2 SYNOPSIS

Sponsor:
Institut Jérôme Lejeune, 37 rue des Volontaires, 75725 PARIS 15, FRANCE

Name of finished products: L-thyroxin and folinic acid

Name of active ingredients: levothyroxine sodium and calcium folinate

Title: Efficacy assessment of systematic folinic acid and thyroid hormone treatment on the psychomotor development of young Down syndrome children. ACTHYF.

Protocol No.	**EUDRACT No.**	**IND**	**Trial Registry & No.**
ACTHYF-IJL-AFHT-TH10	2010-021134-66	119920	ClinicalTrials.gov NCT01576705

Principal Investigator & study centres
Clotilde Mircher, MD (Institut Jérôme Lejeune, France). Patients were included in a single centre in France.

Publication (references): None

Study/reporting periods	**Clinical phase:** 3
First inclusion: 02 April 2012 Last patient/last visit: 14 December 2017 Data cut-off: 31 May 2018	

Background & Rationale:
The fundamental role of thyroid hormones in psychomotor development has been clearly established. A clinical benefit was reported for motor development and height and weight gain in Down syndrome babies treated with thyroxin, however psychomotor development was only significantly influenced in a secondary analysis. Folate deficiency is known to cause cognitive impairment and psychiatric disorders which can be effectively corrected by folic acid supplementation. Various studies have addressed the benefit of systematic folate treatment in Down syndrome children and adults, but none have been conclusive. A possible positive interaction between treatment of Down syndrome children with folate and thyroid hormones has been suggested but has not been directly tested.

Objectives
Primary objective
Evaluation of the following in new born infants and very young children with Down syndrome:

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

Secondary objective
Evaluation of the influence of genetic and biochemical factors (one-carbon and redox metabolism) on the effect of the two studied molecules.

Study Design
Monocentric, randomised, comparative, double-blind, parallel-group, placebo-controlled phase 3 clinical trial. Patients were randomised to one of four treatment groups (stratified by age and sex): folinic acid + L-thyroxin; folinic acid + L-thyroxin placebo; folinic acid placebo + L-thyroxin; folinic acid placebo+ L-thyroxin placebo. Treatment was to be administered for 1 year. Three visits were planned (baseline, 6 and 12 months), with telephone contact at least every 2 months.

Number of patients (planned and analysed)
Planned: 175; Included: 175; Randomised: 175; Treated: 174

Diagnosis and criteria for inclusion
Inclusion criteria

1. Patient with a karyotype demonstrating homogeneous, free and complete or Robertsonian translocation trisomy 21
2. Patient with a cardiac ultrasound showing an absence of severe heart disease or significant mitral valve leak
3. Patient aged 6 to 18 months at inclusion
4. Patient who can be reasonably expected to attend the scheduled study visits, able to take the study treatment and undergo the scheduled tests, in particular the Griffiths test (GMDS) (notably, absence of major hearing, ophthalmological AND behavioural impairments, based on the investigator's judgment)
5. Patient whose parents or legal representative can be contacted by telephone

6. Patient for whom at least one of the two parents speaks to him/her in French
7. Patient whose parents or legal representative have received and understood the information leaflet and signed the informed consent form.

Exclusion criteria

1. Gestational age < 231 days (35 weeks of amenorrhoea = 33 weeks of gestation)
2. Apgar < 7, 5 min after birth
3. Patient having received in the past 3 months, or is still receiving, folic acid or folinic acid
4. Patient having received, or is still receiving, L-thyroxin
5. Patient with a history of known allergy and/or hypersensitivity to calcium folinate or any of its excipients
6. Patient with a history of known allergy and/or hypersensitivity to L-thyroxin or any of its excipients
7. Patient presenting a congenital hypothyroidism
8. Patient with hypothyroidism demonstrated by laboratory tests with TSH >7 mIU/L
9. Patient presenting or having presented hyperthyroidism
10. Patient having presented a leukemoid reaction at birth
11. Patient presenting or having presented with leukaemia
12. Patient presenting or having presented West syndrome or any other form of epilepsy or unstable neurological disease
13. Patient presenting or having presented signs of acute central nervous system distress: stroke, postoperative hypoxia, meningitis
14. Patient presenting severe heart disease or significant mitral valve leak on a cardiac ultrasound, with haemodynamic impact
15. Patient presenting non-controlled cardiac arrhythmia

Investigational product, dose, mode of administration, batch numbers

L-thyroxin (Lévothyrox®, Merck): 25 µg tablets, controlled dose initiated at 3.0 ± 0.2 µg/kg/d, oral; commercial preparation

Folinic acid (Folinoral®,Therabel): 5 mg capsules, 1.0 ± 0.3 mg/kg/d, oral; commercial preparation

Control product, dose, mode of administration, batch numbers

Placebo L-thyroxin tablets (D2M): identical excipients and presentation as active product

Placebo folinic acid capsules (Therabel): identical excipients and presentation as active product

Duration of treatment

Each patient was to receive 1 year of treatment and undergo a last follow-up assessment at 2 and 4 months after the last treatment.

Patients were discontinued prematurely in the event of: withdrawal of consent, any pathology which in the investigator's opinion, could have a significant impact on the child's development and on his/her assessment, a request by the independent physician (responsible for TSH dosage follow-up) due to onset of biological hyperthyroidism, or sponsor / health authorities request.

Assessments

Efficacy

Primary variable: Griffiths Mental Development Scales (GMDS)

Secondary variables:

- Brunet-Lézine psychomotor development scale
- Biometric parameters (height, head circumference)

Exploratory variables:

- Clinical global impression (CGI) and overall evolution of the patient according to the investigating physician

Safety

- Adverse events (AEs), serious adverse events (SAEs), laboratory data (complete blood count, T4, TSH, ferritin, and creatinine at baseline only), vital signs
- Clinical and general examination
- Neurological examination, communication/language assessment, behaviour assessment

Biomarkers

- A panel of one-carbon metabolism markers, oxidative stress markers, and genes involved in folate metabolism

Statistical methods

Sample size calculation

The initial protocol planned for 256 patients with an interim efficacy analysis when 50% of the data were available. The sample size was subsequently increased to 264 patients (Amendment #7, 12 June 2015) with two

interim efficacy analyses at 33% and 66% of planned data. Due to difficulties recruiting patients, the sample size was limited to 175 patients (Amendment #8, 15 December 2015), which was anticipated to give 140 evaluable patients, and the interim analyses were removed.

Study populations

- Randomised: all randomised patients regardless of whether they received study treatment.
- Intent to treat (ITT): all randomised patients with valid informed consent, regardless of whether they received study treatment.
- Modified ITT (mITT): all ITT patients who did not prematurely discontinue the study due to high baseline TSH levels (per exclusion criterion #8)).*
- Safety: all randomised patients receiving at least one dose of study treatment.
- Per protocol (PP): all mITT patients having taken the study medication and without major protocol violations.

* As recruitment was nationwide and patients were included at a single centre, screening and randomisation were performed on the same day to minimise visits to the centre. Eligibility of randomised patients for TSH levels per exclusion criterion #8 was thus confirmed retrospectively, and patients with high TSH levels were discontinued.

Efficacy outcome measures:

Primary efficacy endpoint: Adjusted change from baseline in Global Development Quotient (GDQ) at Visit 3 using the Griffiths Mental Development Scales (GMDS).

Secondary efficacy endpoints:

- Change from baseline in the Brunet-Lézine Revised (BL-R) GDQ at Visit 3.
- Change from baseline in height at Visit 3
- Change from baseline in head circumference at Visit 3

Exploratory efficacy endpoints:

- Overall evolution of CGI at Visit 3 from baseline.
- Correlations between GMDS and BL-R GDQ.

Safety endpoints:

- Incidence, severity and relationship of AEs, SAEs, AEs leading to treatment discontinuation or death, other clinically significant laboratory abnormalities (predefined list), incidence of sleep or transit troubles, general, neurological, communication/language, or behaviour abnormalities/outcomes on treatment
- Changes from baseline in laboratory abnormalities, vital signs at Visits 2 and 3 *Biomarkers*
- Change from baseline in a panel of one-carbon metabolism markers, oxidative s*tress marke*rs, and genes involved in folate and one-carbon metabolism at Visits 2 and 3

Analyses

Primary analyses

Main efficacy analysis

Analysis of the primary endpoint was performed in the mITT using an ANCOVA to compare groups by analysing the change from baseline with adjustment for covariates of sex, age class at randomisation, baseline value of GMDS GDQ and the pair of neuropsychologists performing the Visit 1 and 3 evaluations. A two-step Dunnett step-down procedure was used to assess the treatment effect. In the first step the comparisons of each of the three active treatments versus placebo was considered. In the second step, the comparisons of each single agent treatments versus the combination of treatments were considered. In both steps, a 2.5% level one-sided Dunnett step-down test was used to compare the groups.

Impact of covariates on adjusted change and GMDS GDQ values and changes from baseline at each visit were assessed.

Supportive efficacy analysis

The same analysis was repeated in the per-protocol population.

Sensitivity efficacy analyses

The same model as for the main analysis of the primary endpoint as applied in 3 sub-groups in the mITT: 1) patients not passing baseline items, 2) excluding non-cooperating patients, 3) patients with a baseline TSH level in the upper quartile. In addition, a Mixed-Effect Model Repeated Measure (MMRM) model in change from baseline at Visit 2 and Visit 3 was fit to assess the robustness of the main analysis in the presence of missing values at Visit 3.

Secondary efficacy analyses

Adjusted change from baseline in BL-R GDQ at Visit 3, height at Visit 3, and head circumference at Visit 3 were analysed as per the main analysis.

Exploratory efficacy analyses

Adjusted change from baseline at Visit 3 for GMDS and BL-R DQ sub-scales and adjusted change from baseline at Visit 2 for GMDS Developmental age (global and for each sub-scale) was analysed descriptively for GMDS and BL-R, and adjusted change from baseline in GMDS and BL-R GDQ at Visit 3 for study drugs with a 2-sided test (5% level).and BL-R GDQ were analysed as per the main analysis.

Overall CGI evolution at Visit 3 was evaluated by comparing each treatment group to placebo with a Chi-squared test. Correlations between GMDS and BL/R GDQ values at Visits 2 and 3 and between changes from baseline in GMDS and BL/R GDQ at Visits 2 and 3 were evaluated by Pearson correlation coefficient.

Post-hoc analyses

Additional analyses of the primary endpoint (adjusted change in GMDS GDQ) were performed according to the following sub-groups: age class at randomisation, GMDS GDQ at baseline, patients with baseline TSH level >5 mIU/L, and excluding patient 1016. Analyses of the secondary endpoint adjusted change in height was performed in sub-groups of patients with baseline TSH level >5 mIU/L or in the upper quartile by treatment group and study drug.

Correlations between mean baseline biomarker levels and baseline GMDS GDQ, and between biomarker levels and free T4/TSH were evaluated with the Pearson correlation coefficient, and repeated for changes from baseline to Visit 3.

SUMMARY OF RESULTS

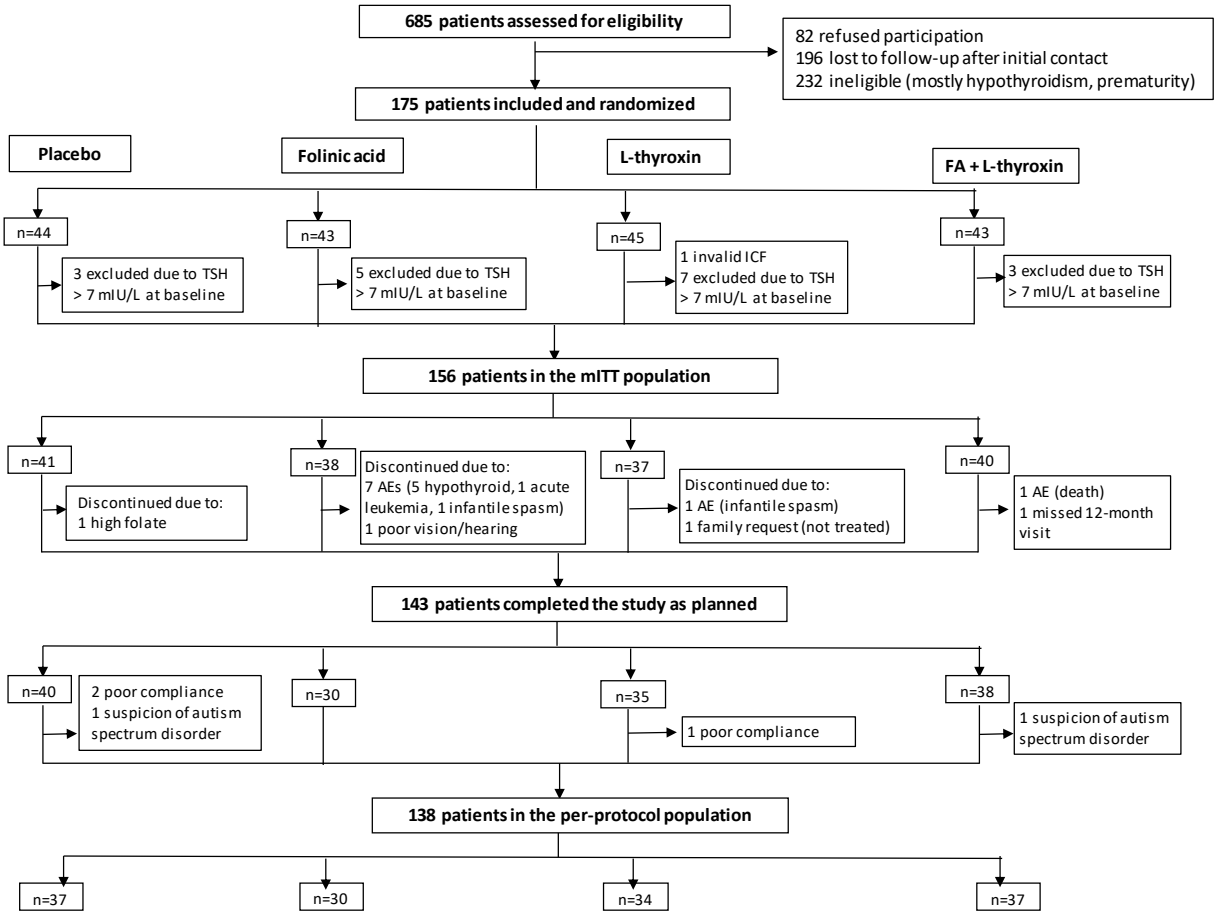
Patient disposition

Randomised: 175 patients; 44 to double placebo (placebo), 43 to folinic acid + L-thyroxin placebo (FA), 45 to L-thyroxin + folinic acid placebo (L-thyroxin), and 43 to folinic acid + L-thyroxin (FA + L-thyroxin).

Completed: 143 patients (81.7%): 40 placebo patients (90.9%), 30 FA patients (69.8%), 35 L-thyroxin patients (77.8%), and 38 FA+L-thyroxin patients (8.4%).

Reasons for premature study withdrawal: 18 patients had baseline TSH levels > 7 mIU/L (retrospectively identified), 9 patients due to AEs (5 hypothyroidism, 2 infantile spasm, 1 acute myeloid leukaemia, 1 sudden death), 5 other reasons (family request, elevated folate, poor vision/hearing, missed Visit 3, non-valid ICF).

37 patients (21.1%) had a total of 44 major deviations: absence of primary criterion assessment (32 patients), autism spectrum (4 patients), bad compliance (3 patients), invalid ICF and not treated (1 patient each).



Demographics

Almost all baseline characteristics were very well balanced between groups in the mITT population. There were 55.3% to 58.5% males across groups, and median age was ~12 months in all groups. Patients had a mean height of ~71 cm, a mean weight of just over 8 kg and a mean head circumference of ~43 cm. Mean birth height was ~48 cm, mean weight was ~3 kg and mean head circumference was ~33 cm, with similar ranges for all parameters. All but 2 mITT patients (98.7%) had a free karyotype. Median 5-min Apgar score was 10 in all groups in the 151 patients assessed (overall range 7 to 10). Foetal disorders were reported in <30% of the population. Median duration of pregnancy was 38 weeks in all treatment groups (overall range 35 to 41). Median age of both parents at birth was ~35 years with both parents attending college or university in ~75% of cases.

Mean free T4 (pmol/L): placebo 17.2 ± 2.8, FA 17.9 ± 2.0, L-thyroxin 17.4 ± 2.3, FA +L-thyroxin 17.3 ± 2.2. Mean TSH (mIU/L: placebo 4.5 ± 1.5, FA 4.8 ± 1.2, L-thyroxin 4.3 ± 1.5, FA +L-thyroxin 4.3 ± 1.7.

The most common medical history events were infection or infestation (~67% of patients), notably bronchiolitis or bronchitis (~25%), gastrointestinal disorders (~40%), congenital disorders (~25%), including cardiac defects (notably septal and respiratory disorders in ~20% of patients).

Rehabilitation therapy at study entry was well balanced between arms; the most common therapies overall were physiotherapy (72.4%), speech therapy (66.0%) and/or psychomotricity treatment (56.4%). Incidence of these therapies on study was similar but with a higher incidence: speech therapy (85.9%), physiotherapy (80.8%), psychomotricity treatment (79.5%), and respiratory physiotherapy (22.4%).

Efficacy

Primary efficacy variable

- Adjusted mean change in GMDS GDQ from baseline at 12 months in the mITT showed similar decreases in all four treatment groups: placebo: -5.1 [95%CI -7.8 to -2.4]; FA: -4.7 [95%CI -7.7 to -1.6]; L-thyroxin: -3.9 [95%CI -6.9 to -0.8]; FA + L-thyroxin: -3.9 [95%CI -6.7 to -1.1]. No significant differences in mean adjusted change were seen for any of the three active treatments compared to placebo (FA, p=0.3919; L-thyroxin p=0.3786; FA + L-thyroxin, p=0.3786), or when comparing each single agent drug with the drug combination.
- The covariates of age class (6-12 vs 12-18 months) and baseline GDQ had a significant impact on adjusted change in GDQ from baseline at 12 months in the mITT (p=0.0043 and p<0.0001 respectively). Neither sex nor the pair of neuropsychologists performing the Visit 1 and 3 analyses significantly impacted the model.
- Mean GMDS GDQs were very well balanced between groups at baseline in the mITT: placebo 56.6 ± 10.6; FA 57.2 ± 9.6; L-thyroxin 53.7 ± 10.6; FA + L-thyroxin: 55.0 ± 8.9. The range of GDQ in all groups was large (26.9 to 89.1 overall).
- Similar results were seen in the per-protocol analysis, with no significant differences in the first or second steps. In this population, the covariates of age class (6-12 vs 12-18 months) and baseline GDQ also had a significant impact on change in GDQ from baseline at 12 months (p=0.0189 and p <0.0001, respectively).

Sensitivity analyses

- The primary analysis was repeated in three sub-groups to account for the impact of potential confounders: 1) patients who could not complete basic items at baseline who have the potential to benefit more from treatment (placebo: 2 patients; FA: 2 patients; L-thyroxin: 7 patients; FA + L-thyroxin: 3 patients); 2) excluding non-cooperating patients (placebo: 27 patients; FA: 19 patients; L-thyroxin: 25 patients; FA + L-thyroxin: 27 patients); and 3) patients with baseline TSH in the upper quartile who have the potential to benefit more from treatment (placebo: 10 patients; FA: 5 patients; L-thyroxin: 9 patients; FA + L-thyroxin: 11 patients). In addition an MMRM model was fit to assess the robustness of the main analysis in the presence of missing values at Visit 3 (placebo: 40 patients; FA: 33 patients; L-thyroxin: 35 patients; FA + L-thyroxin: 40 patients). For all four sensitivity analyses, no significant differences in adjusted mean change in GMDS GDQ from baseline at 12 months were seen for any of the three active treatment groups compared to placebo.

Exploratory analyses of the primary endpoint

- The primary analysis was repeated in the mITT for each GMDS development quotient (DQ) sub-scale (locomotor, personal-social skills, hearing and language, hand-eye co-ordination, and performance) change from baseline at Visit 3. No significant differences in adjusted mean change in GMDS DQ were seen for any of the sub-scales when comparing any of the three active treatments to placebo. Mean changes from baseline at Visit 3 were greater for hearing/language DQ (decreases of 7.3 to 9.7), personal/social DQ (decreases of 5.4 to 6.5) and locomotor DQ (decreases of 4.7 to 6.7) compared to hand/eye coordination DQ (increases of 0.5 to 1.5 in all groups) and performance DQ (decreases of 1.2 to 3.7).
- The primary analysis was repeated for global GMDS development age and for development age according to each DQ sub-scale change from baseline at Visit 3 in the mITT. No significant differences in adjusted mean change in GMDS development age, global or any of the sub-scales, were seen when comparing any of the three active treatments to placebo.
- Adjusted mean change in GMDS GDQ from baseline at 6 months was not significantly different for any of the three active treatments compared to placebo at 6 months in the mITT.

- Analysis of adjusted mean change in GMDS GDQ from baseline at 12 months when comparing the global effect of each study drug (i.e., grouping all patients receiving folinic acid or L-thyroxin, single agent and in combination) versus the absence of the respective study drug, did not show any significant differences in the mITT.

Post-hoc analyses

- The primary analysis was repeated in the mITT according to age class at randomisation (6-12 months and 12-18 months), median baseline GDQ (< and ≥ median GDQ), baseline TSH level > 5 mIU/L. In all analyses no significant differences were seen in adjusted mean change in GMDS GDQ from baseline at 12 months for any of the three active treatment groups compared to placebo.
- No significant differences were seen in the mITT in adjusted mean change in GMDS GDQ from baseline at 12 months for grouped active drug (FA or L-thyroxin) compared to its absence in patients with baseline TSH levels in the upper quartile or > 5 mIU/L.

Secondary efficacy variables

Brunet-Lézine Revised scale

- Adjusted mean change in BL-R GDQ from baseline at 12 months showed similar decreases in all four treatment groups in the mITT (placebo: -8.9 [95%CI -11.7 to -6.1]; FA: -8.3 [95%CI -11.4 to -5.2]; L-thyroxin: -8.0 [95%CI -11.1 to -4.9]; FA + L-thyroxin: -8.7 [95%CI -11.5 to -5.8]). No significant differences were seen for any of the three active treatments compared to placebo, nor when each single agent active drug was compared with the active drug combination.
- The covariates of age class (6-12 vs 12-18 months) and baseline GDQ had a significant impact on adjusted change from baseline in GDQ at 12 months ($p=0.0156$ and $p<0.0001$ respectively) in the mITT. The impact of age class was not significant in the PP population, while baseline GDQ remained significant to the same level.
- Mean BL-R GDQ were well balanced between groups in the mITT at baseline, ranging from 57.0 ± 11.5 to 61.0 ± 11.4. The range of GDQ in each group was large (23.8 to 93.6 overall). Mean GDQ decreased at 6 months with similar decreases in all treatment groups (-4.4 ± 8.1 to -6.9 ± 5.8). Further decreases from baseline in mean GDQ were seen at 12 months in all groups (by -6.9 ± 9.9 to -9.5 ± 7.7).
- *BL-R sensitivity analyses:* Analyses of adjusted change from baseline in BL-R GDQ at 12 months in the mITT were repeated in 1) patients not passing baseline items, 2) excluding non-cooperating patients, 3) in patients with a baseline TSH level in the upper quartile, and 4) using an MMRM model to including patients who discontinued from the study early. For all sensitivity analysis, no significant differences were seen for any of the three active treatments compared to placebo, nor when each single agent active drug was compared with the active drug combination.
- *BL-R exploratory analyses:*
  - The main analysis was repeated in each BL-R DQ sub-scale (postural, coordination, language, and socialisation) in the mITT. No significant differences in adjusted mean change in BL-R DQ were seen for any of the sub-scales when comparing any of the three active treatments to placebo.
  - The main analysis was repeated for global BL-R development age and for development age according to each DQ sub-scale in the mITT. No significant differences in adjusted mean change in BL-R development age, global or any of the sub-scales, were seen when comparing any of the three active treatments to placebo.
  - Adjusted mean change from baseline in BL-R GDQ at 6 months was not significantly different for any of the three active treatments compared to placebo in the mITT.
  - Analysis of adjusted mean change from baseline in BL-R GDQ at 12 months when comparing the global effect of each study drug versus the absence of the respective study drug, did not show any significant differences in the mITT.

Height

- Adjusted mean change from baseline in height at 12 months showed similar increases in all four treatment groups in the mITT (placebo: 9.9 cm [95%CI 9.4 to 10.5]; FA: 10.0 cm [95%CI 9.3 to 10.7]; L-thyroxin: 9.3 cm [95%CI 8.7 to 9.9]; FA + L-thyroxin 9.7 cm [95%CI 9.2 to10.3]). No significant differences were seen for any of the three active treatments compared to placebo, nor when each single agent active drug was compared with the active drug combination.

Head circumference

- Adjusted mean change from baseline in head circumference at 12 months showed similar increases in all four treatment groups in the mITT (placebo: 2.0 cm [95%CI 1.8 to 2.2]; FA: 2.1 cm [95%CI 1.9 to 2.3]; L-thyroxin: 2.1 cm [95%CI 1.9 to 2.3]; FA + L-thyroxin 1.9 cm [95%CI 1.7 to 2.1]). No significant differences were seen for any of the three active treatments compared to placebo, nor when each single agent active drug was compared with the active drug combination.

Exploratory analyses

- *Evolution of clinical global impression (CGI):* Among the 143 evaluable patients in the mITT, 83 (58.0%) were considered to have slightly progressed over the treatment period, and 56 patients (39.2%) had marked progress; there was an improvement in CGI in the FA + L-thyroxin group compared to placebo, with 47.4% (18 of 38) showing slight progress and 52.6% (20 of 38) showing marked progress in the combination group, whereas in the placebo group 67.5% (27 of 40) showed slight progress and 27.5% (11 of 40) showing marked progress. No obvious changes were reported in the investigator's CGI for either single agent active treatment group at 12 months. The improvement seen for the combination group FA + L-thyroxin vs placebo was maintained in the PP population.
- *Correlations between GMDS and BL-R scales:* Correlations between GDQ values at each visit according to the GMDS and BL-R scales were high and equivalent in all four treatment arms in the mITT (0.85 to 0.95), as were correlations between the GDQ change from baseline after 12 months (0.78 to 0.87). This corresponded to a very high positive correlation (0.9 to 1.0) between GMDS and BL-R GDQ at all visits in all groups and a high positive correlation (0.70 to 0.90) between change from baseline in GMDS and BL-R GDQ at Visit 2 or 3 in all groups.

Safety

Exposure

In the safety population, most patients in the placebo group and the FA + L-thyroxin combination group (91% in each group) received at least 11 months of both study drugs, with mean exposures of 10.9 ± 3.4 months and 11.1 ± 3.1 months respectively (for both study drugs/placebo). Exposure was shorter in the two single active agent groups, with 70% of patients in the FA group and 77% of patients in the L-thyroxin group receiving at least 11 months of L-thyroxin/matched placebo and FA/matched placebo, with mean exposures of 9.3 ± 4.4 months and 9.8 ± 4.5 months respectively (for both study drugs/placebo). The number of patients exposed to treatment was relatively stable from 1 month onwards in all treatment groups other than the FA group, reflecting the early discontinuations immediately after inclusion due to elevated TSH which retrospectively rendered patients ineligible (corresponding to the mITT). More patients in the combination and placebo groups had at least one dose adjustment (46.5% and 36.4% respectively) compared to the single agent groups (20.9% FA and 27.3% L-thyroxin).

Adverse events

Almost all patients in the safety population experienced at least one TEAE. The most common TEAEs were infections/infestations, which were reported in more than three-quarters of patients in each group, notably nasopharyngeal infections, gastroenteritis and conjunctivitis, with mostly similar incidences across groups. Respiratory disorders were reported in approximately one-third of patients, with a higher incidence in placebo patients, notably due to cough. Gastrointestinal disorders were reported in approximately one-third to one-half of all patients depending on the group, and were more common in the placebo and combination groups, mainly diarrhoea and GERD. Pyrexia was reported in approximately one-quarter to one-third of patients, with a higher incidence in the FA and combination groups than in the placebo and L-thyroxin groups. Of note, hypothyroidism was reported in the FA group (5 patients; 11.6% of this group).

Related TEAEs were infrequent, and all were mild to moderate. No related events were reported in the L-thyroxin group, one patient in the placebo group (2.3%) reported concurrent single cases of agitation and insomnia considered related, 3 patients (7.0%) in the combination group reported single case of GERD, pyrexia and conjunctivitis considered related, 5 patients in the FA group (11.6%) had events considered related, 4 (9.3%) had hypothyroidism, and 1 had GERD.

Protocol pre-specified treatment-emergent laboratory abnormalities (haematological, metabolic, endocrine, immune) were reported as TEAEs; they occurred in 20.9% to 30.2% of patients overall in the three active treatment groups and 11.4% of placebo patients. Other than hypothyroidism, all were present in <5% of patients overall.

Deaths, SAEs and withdrawals due to AEs

Serious TEAEs were reported in 11.4% of patients in the placebo group, 18.6% in the FA group, 20.5% in the L-thyroxin group, and 18.6% in the FA+L-thyroxin group in the safety population. The most common events were infections/infestation and respiratory, most of which were mild or moderate. None were considered related. A single patient treated in the combination group died while on study and the event was considered not related to study treatment.

TEAEs leading to treatment discontinuation were only reported in the FA group (16.3% of patients in this group): 5 patients (11.6%) due to hypothyroidism (4 related [9.3%]), 1 due to acute myeloid leukaemia and another due to infantile spasms. Of note, another patient withdrew from the study due to infantile spasms and the sudden death on study were not reported as AEs leading to permanent discontinuation.

Laboratory events

No clear clinically significant effects were noted for the laboratory parameters evaluated. Trends towards clinically significant changes in free T4, folate and TSH are described below in post-hoc analyses.

Other assessments

Mean weight and changes in weight at 6 and 12 months were similar between all four treatment groups in the safety population, with patients gaining a mean of ~1 kg at 6 months and ~2 kg at 12 months. Mean heart rate, diastolic and systolic blood pressure at each visit and changes during the study were similar between all four treatment groups, with no clinically significant trends.

Transit troubles were reported in 20% to 40% of the population and sleep troubles in 10% to 30% in the safety population. No clear trends over time were apparent for transit troubles in any of the treatment groups.

In terms of neurological, communication and language assessments, sequential improvements were seen between visits, which were similar across all groups in the safety population.

Post-hoc exploratory safety analyses

Biomarkers

- T4: Possible trends were seen comparing mean values in the L-thyroxin (20.4 ± 2.7) and combination (19.7 ± 3.4) groups, which were higher than the placebo group (16.7 ± 2.1) at Visit 3. Mean changes were also higher in the L-thyroxin group (2.9 ± 3.1) and the combination group (2.5 ± 3.8) than the placebo group (-0.5 ± 2.3) at Visit 3. A similar trend towards higher values was seen when comparing T4 levels at Visit 3 or change at Visit 3 in all patients receiving L-thyroxin to those not receiving it.
- TSH: A trend towards lower mean values at Visit 3 in the FA (3.9 ± 1.9), L-thyroxin (2.4 ± 1.0) and combination groups (2.5 ± 1.7) was seen compared to the placebo group (5.0 ± 2.3). Mean decreases from baseline in TSH levels at Visit 3 were seen in the FA (-0.5 ± 2.0), L-thyroxin (-2.0 ± 1.6) and the combination (-1.9 ± 2.1) groups while the placebo group had a slight increase (0.5 ± 2.0). A trend was seen when comparing TSH levels at Visit 3 or change at Visit 3 in all patients receiving vs those not receiving L-thyroxin.
- Total folate: Possible trends were seen comparing when comparing mean changes in total folate levels from baseline at Visit 3 which were higher in the FA group compared to placebo, and this was confirmed in patients treated with FA versus those not receiving FA at Visit 3 and for changes from baseline.
- Mean homocysteine levels in the FA group were lower than placebo. Differences were also found between patients treated with FA versus those not receiving FA, for mean homocysteine levels at Visit 3 and changes from baseline at Visit 3.
- Similar outcomes were seen for S-adenosyl methionine (SAM), SAM/S-adenosyl homocysteine (SAH).
- Little if any correlation was seen for baseline values of either T4 or TSH with baseline homocysteine in all groups, or for the change from baseline at Visit 3 in T4 or TSH with homocysteine.
- Low or little if any correlation was seen in most groups between baseline GMDS and biomarkers including T4 and TSH. Some exceptions were seen in a range of treatment groups and were both positive and negative.

Conclusions

In the context of robust methodology used in this study, this trial does not support the hypothesis that thyroxin and/or folinic acid improve development of young DS children, nor are they synergistic. The study confirmed large variability of developmental outcome in the DS population at early stages, with a very large range of baseline development quotients, and developmental trajectories in all treatment groups.