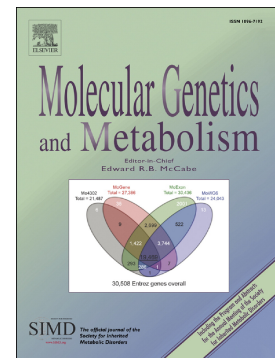


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Intrathecal heparan-N-sulfatase in patients with Sanfilippo syndrome type A: A phase IIb randomized trial

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Abstract

Background: Sanfilippo syndrome type A (mucopolysaccharidosis type IIIA) is a lysosomal disorder wherein deficient heparan-N-sulfatase (HNS) activity results in the accumulation of heparan sulfate in the central nervous system and is associated with progressive neurodegeneration in early childhood. We report on the efficacy, pharmacokinetics, safety, and tolerability of intrathecal (IT) administration of recombinant human HNS (rhHNS) from a phase IIb randomized open-label trial.

Methods: Twenty-one patients, randomized 1:1:1 to rhHNS IT 45 mg administered every 2 weeks (Q2W), every 4 weeks (Q4W), or no treatment, were assessed for amelioration in neurocognitive decline as determined by the Bayley Scales of Infant and Toddler Development®, Third Edition. The primary efficacy goal was defined as ≤ 10 -point decline (responder) in at least three patients in a dosing cohort after 48 weeks. Other efficacy assessments included adaptive behavioral function, assessments of cortical gray matter volume, and glycosaminoglycan (GAG) levels in urine.

Results: A clinical response to rhHNS IT was observed in three treated patients (two in the Q2W group, one in the Q4W group). Cerebrospinal fluid heparan sulfate and urine GAG levels were reduced in all treated patients. However, most secondary efficacy assessments were similar between treated patients (n = 14; age, 17.8–47.8 months) and untreated controls (n = 7; age, 12.6–45.0 months). Treatment emergent adverse events (TEAEs) that occurred with rhHNS IT were mostly mild, none led to study discontinuation, and there were no deaths.

Conclusion: rhHNS IT treatment reduced heparan sulfate and GAG levels in treated patients. Though the primary neurocognitive endpoint was not met, important lessons in the design and endpoints for evaluation of cognitive and behavioral diseases resulted.

Trial Registration: ClinicalTrials.gov NCT02060526; EudraCT 2013-003450-24.

Keywords: Sanfilippo Syndrome type A, intrathecal, heparan-N-sulfatase, clinical trial

Abbreviations: Ab, antibody; ANCOVA, analysis of covariance; BSID-III, Bayley Scales of Infant and Toddler Development[®], Third Edition; CNS, central nervous system; CSF, cerebrospinal fluid; CSHQ, Children's Sleep Habits Questionnaire; DQ, development quotient; ERT, enzyme replacement therapy; GAGs, Glycosaminoglycans; HNS, heparan-N-sulfatase; HS, heparan sulfate; ITQoL, Infant Toddler Quality of Life Questionnaire[™]; IT, intrathecal; IDDD, intrathecal drug delivery device; Q2W, every 2 weeks; Q4W, every 4 weeks; LC-MS, liquid chromatography mass spectrometry; LP, lumbar puncture; MFI, median fluorescence intensity; MPS IIIA, mucopolysaccharidosis type IIIA; MRI, magnetic resonance imaging; NAb, neutralizing antibody; rhHNS, recombinant human heparan-N-sulfatase; SGSH, N-sulfoglucosamine sulfohydrolase; TEAEs, treatment-emergent adverse events; VABS-II, Vineland Adaptive Behavior Scale, Second Edition.

1. Introduction

Mucopolysaccharidosis type IIIA (MPS IIIA; Sanfilippo syndrome), a neurodegenerative genetic disorder of childhood, is a rare autosomal recessive lysosomal disease that causes progressive cognitive and subsequent behavioral decline in affected children [1,2]. It affects ~1:100,000 live births [3].

Subsequent to the original report of Sanfilippo syndrome [1], four different genetic etiologies have been identified, with Sanfilippo syndrome type A (MPS IIIA) being the most prevalent [4]. Defects in the N-sulfoglucosamine sulfohydrolase (*SGSH*) gene result in deficiency of the lysosomal enzyme heparan-N-sulfatase, which is required for the degradation of heparan sulfate (HS). There are > 135 reported mutations for MPS IIIA that can be categorized into severe, intermediate, and attenuated phenotypes [3]. Between the ages of 2–4 years, developmental slowing and behavioral disturbances can be observed, followed by neurodevelopmental decline [5].

In a natural history study of MPS IIIA, Shapiro et al. [5] prospectively evaluated 25 patients aged 1.1–18.4 years, including six sets of siblings, for up to 2 years. Neurocognitive assessments; measures of adaptive behaviors, disability, and sleep disturbances; brain, spleen, and liver imaging; and biomarker levels were recorded at baseline, 6 months, 1 year, and 2 years. The researchers tested the hypothesis that cognitive, imaging, and biomarker changes over 2 years would be reflected in disease stage and severity. From their observations, they concluded that cognitive assessment scores, such as the Bayley Scales of Infant and Toddler Development®, Third Edition (BSID-III), expressed as a development quotient (DQ), and cortical gray matter volumes were sensitive markers of disease progression in MPS IIIA and could be used as clinical study endpoints in patients with rapid progression of MPS IIIA. They also found developmental arrest occurring around age 3–4 years, highlighting the importance of early therapeutic intervention.

Recombinant human heparan-N-sulfatase (rhHNS) is an investigational enzyme replacement therapy (ERT) for MPS IIIA. An intrathecal (IT) formulation of rhHNS (rhHNS IT) was developed to facilitate direct administration of the drug to the central nervous system (CNS) via an implanted IT drug

delivery device (IDDD). IT administration is intended to circumvent issues relating to suboptimal penetration of the central nervous system (CNS) by macromolecules, such as therapeutic proteins administered intravenously, due to the blood–brain barrier.

In an open-label phase I/II dose-escalation safety trial conducted June 2010–September 2012, rhHNS IT was well tolerated in 12 patients with MPS IIIA [6]. Treatment resulted in consistent declines in cerebrospinal fluid (CSF) HS, suggesting *in vivo* activity in the CNS. This report describes the results of a 48-week phase IIb trial (ClinicalTrials.gov NCT02060526; EudraCT 2013-003450-24) that evaluated the efficacy and safety of rhHNS IT 45 mg in MPS IIIA–affected male and female children aged 12–48 months.

2. Material and methods

2.1 Study design

Males and females aged 12–48 months with MPS IIIA, a DQ score of ≥ 60 assessed by the Cognitive Scale of the BSID-III, in stable medical condition and with the ability to accommodate the protocol requirements of travel, assessments, and IDDD implantation surgery were considered for enrollment in the study. Patients were excluded if they tested positive for significant non-MPS IIIA–related CNS impairment or behavioral disturbances, the presence of one or two p.S298P mutations in *SGSH* (associated with attenuated disease), or the presence of relatively attenuated MPS IIIA disease in an older sibling or documentation of the pS298P mutation in a sibling affected by MPS IIIA. Additional exclusion conditions included visual or hearing impairment sufficient to preclude cooperation with neurodevelopmental testing, an unacceptably high risk for anesthesia due to airway compromise, drug hypersensitivity, or other conditions (such as neuroleptic malignant syndrome, malignant hyperthermia, or other anesthesia-related concerns), a history of poorly controlled seizure disorder or a history of bleeding disorder, and current treatment with psychotropic medications.

Enrolled patients were randomized (1:1:1) to no treatment (control), rhHNS IT 45 mg administered Q2W, or rhHNS IT 45 mg Q4W for 48 weeks via an IDDD. The randomization of the study also was stratified by age group (≤ 30 months and > 30 months). Patients randomized to rhHNS IT were scheduled to undergo surgical placement of the Soph-A-Port® Mini S (Sophysa, Orsay, France) device by pediatric or general neurosurgeons. An interval of at least 7 days was allowed for recovery following the placement of the IDDD before the administration of the first dose of rhHNS IT. During this time, the patient received standard perioperative care. Baseline cognitive testing on all patients was performed on days -28 to -8 relative to the first treatment dose on day 0.

2.2 Endpoints

In evaluating the clinical efficacy of rhHNS IT, the trial primarily assessed the number and proportion of responders to treatment, defined as patients with a maximum DQ decline of 10 points on the Cognitive Scale over 48 weeks as assessed by the BSID-III. DQ was the ratio of cognitive age-equivalent score to age at testing (i.e. $[\text{age-equivalent score}/\text{chronological age}] \times 100$). The use of DQ rather than a standard BSID-III score was necessary to identify scores below the floor of the test (which is a score of 55) [7]. Comparison of the rhHNS IT treatment groups and an untreated control group was to be performed using the number of patients who met the responder criterion. For rhHNS IT to be considered effective, an excess of ≥ 3 responders in ≥ 1 of the rhHNS IT treatment groups was proposed.

Several secondary endpoints were evaluated, including the safety and tolerability of rhHNS IT as measured by the number of serious adverse events, the number of treatment-emergent adverse events (TEAEs), and measured antibody response. The neurocognitive effect of rhHNS IT on MPS IIIA progression was assessed by BSID-III age-equivalent and DQ scores, the change from baseline on the Adaptive Behavior Composite of the Adaptive Behavior

Scale, Second Edition (VABS-II), and change from baseline in total cortical gray matter volume as assessed by magnetic resonance imaging (MRI). The pharmacokinetics of rhHNS IT in CSF and serum at each visit, effects of rhHNS IT on the concentration of HS in CSF and GAG in urine, and the emergence of anti-rhHNS Abs in serum also were measured.

PK assessment. A standard sandwich enzyme-linked immunosorbent assay was used to measure rhHNS in serum and CSF with paired rabbit anti-HNS polyclonal Abs.[6] The HNS concentrations in test samples were calculated based on an rhHNS calibration curve in the same assay. To minimize anti-drug antibody (Ab) interference, rhHNS concentrations in serum also were measured by a liquid chromatography mass spectrometry (LC-MS) method. Briefly, the serum proteins were digested with trypsin and the resulting peptides were fractionated by online orthogonal liquid chromatography. The surrogate peptide derived from the rhHNS was subsequently analyzed by multiple-reaction monitoring mass spectrometry using stable isotope labelled peptide as an internal standard.

CSF HS quantification. An LC-MS assay was used to indirectly measure the concentration of HS in CSF [6,8]. The HS was extracted from the CSF by anion exchange and specifically digested by a heparinase cocktail to yield disaccharides. The resultant disaccharides were further derivatized with 4-butylaniline to facilitate isoform separation by liquid chromatography and to enhance the signal intensity of the mass spectrometric detection. Six major disaccharides were subsequently monitored as surrogates for HS by LC-MS.

Urine GAG quantification. The total concentration of GAGs in urine was determined by a dye binding assay using the Blyscan™ Glycosaminoglycan Assay kit (Biocolor Ltd., County Antrim, UK) with dye (1, 9-dimethylmethylene blue) which bound to sulfated proteoglycans and GAGs.

Immunogenicity assessment. Immunogenicity was evaluated in a tiered approach. All samples were screened by a bridging electrochemiluminescent immunoassay using paired biotin- and sulfo-tagged rhHNS. Samples screened positive for anti-drug Ab were confirmed by a

ligand-competition assay. Confirmed anti-drug Ab positive samples were further tested to quantify anti-drug Ab titers and detect the presence of neutralizing Abs (NABs). The NAb inhibited HNS activity was determined by a two-step HNS activity assay. Briefly, samples were incubated with a fixed amount of rhHNS and then with HNS substrate, 4-methylumbelliferyl-2-sulfamino-2-deoxy- α -D-glucopyranoside (MU- α GlcNS). The MU- α GlcNS was converted to MU- α GlcNH₂ by the HNS enzyme. The desulfated substrate was further hydrolyzed during a second incubation with α -glucosidase to release the fluorescent product, 4-methylumbelliferone (4-MU). The 4-MU product was measured at an excitation wavelength of 360 nm and an emission wavelength of 460 nm by a fluorescence plate reader. The relative fluorescence unit of assay controls and test samples were divided by the mean relative fluorescence unit of the negative control and reported as percentage (%) Inhibition. Any sample with a % Inhibition greater than or equal to a NAB assay cut point was reported as NAb positive in the activity assay. The NAb inhibited cell uptake was determined by a flow cytometry method using a primary human fibroblast cell line (HF743B) generated at Shire. Cells were incubated with assay controls and test samples at a minimum required dilution of 1:10 and a fixed amount of AlexaFluor488 conjugated HNS (HNS-ALX488). At the end of incubation, cells were trypsinized and washed by phosphate buffered saline to remove free and cell surface bound HNS-ALX488. The fluorescence intensity of internalized HNS-ALX488 was measured by flow cytometry. The median fluorescence intensity (MFI) of assay controls and test samples were divided by the MFI of the negative control and reported as normalized MFI. The sample was reported NAb positive by cell-based uptake assay if the normalized MFI was less than or equal to the assay cut point.

Exploratory endpoints included rhHNS IT effects on patients' neurocognitive and behavioral subscale assessments using the Infant Toddler Quality of Life Questionnaire™ (ITQoL) and Children's Sleep Habits Questionnaire (CSHQ), and liver and spleen volumetric measurements, as assessed by MRI and normalized to body surface area and weight.

For rhHNS IT treatment groups, each visit during the treatment phase involved electrocardiogram, a urine sample for urinalysis and a CSF sample drawn for biomarker, rhHNS enzyme activity, and antibody analysis before rhHNS IT administration. Vital signs were monitored during the rhHNS IT administration. Post rhHNS IT administration, CSF and blood was drawn for hematology, chemistry, rhHNS pharmacokinetics, and antibody analysis. For the control group, CSF samples were only obtained at baseline and 48 weeks.

Cognitive and behavioral evaluations (BSID-III, VABS-II, ITQoI, CSHQ) and MRIs of the brain, liver, and spleen were performed at baseline, week 24, and week 48 for all study patients. Patients who completed the study and were eligible were offered an opportunity to participate in an extension study (NCT02350816).

2.3 Statistics

To determine statistical significance, the number and proportion of responders and the corresponding exact Clopper Pearson 95% confidence interval (CI) within each treatment group, along with the 95% exact unconditional CI of the difference in proportion between each of the two rhHNS IT treatment groups and the untreated control group, were calculated. Fisher's exact test also was performed. For the BSID-III, change from baseline in DQ scores at week 48 were compared between each of the two rhHNS IT treatment groups versus the untreated control group using an analysis of covariance (ANCOVA) model with baseline score and age group (≤ 30 months and > 30 months) as covariates. The same model also was used to compare the Q2W and Q4W groups.

2.4 Study approval

The study protocol procedures, pertaining to the conduct, evaluation, and documentation of this study, ensure that the sponsor and investigators abided by the International Council for Harmonisation Good Clinical Practice guidelines and the Declaration of Helsinki. Written informed consent was confirmed by the patient's parent(s) or the patient's legally authorized representative(s).

3. Results

Twenty-one patients were enrolled in the trial: nine males and 12 females, with an age range of 12–48 months, and a mean (SD) age of 31.9 (9.3) months. Nine (42.9%) patients were ≤ 30 months of age. Patients were randomized (1:1:1) to no treatment (control; $n = 7$), rhHNS IT 45 mg administered every 2 weeks (Q2W; $n = 7$), or rhHNS IT 45 mg every 4 weeks (Q4W; $n = 7$) for 48 weeks via an IDDD (Soph-A-Port Mini S). The CONSORT diagram for this trial is depicted in Figure 1. Enrollment, patient demographics, and medical history are summarized in Table 1. While all patients had a confirmed diagnosis of MPS IIIA, genotype analysis results were heterogeneous, with no two patients having the same genotype (Appendix A). The mean (SD) age at MPS IIIA diagnosis was 22.0 (7.9) months.

All patients in the study received concomitant medications during the study period. A large number of these drugs were associated with the IDDD surgical procedure, including opioids, general anesthetics, fentanyl, and propofol, or general pain management, e.g., paracetamol/acetaminophen and ibuprofen, or antibiotics such as amoxicillin and cefazolin.

All 14 patients who were treated maintained > 80% treatment compliance with respect to the expected number of rhHNS IT injections received during the treatment period. Patients in the Q2W group (n = 7) received a median of 23 (range, 21–24) doses. Patients in the Q4W group (n = 7) received a median of 13 (range, 12–13) doses. Roughly one-third of the patients (five of 14) received treatments via lumbar puncture (LP) at some point in the study due to IDDD-related issues; however, the frequency of injections by LP in those patients was generally low. There was a mean (SD) of 0.9 (1.9) LPs in the Q2W group; one patient needed a single LP and second patient received five of 22 doses via LP. There was a mean (SD) of 0.6 (0.8) LPs in the Q4W group; two patients received a single LP treatment, and another patient received two LP treatments.

3.1 Primary efficacy endpoint

No patients in the control group met the responder criteria (having a decline of ≤ 10 points in the BSID-III DQ) over the 48-week study period. However, two (28.6%) patients met the responder criteria in the Q2W group (one 12.6-month-old male and one 21.2-month-old male in the Q2W group, and one (14.3%) 31.8-month-old female in the Q4W group met the responder criteria, with no statistical significance between treatment and control groups (Table 2).

3.2 Secondary efficacy endpoints

At baseline, mean (SD) BSID-III cognitive DQ score was generally balanced among the treatment groups: control group, 78.1 (10.5); Q2W group, 79.8 (14.3); Q4W group, 86.0 (17.3). All patients, except one, experienced declines in cognitive capabilities over the study period (Figure 2). Similar declines

from baseline in mean (SD) BSID-III cognitive DQ scores were observed at week 48 in all groups: control group, -19.8 (4.0); Q2W group, -23.8 (14.7); Q4W group, -19.9 (12.7).

From baseline to week 48, individual patient BSID-III DQ scores declined by 15.1–26.6, 6.3–44.8, and 3.1–40.7 points in the control, Q2W, and Q4W groups, respectively.

Of four patients aged 12–24 months, a cognitive response was observed in two males with a starting BSID-III DQ score of 75 and 100 points (Figure 3) and of eight patients aged 24–36 months, one female patient was a responder. None of the patients aged > 36 months responded to treatment. The two non-responder patients aged < 24 months were an untreated male aged 17.8 months who lost 19.4 points from a baseline DQ score of 100.0 and a 20.2-month-old female in the Q4W group who lost 22.5 points from an initial DQ score of 110.0. No patients in the control group declined by ≤ 10 points; five patients declined by 10–20 points and two patients declined by > 20–30 points. In contrast, in the rhHNS IT treatment groups, three patients declined by ≤ 10 points, two patients declined by 10–20 points, six patients declined > 20–30 points, and three patients declined by ≥ 30 points. Most patients aged > 24 months (11 of 17 patients), lost between 15.0–22.5 DQ points. All patients that declined by > 25 points ($n = 5$) were female and aged > 36 months with a baseline BSID-III DQ score of 60.0–94.4 at the start of the study; one patient was in the control group, three patients were in the Q2W group, and one patient was in the Q4W group.

At baseline, mean VABS-II DQ domain scores (communication, daily living skills, socialization, and motor skills) were generally balanced among treatment groups. For all domains and across all groups, mean VABS-II DQ scores from baseline to week 48 declined by 5–38%, with no notable between-group differences. The data were variable within each group. A similar result was seen in the VABS-II DQ subdomain scores. There was no significant change

from baseline over the study in mean VABS-II age-equivalent domain and subdomain scores between either rhHNS IT treatment groups or the control group.

Brain MRI scan data was generally comparable between control and treatment groups at baseline, but declined from baseline in brain gray matter volume at 48 weeks in both rhHNS IT treatment groups; mean (percent) declines were -52.8 cm^3 (-5.4%), -97.8 cm^3 (-13.3%), and -53.1 cm^3 (-7.4%) in control, rhHNS Q2W, and rhHNS Q4W groups, respectively, with no significant difference between the groups after ANCOVA modeling with baseline DQ score and age (≤ 30 and > 30 months) as covariates (Table 3). The range of gray matter volume changes for the control group, however, was much smaller compared with the range for each treatment group (Table 3). For white matter volume, mean changes between baseline and 48-week volumes were variable in each group (control group, $+6.3\%$; Q2W group, $+5.5\%$; Q4W group, -3.7%), with no significant differences between the treatment groups. The range of white matter volume changes was $+15$ to $+32 \text{ cm}^3$, -47 to $+121 \text{ cm}^3$, and -71.1 to $+28.3 \text{ cm}^3$ for the control, Q2W, and Q4W groups, respectively.

3.3 Exploratory efficacy endpoints

There were no significant changes in BSID-III age-equivalent raw scores, scaled scores, or growth scores from baseline over the study period between either rhHNS IT treatment groups or the control group. There were small decreases in mean BSID-III Cognitive Scale and Fine Motor subtest age-equivalent scores from baseline over the study for all groups. Small variable changes in Infant and Toddler Quality of Life (ITQoL) subscale scores (Overall Health, Physical Abilities, Growth and Development, Bodily Pain/Discomfort, Temperament and Moods, General Health Perceptions, Parental Impact-Emotional and Time, and Family Cohesion) from baseline to week 48 were seen in all groups. No notable differences were observed in sleep disturbance score or in the Children's Sleep Habits Questionnaire (CSHQ) subscale scores from baseline to week 48 in either rhHNS IT treatment groups or the control

group. There were no significant differences in these exploratory efficacy endpoints between treatment groups from ANCOVA modeling baseline score using age group (≤ 30 and > 30 months) as covariates.

While variation in individual patient responses over the treatment period was observed, there were no meaningful differences in neurocognitive efficacy results (primary, secondary, or exploratory) between the rhHNS IT treated and control groups, or in any pairwise comparisons of study groups.

3.4 Pharmacodynamics

CSF HS levels declined in patients receiving rhHNS IT Q2W 45 mg or rhHNS IT Q4W 45 mg over 48 weeks, with individual patients meeting or approaching the normal CSF HS level of ≤ 0.648 μM , particularly in the second half of the study. CSF HS levels in untreated controls were largely unchanged during the study (Figure 4). However, using ANCOVA modeling with baseline DQ scores and age group (≤ 30 and > 30 months) as covariates, the differences between the control and treatment groups in CSF HS changes over the study period did not reach statistical significance. At baseline, mean (SD) CSF HS levels for the study groups were 6.07 (1.96) μM in the control group, 8.78 (5.31) μM in the Q2W group, and 8.25 (2.16) μM in the Q4W group. At week 48, mean (SD) CSF HS levels were slightly decreased in the control group (change from baseline, -0.19 [1.32] μM), while substantially reduced in the rhHNS IT treatment groups (Q2W group, -6.89 [5.39] μM ; Q4W group, -5.92 [2.47] μM).

GAG levels in urine were significantly reduced over the course of the study, relative to the control group, for both the Q2W ($P = 0.010$) and Q4W groups ($P < 0.005$). At baseline, mean (SD) urine GAG levels were similar in the three patient groups: control group, 80.43 (41.31) mg/mmol creatinine; Q2W group, 71.49 (15.81) mg/mmol creatinine; Q4W group, 74.97 (36.77) mg/mmol creatinine. At week 48, mean (SD) urine GAG levels were 77.04 (12.16) mg/mmol creatinine in the control group, 39.67 (13.27) mg/mmol creatinine in the Q2W group, and 38.14 (5.81) mg/mmol creatinine in the Q4W group.

The normal range for urine GAG concentration (after creatinine normalization) is age dependent, with higher values occurring at younger ages: 5–18 months, ≤ 31.0 mg/mmol creatinine; 19 months–2 years, ≤ 24.0 mg/mmol creatinine; and 3–5 years, and ≤ 16.0 mg/mmol creatinine [9]. The median age of the treatment groups was 2.8 years at study start and 3.8 years at study end.

As assessed by MRI, from baseline to week 48 in the control group, there were increases in both liver (4.9%) and spleen (19.8%) mean (SD) size of 40.6 (126.8) cm^3 and 28.3 (23.0) cm^3 , respectively. Decreases in mean (SD) liver volume were observed in the rhHNS IT treatment groups: Q2W group, -180.8 (144.0) cm^3 (-20.6%); Q4W group, -183.4 (78.0) cm^3 (-20.4%). The Q2W group mean (SD) spleen volume remained unchanged at 2.7 (14.8) cm^3 ($+2.1\%$), and the Q4W group saw a decrease in mean spleen volume of -21.2 (33.9) cm^3 (-12.3%). The ranges of change from baseline for all groups was very large (liver: control group, -94.3 to $+277.1$ cm^3 ; Q2W group, -405.9 to -38.6 cm^3 ; Q4W group, -287.7 to -89.6 cm^3 ; spleen: control group, $+6.7$ to $+67.0$ cm^3 ; Q2W group, -23.8 to $+22.0$ cm^3 ; Q4W group, -81.0 to $+8.7$ cm^3).

3.5 Pharmacokinetics

At weeks 0 and 48, respectively, mean (SD) CSF fluid concentrations of rhHNS in the 4-hour post-dose CSF sampling were very high: $313,392$ ($142,749$) and $374,544$ ($135,048$) ng/mL in the Q2W group, and $266,022$ ($113,341$) and $335,204$ ($82,919$) ng/mL in the Q4W group. At weeks 0 and 48, respectively, these values were reduced by approximately 40-fold to 3600-fold by 48 hours post-dose: 366 (572) and 104 (66) ng/mL in the Q2W group and 2120 (2068) and 8893 ($19,520$) in the Q4W group.

Enzyme-linked immunosorbent assay analysis showed that rhHNS serum levels increased gradually with a mean time to maximum concentration that ranged from 6.0–9.4 hours after administration, with an apparent clearance of 1.4–3.3 L/h/kg and was eliminated with a mean terminal half-life of 3.6–

4.3 hours. Similar rhHNS serum concentration results were obtained by LC-MS methods with rhHNS serum concentrations peaking between 3.0–8.0 hours, with an apparent clearance of 0.8–3.8 L/h/kg and was eliminated with a mean terminal half-life of 3.6–7.2 hours. Differences in maximum concentration and area under the curve (from 0 to last measured time point) between the Q2W and Q4W groups were variable from baseline to study start and by detection method.

3.6 Safety results

There were no deaths, life-threatening TEAEs, or discontinuations due to a TEAE for any patients during the study period (Table 4). It should be noted that adverse events occurring on or after the time of IDDD implantation or LP procedure or baseline visit for patients in the untreated control group to the end of study visit (+30 days), defined as the last patient's visit in the study, were defined as TEAEs. Most TEAEs that occurred were mild; six (85.7%) patients had a total of 83 TEAEs in the control group, 69 (83.1%) of which were mild, and 14 (100.0%) patients in the rhHNS IT treatment groups had a total of 310 TEAEs, 248 (80.0%) of which were mild. Among rhHNS IT-treated patients, 69 (22.3%) TEAEs were related to the study drug, 36 (11.6%) TEAEs were related to the IDDD, and 41 (13.2%) were related to the IDDD implantation procedure. The most common TEAEs for rhHNS IT-treated patients were vomiting, pyrexia, and upper respiratory tract infection. Three (42.9%) control and 11 (78.6%) rhHNS IT-treated patients experienced four and 33 serious TEAEs, respectively. The most common serious TEAEs in the rhHNS IT-treated groups were CSF leakage in three (21.4%) patients, device failure in two (14.3%) patients, and implant site extravasation in two (14.3%) patients. All of these were considered IDDD-related issues.

No clinically meaningful trends were observed in the clinical laboratory, 12-lead electrocardiogram, or vital sign parameters.

3.7 Antibody results

In the control group, one (14.3%) patient tested positive for anti-rhHNS Abs in serum at baseline but was negative at week 48. In the rhHNS IT treatment groups, no patients tested positive for anti-rhHNS Abs or anti-rhHNS NAbs at baseline. Thirteen (92.9%) patients tested positive for Abs in serum at least once during the study: Q2W group, 7 (100.0%); Q4W group, 6 (85.7%). Seven (50.0%) patients tested positive for CSF Abs at some time during the study: Q2W 45 mg group, four (57.1%); Q4W group, three (42.9%). The enzymatic activity assay measured serum NAbs in 12 of the 13 (92.3%) patients with Ab. Similar results were found in a cell-based assay method. No patients tested positive for CSF NAbs as measured by enzymatic activity, but six of seven (85.7%) CSF Ab-positive patients (four and two in the Q2W and Q4W groups, respectively) tested positive for NAbs in CSF using the cell-based assay. Mean, minimum, and maximum values for each treatment group are presented graphically (Figure 5A–C).

The IDDD device assessments provided further details concerning the safety of rhHNS IT treatments. Four (28.6%) patients required one initial implant surgery with no subsequent revisions. Ten (71.4%) patients required additional surgeries, totaling 14 events. One patient required a device adjustment, another patient required removal and replacement of port only, a patient needed complete removal and replacement of the IDDD implant, four patients had their IDDD completely removed without immediate replacement, three patients required a delayed device implant after previous removal, and some other procedure was needed in four other patients. The overall annual IDDD malfunction and failure rates (95% CI), respectively, were 0.49 (0.10, 1.43) and 0.15 (0.00, 0.82) for the Q2W group and 0.48 (0.10, 1.39) and 0.32 (0.04, 1.14) for the Q4W group. Five malfunction events in four patients occurred in which it was impossible to aspirate CSF before dose administration and two events occurred in two patients wherein the rhHNS dose could not be injected. In addition, there was a single failure of the IDDD due to port leakage or swelling, two events in two patients of failure of the IDDD due to a damaged catheter, and one failure of the IDDD due to an unspecified adverse event.

4. Discussion

The primary objective of this phase IIb trial was to assess the clinical effects and therapeutic potential of rhHNS IT for patients affected with early-stage MPS IIIA (Sanfilippo syndrome type A). At present, no ERT has been approved to treat patients with MPS IIIA, and hematopoietic stem cell transplant is not considered a viable treatment [10]. The isoflavone genistein and related compounds, which may inhibit synthesis of certain GAGs, showed initial promise, but a randomized crossover trial detected no therapeutic effect [11]. Many new therapies for MPS, including gene therapy, are currently under development [12]. In a phase I/II clinical trial of an MPS IIIA gene therapy published in 2014, four children were treated with intracerebral injections of an adeno-associated viral vector serotype rh.10-SGSH-IRE5-SUMF1 vector. The study results showed limited improvements in neuropsychological outcomes with the most positive results coming from the youngest patient and suggested that early intervention may be necessary [13]. To date, no treatments have shown promise in modifying the progression of this exceptionally rare, but ultimately lethal, disease.

Overall, the results of this study indicate that the investigational drug, rhHNS IT, is biochemically active, shows somatic efficacy in all patients, and a cognitive benefit in a small number of patients. The study was intended to provide an indication of the potential of IT administered enzyme on neurocognitive outcomes; however, the primary efficacy goal of at least three responders in a dosing group was not met. Treatment with rhHNS IT was found to reduce CSF GAG levels substantially in some treated patients, although, overall, differences between control and treatment groups were not statistically significant. Following IT administration of rhHNS over 48 weeks, two patients in the Q2W group and one patient in the Q4W group demonstrated a therapeutic benefit in reduction of cognitive decline and important metabolic corrections were reflected in decreased liver volumes for all

treated patients, decreased spleen volumes for a majority of the treated patients, and a decreased urine GAG levels in all treated patients. The three patients who met the responder criteria, among other patients who met the eligibility criteria, continued into the extension study, the results of which will be published elsewhere.

The clinical endpoints for this trial were chosen based on a natural history study in patients with MPS IIIA, which emphasized the importance of cognitive and behavioral endpoints, especially BSID-III, as sensitive markers of disease progression and clinical value [5]. The age of the patients in the study, i.e., between 12 and 48 months at enrollment, and the 48-week duration of the study were chosen to assess treatment effects before the onset of the steep cognitive decline and brain atrophy, which occur at developmental stages in which the disease has a profound impact [2,4]. While the treatment was open label, as necessary, the assessors of neurocognition were blinded to the patient's treatment status.

The DQ point decline range over the study period for the control group was smaller than that for each treatment group. Two patients who had a large (> 40.0-point) decline in BSID-III DQ score had initial DQ scores that were near the lower threshold limit for inclusion in the study ($DQ \geq 60.0$) with DQs of 60.0 and 60.1. In addition, both these patients were aged ≥ 36 months at the start of the study and female, any or all of which may be important factors to consider in future study designs. However, the treatment arms appeared balanced in terms of mean age, sex, and cognitive scores at the study start, and the cause of the greater variation in DQ scores among the treatment groups was not generally reflected in the other measured endpoints. Thus, these anecdotal DQ changes may be a limitation of the study size and treatment period.

The VABS-II DQ data were variable with no apparent trend within each treatment group and no significant differences between groups. Overall, similar declines from baseline to week 48 in mean BSID-III cognitive DQ scores or age-equivalent scores were observed in all treatment arms. Brain MRI scan

data showed mean declines in brain gray matter volume at week 48 versus baseline in all groups, which did not result in significant between-group differences.

Covariate analysis in secondary endpoints for baseline values and age groups (≤ 30 and > 30 months patient age at study start) did not yield any significant differences between the treatment groups. However, anecdotal observations included the fact that two treated males < 24 months of age at the start of the study were responders, large DQ point declines were generally observed for the five patients who were > 36 months of age at the start of the study and in five females (treated and untreated) generally demonstrated the largest DQ point declines. Future studies may benefit from the inclusion of covariate analyses of sex and age group combined, where the study population size permits.

Other behavioral assessments, including sleep disturbance (CSHQ) and quality of life (ITQoI) scores, as well as raw, scaled, growth, and age-equivalent scores for each subtest of the BSID-III, indicated no clinical effect of rhHNS IT over the study period.

Drug concentration measurements, as reflected in pharmacokinetic analysis, showed rhHNS IT biodistribution in serum and CSF to be sufficient to cause pharmacodynamic effects. High CSF rhHNS concentrations were observed at 4 hours, although they had been reduced 40–3600 fold by 48 hours. The maximum concentrations of rhHNS found in serum at 6.0–9.4 hours, suggest a rapid systemic distribution, possibly accounting for the somatic effects observed in this study. These effects were observed as a decrease in mean liver volume in the treatment groups versus the increase in mean liver volume in the control group, a decrease or reduced increase in mean spleen volumes in treatment groups versus the increase in mean spleen volume in the control group, and decreases in urine GAG levels of 45–49% in the treatment groups versus a 4% decrease in the control group).

Anti-rhHNS Abs were detected in the serum of all but one of the treated patients and in the CSF of half of the treated patients. Anti-rhHNS NAbs were detected in the serum of all but one of the treated patients and in the CSF of most treated patients. Sustained Ab titers were reached earlier and were

overall slightly higher in the Q2W group than in the Q4W group, suggesting that less frequent dosing could slow the development of rhHNS-specific Abs. Since immunological reactions to ERT are influenced by many variables, the findings of this study cannot rule out an Ab impact on dose response. The pharmacodynamic effect of rhHNS IT was generally larger for the Q4W group than for the Q2W group when comparing the decreases in liver and spleen volumes and urine GAG levels. The wider diffusion of rhHNS in the general circulation of patients was not investigated in this study. In addition, evaluations were not performed to test whether rhHNS infusions induced a humoral or cell-mediated immune response.

Concerning the safety and tolerability of rhHNS IT, while all but one treated patient experienced a TEAE during the study period, and no deaths or TEAEs that led to study discontinuation occurred. A small proportion of TEAEs that were reported in IT-treated patients were considered serious. However, IDDD malfunction and failure rates, reflecting device issues that interfered with continual treatment, may have contributed to the variability in therapeutic effect of treatment groups. A majority of patients had at least one TEAE considered by study investigators to be related to rhHNS IT, namely the IT treatment regimen, the IDDD, the device surgical procedure, or the IT administration process. Overall, TEAEs that occurred after treatment with rhHNS 45 mg administered via an IDDD Q2W or Q4W for 48 weeks were mostly mild in severity and none led to study discontinuation or death.

The design of this trial posed several challenges. First, a 48-week treatment or observation period may not be sufficient for a neurocognitive effect to be measurable in a significant number of patients. Second, because of the small size of the treatment groups, no attempt was made to analyze the immunogenic effect on cognitive efficacy nor were these data analyzed below the treatment group level. Thus, an analysis of the Ab status of the responders versus non-responders, or the patients with the largest BSID-III DQ point declines (> 25 points) versus Ab status, was not conducted. Additionally, cognition and behavior are complex endpoints and a child with MPS IIIA-caused brain disease may not be able to resume normal cognitive development. The patients with MPS IIIA enrolled in this trial had cognitive dysfunction and pre-existing brain disease, which, ultimately, may irrevocably

limit the effect of therapeutic interventions in cognitive decline. In this view, stabilization of cognitive function, and thus preventing loss of function, may be the most positive achievable therapeutic result in an otherwise invariably progressive brain disorder. Despite the relevance of measures of DQ in the prior MPS IIIA natural history study [5], eventual proof of CNS responsiveness based on these measures may be demonstrated only in very young children, with little or no CNS damage at treatment initiation and when their neurologic and behavioral measures are followed for an extended period. Finally, MPS IIIA arises from a great variety of genetic changes of the *SGSH* gene, resulting in a breadth of disease severities and manifestations that is reflected in a heterogeneous patient population, potentially leading to unavoidable heterogeneous clinical outcomes in small study populations.

While the trial's primary efficacy endpoint of a maximum DQ decline of 10 points for at least three patients in one of the treatment groups was not met, there are several important findings from this study. Notably, rhHNS IT treatment reduced the CSF HS and urine GAG levels in all treated patients, provided metabolic corrections of the liver and spleen, and appears to have resulted in a therapeutic benefit of a slowing of cognitive decline or cognitive stabilization in three of 14 patients, one of which was in a separate treatment group. IDDD performance issues and individual antibody responses were observed that may have had an important impact on the clinical endpoints.

Author contributions

FAW, CBW, JM, SG, MdT, NM, MC, CS, and ES were trial investigators. They participated in the acquisition, analysis, and interpretation of data, and they reviewed and revised the manuscript for scientific content. FAW, CBW, JM, ES, PB, DK, and DA contributed to the design of the trial and analysis of results and reviewed and revised the manuscript for scientific content.

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Conflicts of interest

Dr. Wijburg reports grants, fees for consultations, and speaker fees from Shire, PLC, during the conduct of the study. Dr. Whitley reports grants, fees for consultations, and speaker fees from Shire, PLC, during the conduct of the study. Dr. Muenzer reports grants and fees for consultations from Shire, PLC, during the conduct of the study; personal fees from BioMarin, Denali Therapeutics, Eloxx, Green Cross, Regenxbio, and Sanofi-Genzyme, outside the submitted work. Dr. Gasperini reports grants, fees for consultations, and speaker fees from Shire, PLC, during the conduct of the study; fees for consultations and speaker fees from BioMarin and Sanofi-Genzyme, outside the submitted work. Dr. del Toro reports other from Shire, PLC, during the conduct of the study; fees for consultations and speaker fees from Shire, outside the submitted work. Dr. Muschol reports grants, fees for consultations, and speaker fees from Shire, PLC, during the conduct of the study; grants, personal fees, and other from Shire, PLC, outside the submitted work. Dr. Cleary reports grants, fees for consultations, and speaker fees from Shire, PLC, during the conduct of the study; grants, fees for consultations, and speaker fees from BioMarin outside the submitted work; fees for consultations and speaker fees from Sanofi-Genzyme, outside the submitted work. Dr. Sevin reports other from Shire, PLC, during the conduct of the study; other from Biomarin, personal fees from Bluebird and Shire, PLC, outside the submitted work. Dr. Shapiro reports grants, personal fees, and other from Shire, PLC, during the conduct of the study; other from Shapiro Neuropsychology Consulting, LLC, outside the submitted work. Parul Bhargava was an employee of Shire, PLC, at the time of this study; reports ownership of stock in Shire, PLC, outside of the submitted work. Dr. Kerr was an employee of Shire, PLC, at the time of this study; reports ownership of stock in Shire, PLC, outside of the submitted work. Dr. Alexanderian was an employee of Shire, PLC, at the time of this study; reports ownership of stock in Shire, PLC, outside of the submitted work.

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Fig. 1. CONSORT diagram for this phase IIb randomized clinical trial. Twenty-four patients were screened, 21 eligible patients were randomized, and 3 were excluded. Seven patients received delivered recombinant human heparan-N-sulfatase (rhHNS) intrathecal (IT) 45 mg via an IT drug delivery device (IDDD) every 2 weeks (Q2W) for 48 weeks, seven patients received rhHNS IT 45 mg via an IDDD every 4 weeks (Q4W) for 48 weeks, and seven patients were observed at weeks 0 (baseline), 24, and 48 (control). All patients underwent complete follow-up analysis.

Fig. 2. Bayley Scales of Infant and Toddler Development, Third Edition, cognitive development quotient scores versus patient age in months over the study period: (A) control (no recombinant human heparan-N-sulfatase [rhHNS]), (B) rhHNS intrathecal (IT) every 2 weeks (Q2W), (C) rhHNS IT every 4 weeks (Q4W).

Fig. 3. Bayley Scales of Infant and Toddler Development, Third Edition, cognitive development quotient (DQ) change from baseline to 48 weeks versus patient age at study start.

Fig. 4. Mean concentrations of heparan sulfate (HS) in the cerebrospinal fluid (CSF) over the study period. Q2W, every 2 weeks; Q4W, every 4 weeks; rhHNS, recombinant human heparan-N-sulfatase.

Fig. 5. (A) Mean cerebrospinal fluid (CSF) antibody (Ab) titers for treatment groups over the study period, (B) mean serum Ab titers for treatment groups over the study period, and (C) mean serum neutralizing Ab (NAb) titers for treatment groups over the study period. Q2W, every 2 weeks; Q4W, every 4 weeks; rhHNS, recombinant human heparan-N-sulfatase.

Table 1 Baseline demographics and clinical characteristics of the randomized population.

Characteristic	rhHNS IT			
	Control (n = 7)	Q2W 45 mg (n = 7)	Q4W 45 mg (n = 7)	overall (n = 14)
n	7	7	7	14
Age (months)				
Mean (SD)	32.4 (9.5)	29.6 (10.0)	33.5 (9.3)	31.6 (9.5)
Median	32.7	35.4	31.8	33.6
Min, max	17.8, 47.8	12.6, 39.0	20.2, 45.0	12.6, 45.0
Age groups, n (%)				
≤ 30 months	3 (42.9)	3 (42.9)	3 (42.9)	6 (42.9)

> 30 months	4 (57.1)	4 (57.1)	4 (57.1)	8 (57.1)
Age of diagnosis (months)				
Mean (SD)	19.2 (5.2)	22.5 (7.9)	24.4 (10.1)	23.5 (8.8)
Median	20.0	21.0	25.0	23.5
Min, max	12.0, 28.0	11.0, 34.2	9.0, 37.0	9.0, 37.0
Sex, n (%)				
Male	4 (57.1)	2 (28.6)	3 (42.9)	5 (35.7)
Female	3 (42.9)	5 (71.4)	4 (57.1)	9 (64.3)
Ethnic group, n (%)				
White	6 (85.7)	7 (100.0)	7 (100.0)	14 (100.0)
Black or African American	0	0	0	0
Asian	1 (14.3)	0	0	0

IT, intrathecal; max, maximum; min, minimum; Q2W, every 2 weeks; Q4W, every 4 weeks; rhHNS, recombinant human heparan-N-sulfatase.

Table 2 Primary efficacy analysis: responders per cohort at week 48 according to cognitive DQ (BSID-III).

	rhHNS IT			
	Control	Q2W 45 mg	Q4W 45 mg	overall
	(n = 7)	(n = 7)	(n = 7)	(n = 14)
Responders, n (%)*	0	2 (28.6)	1 (14.3)	3 (21.4)
95% CI	0.0, 41.0	3.7, 71.0	0.4, 57.9	4.7, 50.8
Difference between proportions (95% CI)		0.29 (-0.30, 0.75)	0.14 (-0.42, 0.65)	
<i>P</i> value		0.46	1.00	0.52

*Responders were defined as patients with a decline in BSID-III cognitive DQ of ≤ 10 points over the 48-week treatment period.

BSID-III, Bayley Scales of Infant Development, Third Edition; DQ, development quotient; IT, intrathecal; Q2W, every 2 weeks; Q4W, every 4 weeks; rhHNS, recombinant human heparan-N-sulfatase.

Table 3 Secondary efficacy analysis: change from baseline to week 48 in MRI gray matter volume (cm³).

	rhHNS IT		
	Control (n = 7)	Q2W 45 mg (n = 7)	Q4W 45 mg (n = 7)
n	4	6	6
Min, max	-29, -62	+10, -174	+17, -159
LS mean (SE)	-52.80 (31.83)	-97.82 (25.80)	-53.10 (25.63)
Difference between rhHNS IT treatment and control groups:			
LS mean (SE)		-45.02 (39.67)	-0.30 (39.72)
95% CI		-132.32, 42.29	-87.72, 87.12
<i>P</i> value (1)*		0.28	0.99
<i>P</i> value (2)**		0.11	0.71
Difference between Q2W 45			

mg and Q4W 45 mg:

LS mean (SE)	44.72 (35.50)
95% CI	-33.42, 122.85
<i>P</i> value (1)	0.23
<i>P</i> value (2)	0.23

**P* value (1): comparing treatment and control groups from an analysis of covariance with baseline score and age group (≤ 30 and > 30 months) as covariates.

***P* value (2): Cochran–Mantel–Haenszel row means score test stratified by age group, using rank scores.

IT, intrathecal; LS, least squares; max, maximum; min, minimum; Q2W, every 2 weeks; Q4W, every 4 weeks; rhHNS, recombinant human heparan-N-sulfatase.

Table 4 Overall summary of TEAEs

Category	Q2W 45 mg		Q4W 45 mg		rhHNS IT treated	rhHNS IT	Control	Control; events, n
	patients, n (%) (n = 7)	events, n	patients, n (%) (n = 7)	events, n	patients, n (%) (n = 14)	treated events, n	patients, n (%) (n = 7)	
No TEAE	0		0		0		1 (14.3)	

≥ 1 TEAE	7 (100.0)	174	7 (100.0)	136	14 (100.0)	310	6 (85.7)	83
≥ 1 IT treatment								
regimen-related TEAE	7 (100.0)	56	7 (100.0)	69	14 (100.0)	125	0	0
≥ 1 rhHNS-related TEAE	5 (71.4)	35	6 (85.7)	34	11 (78.6)	69	0	0
≥ 1 IDDD-related TEAE	5 (71.4)	14	7 (100.0)	22	12 (85.7)	36	0	0
≥ 1 IDDD surgical								
procedure-related TEAE	5 (71.4)	20	5 (71.4)	21	10 (71.4)	41	0	0
≥ 1 IT administration								
process-related TEAE	3 (42.9)	6	5 (71.4)	33	8 (57.1)	39	0	0
≥ 1 serious TEAE	5 (71.4)	19	6 (85.7)	14	11 (78.6)	33	3 (42.9)	4
≥ 1 life-threatening TEAE	0	0	0	0	0	0	0	0
Discontinued due to								
TEAE	0	0	0	0	0	0	0	0
Deaths	0	0	0	0	0	0	0	0

Note: adverse events occurring on or after the time of IDDD implantation or lumbar puncture procedure or baseline visit for patients in the untreated control group to the end of study visit (+30 days), defined as the last patient visit in the study, were defined as TEAEs.

IDDD, intrathecal drug delivery device; IT, intrathecal; Q2W, every 2 weeks; Q4W, every 4 weeks; rhHNS, recombinant human heparan-N-sulfatase; TEAE, treatment-emergent adverse event.

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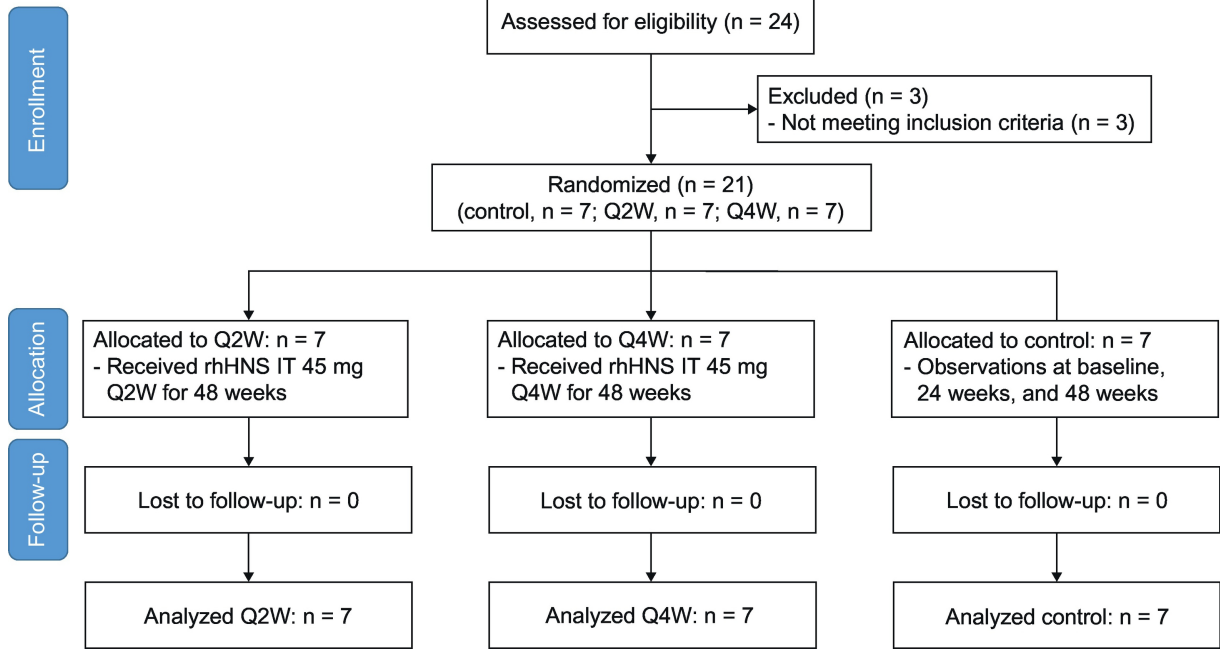


Figure 1

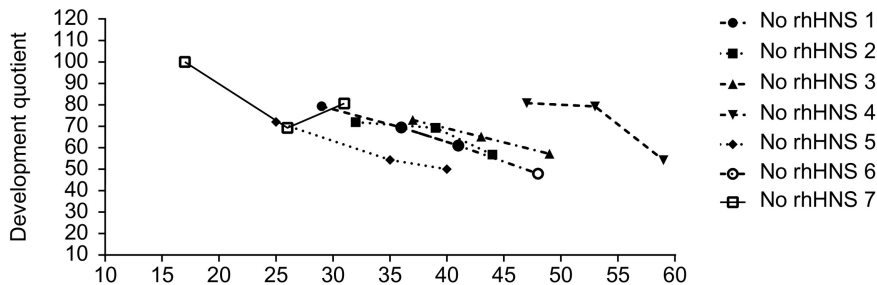
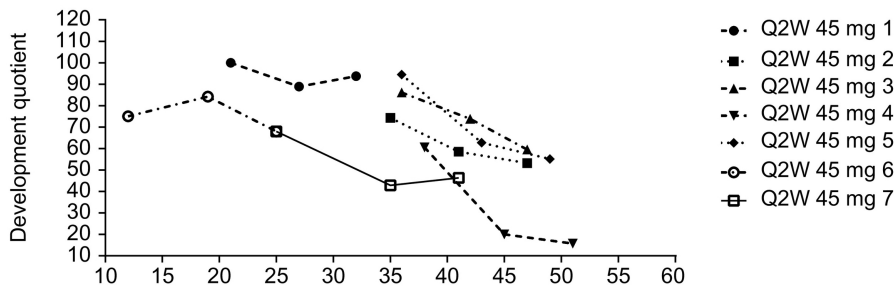
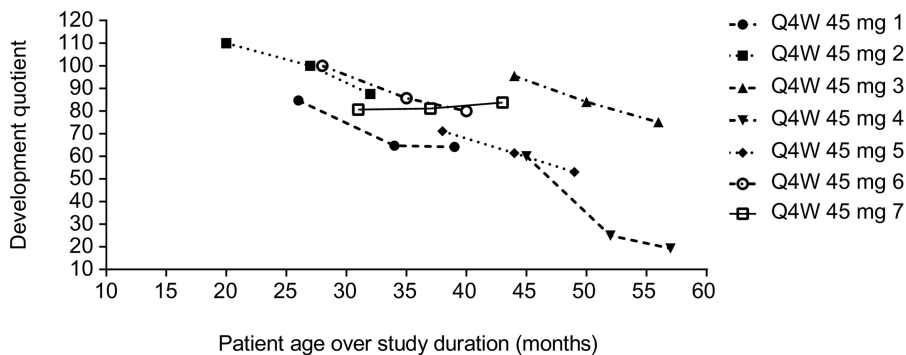
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Figure 2

Age at study start (months)

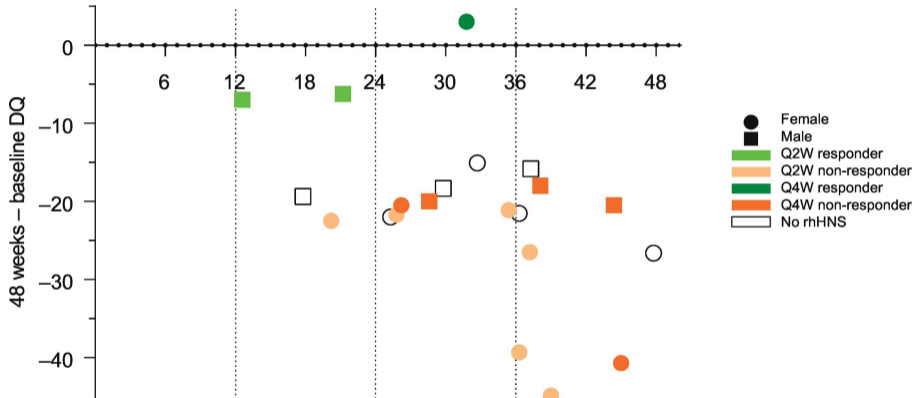


Figure 3

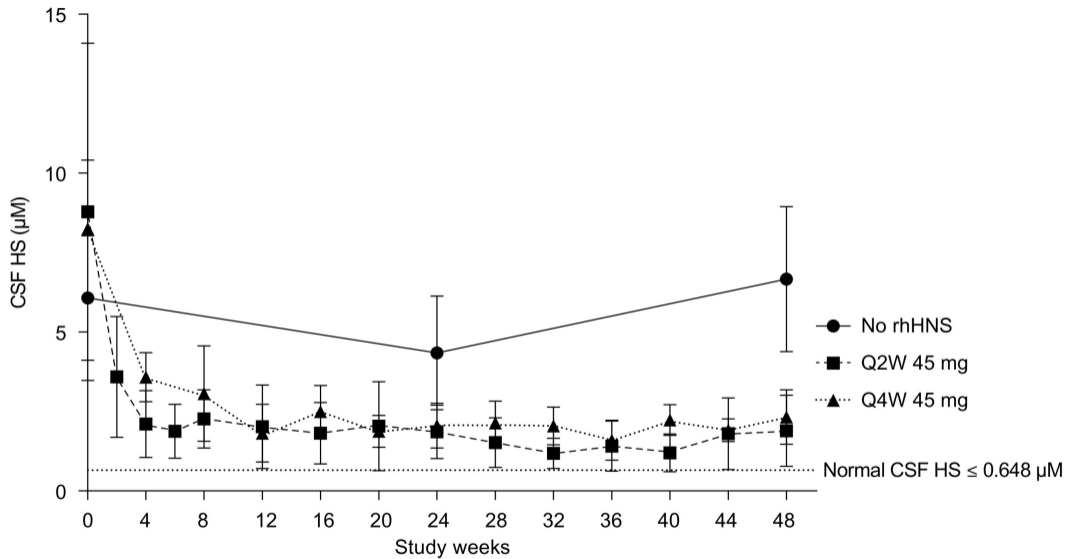


Figure 4

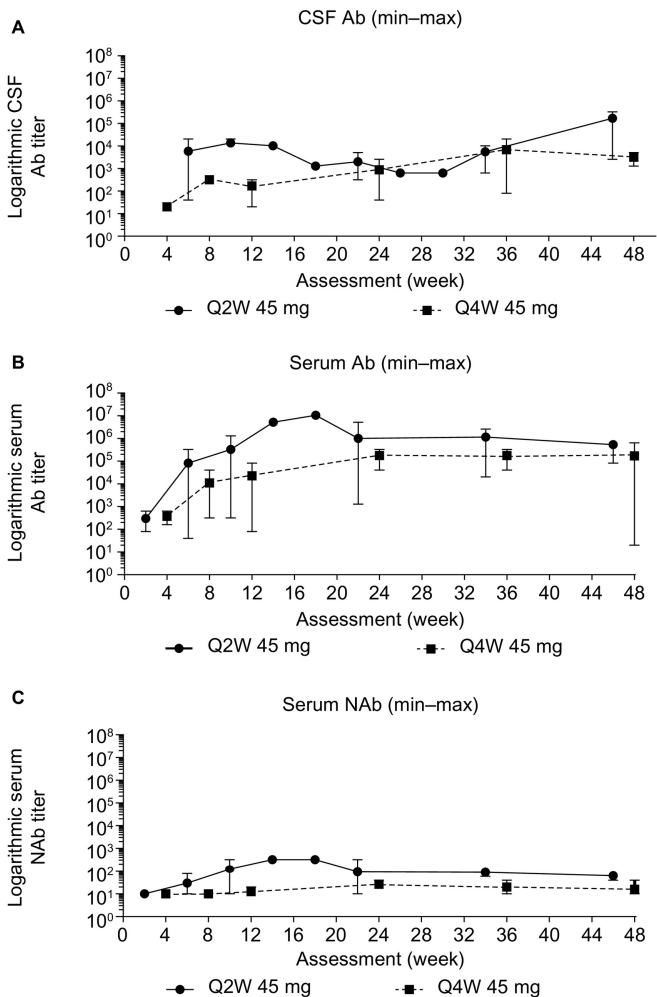


Figure 5