactions.

9.1.4 Unexpected adverse drug reaction

Any adverse reaction for which the nature, severity, or development are not consistent with the information indicated in the documents stipulated in articles R. 1123-20 and R. 1123-30 of the CSP. For the study drugs used in this trial, this information is indicated in the summary of product characteristics for each of the two products used (see annexes).

9.1.5 New information

Any new safety information that might lead to reassessment of the risk-to-benefit ratio of the study or study drug, or which could be sufficient to consider modifications to administration of the study drug, the conduct of the study, or documents relative to the study.

9.2 ADVERSE EVENTS: MONITORING, REPORTING METHOD AND RESPONSIBILITIES

Adverse events must be managed and reported in accordance with the applicable regulations; they must be mentioned in the final study report.

9.2.1 Observation period:

The period for the observation and recording of adverse events runs from signature of the informed consent form until the last visit scheduled by the protocol. Adverse events occurring after the end of this observation period will be reported by the investigator if he considers that there is a causal relationship with the study product.

9.2.2 Investigator's responsibilities

The investigator must respect the legislative and regulatory provisions in force relative to reporting of serious adverse events and abnormal test results scheduled, in particular, in articles L. 1123-10 and R. 1123-54 of the French public health code (CSP).

All adverse events, regardless of the seriousness and causal relationship with the study drug must be recorded in the relevant pages of the case report form. The investigator must specify the date of occurrence of the event, its nature, its severity, the measures taken with respect to the study drug, the treatment initiated, the final outcome and his opinion regarding the causal relationship between the adverse event and the study drug.

The investigator must take all appropriate measures to ensure the safety of patients and, in particular, monitor the course of any adverse event (clinical signs, test results or other) until a return to normal or consolidation of the patient's condition.

Abnormal results for laboratory tests or additional tests must be reported as adverse events only if they are medically relevant: symptomatic or requiring treatment, or leading to withdrawal from the trial and/or meeting a seriousness criterion.

9.2.2.1 Notification

In the event of discontinuation of the study treatment due to a non-serious adverse event, the investigator must send - preferably directly or by fax - the corresponding dated and signed pages of the case report forms within a period of 24 working hours (with "yes" checked for the "discontinuation of study treatment" box in "measures taken", along with the end-of-study form indicating "Adverse event" as the reason checked) to the clinical project manager, whose name, address, and fax number are indicated in the protocol.

9.2.2.2 Serious Adverse Events (SAEs)

Serious adverse events will be recorded during the period running from signature of the informed consent form until the last visit scheduled by the protocol. If a serious adverse event comes to the attention of the investigator after discontinuation of the study drug and if the investigator deems that this event may reasonably have been caused by the study drug, this must be reported.

In the event of a serious adverse event, the patient must be followed up until he/she has recovered and until test results have returned to normal or progression has been stabilised. This means that the follow-up period may be extended beyond the patient's participation in the clinical trial and that additional information may be requested by the sponsor.

The investigator must notify the sponsor of all serious adverse events.

The following situations are not considered to be SAIs:

- Hospitalization of less than 24 hours; the research participant is admitted for less than 24 hours for a non-serious medical reason (see the severity criteria mentioned in chapter 9.1.2)

- Any hospitalization scheduled before the signature of consent, even if it is then carried out after the signature of consent

- Any hospitalization planned after the signature of consent but carried out within the framework of the usual follow-up of the pathology of the patient or associated pathologies (ex. adenoidectomy, transtympanic aerator placement, testicular lowering, etc.)

9.2.2.2.1 Initial notification

In the event of a serious adverse event, the investigator must immediately:

- send (within a period of 24 working hours) the corresponding dated and signed pages of the case report form to the clinical project manager, whose name, address and fax number are indicated in the protocol
- enclose a photocopy of all tests performed and indicate the dates on which these were performed. It is necessary to protect the patient's anonymity and to indicate the patient's identifiers in the context of the clinical trial on any copies of original documents sent to the study sponsor.

9.2.2.2.2 Follow-up

The investigator's initial notification sent to the sponsor must be rapidly followed by one or more additional detailed written reports. The initial report and follow-up reports must identify the patient using the unique number allocated to him/her for the study.

For serious adverse events which caused the patient's death or were life-threatening, a follow-up report must be sent within an additional deadline of one calendar week.

The sponsor may request additional information from the investigator concerning each serious adverse event.

9.2.2.2.3 Overdose

An accidental or deliberate overdose (*i.e.* over two times the maximum daily dose used in this protocol for the study drug), even if this overdose does not meet any seriousness criteria, must be reported to the sponsor within a period of one working day, using the additional SAE form, which must be sent to the clinical project manager, whose name, address, and fax number are indicated in the protocol.

9.2.3 Sponsor's responsibilities

The sponsor must respect the legislative and regulatory provisions in force (scheduled, in particular, in articles L. 1123-10 and R. 1123-38 and subsequent articles of the French public health code (CSP)).

The sponsor must declare any suspected unexpected serious adverse reactions and new information to the ANSM and the relevant ethics committee (CPP) in accordance with the legislative and regulatory provisions in force. The sponsor must also send a safety report taking into account all safety information available to the ANSM and the relevant ethics committee (CPP).

9.2.3.1 Recording and evaluation of adverse events

Each adverse event must be evaluated by the sponsor, including evaluation of the seriousness and causal relationship between the adverse event and the study drug(s) or concomitant treatment(s). The sponsor must also assess whether the adverse reaction is expected or otherwise.

The evaluations will be performed in accordance with the definitions given in § 9.1 of the protocol.

The sponsor must keep detailed records of all the adverse events reported by investigators. These records will be passed on to the ANSM on request.

9.2.3.1.1 Evaluation of the causal relationship

All adverse events for which the investigator and the sponsor consider that a causal relationship with the study drug may reasonably be envisaged must be considered to be suspected adverse drug reactions.

When the evaluation of the causal relationship performed by the investigator and the sponsor differ, both evaluations must be indicated in the declaration sent to the ANSM. The evaluation made by the investigator may not be modified by the sponsor.

9.2.3.2 Notification of safety data

The sponsor must notify the ANSM and the ethics committee (CPP) of all safety data from the date the study is authorised in France. It must also notify the ANSM of any new information emerging during the clinical trial authorisation application assessment period. It will send the ANSM and the ethics committee (CPP) an annual safety update report (ASUR).

9.2.3.2.1 Suspected unexpected serious adverse reactions (SUSARs)

Once the information essential for their notification is obtained, the sponsor must notify the ANSM and the ethics committee (CPP) of any SUSARs potentially due to the study drug that occur during the study:

- within a maximum period of seven calendar days in case of death or a life-threatening event. A follow-up report must be sent to the ANSM and the relevant ethics committee (CPP) within a further period of eight days after the initial seven-day period.
- within a maximum period of 15 calendar days for other SUSARs. Additional relevant information must be sent within a further period of eight days after the initial 15-day period.

Any SUSAR must also be subject to electronic notification by the sponsor, within the same periods, in the Eudravigilance European database relative to adverse drug reactions implemented by the European Medicines Agency (EMEA).

The sponsor will inform the MA holder of the corresponding product about the notification made to the ANSM and the ethics committee CPP, as well as its content.

9.2.3.2.2 Other safety data requiring immediate notification

Any safety data or new information that could significantly modify assessment of the risk-to-benefit ratio of the study drug or the trial, or which could be sufficient to consider modifications to administration of the study drug or the conduct of the study must be declared to the ANSM and the ethics committee (CPP) within a maximum period of 15 days. Additional information must be sent within a further period of 15 days after the initial notification.

9.2.4 Unblinding

Unblinding may be performed by the investigator if a serious adverse event is declared that might be due to one the study treatments.

10. PREMATURE WITHDRAWALS, PATIENTS LOST TO FOLLOW-UP

10.1 GENERALITIES

Exit criteria are defined in §6.3.

10.2 PROCEDURE FOR PREMATURE WITHDRAWAL FROM THE TRIAL

In all cases, the reason and date of discontinuation, when this has been confirmed, must be indicated in the case report form and in the subject's medical records. In case of premature release for an adverse event, the subjects will be followed until recovery from or stabilization of the adverse event that has been followed: this must be reported in accordance with the adverse event notification procedures.

All subjects who have been treated but did not complete the trial in accordance with the protocol must undergo all the tests scheduled for the final visit if possible, as soon as possible.

10.3 PATIENTS LOST TO FOLLOW-UP

The investigator must try to contact subjects lost to follow-up to find out why they did not attend the visit and to obtain information about their state of health and, in particular, any death. This contact attempt must be documented in the subject's medical records (date and time of telephone contact, proof of receipt of a recorded delivery letter, etc.).

Subjects not having completed the trial and for whom the outcome measures are not available must be considered to be "lost to follow-up". The statistical analysis plan will specify how the data for these subjects "lost to follow-up" should be processed.

Subjects having left the trial cannot be included again and will not be replaced. Their inclusion number and treatment number must not be reused.

11. ITEMS RECORDED AND OUTCOME MEASURES

11.1 DATA COLLECTION

The information concerning each patient will be recorded in the case report form provided for this purpose as the study proceeds.

Each case report form will be dated, initialled and signed by the investigator. The investigator may be assisted by an identified contracted person to copy certain data into the case report form, as long as he checks and initials these data.

11.2 FUNCTIONAL AND CLINICAL ITEMS

The following items will be recorded:

- interview, with investigation for the presence of headaches, behavioural or sleep disturbances, the presence of abnormal movements, gastrointestinal disturbances, sleep disorders, or irritability
- general examination, height, weight, head circumference, cardiac auscultation, recording of heart rate, recording of blood pressure (Dynamap)
- neurological assessment, including systematic investigation for abnormal movements, tongue protrusion, tremor, examination of tone, standing, walking, deep tendon reflexes, and sphincter control
- Griffiths test
- Brunet-Lézine test
- concomitant medication and physical therapy
- adverse events

11.3 ADDITIONAL TESTS

The following tests will be performed:

- At the first visit, a portion of the blood sample collected will be used for genotyping of the variants of genes involved in folate and monocarbon radical metabolism by next generation sequencing (NGS)
- At each visit (V1, V2, V3), a blood test will be taken for the following tests:
    - standard laboratory tests: FBC, T4, TSH, Ferritin
    - biochemical analysis of monocarbon metabolism (plasma homocysteine, folate, B12, methylmalonic acid, succinic acid, S- adenosyl methionine, S- adenosyl homocysteine)
    - redox biochemical analysis of oxidative stress markers (total antioxidant status, LDL-TBARS, superoxide dismutase, glutathione peroxidase, catalase, glutathione)
- 4 to 5 weeks after the start of treatment (V1), a blood sample will be taken for assay of TSH, and again after the same interval if necessary. The same assay may be performed in the same conditions after the second visit (V2).

At the last visit (V3), a 2-ml blood sample will be used for transformation of lymphocytes into lymphoblastoid cells for storage with DNA and plasma in a biological sample collection (BSC),

To maintain the double-blind conditions, an independent specialist physician not connected to IJL will be the only recipient of the TSH results after the initial visit (V1). He will make the requests for adjustment of L-thyroxine doses accordingly (see chapter 8).

11.3.1 Methods

Standard laboratory tests (FBC, T4, TSH, Ferritin): the tubes must be taken immediately or centrifuged at 3000 rpm for 10 min at 4°C for treatment by a specialised laboratory, which will centralise analysis and recording of this data:

- FBC: one 2-mL BD EDTA K2 tube (ref: 368841),
- T4, TSH, Ferritin: one 2-mL BD Cat Coagulation Activator tube (ref: 368492).

The interim TSH assays will be performed by the same laboratory, on one 2-mL BD Cat Coagulation Activator tube (ref: 368492), using sampling kits supplied to the patients' parents at the first two visits. The samples will be taken in a laboratory close to the patients' homes and collected by a specialised carrier.

For biochemical analysis of monocarbon metabolism (plasma homocysteine, folate, B12, methylmalonic acid, succinic acid, S- Adenosyl Methionine, S- Adenosyl Homocysteine) and genotyping of monocarbon metabolism by next generation sequencing (NGS) of the variants of genes involved in folate and monocarbon radical metabolism: one 2-mL BD EDTA K2 tube (ref: 368841), will be centrifuged and frozen for subsequent batched shipment to the INSERM U 954 laboratory (Director J. L. GUEANT).

For RedOx biochemical analysis (AOPP, total thioles, carbonyls, total antioxidant status, oxidised LDL- , superoxide dismutase, catalase, glutathione, glutathione reductase, glutathione oxidase): one 2-mL HL tube (ref: 368494) will be centrifuged and frozen for subsequent batched shipment to the biochemistry laboratory at Cochin Teaching Hospital (Dr Didier Borderie) and the biochemistry laboratory at Pitié-Salpétrière University Hospital (Dr Dominique Bonnefont-Rousselot).

To interpret the result of methylmalonic acid (MMA), Pr Didier Borderie (Laboratory of Automated Biology / DBA) recommends the determination of creatinine at V1. No additional sampling will be required from the patient.

For transformation of lymphocytes into lymphoblastoid cells for storage with DNA and plasma, one 2-mL HL tube (ref: 368494) will be sent to the biological resources centre (CRB) of the IJL (BioJel).

11.4 OUTCOME MEASURES

11.4.1 Primary efficacy outcome measure

The primary outcome measure is the Griffiths Mental Development Scale (GMDS) (see chapter 12).

11.4.2 Secondary outcome measures

The secondary outcome measures are:

- The Brunet Lézine test
- Collection of the opinion of the IJL investigating physician concerning the child (CGI)
- Biometric parameters (height, weight, head circumference), standard laboratory data: FBC, T4, TSH, Ferritin

11.4.3 Tolerance of study treatments

This will be assessed clinically and on the basis of laboratory results. The evolution of biological constants will be monitored; these must remain compatible with continuation of treatment, in accordance with the protocol, paragraphs 7 and 8.1.1.3.

12. STATISTICS

For all scheduled analyses, a review of blinding of the first data will be performed after the data-management procedures (recording of data, clean-up, verification of consistency and correction process). The analysis populations will be defined before the database is frozen. No corrections may be made to the data after unblinding.

12.1 ANALYSIS POPULATIONS

Three analysis populations have been defined.

Patients with no values for the primary outcome measure at the initial visit (V1) or the final visit (V3) will be excluded from the analysis population.

- Intent to treat (ITT): this analysis population includes all randomised patients, regardless of whether or not they received the study treatment.
- Safety (S): this analysis population includes randomised patients having received at least one dose of treatment and who have at least one safety value after the initial visit (V1).
- Per protocol (PP): this population includes randomised patients in the ITT population who did not demonstrate any major protocol violation, i.e. without any deviation that might affect assessment of the treatment effect of the primary outcome measure.

12.2 STATISTICAL ANALYSIS PLAN (SAP)

The statistical analysis plan (SAP) will be produced and finalised before the database freeze and unblinding for final analysis.

The SAP will give complete details of the analysis, the results presented, and derivation of data.

The SAP will contain definitions of major and minor protocol deviations, along with the link between the type of deviation and the analysis populations.

Minor and major protocol deviations will be defined with the principal investigator before the final analysis.

12.3 BASELINE

Descriptive statistics will be performed on the criteria recorded at the initial visit (baseline) with no comparison test on the groups.

Quantitative demographic data (age, weight, height, etc.) will be summarised by conventional statistics (mean, standard deviation, median, minimum, maximum, quartiles).

Qualitative demographic data (age in categories. gender, etc.) will be summarised by counts and percentages.

The other baseline characteristics (medical history, previous and concomitant treatments) will be listed by patient.

12.4 PRIMARY OUTCOME MEASURE

The primary outcome measure is the evolution of the developmental quotient (Global Quotient GQ) after one year, determined on the Griffiths Mental Development Scale (GMDS).

The primary analysis of the primary outcome measure will be performed on the ITT population.

12.4.1 Model

The model is a 2-way ANCOVA (analysis of covariance) of the treatment effect (four groups) with covariate adjustment. The developmental quotient (GQ) will be explained by the administration of L-Thyroxine (Thyr) alone, the administration of folinic acid (B9) alone, and the administration of folinic acid and L-Thyroxine together, adjusted based on age (two groups), gender, the psychometric assessor (PA), and the value of the developmental quotient at visit 1 (GQ0).

$GQ=\alpha_0+ \alpha_1*B9+ \alpha_2*Thyr+ \alpha_3*B9*Thyr+ \alpha_4*gender+ \alpha_5*age+ \alpha_6*PA+ \alpha7*GQ0$

In this model, the values α1, α2, and α3 are the respective effects of B9, Thyr, and B9Thyr vs Placebo.

12.4.2 Analysis strategy

The primary outcome measure will be assessed using a two-step procedure.

The bilateral risk of the first type analyses below is 5%, which corresponds to 2.5% of unilateral risk.

Step 1

We will first focus on three tests

- L-Thyroxine vs Placebo
- folinic acid vs placebo
- L-Thyroxine + folinic acid vs placebo

We will perform, in a one-sided logic (only superiority of the active groups vs placebo is considered to be "significant"), the Dunnett "step-down" test of the three comparisons of "L-Thyroxine", "folinic acid", and "acid Folinic + L-Thyroxine "vs Placebo. With the step-down procedure, the significance of the best of the three treatments in the Dunnett test allows Dunnett's test of the two remaining comparisons to be performed in a more powerful way; a new significance finally allows the last comparison to be tested, without further adjustment for multiplicity.

The Dunnett "step-down" test is a direct application of the test method of the multiplicity of tests by the "closed test procedure" [61].

Step 2

We will then focus on the two following comparisons:

- L-Thyroxine + folinic acid vs L-Thyroxine
- L-Thyroxine + folinic acid vs folinic acid

The application of the rules of the "closed test procedure" leads to the following strategy.

It first requires that the previously performed "L-Thyroxine + folinic acid vs placebo" test was significant (step 1).

The Dunnett "step-down" test is then performed on the two comparisons "L-Thyroxine + folinic acid vs L-Thyroxine" and "L-Thyroxine + folinic acid vs folinic acid" at an overall threshold of 2.5%.

However,

- for the comparison "L-Thyroxine + folinic acid vs L-Thyroxine" to be considered significant, it is also necessary that folinic acid alone vs placebo was judged to be significant in step 1

- for the comparison "L-Thyroxine + folinic acid vs. folinic acid" to be considered significant, it is also necessary that L-Thyroxine alone vs placebo was judged to be significant in step 1

- *For purely documentary purposes (i.e. apart from the rules dictated by the "closed-test procedure", the contrasting test "L-Thyroxine vs folinic acid" can be performed.*

- *For complementary and exploratory purposes, after analysis of the complete model, we can seek to simplify the model based on the BIC criterion (so that the model chosen, which is more parsimonious, is the most plausible), and / or the basis of the AIC criterion (so that the model chosen provides the best predictions). In this context, we can also explore possible reparameterization of the treatment effect in the "global" effects of L-Thyroxine and folinic acid and the interaction of the two treatments.*

12.4.3 Number of cases

The initial planned enrolment was 256 patients, with an interim analysis at 50% of the data. The number was increased to 264 patients in Amendment 7 with two interim analyses at 33% and 66% of the data. Enrolment is now limited to 175 patients (without interim analysis), because of the difficulties encountered in recruiting patients, with an expected enrolment of 140 evaluable patients.

12.4.4 Power of the trial

As in the initial protocol, we assume an intra-group standard deviation (SD) of the main criterion of 10.2 and a correlation of 0.5 between the initial measurement of V1 and the final one of V3, which leads to a standard deviation of the model equal to 8.8335 ($10.2 \times \sqrt{(1-0.5^2)}$).

If there is, in fact, a real effect of six points for the main criterion for only one of the three treatments (that is to say for an effect size ES = effect/standard deviation = 6/8.8335 = 0.6792), the power will be 67.9% for an enrolment of 140 patients.

If there is such a six-point effect for two treatments, the best effect observed will be increased on average by $\sigma/\sqrt{(\pi.n_g)}$, with n_g the number of participants per group, and its real variance will be lower (by a factor of $1 - 1/\pi$). The expectation of the test statistic used (with known variance) will be ES.

$\sqrt{(n_g/2)} + 1/\sqrt{(2\pi)}$ (instead of ES $\sqrt{(n_g/2)}$) with a variance of $1-1/(2\pi)$ (instead of 1). By studentising, we therefore make the approximation that $t_{1-\beta} = [ES. \sqrt{(ng / 2)} + 1 / \sqrt{(2\pi)} -VC] / \sqrt{[1-1 / (2\pi)]}$, resulting in a power $(1-\beta)$ of 82.6%.

If this six-point effect exists for three treatments, the best observed effect will be increased by an average of $1.5\ \sigma / \sqrt{(\pi.n_g)}$ and its actual variance will be lower (by a factor of $1 - [[2.25-\sqrt{(0.75)}] / \pi]$). The expectation of the test statistic used (with known variance) will be ES. $\sqrt{(n_g/2)} + 1.5/\sqrt{(2\pi)}$ (instead of ES $\sqrt{(n_g/2)}$) with a variance of $1 - [[2.25-\sqrt{(0.75)}] / (2\pi)]$ (instead of 1). By studentising, we therefore make the approximation that $t_{1-\beta} = [ES. \sqrt{(n_g/2)} + 1.5/\sqrt{(2\pi)} -VC]/\sqrt{\{1 - [[2.25-\sqrt{(0.75)}] / (2\pi)]\}}$, resulting in a power $(1-\beta)$ of 88.5%.

In practice, the Dunnett test on three groups, with 132 degrees of freedom in the analysis model (132 = 140-8, with eight degrees of freedom for the estimation of the four averages and the effects of the four covariates), performed at a unilateral threshold of 0.025 (0.05 bilaterally), would reject, in the case where the number of participants in the four treatment groups was equal, the "best" effect treatment observed vs placebo at a nominal unilateral threshold of 0.0095 (0.0189 bilateral), this threshold

corresponding to a "best" observed effect of approximately 5.0 points (for the case where the standard deviation would be 8.8335).

12.4.5 Robustness analysis

A robustness analysis will be performed on the primary outcome measure. The analysis will be the same as that for the primary outcome measure but without excluding those patients with data for V1 and V2 but not V3 from the ITT population. For these patients, the slope from V1 to V2 will be extended to extrapolate the GQ value at the theoretical time of V3.

12.5 SECONDARY OUTCOME MEASURES

The secondary outcome measures are:
- The Brunet Lézine test,
- Biometric parameters (height, weight, head circumference),
- Collection of the opinion of the IJL investigating physician concerning the child (CGI),
- Standard laboratory data: FBC, T4, TSH, Ferritin,

The evolution of these secondary outcome measures will be assessed between the baseline value (visit V1) and the value at one year (visit V3).

12.6 SAFETY

Adverse events will be coded on the basis of MedDRA terminology.

Events starting after the first dose of treatment or corresponding to an exacerbation of a pre-existing event after the first dose of treatment will be considered to be "treatment-emergent" events.

"Treatment-emergent" events will be tabulated by SOC (System Organ Class) and Preferred Term within each SOC. They will also be tabulated according to their seriousness and causal relationship with the treatment.

"Treatment-emergent" events leading to premature treatment discontinuation will also be tabulated by SOC and Preferred Term within each SOC.

Serious "Treatment-emergent" events will also be tabulated by SOC and Preferred Term within each SOC.

12.7 INTERIM ANALYSIS

We expect to reach the figure of 175 patients in the last quarter of 2016 and complete the study by the end of 2017. As a result, there is little interest in conducting interim analyses. In addition, the safety assessment of the study is performed each year by an independent data monitoring committee, which this year has not raised any objections to the continuation of the study.

As a result, no interim analysis is planned.

12.8 EXPOSURE TO TREATMENT

Exposure to treatment will be described in terms of duration and dose.

The exposure duration is defined as the time between the start and end of treatment.

12.9 EXPLORATORY ANALYSES

The different characteristics of genotyping of the variants of genes involved in folate and monocarbon radical metabolism by next generation sequencing (NGS) will be be analysed to define their potential influence on the effect of the treatments studied.

Any incidence of each of the following factors on the effect of the treatments studied will be assessed between the baseline value (visit V1) and the value at one year (visit V3):

- biochemical markers of monocarbon metabolism (plasma homocysteine, folate, B12, methylmalonic acid, succinic acid, S- adenosyl methionine, S- adenosyl homocysteine)
- redox biochemical factors: total thioles, carbonyls, AOPP, total antioxidant status, oxidised LDL, superoxide dismutase, glutathione oxidase, glutathione reductase, catalase, glutathione

13. ETHICAL AND REGULATORY CONSIDERATIONS

This trial will be conducted in accordance with:

- the appended recommendations of the Helsinki Declaration
- European Good Clinical Practices
- the French regulations in force, in particular:
  - amended Law 2004-806, dated August 9, 2004, relative to public health policy,
  - The French Public Health Code, article 1121-1 and subsequent articles,
  - amended Law No. 78-17, dated January 6, 1978, relative to computerised data and freedom of access (CNIL),
  - directive 2001/20/EC of the European Parliament and of the Council dated April 4, 2001.

The sponsor is defined by French law 2004-806, dated August 9, 2004 and performs regulatory tasks.

Before starting the study, each investigator must provide the study sponsor's representative with a **dated and signed copy of his/her curriculum vitæ**, including his medical association number (Ordre des Médecins) and **ADELI number.**

To start the study, the sponsor must submit an application for authorisation to ANSM, which must issue its opinion relative to the safety of individuals taking part in the study, taking into consideration, in particular, the safety and quality of the products used during the study, in accordance, as applicable, with the reference systems in force, their conditions of use and the safety of individuals with respect to the procedures performed and methods used, as well as the conditions scheduled for the follow-up of individuals.

The investigator must undertake to conduct the research in accordance with these ethical and regulatory provisions. All the documents, along with all the data relative to the study, must be the subject of audits and inspections conducted in accordance with professional secrecy and without breaking medical confidentiality. The study results will be the property of the sponsor.

13.1 ETHICS COMMITTEE (C.P.P.), ANSM

13.1.1 Before initiation of the study

The sponsor submitted a clinical trial authorisation application to the ANSM, which issued its authorisation on 02 September 2011.

In compliance with article L.1123-6 of the French Public Health Code, the sponsor submitted this protocol to the opinion of the Ile de France III Ethics Committee and supplied all required information (study protocol, information and consent forms, etc.) and any other relevant documents required for submission to the Committee.

The unreservedly favourable opinion of the Ethics Committee was issued on 27 September 2011. This opinion indicates the protocol title and number allocated by the sponsor, the documents examined and

the date on which they were examined, and the list of Ethics Committee members who took part in the deliberations.

The sponsor notified the ANSM of the Ethics Committee's opinion before the start of the study.

13.1.2 Substantial amendment

Any substantial amendment to the protocol must be made with the agreement of the parties concerned (sponsor, principal investigator), then submitted to the ANSM and the Ethics Committee for approval, in the event of a substantial amendment, or for notification purposes in the case of a minor amendment.

A substantial amendment is any change liable to modify the guarantees made to the individuals taking part in the study in any way (modification of an inclusion criterion, extension of inclusion duration, participation of new centres, etc.).

After the start of the study, any substantial amendment initiated by the sponsor must be submitted **by the sponsor** to the ANSM for its authorisation and/or to the Ethics Committee for an opinion prior to implementation.

13.1.3 Safety data

The study safety data must be declared by the sponsor to the ANSM and Ethics Committee in line with the regulations in force, in accordance with the provisions mentioned in chapter 9 of the present protocol.

13.2 PATIENT INFORMATION AND INFORMED CONSENT

In accordance with the French Public Health Code, article L1122-1, the written informed consent of all individuals taking part in a biomedical research study must be obtained by the investigator before any procedures are conducted within the context of the study protocol, regardless of the procedure.

The investigator must be a Doctor of Medicine and registered in the French Medical Association *(Ordre des Médecins)*.

The information must be given verbally and in writing to the parent or legal representative of the child taking part in the study, using the information letter accompanying the informed consent form.

The parent or legal representative of the child must be allowed a period of reflection between receiving the information from the investigator and giving written consent, leaving him/her the time required to freely reach a decision and read and understand the information letter and consent form.

Consent must be given by signature of the parent or legal representative of the child taking part in the study along with his/her first name, surname and the date on which consent was given, written by hand.

In addition, the investigator collecting the consent must date and sign the form in the space provided.

The investigator must ensure:

- that the form is in compliance and that no information or dates are missing
- that an original copy of this document is given to the parent or legal representative of the child taking part in the study. A second original copy will be kept in a secure location, with controlled access

The information letter and consent form were approved by the Ile de France III Ethics Committee on 27 September 2011.

The text of the information letter and the consent form is appended.

The presence of consent will be verified during monitoring of the trial, preserving medical confidentiality; the consent form will be kept by the investigator until the end of the study. The consent forms will be sent in a sealed envelope to the sponsor for archiving at the end of the study.

13.3 ANONYMITY - CONFIDENTIALITY

All personnel involved in implementation of this trial are bound by professional secrecy. In addition, the patients' anonymity will be protected.

All the information collected during this study is strictly confidential. It may only be consulted by personnel involved in the study, appointed by the sponsor and, if applicable, a representative of the health authorities. There will be direct access to the clinical data and source documents for the purposes of monitoring, audits commissioned by the sponsor, and inspections conducted by the competent administrative authorities. All the personnel involved in this study will be required to protect the confidentiality of this information. In addition, no scientific publication resulting from this study may mention the identity of participating patients.

All the data collected during this study will be computerised. It will be coded to make it anonymous and protect the confidentiality of the individuals taking part in the study. This data will be processed in accordance with the laws in force, including, in particular, the amended French *"Informatique et Libertés"* data protection law of 6 January 1978 and its application decrees.

The Institut Jérôme Lejeune has undertaken to comply with the reference methodology (MR001, January 2006) of the CNIL (French data protection body) for data processing implemented in the context of its biomedical research studies. Named clinical data will be kept in the patient's clinical records.

13.4 SUITABILITY OF THE PREMISES FOR THE STUDY

The Institut Jérôme Lejeune (IJL) exerts medical expert and assessment activities in the field of genetics, as part of a health resources cooperation group with the *Groupe Hospitalier Paris Saint-Joseph* (GHPSJ). This consultation centre treats patients of all ages suffering from mental disabilities of genetic origin within the context of the principles identified by law No. 2005-102 of 11 February 2005 on disability and in accordance with the provisions of article L6133-1 of the French Public Health Code. Over 75% of the patients monitored at the IJL are Down syndrome patients.

The IJL premises include a first aid room with an emergency first aid kit, especially designed for clinical trials. If necessary, the closest paediatric intensive care unit is located at Necker-Enfants-Malades Hospital. The premises are therefore suitable for this clinical trial.

13.5 DATA MONITORING COMMITTEE (CISD/DMC)

A Data Monitoring Committee (DMC) will be responsible for reviewing the safety data throughout the study. The CISD/DMC has the power to stop the study for safety reasons if it deems that patients' health is being jeopardised.

The procedures specific to the CISD/DMC are described in a separate charter, appended to the protocol.

13.6 DECLARATION OF HUMAN BIOLOGICAL SAMPLE COLLECTION (BSC) TO THE ANSM.

Human biological sample collection (BSC), as defined by article L. 1243-3 of the CSP, is scheduled for the sole purpose of biomedical research, as defined in the protocol. This collection will be declared to the ANSM, this declaration being appended to the clinical trial authorisation application.

14. PRACTICAL CONSIDERATIONS

14.1 FOLLOW-UP AND MONITORING

Regular contact will be made by telephone (at least every two months) in addition to the visits by the investigators and/or the monitor and/or IJL nurse. Checking of the data will require direct access to patient source data (medical records), which the investigator will make available to the monitor for consultation and comparison with the data recorded in the case report form.

14.2 PLASMA ASSAYS

Standard laboratory tests (FBC, T4, TSH, Ferritin): sample taken at the IJL at each visit and transported to a specialised laboratory for analysis.

In case of difficulties related to drawing of the blood sample, it may be performed outside the IJL in a biological analysis laboratory specialized in the drawing of paediatric blood samples. Patients will go there by taxi, paid for by the IJL.

Interim TSH assays will be performed by the same laboratory, using sampling kits supplied to the patients' parents at the first two visits. The samples will be taken in a laboratory close to the patients' homes and collected by a specialised carrier.

- For biochemical analysis of monocarbon metabolism (plasma homocysteine, folate, B12, methylmalonic acid, succinic acid, S- adenosyl methionine, S- adenosyl homocysteine) and genotyping of monocarbon metabolism by next generation sequencing (NGS): samples taken at IJL and subsequent batched shipment to the INSERM U 954 laboratory (Director J. L. GUEANT).

For redox biochemical analysis (AOPP, total thioles, carbonyls, total antioxidant status, oxidised LDL-, superoxide dismutase, catalase, glutathione, glutathione reductase, glutathione oxidase): samples will be taken at the IJL and subsequently shipped in batches to the biochemistry laboratory at Cochin Teaching Hospital (Dr Didier Borderie) and the biochemistry laboratory at Pitié-Salpétrière University Hospital (Dr Dominique Bonnefont-Rousselot).

For transformation of lymphocytes into lymphoblastoid cells for storage with DNA and plasma, samples will be taken at IJL and transported to the CRB of the IJL (BioJel).

14.3 AUDIT, QUALITY ASSURANCE

An audit of files relative to the study may be conducted independently of the follow-up visits at the request of the sponsor.

14.4 RECORDING OF DATA AND ARCHIVING

The investigator must keep source records for a period of at least 15 years after the end of the trial, along with the originals of the informed consent forms. The sponsor must archive and ensure accessibility to all the data relative to the trial for a period of 15 years after the end of the trial,

This indexed archiving must include:

- Copies of the ANSM authorisation letter and the opinion of the Ethics Committee (CPP)

- Successive protocol versions (identified by the version number and the version date)
- Correspondence,
- The subjects' signed consent forms in a sealed envelope (signed by the holders of parental authority), along with the corresponding inclusion list or register
- The completed and validated case report form for each patient included
- All the annexes specific to the study
- The final study report resulting from statistical analysis and quality control of the study
- The certificates for any audits performed during the study

The database having used for statistical analysis must also be archived by the sponsor.

14.5 STUDY DURATION

The total scheduled inclusion duration is four years. The duration for participation of a given child in the study is one year.

14.6 FINAL REPORT, PUBLICATION, AND CONFIDENTIALITY

This trial may not be the subject of any written or oral commentary (publication, paper) without the prior permission of the sponsor (French Public Health Code).

In the event of publication, the order of the authors will be as follows: the principal investigator, followed by the other investigators as a function of the number of patients participating in the protocol who completed the study in its entirety and, in the event of equal numbers, in alphabetical order.

No information concerning the protocol may be passed on to a third party, published, or communicated without the prior written permission of the sponsor.

The results obtained during this study are the property of the sponsor.

15. ADMINISTRATIVE PROCEDURES

These must comply with the French legislation.

15.1 INSURANCE

The conditions of the civil liability insurance contract taken out by the sponsor apply in accordance with French Law (civil liability insurance contract for biomedical research sponsors taken out with an insurance company specialising in health risks: SM Biomédic, ZA de Luscanen Ploeren, BP 220, 56006 VANNES Cedex, France).

15.2 FINANCIAL AGREEMENTS, FEES

The investigators will receive no fees or compensation for participating in this study. The costs related to the study will be covered by the sponsor: additional tests (laboratory tests, genotyping), insurance, study products.

Any financial agreements with co-investigators (laboratory tests, etc.) will be submitted by the sponsor to the French National Medical Association *(Conseil National de l'Ordre des Médecins)* and, for each investigator concerned, the relevant Regional Medical Association *(Conseil Départemental de l'Ordre)*.

16. USEFUL ADDRESSES

Each participant will supply his/her curriculum vitae to the sponsor.

16.1 SPONSOR

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16.3 INVESTIGATORS

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17. APPENDED DOCUMENTS

- Declaration of Helsinki
- Information sheet (Art. L. 1122-1 of the French Public Health Code)
- Informed consent form (Art. L. 1122-1 of the French Public Health Code)
- Investigators CVs
- Insurance certificate
- Opinion of the Ethics Committee (CPP)
- Case report forms
- Charter of the Data Monitoring Committee (DMC)
- Summary of product characteristics for L-thyroxine
- Summary of product characteristics for folinic acid

18. RESPONSIBILITIES OF THE SPONSOR AND THE INVESTIGATORS

The sponsor's responsibilities are defined by French law 2004-806 of 9 August 2004. In particular, it must ensure the following:

- provision of prerequisites and an investigator file
- provision of the material required to conduct the trial
- follow-up of study conduct, the end-of-study visit, verification of case report forms, verification that subjects' consent has been collected
- a commitment to communicate any new information concerning the product's safety to each investigator and to supply any documentation related to the product to investigators on request
- forwarding of safety data to the Ethics Committee (CPP) and the ANSM

The investigators must ensure the following:

- provision of a curriculum vitae to IJL
- confidentiality and compliance with publication rules with respect to this trial
- storage of documents, products, and material related to this trial in a secure place
- the supply of information to subjects and collection of their freely-given, informed consent
- their availability for monitor visits and audits, access to source records
- protection of subject anonymity
- completion of case report forms and conduct of the trial in accordance with the protocol and within the stipulated time-frames and notification of adverse events within the required time-frames
- return of unused material to the sponsor at the end of the trial (in particular, unused drugs)

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