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RESEARCH ARTICLE

Efficacy and Safety of N-Acetyl-L-Leucine in Children and Adults With GM2 Gangliosidoses

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Abstract

Background and Objectives

GM2 gangliosidoses (Tay-Sachs and Sandhoff diseases) are rare, autosomal recessive, neurodegenerative diseases with no available symptomatic or disease-modifying treatments. This clinical trial investigated N-acetyl-L-leucine (NALL), an orally administered, modified amino acid in pediatric (≥6 years) and adult patients with GM2 gangliosidoses.

Methods

In this phase IIb, multinational, open-label, rater-blinded study (IB1001-202), male and female patients aged ≥6 years with a genetically confirmed diagnosis of GM2 gangliosidoses received orally administered NALL for a 6-week treatment period (4 g/d in patients ≥13 years, weight-tiered doses for patients 6–12 years), followed by a 6-week posttreatment washout period. For the primary Clinical Impression of Change in Severity analysis, patient performance on a predetermined primary anchor test (the 8-Meter Walk Test or the 9-Hole Peg Test) at baseline, after 6 weeks on NALL, and again after a 6-week washout period was videoed and evaluated centrally by blinded raters. Secondary outcomes included assessments of ataxia, clinical global impression, and quality of life.

Results

Thirty patients between the age of 6 and 55 years were enrolled. Twenty-nine had an on-treatment assessment and were included in the primary modified intention-to-treat analysis. The study met its CI-CS primary end point (mean difference 0.71, SD = 2.09, 90% CI 0.00, 1.50, $p = 0.039$), as well as secondary measures of ataxia and global impression. NALL was safe and well tolerated, with no serious adverse reactions.

Discussion

Treatment with NALL was associated with statistically significant and clinically relevant changes in functioning and quality of life in patients with GM2 gangliosidosis. NALL was safe and well tolerated, contributing to an overall favorable risk:benefit profile. NALL is a promising, easily administered (oral) therapeutic option for these rare, debilitating diseases with immense unmet medical needs.

Trial Registration Information

The trial is registered with ClinicalTrials.gov (NCT03759665; registered on November 30, 2018), EudraCT (2018-004406-25), and DRKS (DRKS00017539). The first patient was enrolled on June 7, 2019.

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Class of Evidence
Criteria for rating therapeutic and diagnostic studies
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Go to Neurology.org/N for full disclosures. Funding information and disclosures deemed relevant by the authors, if any, are provided at the end of the article.

Glossary

8MWT = 8-Meter Walk Test; **AE** = adverse event; **CGI** = Clinical Global Impression; **MCL** = Medpace Core Laboratories; **NPC** = Niemann-Pick disease type C; **SARA** = Scale for the Assessment and Rating of Ataxia; **SCAFI** = Spinocerebellar Ataxia Functional Index; **SAP** = statistical analysis plan; **TEAE** = treatment-emergent adverse event; **VAS** = visual analog scale.

Classification of Evidence

This study provides Class IV evidence that NALL improves outcomes for patients with GM2 gangliosidoses.

GM2 gangliosidoses, that is, Tay-Sachs and Sandhoff diseases, are rare (incidence 0.28:100,000), autosomal recessive lysosomal disorders.[1] GM2 gangliosidoses most commonly affect infantile and pediatric patients and are characterized by progressive neurodegeneration, which significantly affects quality of life and results in premature death.[2] GM2 gangliosidoses feature a wide spectrum of heterogeneous, debilitating symptoms, including cerebellar ataxia, dysphagia, and dysarthria.[2] No treatments for GM2 gangliosidoses are currently approved in any jurisdiction worldwide.

N-acetyl-L-leucine (NALL) is a modified amino acid and the L-enantiomer of the raceme, approved since 1957 in France as a treatment for acute vertigo (Tanganil).[3,4] In observational studies, acetyl-leucine has been demonstrated to have symptomatic and long-term, disease-modifying effects in patients with GM2 gangliosidoses and other lysosomal disorders like Niemann-Pick disease type C (NPC).[3,5-8] Recently, a parallel, multinational, phase IIb clinical trial with NALL for NPC showed a statistically significant (primary and secondary end points) and clinically meaningful improvement in symptoms, functioning, and quality of life for children and adults with NPC.[3] NALL was observed to be well tolerated in all observational and clinical studies completed to date, with no reports of serious adverse reactions.

Animal studies in the GM2 mouse model (*Hexb*$^{-/-}$) and related NPC mouse model (*NPC* $^{-/-}$) have shown that N-acetyl-leucine significantly improved ataxia when administered presymptomatically or symptomatically.[3,8,9] In these studies, acetyl-leucine–treated animals exhibited slowed disease progression and an extended lifespan. These studies specifically identified the L-enantiomer as the active isomer of the racemate, responsible for the neuroprotective effect, and suggested superior clinical effects when administered independently.[3,8,9] Furthermore, pharmacokinetic studies in mice indicate that during chronic dosing of the racemate, the D-enantiomer may accumulate, with the potential for unwanted effects.[10] Recently, it was reported that NALL is taken up and distributed to all tissues including the CNS by the monocarboxylate transporter (MCT1) and hydrolyzed to L-leucine,[11] thereby functioning as a prodrug for the delivery of L-leucine, a powerful intracellular metabolic signal of pathways such as mTORC1.[12] Therefore, in this clinical trial, we aimed to investigate the safety and efficacy of NALL on symptoms, functioning, and quality of life for pediatric and adult patients with GM2 gangliosidoses.

Methods

Standard Protocol Approvals, Registrations, and Patient Consents

Approval for the study (clinicaltrials.gov identifier NCT03759665, EudraCT number 2018-004406-25, and DR KS-ID: DRKS00017539) was obtained by National Regulatory Authorities in each country (German Federal Institute for Drugs and Medical Devices, Spain Agency of Medicines and Medical Devices, UK Medicines and Healthcare products Regulatory Agency, and US Food and Drug Administration) and the applicable responsible central research ethics committees/institutional review boards for each center (Ethics Committee of Ludwig Maximilian University of Munich (19-119), Bellvitge Hospital University Clinical Research Ethics Committee (AC004/19), North West–Greater Manchester South (260774), Mayo Clinic Institutional Review Board (19-000373), Office of Science and Research Institutional Review Board, New York University School of Medicine (i17-01666), and University of California Los Angeles Institutional Review Board (19-000348)). Written informed consent was obtained for all study participants by the patient or, if applicable, their parent or legal representative.

Study Design

The IB1001-202 clinical trial was conducted using a master protocol, which was also used to assess the efficacy of NALL (Sponsor Code IB1001) for symptoms, functioning, and quality of life in 2 related rare, neurodegenerative diseases (NPC [NCT03759639] and Ataxia-Telangiectasia [NCT03759678]).[3,4] Details of the master protocol including its rationale, methods, study design and procedures, and oversight-have been previously published. The results of the IB1001-201 clinical trial for NPC have also previously been reported.[3,4]

Participants

Adults and children aged 6 years and older with a confirmed genetic diagnosis of GM2 gangliosidoses were eligible to participate at 8 clinical research universities and hospitals in 4 countries (Germany, Spain, the United Kingdom, and the United States). Patients using prohibited medications at screening (i.e., medications that may have confounded the

safety or efficacy analysis of the trial, including N-acetyl-DL-leucine, N-acetyl-L-leucine, or aminopyridines [prohibited if not provided as the investigational medicinal product], varenicline, riluzole, sulfasalazine, chlorzoxazone, gabapentin, or rosuvastatin) were required to complete a 42-day washout prior to their first baseline visit. The eligibility criteria were previously published.[4]

Procedures

The IB1001-202 trial consisted of 3 consecutive study periods: a 2-week (+7 days) baseline period, a 6-week (+7 days) treatment period (in which all patients were to receive NALL), and a 6-week (+7 days) posttreatment washout period. The schedule of events is presented in Table 1. Patients were assessed twice during each study period: that is, pretreatment at visits 1 and 2 (baseline 1 and 2), during treatment at visits 3 and 4 (treatment 1 and 2), and during washout at visits 5 and 6 (washout 1 and 2). After the final visit of the parent study (visit 6), patients may have entered an open-label extension phase to explore the long-term benefit of NALL. The parent study has been completed, and the results are reported below. Because of the exceptional circumstances caused by the coronavirus pandemic (COVID-19), necessary deviations from the schedule of events were made for some

Table 1 Schedule of Assessments

Period	Baseline period		Treatment period		Washout period		Early termination
Duration of the whole period	**1 d**	**2 wk**	**6 wk**		**6 wk**		**1 d**
Visit number	**Visit 1**	**Visit 2**	**Visit 3**	**Visit 4**	**Visit 5**	**Visit 6/ EOS**	**ET**
Name of the visit	**Screening/ Bsl 1**	**Baseline 2**	**Treatment 1**	**Treatment 2**	**Washout 1**	**Washout 2**	**ET**
Timeline, d	*Day 14*	*Day 1, start NALL*	*Day 28*	*Day 42*	*Day 70*	*Day 84*	*XX*
Visit window allowed	*NA*	*+7 d*	*+7 d*	*+7 d*	*+7 d*	*+ 7 d*	*NA*
Patient information and informed consent process	X						
Inclusion/exclusion criteria, medical history, and patient demographics	X	X					
Classify patient as naive or non-naive[a]	X						
Documentation of therapy and concomitant medications	X	X	X	X	X	X	X
Vital signs	X	X	X	X	X	X	X
12-lead ECG	X		X		X		X
Urine test for N-acetyl-D-leucine	X	X			X	X	X
Blood safety laboratory tests and urinalysis	X	X	X	X	X	X	X
Blood sample for sparse PK	X	X	X	X	X	X	X
Quality of Life EQ-5D	X	X	X	X	X	X	X
SARA	X	X	X	X	X	X	X
mDRS	X	X	X	X	X	X	X
SCAFI	X	X	X	X	X	X	X
CI-CS anchor test video record	X	X	X	X	X	X	X
CGI-S	X	X	X	X	X	X	X
CGI-C by the physician, caregiver, and patient				X		X	X
Documentation of AEs	X	X	X	X	X	X	X

Abbreviations: AE = adverse event; CGI-C = Clinical Global Impression of Change; CGI-S = Clinical Global Impression of Severity; CI-CS = Clinical Impression of Change in Severity; EQ = EuroQol; mDRS = modified Disabling Rating Score; NALL = N-acetyl-LL-leucine; SARA = Scale for Ataxia Rating; SCAFI = Scale for Spinocerebellar Ataxia Functional Index.
[a] Patients using prohibited medication at screening (versions of the investigational medicinal product, aminopyridines, riluzole, gabapentin, varenicline, chlorzoxazone, sulfasalazine, or rosuvastatin) were classified as non-naive and allowed to perform a 6-week washout from the medication before returning for the baseline assessment.

patients as permitted by national guidance to safeguard patients, their families, and study teams.

Treatment

Patients aged ≥13 years or 6–12 years weighing ≥35 kg received 4 g/d 3 times per day (2 g in the morning, 1 g in the afternoon, and 1 g in the evening). Patients aged 6–12 years weighing <35 kg received weight-tiered doses 2 or 3 times per day based on approximately 0.1 g/kg/d. In the parent study, NALL was provided as a powder for suspension, suspended in 40 mL ORA-Blend to be administered orally at least 30 minutes before or 2 hours after a meal. After finishing the parent study, patients were allowed to be involved in a 1-year long, open-label extension study that is ongoing.

Outcomes

For the primary end point, the Clinical Impression of Change in Severity (CI-CS), the patient's performance on either the 9 Hole Peg Test–Dominant Hand (9HPT-D), or the 8-Meter Walk Test (8MWT) was compared based on video recordings taken at baseline (visit 2), the end of treatment (visit 4), and the end of the washout period (visit 6). For each patient, either the 9HPT-D or 8MWT was chosen as the primary assessment measure by the principal investigator at visit 1, based on their unique individual symptoms. Sites were trained on a standardized protocol to ensure that the 9HPT-D and 8MWT were filmed consistently, and the videos were uploaded to be centralized assessed by a team of 3 certified neurologists. Two of these neurologists reviewed randomized, blinded video pairs as follows: baseline vs end of treatment (pair A), end of treatment vs end of washout (pair B), and baseline vs end of washout (pair C). For each pair, the raters had to assess the change of severity of the patient's signs using a 7-point Likert scale. The third rater acted as an adjudicator, when the results of the assessment of the 2 primary raters differed by more than 1 point on the Likert scale. The CI-CS was defined as the change from pair A minus pair B; thus, the washout period served as the control arm to the treatment period.

Secondary efficacy measurements of ataxia and functioning included the Scale for the Assessment and Rating of Ataxia (SARA) and Spinocerebellar Ataxia Functional Index (SCAFI)[13] and the modified Disability Rating Scale (mDRS)[14]—a measurement of overall neurologic status was applied. In addition, subjective impairment and quality of life were evaluated using the Clinical Global Impression (CGI) scale (completed by the investigator, caregiver, and patient)[15] and the EuroQol (EQ) 5Q-5D-5L/-Y, consisting of a descriptive part and a visual analog scale (VAS).[16] Secondary assessments compared the change over the treatment period, that is, baseline (visit 2) to the end of treatment (visit 4) and the change over washout period, that is, the end of treatment (visit 4) to the end of posttreatment washout (visit 6).

Safety was assessed via the monitoring of adverse events (AEs), vital signs, 12-lead ECGs, blood safety laboratory tests, and urinalysis. Treatment-emergent adverse events (TEAEs) were defined as AEs that appeared or worsened during or after study treatment.

Randomization and Masking

This study was open label. To reduce bias, videos of the 9HPT-D and 8MWT from the baseline, end of treatment, and end of washout visits were randomized by Medpace Core Laboratories (MCL) to create 3 video pairs. These randomized video pairs were released for review to 2 central, blinded raters via the secure MCL Clintrak Imaging System Portal. Access to the randomized sequences was restricted to the MCL IB1001 study team.

Statistical Analysis

The primary end point was defined as the CI-CS comparing performance at the end of treatment with NALL (visit 4) with the performance at baseline (visit 2) minus the CI-CS performance at the end of washout (visit 6) with the one at the end of treatment with NALL (visit 4). The CI-CS end point was designed to capture clinical improvement during treatment with NALL and deterioration once treatment was stopped. It was estimated that a sample size of 30 patients would be needed to provide the trial with 76% power at a 1-sided significance level of 5% to detect a mean effect of at least 0.45 in the primary end point (assuming an SD of 1.02). Because of the rarity of GM2 gangliosidosis and the resulting limited potential pool of patients, it was not feasible to specify a higher level for the power. The data analysis though was not dependent on the value chosen for study power.

The analysis of the primary end point was performed on the modified intention-to-treat (mITT) population, comprising all patients who received at least 1 dose of the study drug and who had 1 baseline video (visit 1 or 2 or both) and 1 treatment period video (visit 3 or visit 4 or both). A last observation carried forward approach was used for the primary CI-CS end point, in which the CI-CS value for visit 4 to visit 6 was assigned the value 0 (stable), if both videos from the washout period (i.e., both visit 5 and visit 6) were missing. The null hypothesis was that the mean is 0 (no change), with the alternative hypothesis that this mean was >0. A single-sample, 1-tailed t test was used to compare the mean of the CI-CS differences with zero at a significance level of 5%. Nonparametric 90% CIs were calculated using the Hodges-Lehmann method.[17] Secondary end points were evaluated either statistically based on a single-sample t test or a single-sample Wilcoxon signed-rank test or descriptively. No formal hierarchical structure was defined for the secondary end points, and analyses presented for these end points should be considered exploratory only. Separate analyses were performed for key subgroups as predefined in the statistical analysis plan (SAP). The safety population included all patients who received at least 1 dose of study drug. An independent data safety monitoring board (DSMB) consisting of 3 independent, nonparticipating members (including 2

clinicians and a statistician) monitored safety, study conduct, and progress and was involved in risk assessments of the effect of COVID-19.

Data Availability

All authors were provided with full access to all the data in the study and were responsible for the final decision to submit for publication. The study Sponsor, IntraBio Ltd., is dedicated to sharing anonymized data and information about the clinical study information, which supports further scientific research. Requests for these data will be considered in the context of the basis of the request, how the data will be used, and how the data will be analyzed to be of value to the scientific community. Therefore, there are circumstances that may prevent IntraBio from sharing the requested data at this time. Request for anonymized data from the clinical trial or additional information about the trial can be submitted after the product is approved in the United States and European Union, or if development of the product is ceased, or as otherwise required by law or regulation. At the time of publication, the product remains under development.

Results

Study Population

Thirty-six participants were screened between June 7 2019, and October 1, 2020, and 30 patients qualified for inclusion (Figure 1). Patient baseline demographic and clinical characteristics are shown in Table 2. The mean age was 27 (SD 15.2) years, with a range of 6–55 years. Twenty-seven patients (90%) completed the parent study (visit 6). A patient was withdrawn after visit 2 due to a self-reported tremor they believed was related to the investigational medicinal product. The patient did not participate in a follow-up or early termination visit, and thus, the principal investigator was unable to assess the patient in person/further evaluate the causality of this AE. Another patient was withdrawn after visit 3, as they were unwilling to travel due to the COVID-19 pandemic outbreak.

Patients were exposed to NALL for a median (range) duration of 49 (16–132) days and a mean duration of 58.8 days. The range varied widely due to the urgent measures implemented to safeguard patients from exposure to COVID-19.

Figure 1 IB1001-202 Study Flow Diagram

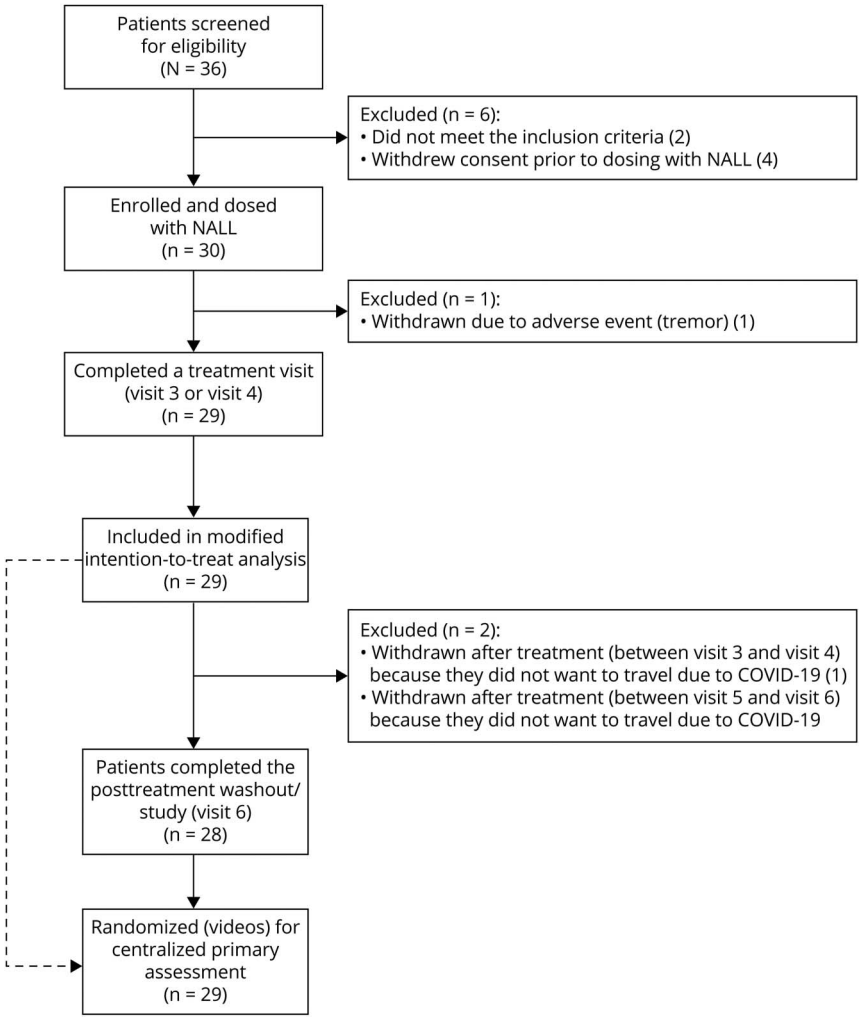
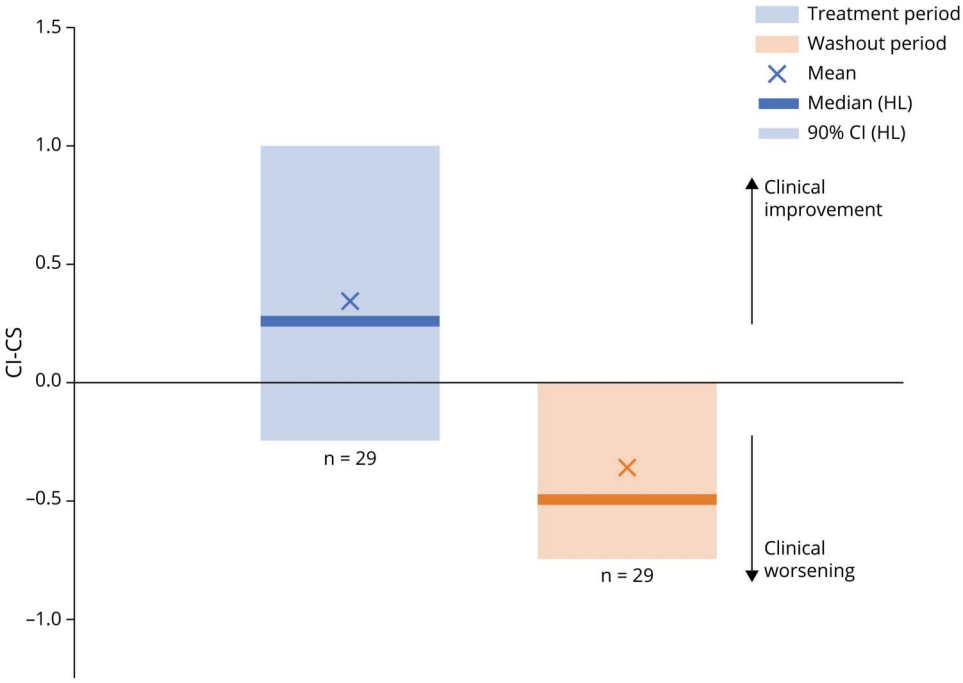


Table 2 Subject Disposition and Baseline Information (Safety Analysis Set Population)

Age (y)	Mean (SD)	27.0 (15.2)
	Median	28.5
	Range	6.0–55.0
Ethnicity, n (%)	Asian	1 (3.3)
	White	29 (96.7)
Sex, n (%)	Male	11 (36.7)
	Female	19 (63.3)
Age group, n (%)	Pediatric (<18 y)	10 (33.3)
	Adult (≥18 y)	20 (66.7)
Dose, n (%)	Age 6–12 y—15 to <25 kg—2g per day	3 (10.0)
	Age 6–12 y—25 to <35 kg—3g per day	4 (13.3)
	Age 6–12 y—≥35 kg—4g per day	1 (3.3)
	Age ≥13 y—4g per day	22 (73.3)
Geographic location, n (%)	United States	10 (33.3)
	Europe	20 (66.7)
Disease, n (%)	Tay-Sachs	27 (90.0)
	Sandhoff	3 (10.0)
Selected primary anchor test, n (%)	8-Meter Walk Test (8MWT)	12 (40.0)
	9 Hole Peg Test–Dominant Hand (9HPT-D)	18 (60.0)

Figure 2 Primary End Point: Clinical Impression of Change in Severity (CI-CS) (mITT)



The CI-CS was calculated from a 7-point scale, ranging from −3, "significantly worse", to 0, "no change", to +3 "significantly improved." The panel compares the CI-CS score calculated for the end of the treatment period vs end of baseline (left-hand column, in blue) with the CI-CS score calculated for the end of the washout period vs end of treatment (right-hand column, in orange). The vertical length of the column represents the 90% Hodges-Lehmann (HL) CI of the CI-CS. Solid lines are used to denote the Hodges-Lehmann Median Estimator, and cross symbols are used to denote the mean response.

Efficacy

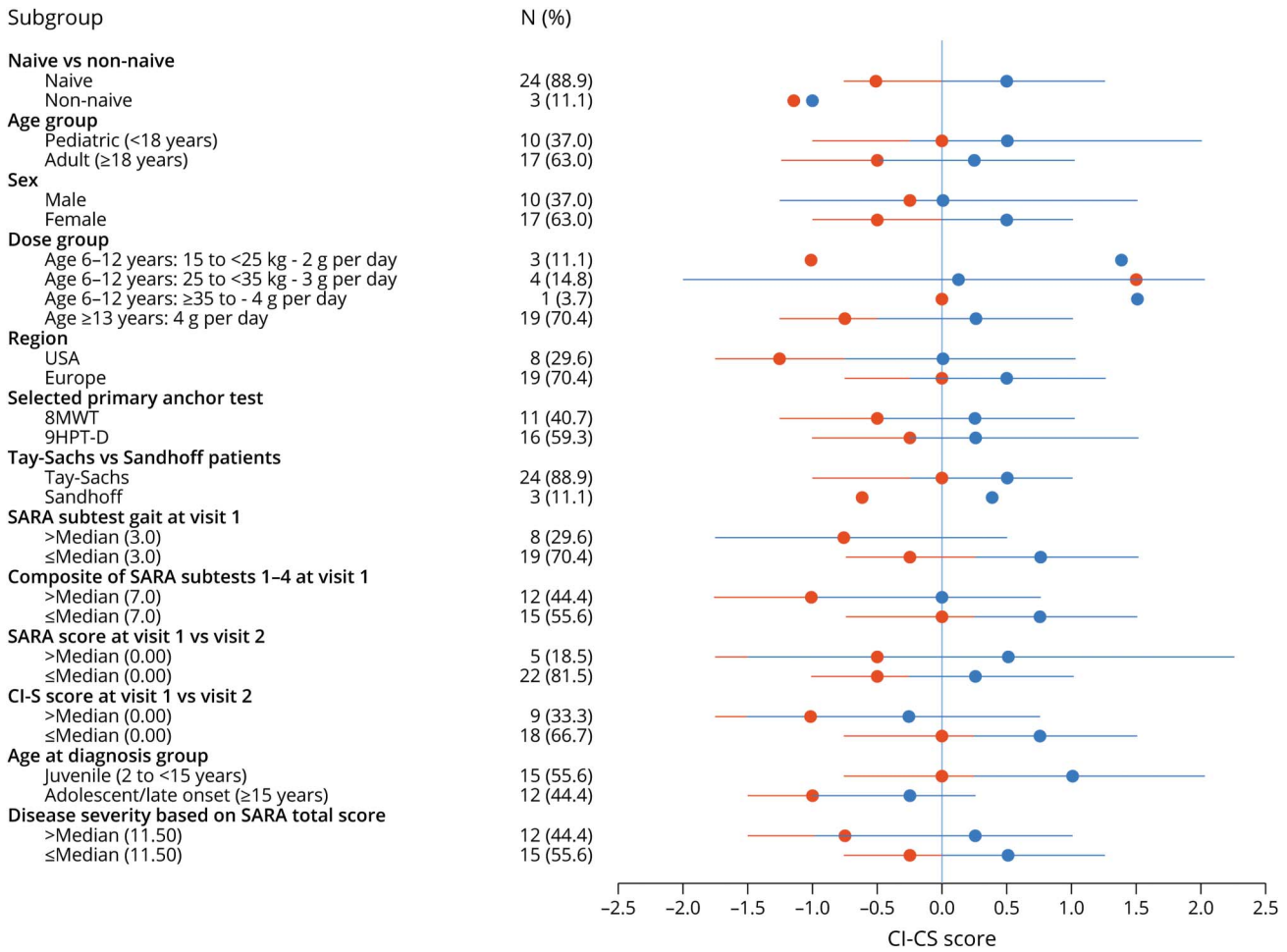
Overall, patient performance on their primary anchor test, as evaluated by the blinded, independent rates, improved on NALL with a mean difference of 0.34 (SD = 1.59, median = 0.5). Conversely, patient performance worsened during the washout period, with a mean value of −0.36 (SD = 1.33, median = −0.50). No difference was observed between the CI-CS comparing the baseline and washout visits (visit 6 vs visit 2), with a mean value 0.063 (SD = 1.32, median = 0, n = 30). This demonstrated the absence of a learning effect on the CI-CS anchor tests. The CI-CS primary end point of the study reached statistical significance with $p = 0.039$, with mean value = 0.71 (SD = 2.09, median = 1.0) and Hodges-Lehmann 90% CI of 0.00, 1.50 (Figure 2).

The interrater correlation of the CI-CS scores was calculated for 81 pairs of videos recorded in the trial. The Spearman rank correlation was 83%, indicating a high degree of consistency in the blinded raters' assessment of the videos. This result was comparable with the interrater correlation of 70% in the NALL clinical trial for NPC (IB1001-201).[3] In the IB1001-201 (NPC) and IB1001-202 (GM2 gangliosidoses) trials, different primary and adjudication raters were used. That this degree of consistency was observed between raters is supportive of the robustness of the CI-CS methodology.

Subgroup analysis conducted on the primary CI-CS end point for the key predefined populations, including age, age at disease onset, and disease severity, indicated the benefits of treatment applied to the entire study population (Figure 3). As expected with the small sample sizes, some variation can be observed; however, the analysis shows that NALL is similarly efficacious across all demographics, with consistent improvement under treatment and a return to baseline after the posttreatment washout.

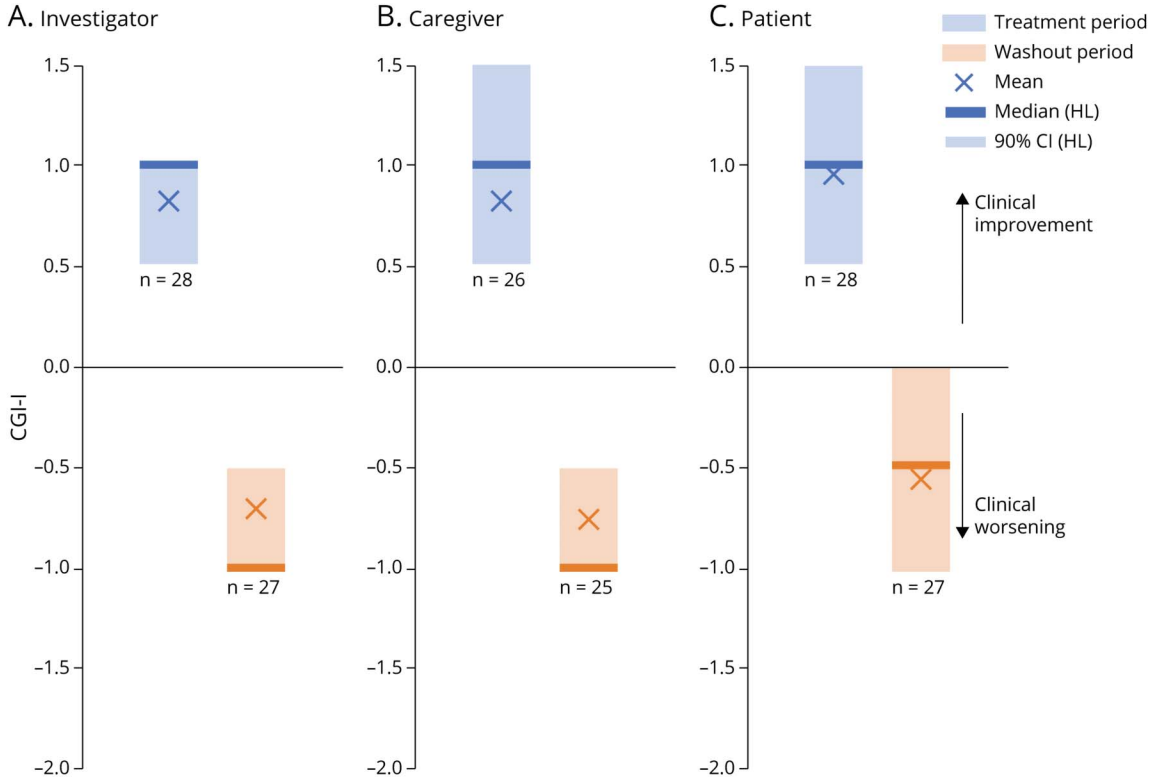
The change of the CGI was evaluated by the investigator, caregiver, and patient to overall assess the patient's physical

Figure 3 Forest Plot Subgroup Analyses for the Primary End Point: CI-CS Scores (mITT)



The change in the CI-CS scores during the treatment period (end of treatment vs baseline; depicted in blue) and during the washout period (end of washout vs end of treatment; depicted in orange) is displayed for individual, predefined subgroups. The dots represent the pseudo-medians or Hodges-Lehmann estimators. Some subgroups were large enough to calculate 90% CIs (represented by the horizontal lines). Only values from patients with reported data were included in the subgroup analyses—no last observation carried forward (LOCF) approach was applied.

Figure 4 Secondary End Point: Clinical Global Impression of Change (mITT)



(A) Physician's CGI-C. (B) Caregiver CGI-C. (C) Patient's CGI-C. For each scale, the results comparing baseline to end of treatment (left-hand column, blue) are compared with the results comparing the end of the treatment period to the end of the washout period (right-hand column, orange). The vertical length of the column represents the 90% Hodges-Lehmann (HL) CI of the CGI-C. Solid lines are used to indicate the Hodges-Lehmann Median Estimator, and cross symbols are used to denote the mean response.

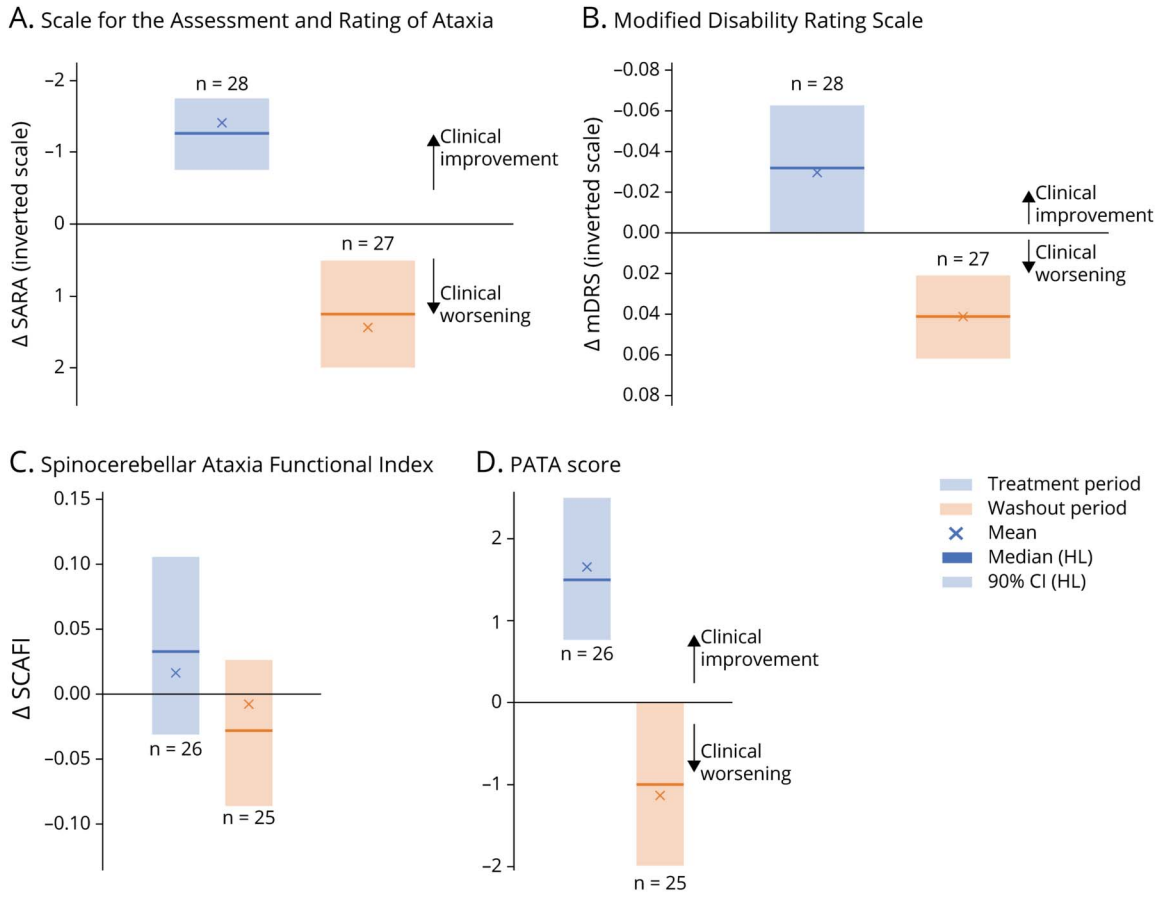
and cognitive status. Following the 6-week treatment period with NALL, there was consensus between the 3 evaluating groups that the patient had significantly improved during the treatment period (investigators: 90% CI (0.5, 1.0), $p < 0.001$; caregivers: 90% CI (0.5, 1.5), $p = 0.001$; patients: 90% CI (0.5, 1.5), $p < 0.001$). Comparably, following the posttreatment washout, a mutual deterioration was observed (investigators: 90% CI (−1.0, −0.5), $p < 0.001$; caregivers 90% CI (−1.0, −0.5), $p < 0.001$; patients: 90% CI (−1.0, 0.0), $p = 0.01$) (Figure 4, A–C).

The SARA score was applied to evaluate cerebellar function and overall neurologic status. At baseline, the study population displayed a full range of disease severity. The respective minimum and maximum individual SARA scores were 5 and 33 out of a maximum of 40 points (mean baseline score 14.24). Under treatment with NALL, the average SARA score decreased −1.41 points compared with baseline (90% CI (−1.75, −0.75), $p < 0.001$), indicating an improvement in cerebellar ataxia. After the posttreatment washout, the SARA score increased by 1.43 points compared with the end of the treatment period (90% CI (0.50, 2.00), $p < 0.001$), thus returning to the baseline value and once again showing that the benefits gained during the treatment period were lost on washout (Figure 5A).

The mDRS scores for overall neurologic function tracked consistently with the SARA scores for cerebellar ataxia: After the 6-week treatment period with NALL, the mDRS score changed significantly (90% CI (−0.063, 0.00), $p = 0.020$), the benefits of which were lost after patients stopped medication with NALL during the washout period (90% CI (0.021, 0.0635, $p < 0.001$) (Figure 5B).

The SCAFI scale lacked sensitivity because of the heterogeneity of symptoms of the enrolled cohort, compromising the individual domains. Only 17 patients were able to complete the 8MWT, and 23 patients were able to complete the 9HPT-D or 9HPT-ND at the treatment and washout visits. No overall changes were therefore seen for the SCAFI total score or these single domains (Figure 5C). However, 29 patients were able to complete the PATA at baseline, and a significant change was observed over the treatment and washout period. The total PATA score was 18.96 at baseline, 20.56 on medication, and 19.28 after the posttreatment washout (Figure 5D), replicating the SARA and mDRS results, respectively. These changes were statistically significant (treatment: 90% CI 0.75, 2.50, $p < 0.001$; washout: 90% CI −2.00, 0.00, $p = 0.027$), demonstrating a clear improvement in speech under NALL.

Figure 5 Secondary Functional End Points (mITT)



(A) Scale for the Assessment and Rating of Ataxia (SARA). (B) Modified Disability Rating Scale (mDRS). (C) Spinocerebellar Ataxia Functional Index (SCAFI). (D) PATA Speech Test. For each test, the results comparing baseline to end of treatment (left-hand column, blue) are compared with the results comparing the end of the treatment period to the end of the washout period (right-hand column, orange). The vertical length of the column represents the 90% Hodges-Lehmann (HL) CI. Solid lines are used to denote the Hodges-Lehmann Median Estimator, and cross symbols are used to denote the mean response.

The results of EQ-5D-5L/EQ-5D-Y and EQ-VAS were summarized by visit using descriptive statistics, consistent with the questionnaires. Of the domains, there was a trend for improvement in patient's mental health, as evaluated by the anxiety/depression domain.

Safety

Five AEs assessed as related to treatment were reported in 10% of patients (3 patients in total), which were flatulence, asthenia, acne, and 2 accounts of tremor. None of the related AEs were serious. No deaths occurred during the study. No clinically relevant changes were observed in safety laboratory tests, urine analysis, vital signs, or ECG recordings. In total, this study provides Class IV evidence that NALL improves outcomes for patients with GM2 gangliosidoses.

Discussion

In this phase IIb clinical trial in patients aged 6 years or older with GM2 gangliosidosis, NALL improved cerebellar signs, fine motor skills, ambulation (gait) and stance, and speech. These improvements were observed irrespective of age, sex, age at disease onset, and baseline disease severity, suggesting NALL's applicability as a treatment for all patients with GM2 gangliosidoses. Improvements in neurologic status correlated with improvements in functioning and quality of life. NALL was well tolerated with a low frequency of related AEs, none of which were serious.

IB1001-202 is the second multinational, phase IIb clinical trial to be completed with NALL. The results of the trial are consistent with a parallel trial completed for NPC, where treatment also had a statistically significant and clinically meaningful effect on the primary CI-CS and secondary (SARA, CGI-C) end points.[3]

As briefly described, these clinical findings correlate directly with studies in the GM2 gangliosidoses mouse model ($Hexb^{-/-}$) and NPC mouse model ($NPC^{-/-}$), where acetyl-leucine reduced ataxia when treatment was commenced pre-symptomatically (from 3 weeks of age onward) or symptomatically (for 1-week treatment, starting at 8 weeks of age).[3,8,9] NALL restored aerobic (pyruvate dehydrogenase

dependent) and enhanced anaerobic (lactate dehydrogenase dependent) glycolysis and returned the glutamate-metabolizing enzyme, glutamate dehydrogenase, to levels observed in *Hexb*$^{+/+}$ and *NPC* $^{+/+}$ null mice. In general, considering the normalization of the altered glucose and glutamate metabolism, NALL improves energy production, as well as cellular functions and signaling.[3,8,9] This is manifested via the drugs modulation of multiple secondary therapeutic targets, including a reduction in lipid and cholesterol accumulation and lysosomal volume,[8,9,18] a reduction in neuroinflammation,[19] and a normalization of neuronal membrane potential.[20] These multimodal actions lead to the restoration of neuronal function and improvement of overall brain health, including throughout the cerebellum. These effects correlate with NALL's symptomatic effects (e.g., improvement of postural stability, gait, fine motor skills with diadochokinesia, and speech). These effects also indicate NALL's neuroprotective action—and ability to slow or even stabilize neurodegeneration—which is being investigated in the ongoing extension trial.

As previously described,[3,4] the master protocol used has several limitations. This study was single armed, and participants, caregivers, and site staff were aware of the current study phase and treatment. This approach was chosen due to the rarity of the condition and the documented, widespread, unlicensed use of the commercially available racemate (N-acetyl-DL-leucine; Tanganil) or even chemical grade NALL within the GM2 gangliosidoses community.[21] Patients and caregivers were reluctant to discontinue treatment with N-acetyl-leucine to take an inactive placebo, and hence, the open-label, rater-blinded paradigm was implemented. The primary CI-CS assessment minimized the bias of the single-arm, open-label approach through the intrapatient, internal control of each patient's washout arm and the centralized, blinded ratings of randomly paired videos. Moreover, the majority of patients enrolled in the trial were severely physically impaired with mild to significant levels of cognitive impairment. Given the extent of impairment at baseline, the potential for a placebo effect on neurologic signs and symptoms is fundamentally reduced. However, to further reduce bias and thus to ensure the interpretability of the assessment, execution and video recording of the anchor tests were standardized across sites, with site personnel trained on detailed protocols, detailing precise verbal instructions, encouragement, break times between test trials, and instructions on which trial to record. This single-arm approach is in agreement with principles aiming to minimize the exposure of patients with orphan diseases, and in particular pediatric patients, to placebo.[22]

A further limitation was that the novel CI-CS primary end point is not yet validated. The CI-CS was chosen as the primary end point over preexisting scales due to the methodological limitations of applying preexisting ataxia scales in diseases in which neurodegeneration is widespread with a variety of symptoms and the neurologic progression and severity differ greatly between individuals. In such cases, the scales may be too specific regarding the respective neurologic functions, for example, cerebellar, and therefore not sensitive enough to capture small but meaningful functional changes.[4,23] Accordingly, the CI-CS was developed as a more clinically relevant end point capable of recognizing clinically meaningful treatment effects across the whole patient population. The primary efficacy analysis was supported by significant improvements observed in SARA and CGI, both validated, established scales. Finally, because of the small size sample characteristic of the ultra-orphan setting, a 1-tailed p value with significance level of 5% was used for the primary end point.

This study demonstrates statistically significant and clinically meaningful improvements in GM2 gangliosidoses patients treated with NALL. NALL at a dose of up to 4 g/d was safe and well tolerated. The findings from IB1001-202 in GM2 are in agreement with those from previous studies and provide further evidence that NALL may improve symptoms in patients with GM2 gangliosidoses, as well as similar progressive, life-threatening conditions.

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