Safety, tolerability, and pharmacokinetics of antisense oligonucleotide BIIB078 in adults with *C9orf72*-ALS: a phase 1, randomised, double blinded, placebo-controlled, multiple ascending dose study

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Summary

Background Hexanucleotide repeat expansion of chromosome 9 open reading frame 72 (*C9orf72*) is a common genetic cause of amyotrophic lateral sclerosis (ALS). Currently, no *C9orf72*-targeted treatments are available. BIIB078 is an investigational antisense oligonucleotide targeting *C9orf72* sense RNA. This phase 1 study assessed the safety, tolerability, and pharmacokinetics of BIIB078 in participants with *C9orf72*-ALS.

Methods This was a randomised, double blinded, placebo-controlled, multiple ascending dose study of adults with *C9orf72*-ALS. Participants were enrolled at 22 sites in six countries: USA, Canada, Netherlands, UK, Switzerland, and Ireland. Adults with ALS and a pathogenic repeat expansion in *C9orf72* were randomly assigned via Interactive Response Technology in a 3:1 ratio (per cohort) to receive BIIB078 (5, 10, 20, 35, 60, or 90 mg in cohorts 1–6, respectively) or placebo (all cohorts) via an intrathecal bolus injection. The treatment period consisted of three loading doses of study treatment, administered approximately once every 2 weeks, followed by monthly maintenance doses comprising a treatment period of ~3 months for Cohorts 1-3 and ~6 months for Cohorts 4-6. The primary endpoint was the incidence of adverse events (AE) and serious AEs. Secondary endpoints included serum BIIB078 concentration, serum pharmacokinetic parameters, and clinical assessments.

Findings A total of 106 participants were enrolled with 27 receiving placebo and 79 receiving BIIB078: 90 mg (n=18), 60 mg (n=18), 35 mg (n=19), 20 mg (n=9), 10 mg (n=9) and 5 mg (n=6). All participants had at least one AE; most AEs were mild or moderate in severity and were not treatment limiting. The most common AEs in BIIB078-treated participants were falls, procedural pain, headache, and post lumbar puncture syndrome. Fourteen participants (18%) receiving BIIB078 reported SAEs, while nine participants (33%) receiving placebo reported SAEs. Five participants who received BIIB078 and three participants who received placebo had fatal AEs: respiratory failure (n=1) in the 10 mg cohort; ALS (n=2) and traumatic intracranial haemorrhage (n=1) in the 35 mg cohort; pulmonary embolism (n=1) in the 60 mg cohort; and respiratory failure (n=3) in the placebo group. All deaths were assessed as not related to the study treatment by the investigator. Though target engagement was demonstrated via reductions in poly(GP) and poly(GA) levels in the CSF, the top dose BIIB078 cohort experienced increases in neurofilament levels and worsening on clinical assessments relative to placebo.

Interpretation Based on these phase 1 study results, namely lack of reduction in neurofilament levels and lack of benefit on clinical outcomes relative to the placebo group, BIIB078 clinical development has been discontinued. However, these results will be informative in evolving our understanding of the complex pathobiology of *C9orf72*-ALS.

Funding This study (NCT03626012, Eudra CT 2017-000294-36) was sponsored by Biogen.

Research in Context

Evidence before this study: Amyotrophic lateral sclerosis (ALS) due to mutations in chromosome 9 open reading frame 72 (*C9orf72*-ALS) is the most common known genetic cause of ALS among those with and without a family history of ALS. We searched PubMed for clinical trial articles published in English before Jan 15, 2024, using the terms "amyotrophic lateral sclerosis" and "C9orf72". As of that date, no *C9orf72*-targeted treatments are available and there is a high unmet need given the life-threatening nature of this disease. BIIB078 is designed to reduce the toxic gain of function in *C9orf72*-ALS.

Added value of this study: We present results from a phase 1, randomised, double blinded, placebo-controlled, multiple ascending dose study conducted to evaluate the effects of BIIB078 administered intrathecally in adults with *C9orf72*-ALS. A total of 106 participants were enrolled with 27 receiving placebo and 79 receiving BIIB078: 5 to 90 mg. Most adverse events were mild or moderate in severity and were not treatment limiting. While target engagement was achieved, administration of BIIB078 at all dose levels did not slow disease progression. At the highest dose tested, an increase in neurofilament light chain and numerical clinical decline were observed.

Implications of all the available evidence: Based on these phase 1 study results, namely the elevations in NfL and consistent numerical declines in clinical outcome measures, BIIB078 clinical development has been discontinued, including the open-label extension study. However, these results will be informative in evolving understanding of the pathobiology of *C9orf72*-ALS. These results show the heterogeneity of pathology among different genetic mutations causing ALS such as *C9orf72*, and the importance of conducting well-designed phase 1 studies, including biomarkers of target engagement and efficacy.

Introduction

In ~10% of people living with amyotrophic lateral sclerosis (ALS), the disease is thought to be caused by a hexanucleotide repeat expansion (HRE) in the intron 1 non-coding region of chromosome 9 open reading frame 72 (*C9orf72*). *C9orf72*-ALS is the most common known genetic cause of ALS among many North American and European people, and can occur in individuals with and without a known family history of ALS.[1-5] The dominant hypothesis of pathogenicity suggests that the HRE leads to a toxic gain of function through: (i) the production of sense and antisense repetitive RNA species that form RNA foci and (ii) dipeptide repeat (DPR) proteins that are translated from the sense and antisense RNA transcripts through a mechanism known as repeat-associated non-AUG (RAN) translation. It has also been proposed that loss of *C9orf72* function may contribute to the pathophysiology of *C9orf72*-ALS or the toxicity driven directly by the DNA expansion.[6] Currently, no *C9orf72*-targeted treatments are available, and there is a high unmet need given the life-threatening nature of this disease.

In *C9orf72*-ALS, the hexanucleotide repeats reside between exon 1a and exon 1b of the *C9orf72* gene. Transcription of this *C9orf72* gene may initiate at either exon 1a or exon 1b, giving rise to three common RNA sense transcripts, including V1 and V3 (both initiated at exon 1a, and therefore including hexanucleotide repeats), and V2 (initiated at exon 1b, and therefore not including the hexanucleotide repeats). BIIB078 is an investigational antisense oligonucleotide (ASO) designed to bind to a site in intron 1 that is adjacent to the 5' end of hexanucleotide repeat-containing sense RNA transcribed from the mutant *C9orf72* gene, resulting in its degradation via RNase H. BIIB078 targets exon 1a-initiated RNA transcripts (V1 and V3) generated from both mutant and normal *C9orf72* alleles, and is designed with the goal of decreasing production of toxic RNA and DPRs, with the intent of reducing the toxic gain of function in *C9orf72*-ALS without altering V3 transcript levels. While BIIB078 targets the hexanucleotide repeat regions in V1 and V3 sense transcripts, it does not target the antisense transcripts generated between exon 1a and exon 1b, therefore not altering production of both DPR and RNA foci from the antisense strand (figure 1). Moreover, BIIB078 does not target exon 1b-initiated transcripts, which have been shown to be the dominant RNA transcript produced from the *C9orf72* gene. Therefore, it is expected

that total *C9orf72* RNA and protein expression will be largely preserved with BIIB078 treatment, thus not markedly exacerbating known reductions of *C9orf72* expression. In a preclinical study of mice expressing *C9orf72* RNAs with up to 450 GGGGCC repeats or with one or both *C9orf72* alleles inactivated, administration of a single dose ASO that targets repeat-containing sense C9orf72 transcripts was shown to significantly reduce the accumulation of repeat-containing *C9orf72* and poly(GP) and poly(GA) proteins in the cortex and spinal cord, RNA foci in the dentate gyrus, and the development of behavioural deficits.[7]

A phase 1, randomised, double blinded, placebo-controlled, multiple ascending dose (MAD) study was conducted to evaluate the effects of BIIB078 administered intrathecally in adults with *C9orf72*-ALS. Dose levels for the MAD were selected based on the expected target tissue concentrations of BIIB078 that were projected to achieve various levels of biological activity, derived from the identified exposure response relationships in human *C9orf72* transgenic mice and the predicted human tissue profiles from a physiologically-based PK model.[7,8]

Following completion of the MAD study (NCT03626012, Eudra CT 2017-000294-36), participants had the opportunity to screen for enrolment in an open-label extension (OLE) study evaluating the long-term safety, tolerability, pharmacokinetics (PK), pharmacodynamics (PD), and effect on disease progression of BIIB078. Here, we describe the results from the MAD study.

Methods

Study design

This was a phase 1, randomised, double blinded, placebo-controlled MAD study (NCT03626012) designed to evaluate the safety, tolerability, and PK of BIIB078 administered intrathecally to adults with *C9orf72*-ALS and conducted from September 2018 to November 2021. Participants were enrolled at 22 sites in six countries: USA, Canada, Netherlands, UK, Switzerland, and Ireland (see clinicaltrials.gov for details on included sites). The trial lasted up to ~52 weeks, and included a 6-week screening period, a 12-week (cohorts 1–3) or 24-week (cohorts 4–6) treatment period, and a follow-up period of 22 weeks for

those who did not enrol directly in the OLE. Informed consent was obtained during the Screening Period, prior to performing any study assessments.

Participants were randomly assigned via Interactive Response Technology system in a 3:1 ratio of BIIB078:placebo to receive a 15mL intrathecal bolus injection via lumbar puncture of BIIB078 (5, 10, 20, 35, 60, or 90 mg in cohorts 1–6, respectively) or placebo (artificial CSF). The treatment period consisted of three loading doses of study treatment, administered approximately once every 2 weeks, followed by two maintenance doses for cohorts 1–3 and five maintenance doses for cohorts 4–6, administered approximately once every 4 weeks. The sample size in cohorts 4 through 6 (n=15) allowed for more than 80% power to detect a reduction in key biomarkers, such as RAN dipeptides in the CSF, of an effect size of 0.89, at an α level of 0.1 (2-sided) based on 2-sample t-test. For each participant, a review of all available safety and tolerability data was performed, by the sponsor with the site Investigator, ~7 days after the first dose was administered and before administration of the second planned dose on Day 15 to allow continuation to subsequent doses. The second dose was not administered until this review was complete.

Prior to escalating to a higher dose level, a safety surveillance team reviewed all available blinded safety data from all randomised participants with the Investigators from sites where participants were dosed. In addition to review of the safety data, there was a review of trough ASO levels after the fourth dose (samples collected pre-dose on Day 85). Participants who completed the phase 1 study had the opportunity to participate in the OLE study (NCT04288856).

Participants

This trial enrolled adults (≥18 years of age) diagnosed with ALS according to the World Federation of Neurology revised El Escorial criteria[9] and documentation of a clinical genetic test demonstrating the presence of a pathogenic repeat expansion in *C9orf72*. Key inclusion criteria included: slow vital capacity (SVC) ≥50% of predicted normal adjusted for sex, age, and height (from the sitting position); and ALS Cognitive Behavioural Screen (ALS-CBS) score ≥11 for the cognitive portion and ≥33 for the behavioural portion. Participants taking concomitant riluzole at study entry were required to be on a stable

dose for ≥30 days prior to the first dose of study treatment (day 1); and participants taking concomitant edaravone at study entry were required to be on a stable dose for ≥60 days prior to the first dose of study treatment (day 1). Key exclusion criteria included: tracheostomy; pre-screening Amyotrophic Lateral Sclerosis Functional Rating Scale – Revised (ALSFRS-R) slope (ΔFRS score <0.4 points/month (defined as: (48 - ALSFRS-R score at screening) / months from date of symptom onset to date of screening); and concurrent enrolment in any other interventional study. A full list of inclusion and exclusion criteria is available in the protocol (Supplementary Materials).

Trial oversight

This trial was conducted in accordance with Good Clinical Practice Guidelines of the International Council for Harmonisation, and according to the ethical principles outlined in the Declaration of Helsinki. The protocol (Supplementary Materials) was approved by relevant ethics committees. Written informed consent was provided by the participants or their legal representatives (in the event of the participant's physical incapacity to sign, in accordance with local practice and regulations). The background of the proposed study, the procedures, the benefits and risks of the study, and that study participation is voluntary for the participant was explained to the participant (or the participant's legal representative), and the participant was required to be given sufficient time to consider whether to participate in the study. The sponsor (Biogen) and authors designed the trial. Biogen provided study treatment (BIIB078 and placebo) and oversaw the trial. Data were collected by the investigators and analysed by the sponsor. No independent statistician was involved with this study. To maintain the integrity of the trial conduct and analyses, the study management team were blinded and all analyses were prespecified prior to unblinding to eliminate potential bias.

Dose selection

Selection of dose levels was driven by the prediction of human exposures informed by a physiologically-based pharmacokinetic (PBPK) model developed based on non-human primate (NHP) PK data, as well as potencies derived from humanized *C9orf72* transgenic mice experiments.[7,8] Concentrations of BIIB078 in the human spinal cord and cortex were derived by dividing the concentrations predicted with the NHP-

based model by factors of 6.5 and 20, respectively. The scaling factors reflect approximate ratios in physiological volumes of the spinal cord and brain between cynomolgus monkeys and humans. This approach assumes that the compartmental model structure and especially CNS-related transfer rates in human are similar to those in NHP.

The exposure-response relationship was established in *C9orf72* transgenic mice and further combined with PK predictions scaled to human to predict the expected mRNA reduction in human cortex and spinal cord at different dose regimens. The EC_{50} values for BIIB078 were determined to be 0·32 μg/g in the spinal cord and 1.56 μg/g in the cortex. Doses of BIIB078 10 mg, 20 mg, 35 mg, 60 mg, and 90 mg were respectively projected to result in average *C9orf72* mRNA knockdown (at steady state) corresponding to 45%, 62%, 77%, 85%, 91%, and 94% in the cervical spinal cord, and 7%, 14%, 24%, 36%, 49%, and 59% in the cortex.

Randomisation and masking

Randomisation numbers were generated by Signant Health (Bluebell, PA, USA) Interactive Response Technology system for each cohort according to the randomisation specifications, in ascending order, as participants enrolled into the study. No treatment or dose information were shared with participants, their families, or any member of the blinded study team, either at the study site or at the sponsor or its representatives.

Endpoints

The primary endpoint was the incidence of adverse events (AEs) and serious AEs (SAEs). Secondary endpoints were serum PK parameters and measures of clinical function, including changes in ALSFRS-R scores (measure of clinical function that comprises 12 items across bulbar, fine motor, gross motor, and respiratory domains), percent-predicted SVC (measure of ventilatory strength)**,** handheld dynamometry (HHD) megascore (measure of muscle strength)**,** and Iowa Oral Pressure Instrument (IOPI; measure of tongue strength). Exploratory endpoints included assessments of cerebrospinal fluid (CSF) PK**,** PD (including poly-GP and poly-GA as indirect measures of target engagement), neurofilament light (NfL; measure of axonal injury and neurodegeneration),[10] quality of life measures (ALS Assessment

Questionnaire – 5 Item Form [ALSAQ-5], Fatigue Severity Scale [FSS], 36-Item Short Form Health Survey, and Zarit Burden Interview). The treatment duration and the frequency of the ALSFRS-R, HHD, and IOPI assessments for Cohorts 4 -6 were increased to improve the likelihood of detection of any effects of BIIB078 on amyotrophic lateral sclerosis (ALS) disease progression. Detection of such putative clinical effects during the study could accelerate development, potentially leading to the earlier availability of an effective therapy without jeopardizing subject safety. NfL concentration in both plasma and CSF were determined utilising the NF-light Advantage Kit immunoassay on the Quanterix single molecule array (Simoa) platform (Quanterix, Billerica, MA, USA) and separately using the Atellica® IM Neurofilament Light (NfL) immunoassay (Siemens Healthineers, Malvern, PA, USA). Study samples were analysed using analytically validated manufacturer's protocols at the respective manufacturer's laboratories. NfL results generated on the Siemens assay are presented in the main body, and those generated on the Quanterix assay are presented in the Supplementary Appendix. CSF samples for the assessment of *C9orf72*-RAN dipeptide proteins were scheduled to be taken pre-dose at Days 1, 15, 29, 57, 85 (cohorts 1–3) and Days 1, 15, 29, 57, 85, 113, 141, 169 (cohorts 4–6). In addition, samples were also taken at non-dosing visits on Day 134 and EOS/Day 176 (cohorts 1-3) and Day 218 and EOS/Day 260 (cohorts 4-6). To account for natural disease progression in this study population, baseline NfL, a prognostic marker for disease progression, was adjusted as a covariate. The size of expanded GGGGCC repeats was not recorded in the DNA sample collection form. Ventilatory support data was not collected in this study.

Statistical analysis

The sample size of the study was typical for first-in-human clinical studies, with the goal of balancing safety considerations for study participants and the need to obtain sufficient information to assess the safety and biological activities of BIIB078. For cohorts 4–6, a sample size of 15 participants in each cohort exposed to the experimental ASO was calculated to allow for an ≥80% probability of observing at least one occurrence of an AE with an event rate of 11%.

The analysis population included all randomised participants who received at least one dose of study treatment (BIIB078 or placebo). The actual scores and changes from baseline in secondary and exploratory clinical endpoints were summarised by visit and dose level using descriptive statistics. The change from baseline in each of these clinical function scores at day 176 for the BIIB078 60 mg and 90 mg dose levels was estimated separately versus the pooled placebo group and presented along with their 95% CIs based on a mixed-effects repeated-measures model (MMRM). The model included baseline score, scheduled visit day for data collection (days 15, 29, 57, 85, 113, 141, and 169/176 for NfL and SVC; days 36, 64, 92, 120, 148, and 176 for ALSFRS-R and HHD), treatment, treatment-by-visit interaction (to account for potential differences in response over time between the two groups), baseline-by-visit interaction, ΔFRS, and CSF NfL baseline value. In consideration of the consistency with protocol planned analyses for CSF NfL and high correlation between plasma and CSF NfL baseline, CSF NfL baseline was included in the model for analysing clinical efficacy endpoints to account for predicted patient disease progression.

Role of the funding source

The study funder was involved in study design, study monitoring, data collection and management, statistical analysis, data interpretation, and writing of the draft report of this study. All authors were able to have full access to the data, and had full responsibility for the decision to submit for publication. Authors Leonard H. van den Berg, Susie Sinks, Steve Garafalo, and Stephanie Fradette verified the underlying data in the study.

Results

A total of 106 participants were enrolled, with 27 receiving placebo across dose cohorts. Among participants who were randomised to BIIB078, 18 received 90 mg, 18 received 60 mg, 19 received 35 mg, nine received 20 mg, nine received 10 mg, and six received 5 mg (figure 2). All participants received at least one dose of study treatment and were included in the intention to treat, safety, and PK analysis populations. Overall, the clinical characteristics of the participants at baseline were generally similar in the trial groups for use of riluzole and edaravone, time since symptom onset, baseline ALSFRS-R score, and percentage predicted SVC, with some variations observed across treatment groups (table 1). Relative to placebo, baseline plasma and CSF NfL levels were higher, ΔFRS was greater, and time from symptom onset was shorter in the top-dose BIIB078 cohort (90 mg), all suggesting a faster rate of disease progression. All participants receiving BIIB078 or placebo had at least one AE, and most AEs were mild or moderate in severity (table 2). No statistically significant differences in overall adverse events were found between the high dose groups (BIIB078 60 mg and 90 mg) versus pooled placebo. In general, no dose-responsive differences were observed in the safety results across the different BIIB078 dose groups; therefore, safety results will be presented as a total BIIB078 treatment group (n=79). The most common AEs in BIIB078-treated participants by Medical Dictionary for Regulatory Activities preferred term were fall, procedural pain, headache and post lumbar puncture syndrome. Most AEs were not treatment limiting. Fourteen participants (17.7%) receiving BIIB078 reported SAEs, while nine participants (33.3%) receiving placebo reported SAEs. The most common SAEs in BIIB078-treated participants were respiratory failure, ALS (verbatim term: ALS worsening), dysphagia, fall, pneumonia aspiration, and pulmonary embolism. All SAEs were assessed as unrelated to study treatment by the investigator, and most were secondary to ALS according to investigators. There were no clinically meaningful changes in laboratory tests, including CSF white blood cells or protein in participants treated with BIIB078.

Five participants who received BIIB078 and three participants who received placebo had fatal AEs: respiratory failure (n=1) in the 10 mg cohort; ALS (n=2) and traumatic intracranial haemorrhage (n=1) in the 35 mg cohort; pulmonary embolism (n=1) in the 60 mg cohort; and respiratory failure (n=3) in the

placebo group. All deaths were assessed as not related to the study treatment by the investigator, and most fatal events were common causes of death in the ALS population. Both CSF and serum PK data from participants exposed to multiple doses in a range of 5–90 mg indicate that median concentrations of BIIB078 in CSF and blood increased in a dose-dependent manner (figure S1). A nearly linear dependence on dose was observed for median serum exposure parameters (maximum concentration [C_{max}] and area under the curve [$AUC_{0\text{-}24hr}$] after the first dose) and CSF trough levels (pre-5th dose on day 85) up to 35 mg. The dependence of median serum C_{max} and CSF trough levels on dose was slightly under-proportional above 35 mg, whereas the dose-linearity for serum $AUC_{0\text{-}24hr}$ persisted over the entire dose range. Although the BIIB078 exposures in serum and CSF were highly variable across the 5- to 90 mg dose range, the 5th and 95th percentiles of the exposure parameters increased in a dose-dependent manner. Although some individual serum C_{max} and $AUC_{0\text{-}24hr}$ values exceeded the median values achieved at the no observed AE level (NOAEL in mice (median C_{max} = 5380·0 ng/mL and AUC = 15·2 μg/mL*h. at 5 mg/kg), the cohort median values were below the median threshold values at all doses.

BIIB078 serum concentrations peaked at a median time (T_{max}) ranging between 4–6 hours after the first dose (day 1) across study groups. In all dose groups, BIIB078 serum concentrations declined below the limit of quantitation (1 ng/mL) by the end of each dosing cycle (2 or 4 weeks post-dose). No dose-related change was observed in the median T_{max} for BIIB078 serum concentrations. Mean BIIB078 levels in CSF demonstrated a steady increase until day 29, followed by a slight decrease indicating adjustment of PK due to switching from biweekly to monthly dosing (figure S1). This transient decrease was observed until day 57, then remained stable for the rest of the monthly treatment maintenance period in each dose group.Reductions in both CSF poly(GP) and poly(GA) protein levels were relatively similar, and observed across BIIB078 treatment groups, consistent with BIIB078-induced reduction of *C9orf72* RAN transcripts. Similar reductions in CSF poly(GP) and poly(GA) were observed over 6 months in the two highest dose cohorts (60 mg cohort: 65% and 44%, respectively; 90 mg cohort: 54% and 49%, respectively; figure 3). Across secondary efficacy endpoints including ALSFRS-R, SVC, HHD, and IOPI anterior or posterior assessments, no significant slowing of decline was observed with any BIIB078

treatment group compared to placebo (BIIB078 60 mg and 90 mg *vs* pooled placebo shown in figure 4, all cohorts shown in figure S3). Secondary efficacy endpoints analyzed with all-dose MMRM were generally consistent with BIIB078 60 mg and 90 mg vs pooled placebo results, showing no significant slowing of decline with any BIIB078 treatment group compared to placebo (data not shown). Greater numerical clinical declines were observed in the 90 mg BIIB078 (n=18) group compared with placebo (n=27; difference [BIIB078 *vs* placebo] in adjusted means [95% CI] –2·84 [–7·13 to 1·46]). In addition, no improvement was observed in any of the BIIB078 treatment groups when evaluating changes from baseline in ALSAQ-5 scores, FSS scores, or SF-36 scores, consistent with results of the clinical functional measures (figure S5). Consistent results were observed for each clinical endpoint with and without adjustment for covariates, including adjustment for a combination of different covariates (including age, sex, time since symptom onset to first dose of study treatment, site of onset, pre-screening slope, plasma NfL, CSF NFL, concomitant use of edaravone and/or riluzole use). Consistent results were also observed for each clinical endpoint with all dose groups included in the MMRM model. BIIB078 60 mg and 90 mg treatment groups experienced a significant increase from baseline in CSF NfL levels at 6 months compared with the placebo group (Siemens assay results: geometric mean ratio [GMR] [95% CI] to baseline: 1·32 [1·09-1·60], p=0·01 and 1·31 [1·07-1·60], p=0·01 for BIIB078 60 mg and 90 mg *vs* pooled placebo, respectively) (Siemens assay results shown in figure 5, Quanterix assay results shown in figure S2). Consistently, the BIIB078 90 mg treatment group also experienced a significant increase from baseline in plasma NfL levels at 6 months compared with the pooled placebo group (Siemens assay results: 1·23 [1·04–1·46], p = 0·02 for BIIB078 90 mg vs pooled placebo). The Siemens assay NfL levels results for BIIB078 60 mg and 90 mg versus pooled placebo are shown in figure 5, and Siemens assay results for all cohorts are shown in figure S4.

Discussion

In this trial of BIIB078 administered intrathecally to people with *C9orf72*-ALS, the most common AEs in BIIB078-treated participants were falls, procedural pain, headache, and post-lumbar puncture syndrome. Most AEs were mild-to-moderate in severity and were not treatment limiting. All SAEs and fatal events in BIIB078-treated participants were assessed as unrelated to study treatment, and most were consistent with ALS disease progression. There were no clinically relevant changes in CSF white blood cells or protein in BIIB078-treated participants, in contrast to results observed in other intrathecal ASO clinical trials.[11,12]

BIIB078 exposure generally increased in a nearly linear dose-proportional manner across the 5- to 90 mg dose groups; however, a plateauing effect of CSF and plasma exposures appeared to emerge at doses above 35 mg. Administration of BIIB078 treatment led to robust reduction of CSF poly(GP) and poly(GA) proteins (53% and 49% reduction at day 169/176 compared with baseline, respectively), consistent with BIIB078-induced reduction of *C9orf72* sense strand RNA transcripts. This reduction was predicted based on the BIIB078 concentrations in *C9orf72* transgenic mouse. The magnitude of CSF poly(GP) and poly(GA) reduction was similar in the 60- and 90 mg cohorts, suggesting the pharmacodynamic response may have been saturated at 60 mg. Notably, greater increases in NfL and numerical clinical decline were seen in the BIIB078 90 mg cohort. Poly PR (or PA), which would be produced by the antisense, C_4G_2, RNA strand, were not measured due to the unavailability of a commercial or academic assay with appropriate analytical parameters to at this time.

The dose nonlinearity observed in CSF and serum PK was mild (figure S1). However, the plateauing effect of poly(GP) and poly(GA) with increasing dose was much more remarkable and persistent.

There was no improvement observed in the BIIB078 treatment groups on any of the clinical outcomes, including measures of physical function (ALSFRS-R), respiratory function (SVC), muscle strength (HHD), and tongue strength (IOPI). Although this study was not powered to detect changes in these efficacy measures, numerically greater clinical worsening was observed for the 90 mg group, as compared with placebo across all outcome measures. Consistent with these clinical findings, increases in CSF and

plasma NfL were observed for the BIIB078 90 mg group, and increases in CSF NfL were observed for the BIIB078 60 mg group. Given the importance of this finding, samples were tested on both the Siemens and Quanterix NfL assays, and results were concordant.

One possible confounding factor for interpretation of the clinical worsening and increase in mean NfL observed in the BIIB078 90 mg group is that the participants randomized to this treatment group had more quickly progressing disease at baseline. Specifically, baseline plasma and CSF NfL levels were numerically higher in the BIIB078 90 mg group compared to the placebo group. Given NfL levels have been found to be strongly prognostic for disease progression and survival in ALS,[12-17] baseline CSF NfL levels were prospectively incorporated as a covariate across analyses to account for potential imbalances. As such, these potential differences in natural disease progression do not explain the worsening observed in the BIIB078 90 mg group.

Ultimately, this Phase 1/2 study was conducted to inform whether to move BIIB078 (and at what dose level) into later stages of development. While the treatment period and cohort size were sufficient to assess safety, PK, changes in poly(GP), poly(GA), and neurofilament levels, clinical outcome data (e.g., ALSFRS-R) should be interpreted with caution in studies of this size/duration due to heterogeneity in the population and limitations of available outcome measures. The development of BIIB078 was discontinued based on the results of this study, namely increases in NfL (generally accepted as evidence that the drug was worsening axonal injury and neurodegeneration) and lack of benefit on clinical outcomes relative to the placebo group across BIIB078 dose groups. These signals were further confirmed with longer-term follow-up in the OLE study.

This phase 1 study of BIIB078 was designed to test the therapeutic value of targeting the *sense* hexanucleotide repeat-containing *C9orf72* transcripts (initiated from exon 1a). While target engagement was achieved, as reflected by reduced poly(GP) and poly(GA) protein levels, administration of BIIB078 did not slow clinical disease progression or lower NfL. This was an unexpected outcome and not predicted by the preclinical human cellular or rodent transgenic model systems, employing single dose ASO treatment paradigms. A null or negative effect could be attributed to several potential factors

including, but not limited to: (i) a more prominent role of *antisense* hexanucleotide repeat-containing *C9orf72* transcripts (initiated from exon 1a) and translated *antisense* DPRs (i.e. PR and PA) in disease pathogenesis, (ii) the role of haploinsufficiency (failure to correct existing haploinsufficiency or exacerbation of existing haploinsufficiency by knockdown of exon 1a-initiated transcripts), (iii) suppression of *C9orf72* expansion repeat-mediated toxicity not suitably addressing downstream disease propagation (mediated, for example, via TDP-43 dysfunction), (iv) toxicity induced by DNA repeat expansion, and/or (iv) effect of ASOs on yet unknown pathogenic mechanisms in *C9orf72*-ALS.[18] In addition, it is possible that effects specific to this ASO, particularly at higher doses, may have impacted the clinical outcomes observed in this study.

Importantly, since the initiation of the BIIB078 trial, new work has revealed that an ASO targeting the *C9orf72 sense* strand does not correct TDP-43 functional deficits as measured by downstream splicing changes in human iPSC models, yet an experimental ASO targeting the *antisense* strand was able to mitigate TDP-43 mediated loss of function.[19-21] Similar to the results of this study, administration of another ASO targeting V1 and V3 transcripts (WVE-004), led to significant increases in NfL with greater clinical decline in some treatment groups versus placebo in the phase 1b/2a FOCUS-C9 study.[22] In addition, arginine-rich DPRs, including the antisense poly(PR), have been implicated in the pathobiology of *C9orf72* within both in vitro and in vivo models of disease. These data suggest that *antisense* hexanucleotide repeat-containing *C9orf72* transcripts (and/or downstream *antisense* DPRs) may be an important therapeutic target in *C9orf72*-ALS. Ongoing analysis of autopsy tissue from trial participants may help further our understanding of the underlying disease pathophysiology and effects of BIIB078.

Declaration of interests

LHvdB: advisory board for Amylyx, Biogen, Cytokinetics, Denali, QurAlis, Corcept, Novartis, Ferrer, and Sanofi.
JDR: advisory board for Expansion Therapeutics and Sanofi. Receives support from AbbVie Foundation, ALS Association, ALS Finding a Cure Foundation, American Airlines, Answer ALS Foundation, Aviators Against ALS, Bruce Edwards Foundation, Calico, Caterpillar Foundation, Chan Zuckerberg Initiative, Fishman Family Foundation, F Prime, Glaxo Smith Kline, Microsoft, M. Armstrong, Muscular Dystrophy Association, National Football League, National Institutes of Health, Stay, Strong Vs. ALS, Team Gleason, The Judith and Jean Pape Adams Charitable Foundation, Travelers Insurance, and the US Dept. of Defense
PJS: advisory board member for Aclipse Therapeutics, Benevolent AI, Biogen, Bristol Meyers Squibb, Darby Rimmer Foundation, Lilly, Novartis, Quell Therapeutics, QurAlis, Samsara Therapeutics, Pangea Botanica; receives research support from: Dept. of Defense, EU Horizon 2020, EU Innovative Medicines Initiative, Fight MND, LifeArc, the Medical Research Council, MND Association, My Name'5 Doddie Foundation, NIHR, Pfizer, Quell Therapeutics, SwanBio, and Wolfson Foundation, support for clinical trials participation has been received from Alexion, Biogen, the EU Horizon programme, and UK NIHR
SB: received research funding from AI Therapeutics, AAN, AANEM, Biogen, Ionis, MDA, Medicinova, Novartis, Orion, and Voyager Therapeutics; honoraria for patient and clinician educational activities related to ALS from AANEM Foundation, McCourt Foundation, and Medscape; institutional consulting/advisory board fees from AI therapeutics and Biogen; and platform trial coordination centre activities from HEALEY ALS and NIH-NINDS
MB: consultant to Alector, Annexon, Arrowhead, Biogen, Denali, Eli Lilly, Novartis, Roche, Sanofi, UniQure, Woolsey; clinical trial site investigator for Biogen and Orphazyme; intellectual property licensed to Biogen and Orphazyme
RCB: advisory board for MT Pharma, has a consulting role with Biogen, has Equity in Neuroquestions LLC and receives a recurring annual gift from a patient's family for research on neuralgic amyotrophy
AG: ad hoc consultant for genetic testing for Biogen; consultant on ALS trial design for Alexion, AL-S Pharma, Calico, Cytokinetics, and Sanofi; and CMO at QurAlis
JDG: institution was contracted by Biogen as a trial site and was paid for those services; received grants from MDA and NIH ALSA; and received commercial sponsorships for clinical trials from Amylyx, Biogen, Cytokinetics, and Neuralstem
OH: funding from Science Foundation Ireland grants SFI 16/RC/3948 and 20/SP/8953; received consulting fees from Accelsior, Biogen, Cytokinetics, Denali, Novartis, Orion, and Wave Pharmaceuticals; and is Editor-in-Chief of the journal ALS and Frontotemporal Degeneration
VL: institution was contracted by Biogen as a trial site and was paid for those services; Chair of iDMC at Vico Therapeutics and member of iDMCs at QurAlis and Cyclo Therapeutics
TM: consultant to Amylyx, Biogen, Cytokinetics, and QurAlis
BO: serves as a consultant for Columbia University/Tsumura Inc, MediciNova, Biogen, UniQure, Amylyx and Mitsubishi; and has research grants from Columbia University/Tsumura Inc, Biogen, MediciNova, Cytokinetics, Mitsubishi, Calico, Novartis, Sanofi, Ashvattha and TARGET ALS.
GLP: medical advisory roles for Amylyx, Avanir, Biogen, Catalyst, Cytokinetics, MT Pharma, Otsuka, and the Dept. of Defense
JR: serves on advisory boards to Libra, Inc, Golgi Inc and Transposon, Inc; consultant to Verge, Iris and Zydus, Inc
CES: nothing to disclose
MW: consultant to Allmiral, Biogen, Mitsubishi Tanabe Pharma, Neuraxpharm, and Novartis
LZ: received honorarium for being on a Biogen advisory board
PJ, FR: paid employees of Ionis Pharmaceuticals
LL, DG, MM, JI, SS, SG, SF: employees of and may hold stock in Biogen
TAF, ALG, SE: employees of Biogen at the time the study was conducted

Contributors

Study concept/design: LHvdB, JDR, PJS, LL, TAF, ALG, DG, MM, JI, SS, SE, SG, SF; Major role in acquisition of data: LHvdB, JDR, PJS, SB, MB, RCB, AG, JDG, OH, VL, TM, BO, GLP, JR, CES, MW, LZ; Analysis/interpretation of data: all authors; Drafting/revision of the manuscript for content: all authors.

Data sharing statement

Individual participant data collected during the trial may be shared after anonymization and on approval of the research proposal. Biogen commits to sharing patient-level data, study-level data, CSRs, and protocols with qualified scientific researchers who provide a methodologically sound proposal. Biogen reviews all data requests internally based on the review criteria and in accordance with our Clinical Trial Transparency and Data Sharing Policy. Deidentified data and documents will be shared under agreements that further protect against participant reidentification. To request access to data, please visit https://vivli.org/.

Acknowledgments

This study was funded by Biogen. Biogen provided funding for medical writing support in the development of this paper; Yien Liu, PhD (Excel Scientific Solutions, Fairfield, CT) wrote the first draft of the manuscript based on input from the authors, and Cara Farrell (Excel Scientific Solutions, Fairfield, CT) copyedited and styled the manuscript per journal requirements. Biogen reviewed and provided feedback on the manuscript. The authors had full editorial control of the manuscript, providing critical input on the draft manuscript, and provided their final approval of the final manuscript.

The authors would like to thank the study participants and their caregivers and families, the Study Steering Committee, Investigators (full list of Principal Investigators provided in Supplement) and site

staff, and the entire community, without whom this important study could not have been conducted.

References

1 Renton AE, Chiò A, Traynor BJ. State of play in amyotrophic lateral sclerosis genetics. *Nat Neurosci* 2014; **17:** 17-23.

2 Jiang J, Ravits J. Pathogenic mechanisms and therapy development for C9orf72 amyotrophic lateral sclerosis/frontotemporal dementia. *Neurotherapeutics* 2019; **16:** 1115-32.

3 Majounie E, Renton AE, Mok K, et al. Frequency of the C9orf72 hexanucleotide repeat expansion in patients with amyotrophic lateral sclerosis and frontotemporal dementia: a cross-sectional study. *Lancet Neurol* 2012; **11:** 323-30.

4 Renton AE, Majounie E, Waite A, et al. A hexanucleotide repeat expansion in C9ORF72 is the cause of chromosome 9p21-linked ALS-FTD. *Neuron* 2011; **72:** 257-68.

5 Zou ZY, Zhou ZR, Che CH, Liu CY, He RL, Huang HP. Genetic epidemiology of amyotrophic lateral sclerosis: a systematic review and meta-analysis. *J Neurol Neurosurg Psychiatry* 2017; **88:** 540-49.

6 Liu Y, Huang Z, Liu H, et al. DNA-initiated epigenetic cascades driven by C9orf72 hexanucleotide repeat. *Neuron* 2023; **111:** 1205-21.e9.

7 Jiang J, Zhu Q, Gendron TF, et al. Gain of Toxicity from ALS/FTD-Linked Repeat Expansions in C9ORF72 Is Alleviated by Antisense Oligonucleotides Targeting GGGGCC-Containing RNAs. *Neuron* 2016; **90:** 535-50.

8 Monine M, Norris D, Wang Y, Nestorov I. A physiologically-based pharmacokinetic model to describe antisense oligonucleotide distribution after intrathecal administration. *J Pharmacokinet Pharmacodyn* 2021; **48:** 639-54.

9 Brooks BR, Miller RG, Swash M, Munsat TL. El Escorial revisited: revised criteria for the diagnosis of amyotrophic lateral sclerosis. *Amyotroph Lateral Scler Other Motor Neuron Disord* 2000; **1:** 293-9.

10 Kuhle J, Barro C, Andreasson U, et al. Comparison of three analytical platforms for quantification of the neurofilament light chain in blood samples: ELISA, electrochemiluminescence immunoassay and Simoa. *Clin Chem Lab Med* 2016; **54:** 1655-61.

11 Miller T, Cudkowicz M, Shaw PJ, et al. Phase 1-2 trial of antisense oligonucleotide tofersen for SOD1 ALS. *N Engl J Med* 2020; **383:** 109-19.

12 Miller TM, Cudkowicz ME, Genge A, et al. Trial of antisense oligonucleotide tofersen for SOD1 ALS. *N Engl J Med* 2022; **387:** 1099-110.

13 Lehnert S, Costa J, de Carvalho M, et al. Multicentre quality control evaluation of different biomarker candidates for amyotrophic lateral sclerosis. *Amyotroph Lateral Scler Frontotemporal Degener* 2014; **15:** 344-50.

14 Thompson AG, Gray E, Verber N, et al. Multicentre appraisal of amyotrophic lateral sclerosis biofluid biomarkers shows primacy of blood neurofilament light chain. *Brain Commun* 2022; **4:** fcac029.

15 Weydt P, Oeckl P, Huss A, et al. Neurofilament levels as biomarkers in asymptomatic and symptomatic familial amyotrophic lateral sclerosis. *Ann Neurol* 2016; **79:** 152-8.

16 Benatar M, Zhang L, Wang L, et al. Validation of serum neurofilaments as prognostic and potential pharmacodynamic biomarkers for ALS. *Neurology* 2020; **95:** e59-e69.

17 Saracino D, Dorgham K, Camuzat A, et al. Plasma NfL levels and longitudinal change rates in C9orf72 and GRN-associated diseases: from tailored references to clinical applications. *J Neurol Neurosurg Psychiatry* 2021; **92:** 1278-88.

18 Liu F, Rossoll W. The DAXX tax: C9orf72 DNA repeat expansions drive gain- and loss-of-function pathology in c9FTD/ALS. *Neuron* 2023; **111:** 1165-67.

19 Zaepfel BL, Rothstein JD, Coyne A. Pathomechanisms of distinct repeat RNA species in C9orf72 ALS. Society for Neuroscience; 2022; San Diego, CA, USA; 2022.

20 Mayl K, Shaw CE, Lee YB. Disease mechanisms and therapeutic approaches in C9orf72 ALS-FTD. *Biomedicines* 2021; **9:** 601.

21 Rothstein JD, Baskerville V, Rapuri S, et al. G(2)C(4) targeting antisense oligonucleotides potently mitigate TDP-43 dysfunction in human C9orf72 ALS/FTD induced pluripotent stem cell derived neurons. *Acta Neuropathol* 2023; **147:** 1.

22 Wave Life Sciences announces topline results from phase 1b/2a FOCUS-C9 study of WVE-004 for C9orf72-associated amyotrophic lateral sclerosis and frontotemporal dementia. https://ir.wavelifesciences.com/news-releases/news-release-details/wave-life-sciences-announces-topline-results-phase-1b2a-focus-c9.

***Figure 1*: *C9orf72* gene structure and transcripts. Figure adapted from Jiang and Ravits 2019[2]**

(A) Structure of the *C9orf72* gene. Yellow boxes indicate untranslated regions and blue boxes indicate translated regions; exon 2 contains the start codon for translation and is an obligatory exon for all transcripts. The red triangle indicates the location of the disease-associated hexanucleotide repeats between exons 1a and 1b.
(B) Transcripts originating from the *C9orf72* locus. Transcription initiates at either exon 1a or 1b; 1a-initiated primary transcripts but not 1b-initiated primary transcripts contain the hexanucleotide repeats (indicated by the green resistor symbols). The red box indicates the binding site for BIIB078 in intron 1, upstream of the hexanucleotide repeat region. Given the location of its binding site in intron 1, BIIB078 targets only exon 1a-initiated, repeat-containing *C9orf72* RNAs.
C9orf72 = chromosome 9 open reading frame 72; G4C2 = GGGGCC; RNA = ribonucleic acid.

***Figure 2:* Participant disposition**

AE=adverse event. ALS= amyotrophic lateral sclerosis.
*Receiving at least one dose. All deaths assessed as unrelated to study drug. Deaths in placebo were respiratory failure (n=2) and chronic respiratory failure. Deaths in the 10 mg group were due to respiratory failure. Deaths in the 35 mg group were due to traumatic intracranial haemorrhage and ALS (n=2). Death in the 60 mg group was due to pulmonary embolus. Two AEs in the 90 mg group leading to withdrawal were pulmonary embolus and respiratory failure, both assessed as unrelated to BIIB078.

***Figure 3:* Ratio of CSF poly(GP) and poly(GA) to baseline values on observed data for all cohorts**

CSF=cerebrospinal fluid. GMR=geometric mean ratio.

*Indicates common visits between cohorts 1–3 and cohorts 4–6.

***Figure 4:* Clinical outcome measures: (A) ALSFRS-R total score, (B) Percent-predicted SVC, (C) HHD megascore, (D) IOPI anterior, (E) IOPI posterior**

ITT=intention to treat.

*Indicates common visits between cohorts 1–3 and cohorts 4–6.

P values on figure represent difference in adjusted means (BIIB078 versus placebo) at last study visit.

***Figure 5:* Siemens assay results for (A) CSF NfL and (B) plasma NfL**

LS mean estimates are different between placebo groups as the model was fitted separately by dose cohort against placebo.
CSF=cerebrospinal fluid. GMR=geometric mean ratio. LS=least squares. NfL=neurofilament light chain.

*Indicates common visits between cohorts 1–3 and cohorts 4–6.

P values on figure represent ratio of GMR (BIIB078 versus placebo) at last study visit.