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RESEARCH ARTICLE

A Phase 1 study of GDC-0134, a dual leucine zipper kinase inhibitor, in ALS

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Abstract

Objective: Dual leucine zipper kinase (DLK), which regulates the c-Jun N-terminal kinase pathway involved in axon degeneration and apoptosis following neuronal injury, is a potential therapeutic target in amyotrophic lateral sclerosis (ALS). This first-in-human study investigated safety, tolerability, and pharmacokinetics (PK) of oral GDC-0134, a small-molecule DLK inhibitor. Plasma neurofilament light chain (NFL) levels were explored in GDC-0134-treated ALS patients and DLK conditional knockout (cKO) mice. **Methods**: The study included placebo-controlled, single and multiple ascending-dose (SAD; MAD) stages, and an open-label safety expansion (OLE) with adaptive dosing for up to 48 weeks. **Results**: Forty-nine patients were enrolled. GDC-0134 (up to 1200 mg daily) was well tolerated in the SAD and MAD stages, with no serious adverse events (SAEs). In the OLE, three study drug-related SAEs occurred: thrombocytopenia, dysesthesia (both Grade 3), and optic ischemic neuropathy (Grade 4); Grade ≤2 sensory neurological AEs led to dose reductions/discontinuations. GDC-0134 exposure was dose-proportional (median half-life = 84 h). Patients showed GDC-0134 exposure-dependent plasma NFL elevations; DLK cKO mice also exhibited plasma NFL compared to wild-type littermates. **Interpretation**: This trial characterized GDC-0134 safety and PK, but no adequately tolerated dose was identified. NFL elevations in GDC-0134-treated patients and DLK cKO mice raised questions about interpretation of biomarkers affected by both disease and on-target drug effects. The safety profile of GDC-0134 was considered unacceptable and led to discontinuation of further drug development for ALS. Further work is necessary to understand relationships between neuroprotective and potentially therapeutic effects of DLK knockout/inhibition and NFL changes in patients with ALS.

Introduction

Amyotrophic lateral sclerosis (ALS) is an incurable neurodegenerative disease affecting approximately 5 in every 100,000 adults in the United States.[1] Degeneration of upper and lower motor neurons leads to progressive weakness and functional disability, and most patients die within 3–5 years of onset.[2,3] The exact mechanisms underlying neurodegeneration in ALS remain poorly understood.[4–6] The only FDA-approved treatments are riluzole, which modestly improves survival, and intravenous edaravone, which can reduce the rate of functional decline.[7,8]

GDC-0134 is a potent, selective, orally available, brain-penetrant small-molecule inhibitor of dual leucine zipper kinase (DLK). GDC-0134 blocks DLK activity in cellular assays and in animal models of neuronal injury (Genentech, data on file). DLK lies upstream of the c-Jun N-terminal kinase (JNK) pathway and contributes to both axon degeneration and neuronal apoptosis following axotomy or excitotoxic injury.[9–12] Genetic deletion of DLK or DLK inhibition with small molecules blocks JNK activation and protects neurons, particularly motor neurons and their axons, from degeneration.[9,11,13,14] In a transgenic ALS mouse model ($SOD1^{G93A}$) that exhibits stress-induced activation of the DLK/JNK pathway in motor neurons, conditional knockout of DLK (DLK cKO) protected motor neurons and extended survival.[15,16] Additionally, the DLK cKO mouse model has shown that injury-induced JNK activation in neurons, but not physiological JNK activity, requires DLK.[9,10] DLK expression is largely limited to central and peripheral neurons, and inhibiting DLK provides more specificity than inhibiting JNK, which has broader expression.[17]

There are ongoing efforts to identify biomarkers to help monitor disease progression, evaluate treatment effectiveness, or improve patient stratification for clinical trials. Neurofilament light chain (NFL) is a structural component of neurons that is elevated in various neurodegenerative diseases, including ALS.[18–20] Patients with ALS exhibit increased levels of NFL both in cerebrospinal fluid (CSF) and plasma and these may correlate with the rate of disease progression.[20–22] Reductions in NFL levels theoretically correlate with a positive functional effect; however, few human trials support this hypothesis.[22] Therefore, measuring the effects of putative treatments on NFL levels is becoming common practice in ALS clinical trials.

This first-in-human trial was designed to evaluate the safety, tolerability, and pharmacokinetics (PK) of GDC-0134 in patients with ALS and explored effects of DLK inhibition on levels of plasma NFL, both in the clinical trial and in a mouse model of conditional DLK deletion. Given the novelty of this target, we included a 48-week OLE to gather longer-term safety data early in drug development.

Methods

Clinical study design

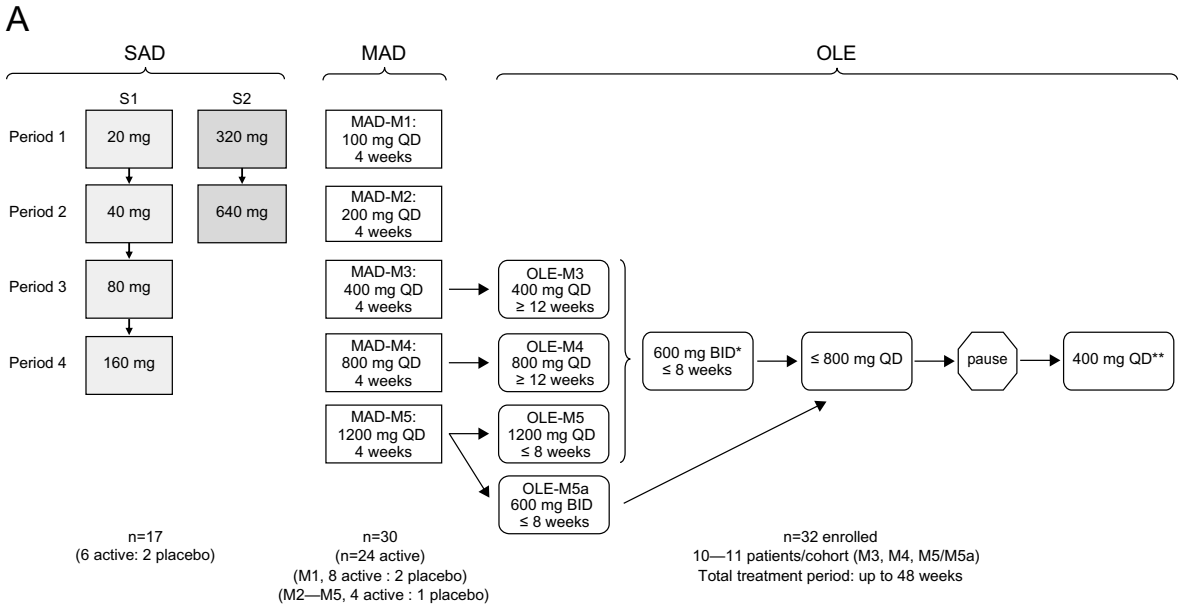
This Phase 1 study of GDC-0134 (EuDRACT: 2017–002931-41; ClinicalTrials.gov: NCT02655614) in patients with ALS included a randomized, double-blind, placebo-controlled, single ascending-dose (SAD) stage; a randomized, double-blind, placebo-controlled multiple ascending-dose (MAD) stage lasting 28 days, and an optional open-label extension (OLE) study designed to evaluate safety up to 48 weeks of dosing (Fig. 1). The study was conducted at 10 clinical sites in the United States, and one each in Canada and the Netherlands. GDC-0134 and matching placebo were administered orally as a powder-in-capsule formulation.

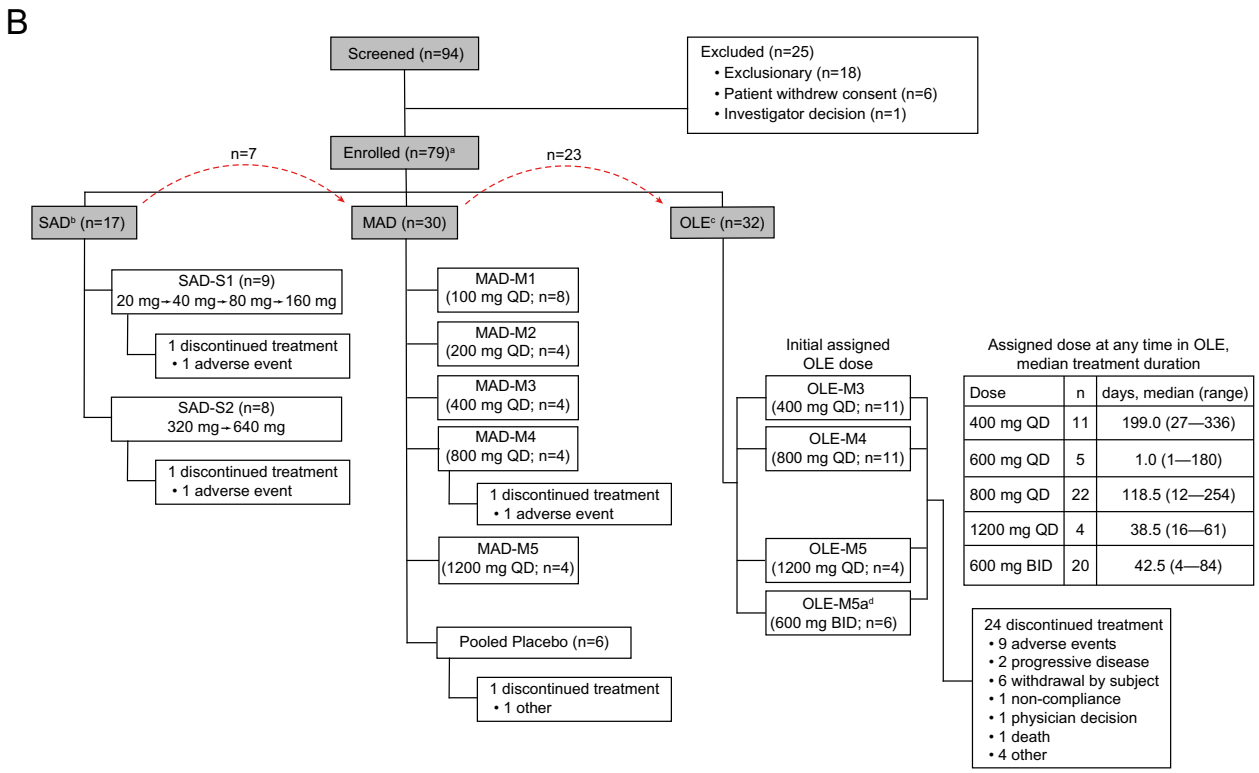
In the SAD stage, 17 patients were enrolled across two cohorts and received up to five single doses of GDC-0134

Figure 1. Study design. (A) In the SAD, in each cohort, patients were re-randomized for each dosing period; all patients received GDC-0134 at one or more periods. Patients from the SAD could participate in the MAD if no safety or tolerability concerns arose in the SAD and if at least 1 month had elapsed since their last dose. The OLE included patients who continued directly from the MAD, previously enrolled SAD or MAD patients, and treatment-naive patients. The OLE was designed to enable adaptive dosing based on the MAD; however, patients were required to remain at their initial dose level for at least 12 weeks before escalating. One dose escalation to 600 mg BID was offered in OLE after MAD M5 was completed; some but not all patients escalated. Due to subsequent safety/tolerability concerns, the highest allowable OLE dose was reduced to 800 mg QD shortly after escalation. *Not all patients escalated to 600 mg BID, and patients may have received doses below 800 mg QD before the study was paused. **After the pause, one patient resumed dosing at 400 mg QD for 11 days. (B) Study flow and treatment discontinuations. [a]Seventy-nine patients were enrolled and evaluated across the SAD, MAD and OLE. Patients appear in the summary for each stage in which they participated. In total, 49 unique patients were enrolled in the study (7 patients from SAD also participated in the MAD; 23 patients from SAD and/or MAD participated in the OLE). [b]Single ascending doses of GDC-0134 were explored in a serial fashion; each patient within a SAD cohort received a single dose of GDC-0134 or placebo on up to 4 occasions. [c]Doses assigned at OLE entry and the number of patients who received each dose throughout the OLE are indicated, along with the median (range) duration of dosing. [d]Patients who entered the OLE from MAD-M5 began dosing at 1200 mg QD and later switched to 600 mg BID, whereas patients who enrolled in the OLE after review of MAD-M5 data began dosing at 600 mg BID (OLE-M5a). NFL, neurofilament light chain; SAD, single ascending dose; MAD, multiple ascending does; OLE, open-label safety expansion; QD, once daily; BID, twice daily.

or placebo. Each dose was separated by a washout period of at least 2 weeks. Cohort SAD-S1 evaluated 20, 40, 80, and 160 mg; and cohort SAD-S2 evaluated 320 and 640 mg of GDC-0134. Patients were re-randomized for each treatment period. Safety follow-up visits occurred on Days 8 and 15 post-dose.

In the MAD stage, subjects received once daily (QD) dosing of GDC-0134 or placebo for 28 days. Thirty patients were randomized to each of five cohorts: MAD-M1 through MAD-M5 (100, 200, 400, 800, and 1200 mg). Safety follow-up visits occurred on Days 42 and 56. Safety data through the Day 42 visit were





reviewed for dose escalation decisions. A 28 day follow-up period following the final dose administration was included to ensure the safety profile could be adequately characterized.

The 48-week OLE enabled longer-term dosing, collection of extended safety data, and exploration of biomarkers and measures of functional change. Beginning with MAD-M3, each MAD dose level was extended into three corresponding OLE arms (OLE-M3 [$n = 11$], OLE-M4 [$n = 11$], and OLE-M5/M5a [$n = 10$]). Doses could be increased in individual patients after safety evaluation of subsequent MAD doses if the patient tolerated ≥12 weeks of GDC-0134 at their initial dose level. After MAD-M5 completion, the maximum OLE dose was modified from 1200 mg QD to 600 mg twice daily (BID) to minimize the peak-to-trough concentration ratio. Patients from MAD-M5 who initiated dosing at 1200 mg QD were designated as OLE-M5, while patients who later started at 600 mg BID were designated as OLE-M5a. All other patient cohorts were labeled according to the initial OLE dose. Patient completion visits occurred ~28 days after the final dose.

Randomization and blinding

Randomization lists were generated using SAS software, version 9.2 or higher (SAS Institute, Cary, NC). In the SAD, patients within a cohort were randomized 3:1 to GDC-0134 or placebo for each treatment period. Each MAD cohort was randomized 4:1 without consideration of prior randomization if a patient re-enrolled from the SAD. All patients and study site personnel were blinded to treatment assignments throughout the SAD and MAD with the exception of an unblinded pharmacist and unblinded drug monitor at each site. Except for the study statistician, who was fully unblinded for the entire study, the Sponsor Safety Review Group was blinded throughout dosing unless there was a potential dose-limiting AE. In the OLE, all patients received open-label GDC-0134, and patients and investigators were not informed of treatment assignments from previous stages.

Patients

Patients were ≥18 years of age with possible, laboratory-supported probable, probable, or definite ALS according to the modified El Escorial criteria.[23] Inclusion criteria also required the ability to swallow up to 12 capsules with water or ingest ~120 mL of applesauce-containing drug suspension within 5 min; upright forced vital capacity (FVC) of at least 50% of predicted value; and the ability to fast from food for 8 h before dosing and 2 h after dosing during the SAD and MAD. Enrollment was not restricted based on disease duration, genetic variant, progression rate, or site of onset. Stable doses of riluzole and edaravone were permitted. Patients were excluded if they had any medical condition or laboratory result that might preclude safe participation or had received any investigational therapy within the prior month or within 5 half-lives of that agent. Finally, we also excluded patients with visual acuity below 20/40, a history of optic neuropathy, optic disc swelling, or atrophy or intraocular eye disease.

Clinical outcomes

The primary outcome in all stages was safety and tolerability of GDC-0134 as assessed by the nature, frequency, and severity of AEs, effects on laboratory values, vital signs, electrocardiogram parameters, other safety biomarkers, and neurological and comprehensive ophthalmalogic exam findings, using the Medical Dictionary for Regulatory Activities (MedDRA), version 23.0. We conducted comprehensive eye exams throughout the study because earlier nonhuman primate (NHP) toxicology studies of GDC-0134 had identified asymptomatic and reversible retinal nerve fiber layer thickening (Genentech, data on file). AE severity was determined using the Division of Microbiology and Infectious Diseases (DMID) severity scale.[24] A systematic review of the incidence and severity of AEs relevant to peripheral neuropathy, retinal disorders, and optic disc disorders using standardized MedDRA queries (SMQs)[25] was performed.

In the SAD and MAD stages, the primary PK outcome was the plasma PK of single and multiple oral doses of GDC-0134 in the fasted state. For all stages, blood draws for PK analyses occurred pre-dose and at multiple times throughout the study. GDC-0134 drug concentrations were measured in plasma using liquid chromatography–tandem mass spectrometry.

Exploratory outcomes included the revised ALS Functional Rating Scale (ALSFRS-R)[26] a measure of disease progression, and plasma levels of NFL. In addition to evaluating longitudinal changes in plasma NFL, we also compared baseline NFL levels in 16 healthy donors, age- and sex- matched to the patients in the SAD. In the OLE cohort, plasma NFL levels were evaluated during the first 12 weeks (through Day 85), before patients were eligible to dose escalate. Donors provided blood samples through Genentech Samples for Science, an institutional review board-approved research program that collects samples from donors providing self-reported health data and written, informed consent. Plasma was collected in EDTA vacutainers from non-fasted healthy donors in the morning. Blood was spun at 1300 RCF for 15 min at 4°C and plasma was aliquoted and stored at −80°C until use. Plasma NFL was measured by a high-sensitivity

immunoassay (Quanterix, Inc., Billerica, MA) per the manufacturer's instructions.

Statistical analyses

Statistical analysis was performed by C.C. A sufficient number of patients were screened to ensure enrollment of 8 patients at each dose level for single-dose cohorts and for 5–10 patients for the multiple-dose cohorts. Each OLE cohort was designed to accommodate roughly 10 patients, based on the maximum size of the associated MAD cohort.

All patients who received at least one dose of study drug were included in the safety analysis. In the OLE, individuals who enrolled in more than one cohort were analyzed separately in each group. For patients who changed doses or discontinued treatment, AEs were assigned to the last dose that they received.

PK analyses included all patients who had at least one measurable post-dose concentration of GDC-0134. PK endpoints, determined by non-compartmental analysis, were maximum plasma concentration (C_{max}), minimum plasma concentration (C_{min}), area under the curve (AUC), and terminal elimination half-life ($t_{1/2}$). Descriptive statistics, including mean, standard deviation (SD), geometric mean, geometric coefficient of variation (CV), and n, were calculated by dose level for all GDC-0134 PK parameters. PK parameters were analyzed using Phoenix 64 WinNonlin (Certara, Princeton, NJ).

For exploratory analyses of effects on ALSFRS-R and NFL, OLE patients were grouped according to assigned dose at OLE entry. Baseline ALSFRS-R scores and change from baseline were calculated with means and SDs. Baseline was defined as the ALSFRS-R measure at the visit prior to the first OLE dose. For patients who continued to the OLE from the MAD, this was the last ALSFRS-R recorded in the MAD. The progression rate of patients entering the OLE was estimated using the recalled date of first symptom onset: (ALSFRS-R score at OLE baseline−48)/(months from first symptom onset to OLE baseline). This estimated pre-study progression rate provides a general sense of the baseline characteristics but is not suitable for comparison to the on-study measurement of ALSFRS-R slope change given: (1) ALSFRS-R slope may not be linear[27] and there was a wide pre-OLE duration across patients entering the OLE (10.8–97.4 months); (2) the pre-study slope estimate is less precise than the on-study slope generated by ALSFRS-R measurements obtained by trained raters, and (3) pre-OLE slope estimate does not account for prior treatment, including prior exposure to GDC-0134.

For baseline NFL levels in the OLE, the mean of two pre-dose samples for each patient (either pre-MAD or pre-OLE samples were used) was calculated. NFL elevation was defined as greater than a 1.5-fold increase above the baseline and a decrease as a decline of more than 0.5-fold below baseline. The 50% threshold was established using the longitudinal intrasubject variability of plasma NFL levels obtained during off-drug timepoints in the SAD. Analysis of plasma NFL change from baseline included timepoints up to OLE Day 85 (Week 12) or the first dose modification. OLE-M5 and OLE-M5a were combined in the analysis. MAD data were included in the OLE analysis when patients proceeded directly from active treatment in the MAD to the same dose level in the OLE, without dose interruption.

Animal studies

DLK cKO mice have been generated and described previously.[10,16] Animals were housed in specific pathogen-free conditions with 12-h light/12-h dark per day and maintained on regular chow diets. Because DLK deletion is lethal during embryonic development, a cKO model was used. A tamoxifen diet from weeks 4–7 was used to induce Cre deletion of the DLK gene, and blood was collected from 9-week-old mice by retro-orbital bleed into K2EDTA microtainers and spun at 10,000 RCF to isolate plasma. NFL was measured using a high-sensitivity immunoassay (Neurofilament-light Advantage, Quanterix Inc., Billerca, MA) according to manufacturer's instructions. Plasma NFL levels in DLK cKO homozygous (−/−) mice, DLK cKO heterozygous (−/+) mice and age-matched, wild-type (+/+) littermates were compared using the Mann–Whitney nonparametric t-test.

Ethics

The clinical trial was conducted in full conformance with the ICH E6 guideline for Good Clinical Practice and the principles of the Declaration of Helsinki or the laws and regulations of the country where the research was conducted. The study complied with the ICH E2A guideline. Study sites in the United States or under a US Investigational New Drug application complied with US Food and Drug Administration regulations and applicable local, state, and federal laws. The study site in the European Union/European Economic Area complied with the EU Clinical Trial Directive (2001/20/EC); the site in Canada complied with Health Canada regulations governing Clinical Trial Applications. All patients provided written, informed consent. Before study initiation, an institutional review board reviewed and approved the protocol and study procedures.

The Genentech Institutional Animal Care and Use Committee (IACUC) reviewed and approved all animal

care and handling procedures, which were conducted in full compliance with regulatory statutes, IACUC policies, and National Institutes of Health guidelines.

Results

Patient demographics, disposition, and study flow

From 25 April 2016 to 17 December 2018, 94 ALS patients were screened and 49 unique participants were enrolled across the SAD, MAD, and OLE stages (79 considered enrolled due to participation in multiple stages).

Patients could participate in multiple stages, provided they did not withdraw from prior stages. Overall, 17 participated in the SAD, 30 in the MAD (7 from the SAD), and 32 in the OLE (23 from the SAD and/or MAD) (Fig. 1B). All patients in the SAD received GDC-0134 at one or more treatment periods. In the MAD, the mean treatment duration was 28 days. One patient on GDC-0134 (MAD-M4) and one on placebo (MAD-M2) discontinued before completing the 28-day treatment period. In the OLE, 8 patients completed the 48-week treatment period and follow-up, while 24 patients discontinued treatment early. The OLE median duration of GDC-0134 treatment (any dose) was 214 days (range 4–344 days).

In the SAD, MAD, and OLE, the mean age was 56.9 ± 9.8, 56.3 ± 9.3, and 57.4 ± 9.2 years, respectively (Table 1). There was a higher proportion of men (69%), and most patients were white (94%). The mean time from diagnosis for OLE was 23.8 ± 18.8 months, the mean time from symptom onset was 36.8 ± 22.0 months and the mean FVC was 82.4 ± 17.9%. Four (12.5%) OLE patients had bulbar onset ALS, and one had familial ALS (Table 2). Genetic screening was not performed.

In total, 20 patients received 600 mg BID in the OLE, which was the highest dose level in this trial (Fig. 1B). The sponsor reduced the maximum dosing to 800 mg QD after a patient developed blindness from optic ischemic neuropathy while taking 600 mg BID (see Safety). In follow-up, this finding proved irreversible and led to a pause of all dosing to re-evaluate safety. Dosing was subsequently planned to resume at 400 mg QD. Of eight eligible patients, only one resumed, but withdrew from the study after 11 days of treatment (Fig. 1A).

Safety

In the SAD stage, the most common AEs (in ≥20% overall) were headache, falls, and occult blood in stool (Table 3). In the MAD, the most common AEs (in ≥20% overall) were falls and fatigue (Table 3). Combined, three Grade 3 AEs occurred in the SAD and MAD that were considered unrelated to study drug. There were no serious adverse events (SAEs) (Table 4). One patient (MAD-M4) discontinued the study because of bilateral blurred vision, assessed (by the investigator) as related to treatment, that was resolving at the safety follow-up visit.

All patients in the OLE reported AEs; the most common (in ≥20% overall) were falls, constipation, headache, fatigue, burning sensation, dizziness, and paresthesia (Table 3). Most AEs were Grade 1 or 2. Nine patients discontinued OLE treatment because of AEs (Fig. 1B; Table 3). Three SAEs considered to be related to study drug occurred in 3 patients, including dysesthesia, thrombocytopenia (both Grade 3), and the case of optic ischemic neuropathy (Grade 4) (Table 4). One patient (OLE-M5a) had a fatal myocardial infarction 17 days after taking the last dose (800 mg QD). This was considered unrelated to GDC-0134.

The case of dysesthesia started as Grade 2 pain in bilateral extremities approximately 10 days after discontinuing OLE treatment (last dose level was 600 mg QD) that worsened into generalized body aches rated as a Grade 3 SAE approximately 8 days later. The patient required hospitalization with treatments including opiates and multidisciplinary pain care. Electrophysiological testing (EMG/NCS) and epidermal nerve fiber counting did not demonstrate a peripheral neuropathy. The symptoms gradually subsided over 1 month after discontinuation of exposure.

The case of Grade 3 thrombocytopenia was preceded by an erythematous petechial rash, leading to discontinuation of OLE treatment (400 mg QD). Six days later, the patient was hospitalized with a platelet count near zero and required transfusions. The thrombocytopenia resolved 31 days later. Antiplatelet antibodies were not detected.

The case of optic ischemic neuropathy occurred in a 69-year-old woman with bulbar onset ALS who developed bilateral irreversible vision loss while receiving 600 mg BID GDC-0134 during the OLE. She noticed painless blurry vision on OLE Day 68, approximately 1 week after completing a study visit where the visual examination, including spectral domain optical coherence tomography (SD-OCT) and visual acuity, was normal. The blurry vision was reported to investigators 15 days later, on OLE Day 83, at which time SD-OCT revealed Grade 4 optic disc edema with bleeding in the right eye and Grade 1 optic disc edema in the left eye. On examination, 4-meter best corrected visual acuity was not significantly impaired in either eye. GDC-0134 was discontinued. Ten days later, right eye visual acuity was worse and SD-OCT indicated Grade 3–4 optic disc edema in the left eye and Grade 4 optic disc edema on the right. Over the course of several weeks, the patient developed near complete bilateral vision loss that had not improved by the last reported follow-up, approximately 9 months from the onset of the

Table 1. Patient demographics in the SAD, MAD, and OLE.

	SAD			MAD							OLE				
	SAD-S1 (*n* = 9)	SAD-S2 (*n* = 8)	All SAD patients (*n* = 17)	MAD-M1 100 mg (*n* = 8)	MAD-M2 200 mg (*n* = 4)	MAD-M3 400 mg (*n* = 4)	MAD-M4 800 mg (*n* = 4)	MAD-M5 1200 mg (*n* = 4)	MAD pooled placebo (*n* = 6)	All MAD patients (*n* = 30)	OLE-M3 (*n* = 11)	OLE-M4 (*n* = 11)	OLE-M5 (*n* = 4)	OLE-M5a (*n* = 6)	All OLE patients (*n* = 32)
Age (y) at baseline, mean (SD)	53 (10)	61 (8)	57 (10)	57 (12)	60 (8)	50 (4)	60 (8)	49 (9)	60 (6)	56 (9)	55 (10)	57 (9)	57 (9)	64 (9)	57 (9)
Sex, female, *n* (%)	3 (33)	3 (38)	6 (35)	4 (50)	1 (25)	1 (25)	1 (25)	1 (25)	4 (67)	12 (40)	3 (27)	2 (18)	2 (50)	3 (50)	10 (31)
Race															
Black or African American, *n* (%)	1 (11)	1 (12)	2 (12)	–	–	–	–	1 (25)	0	1 (3)	–	–	1 (25)	–	1 (3)
White, *n* (%)	7 (78)	7 (88)	14 (82)	8 (100)	4 (100)	4 (100)	4 (100)	3 (75)	6 (100)	29 (97)	11 (100)	11 (100)	3 (75)	5 (83)	30 (94)
Unknown, *n* (%)	1 (11)	–	1 (6)	–	–	–	–	–	–	–	–	–	–	–	–
Multiple, *n* (%)	–	–	–	–	–	–	–	–	–	–	–	–	–	1 (17)	1 (3)
Ethnicity, not Hispanic or Latino, *n* (%)	9 (100)	8 (100)	17 (100)	8 (100)	4 (100)	3 (75)	4 (100)	4 (100)	6 (100)	29 (97)	10 (91)	10 (91)	4 (100)	6 (100)	30 (94)
Weight (kg), mean (SD)	84 (24)	73 (18)	79 (22)	87 (19)	81 (19)	83 (8)	80 (14)	64 (14)	72 (19)	79 (17)	85 (18)	80 (13)	64 (12)	68 (11)	77 (16)
Height (cm), mean (SD)	170 (8)	175 (14)	172 (11)	171 (8)	175 (10)	172 (6)	176 (8)	178 (10)	164 (15)	172 (10)	172 (13)	176 (8)	176 (11)	170 (13)	173 (11)
BMI (kg/m^2), mean (SD)	29 (7)	24 (3)	26 (6)	30 (7)	26 (4)	28 (3)	25 (3)	20 (4)	27 (4)	27 (5)	29 (4)	26 (3)	21 (4)	24 (4)	26 (4)
Temperature (°C), mean (SD)	36.6 (0.2)	36.5 (0.4)	36.6 (0.3)	36.6 (0.2)	36.6 (0.5)	36.8 (0.1)	36.4 (0.3)	36.6 (0.3)	36.4 (0.6)	36.6 (0.4)	36.6 (0.3)	36.6 (0.2)	36.5 (0.7)	36.6 (0.2)	36.6 (0.3)
Riluzole use, *n* (%)	5 (56)	4 (50)	9 (53)	4 (50)	3 (75)	4 (100)	3 (75)	4 (100.0%)	4 (67)	22 (73)	8 (73)	9 (82)	4 (100)	4 (67)	25 (78)
Edaravone use, *n* (%)	–	–	–	2 (25)	2 (50)	2 (50)	1 (25)	1 (25)	1 (17)	9 (30)	4 (36)	3 (27)	–	2 (33)	9 (28)
Treatment status															
Previously enrolled, *n* (%)	–	–	–	3 (38)	3 (75)	–	–	–	1 (17)	7 (23)	10 (91)	7 (64)	3 (75)	–	20 (63)
Study naive, *n* (%)	9 (100)	8 (100)	17 (100)	5 (63)	1 (25)	4 (100)	4 (100)	4 (100)	5 (83)	23 (77)	1 (9)	4 (36)	1 (25)	6 (100)	12 (38)

Patients who participated in multiple stages of the study (e.g., both the SAD and MAD) appear in the summary for each study stage. Patients who were exposed to multiple dose levels in the OLE are shown once, and OLE demographics are summarized by the dose level assigned at OLE entry. Values and percentages, except for temperature, are rounded to the nearest integer. SAD, single ascending dose; MAD, multiple ascending does; OLE, open-label safety expansion; SD, standard deviation; BMI, body mass index.

Table 2. ALS history of patients in the OLE.

	OLE-M3 (*n* = 11)	OLE-M4 (*n* = 11)	OLE-M5 (*n* = 4)	OLE-M5a (*n* = 6)	All OLE patients (*n* = 32)
Symptoms onset, *n* (%)					
Bulbar onset	1 (9)	1 (9)	2 (50)	0	4 (12)
Limb onset	10 (91)	10 (91)	2 (50)	6 (100)	28 (88)
Familial/sporadic, *n* (%)[1]					
Familial disease	0	1 (9)	0	0	1 (3)
Sporadic disease	10 (91)	9 (82)	3 (75)	6 (100)	28 (88)
Not known	1 (9)	1 (9)	1 (25)	0	3 (9)
Modified El Escorial, *n* (%)					
Definite	7 (64)	5 (46)	1 (25)	2 (33)	15 (47)
Probable	2 (18)	5 (46)	1 (25)	3 (50)	11 (34)
Laboratory-supported probable	1 (9)	1 (9)	1 (25)	1 (17)	4 (12)
Possible	1 (9)	0	1 (25)	0	2 (6)
Baseline ALSFRS-R score, mean (SD)	36.5 (5.5)	33.0 (5.5)	38.5 (2.4)	35.8 (3.7)	35.4 (5.1)
Pre-OLE ALSFRS-R progression rate	−0.45 (0.25)	−0.36 (0.17)	−0.26 (0.07)	−0.61 (0.47)	−0.42 (0.28)
On-OLE ALSFRS-R progression rate	−0.69 (0.89)	−0.51 (0.27)	−1.86 (1.75)	−0.83 (0.88)	−0.82 (0.97)
Months since disease diagnosis, mean (SD)	24.4 (16.9)	32.2 (24.5)	20.8 (11.8)	11.2 (4.9)	24.0 (18.9)
Months since symptoms onset, mean (SD)	32.9 (22.9)	50.4 (26.2)	38.7 (10.0)	22.4 (6.8)	37.0 (22.1)
Baseline FVC[2], % predicted, mean (SD)	81.5 (20.9)	83.3 (16.6)	83.1 (12.3)	82.6 (19.4)	82.5 (17.5)

Pre-OLE ALSFRS-R progression rate = (ALSFRS-R score at OLE baseline−48)/duration in months from symptom onset to OLE baseline. On-OLE ALSFRS-R progression rate = (ALSFRS-R score at last available ALSFRS-R timepoint - ALSFRS-R score at OLE baseline)/duration in months from OLE baseline to last available ALSFRS-R timepoint. OLE, open-label safety expansion; ALSFRS-R, ALS Functional Rating Scale-Revised; SD, standard deviation; FVC, forced vital capacity.

[1]Genetic screening was not performed.

[2]The average of multiple FVC measurements collected at the OLE screening visit and OLE Day 1 visit was used to calculate summary statistics. Percentages are rounded to the nearest integer.

visual symptoms. Her findings were believed to be due to non-arteritic ischemic optic neuropathy (NAION) related to GDC-0134.

Analysis of the comprehensive ocular exams performed throughout the study found a potential association between GDC-0134 and low-grade retinal or optic nerve toxicity using the SMQs of "optic nerve disorders" and "retinal disorders" (Table 5). Six patients had 10 AEs of optic nerve disorders, and 12 patients had 19 AEs of retinal disorders (Table 5). Except for the case of NAION, these were low grade, non-serious, and resolved without sequelae.

In the OLE, the 1200 mg QD/600 mg BID regimens were associated with an increased frequency of sensory neurological complaints (Table 3). To evaluate the frequency of relevant events across the study population, an SMQ of "peripheral neuropathy" was performed. Overall, 16 patients experienced 28 AEs in this SMQ (Table 5). Except for the patient with Grade 3 SAE of painful dysesthesia, these were deemed to be non-serious Grade 1 or 2 AEs. However, they did result in dose modifications, interruptions, or discontinuations in 13 (41%) OLE patients and were important in determining that GDC-0134 doses above 800 mg QD were poorly tolerated.

Pharmacokinetics

Across the range of single doses tested (20–640 mg), GDC-0134 C_{max} and $AUC_{0\text{-}24}$ increased with dose and were approximately dose proportional (Fig. 2A and B). GDC-0134 was rapidly absorbed, with a median t_{max} of 1.5 h following oral dosing. GDC-0134 elimination was apparently biphasic with a mean $t_{1/2}$ of 84 h across all dose groups. The inter-subject variability (geometric %CV) for C_{max} was ~42–155% and for $AUC_{0\text{-}inf}$ was ~27–106%.

Increases in steady-state C_{max} and AUC of GDC-0134 were dose-proportional across the range of multiple doses administered QD in the MAD (100–1200 mg) (Fig. 2C). At the highest dose tested (1200 mg QD), geometric mean steady-state C_{max} was 4520 ng/mL, $AUC_{0\text{-}24}$ was 55,700 ng/mL×h, and C_{min} was 1910 ng/mL. Large inter-subject (geometric %CV for C_{max}, $AUC_{0\text{–}24}$, and C_{min} were 119, 103, and 55%, respectively) and intra-subject variability (~50% CV for C_{min}) were observed at this dose level. In general, GDC-0134 steady-state exposures in the OLE were within the range of the exposures observed in the MAD at a given QD dose level. Following the 600-mg BID dose in the OLE, the geometric mean steady-state C_{max} was 3730 ng/mL and C_{min} was 2080 ng/mL.

Table 3. Adverse events in the SAD, MAD, and OLE stages.

	SAD			MAD							OLE				
	SAD-S1 (*n* = 9)	SAD-S2 (*n* = 8)	All SAD patients (*n* = 17)	MAD-M1100 mg QD (*n* = 8)	MAD-M2200 mg QD (*n* = 4)	MAD-M3400 mg QD (*n* = 4)	MAD-M4 800 mg QD (*n* = 4)	MAD-M5 1200 mg QD (*n* = 4)	Pooled placebo (*n* = 6)	All MAD patients (*n* = 30)	OLE-M3 (*n* = 11)	OLE-M4 (*n* = 11)	OLE-M5 (*n* = 4)	OLE-M5a (*n* = 6)	All OLE patients (*n* = 32)
Overall total number of AEs[1]	85	39	124	24	8	9	18	23	11	93	126	169	34	59	388
No. of patients with ≥1 AE	9 (100)	8 (100)	17 (100)	5 (62)	4 (100)	3 (75)	4 (100)	3 (75)	5 (83)	24 (80)	10 (91)	11 (100)	4 (100)	6 (100)	31 (97)
No. of deaths	–	–	–	–	–	–	–	–	–	–	–	–	–	1 (17)	1 (3)
No. of patients with ≥1 SAE	–	–	–	–	–	–	–	–	–	–	1 (9)	2 (18)	2 (50)	1 (17)	6 (19)
No. of patients withdrawn from study due to an AE	1 (11)	1 (12)	2 (12)	1 (12)	–	–	1 (25)	–	1 (17)	3 (10)	1 (9)	0	1 (25)	3 (50)	5 (16)
No. of patients who discontinued treatment due to an AE	1 (11)	1 (12)	2 (12)	–	–	–	1 (25)	–	–	1 (3)	1 (9)	4 (36)	1 (25)	3 (50)	9 (28)
No. of patients with a related AE	2 (22)	2 (25)	4 (24)	1 (122)	1 (25)	1 (25)	2 (50)	3 (75)	3 (50)	11 (37)	7 (64)	8 (73)	1 (25)	4 (67)	20 (62)
No. of patients with a severe AE	9 (100)	8 (100)	17 (100)	5 (62)	4 (100)	3 (75)	4 (100)	3 (75)	5 (83)	24 (80)	10 (91)	11 (100)	4 (100)	6 (100)	31 (97)
Most common AEs (preferred term) in ≥2 patients overall per stage															
Fall	2 (22)	3 (38)	5 (29)	3 (38)	2 (50)	–	1 (25)	3 (75)	–	9 (30)	7 (64)	5 (46)	3 (75)	4 (67)	19 (59)
Constipation	–	–	–	1 (12)	1 (25)	–	–	–	–	2 (7)	2 (18)	5 (46)	1 (25)	1 (17)	9 (28)
Headache	3 (33)	4 (50)	7 (41)	1 (12)	1 (25)	–	1 (25)	–	1 (17)	4 (13)	3 (27)	3 (27)	–	2 (33)	8 (25)
Fatigue	1 (11)	1 (12)	2 (12)	–	–	1 (25)	2 (50)	1 (25)	2 (33)	6 (20)	5 (46)	1 (9)	–	2 (33)	8 (25)
Burning sensation	–	–	–	–	–	–	–	–	–	–	1 (9)	3 (27)	1 (25)	2 (33)	7 (22)
Dizziness	–	–	–	–	–	–	–	–	–	–	–	2 (18)	1 (25)	4 (67)	7 (22)
Paresthesia	–	–	–	–	–	–	–	–	–	–	1 (9)	4 (36)	–	2 (33)	7 (22)
Muscle spasms	–	–	–	1 (12)	–	1 (25)	1 (25)	1 (25)	–	4 (13)	2 (18)	2 (18)	1 (25)	1 (17)	6 (19)
Musculoskeletal pain	–	–	–	–	–	–	–	–	–	–	4 (36)	–	2 (50)	–	6 (19)
Rash	–	–	–	–	–	–	–	–	–	–	2 (18)	1 (9)	–	2 (33)	5 (16)
Upper respiratory tract infection	2 (22)	–	2 (12)	–	–	–	–	–	–	–	3 (27)	1 (9)	–	–	4 (12)
Arthralgia	–	–	–	–	–	–	1 (25)	1 (25)	–	2 (7)	1 (9)	2 (18)	–	1 (17)	4 (12)
Tinnitus	–	–	–	–	–	–	–	–	–	–	–	3 (27)	–	1 (17)	4 (12)
Asthenia	–	–	–	–	–	–	–	–	–	–	3 (27)	1 (9)	–	–	4 (12)

(*Continued*)

Table 3 Continued.

	SAD			MAD							OLE				
	SAD-S1 (*n* = 9)	SAD-S2 (*n* = 8)	All SAD patients (*n* = 17)	MAD-M1100 mg QD (*n* = 8)	MAD-M2200 mg QD (*n* = 4)	MAD-M3400 mg QD (*n* = 4)	MAD-M4 800 mg QD (*n* = 4)	MAD-M5 1200 mg QD (*n* = 4)	Pooled placebo (*n* = 6)	All MAD patients (*n* = 30)	OLE-M3 (*n* = 11)	OLE-M4 (*n* = 11)	OLE-M5 (*n* = 4)	OLE-M5a (*n* = 6)	All OLE patients (*n* = 32)
Nasopharyngitis	–	–	–	–	–	–	–	–	–	–	–	3 (27)	–	1 (17)	4 (12)
Muscular weakness	–	–	–	–	–	–	–	–	–	–	1 (9)	2 (18)	–	1 (17)	4 (12)
Insomnia	–	–	–	–	–	–	–	–	–	–	–	2 (18)	1 (25)	1 (17)	4 (12)
Cough	–	–	–	–	–	–	–	–	–	–	1 (9)	2 (18)	1 (25)	–	4 (12)
Skin abrasion	–	–	–	2 (25)	–	–	–	2 (50)	–	4 (13)	–	–	–	–	–
Diarrhea	–	–	–	–	–	–	1 (25)	–	1 (167)	2 (7)	–	–	–	–	–
Dysarthria	–	–	–	–	–	–	–	2 (50)	–	2 (7)	–	–	–	–	–
Back pain	–	–	–	–	1 (25)	–	1 (25)	–	–	2 (7)	–	–	–	–	–
Erythema	–	–	–	1 (12)	–	1 (25)	–	–	–	2 (7)	–	–	–	–	–
Occult blood positive	2 (22)	1 (12)	3 (18)	–	–	–	–	–	–	–	–	–	–	–	–
Eye pruritus	1 (11)	1 (12)	2 (12)	–	–	–	–	–	–	–	–	–	–	–	–
Visual acuity reduced	2 (22)	–	2 (12)	–	–	–	–	–	–	–	–	–	–	–	–
Nausea	1 (11)	1 (12)	2 (12)	–	–	–	–	–	–	–	–	–	–	–	–
Edema peripheral	2 (22)	–	2 (12)	–	–	–	–	–	–	–	–	–	–	–	–
Skin laceration	2 (22)	–	2 (12)	–	–	–	–	–	–	–	–	–	–	–	–
Nasal congestion	2 (22)	–	2 (12)	–	–	–	–	–	–	–	–	–	–	–	–

Patients who participated in multiple stages of the study appear in the summary for each study stage. Patients who were exposed to multiple dose levels in the OLE are shown once. AEs occurring in the OLE are summarized by OLE dose level assigned at OLE entry. SAD, single ascending dose; MAD, multiple ascending does; OLE, open-label safety expansion; QD, once daily; AE, adverse event; SAE, serious adverse event.

[1]Data are no. of patients (%), except for the overall total number of AEs. Percentages are rounded to the nearest integer.

Table 4. Grades 3 and 4 AEs and SAEs.

SAD	SAD-S1 (n = 9)	SAD-S2 (n = 8)	All SAD patients (n = 17)	Related to study treatment?	Grade	SAE?
Tibia fracture	1 (11)	0	1 (6)	No	3	No
Nephrolithiasis	0	1 (12)	1 (6)	No	3	No

MAD	MAD-M1 100 mg QD (n = 8)	MAD-M2 200 mg QD (n = 4)	MAD-M3 400 mg QD (n = 4)	MAD-M4 800 mg QD (n = 4)	MAD-M5 1200 QD (n = 4)	Pooled placebo (n = 6)	All MAD patients (n = 30)	Related to study treatment?	Grade	SAE?
Muscle spasticity	1 (12)	0	0	0	0	0	1 (33)	No	3	No

OLE	OLE-M3 (n = 11)	OLE-M4 (n = 11)	OLE-M5 (n = 4)	OLE-M5a (n = 6)	All OLE patients (n = 32)	Related to study treatment?	Grade	SAE?
Patient 1								
Dysesthesia	0	1 (9)[1]	0	0	1 (3)	Yes	3	Yes
Patient 2								
Face injury	1 (9)[2]	0	0	0	1 (3)	No	3	No
Patient 3								
Rash erythematous	1 (9)	0	0	0	1 (3)	Yes	3	No
Pneumonia aspiration	1 (9)	0	0	0	1 (3)	No	3	No
Proteinuria	1 (9)	0	0	0	1 (3)	Yes	3	No
Thrombocytopenia	1 (9)	0	0	0	1 (3)	Yes	3	Yes
Patient 4								
Hyponatremia	0	1 (9)	0	0	1 (3)	No	3	No
Patient 5								
Traumatic liver injury	0	1 (9)[2]	0	0	1 (3)	No	4	Yes
Acute respiratory failure	0	1 (9)[2]	0	0	1 (3)	No	3	Yes
Patient 6								
Optic ischemic neuropathy	0	0	1 (25)[2]	0	1 (3)	Yes	4	Yes
Patient 7								
Dyspnea	0	0	1 (25)[3]	0	1 (3)	No	4	No
Acute respiratory failure	0	0	1 (25)[3]	0	1 (3)	No	4	Yes
Chronic respiratory failure	0	0	1 (25)[3]	0	1 (3)	No	4	Yes
Patient 8								
Myocardial infarction	0	0	0	1 (17)[3]	1 (3)	No	4	Yes

Data are n (%). Each group of rows indicates AEs occurring in the same patient. Percentages are rounded to the nearest integer. AE, adverse event; SAE, serious adverse event; SAD, single ascending dose; MAD, multiple ascending does; OLE, open-label safety expansion; QD, once daily; BID, twice daily.

The last dose a patient had received before the AE was reported, if different from their starting dose, is indicated as follows: [1]600 mg QD; [2]600 mg BID; [3]800 mg QD.

ALSFRS-R disease progression

In the OLE, the ALSFRS-R was evaluated approximately every 12 weeks. The mean baseline ALSFRS-R score for the 32 patients was 35.4 ± 5.1 (Table 2). The mean time from symptom onset to OLE baseline was 37.0 ± 22.1 months among patients where it was available. The mean pre-treatment progression rate was estimated to be −0.42 ± 0.28 points/month irrespective of prior therapies, including GDC-0134 exposure in the SAD or MAD. The mean ALSFRS-R progression rate measured during the OLE treatment period (mean 238.5 ± 38.8 days) was −0.82 ± 0.97 points/month among the 28 patients with at least one post-baseline measure who were included in the analysis.

Clinical and preclinical NFL analyses

Sixteen healthy controls were age- and sex-matched to patients in the SAD (age, mean ± SD: 55.4 ± 8.8 years old). Among ALS patients, baseline plasma NFL levels (mean ± SD: 55.2 ± 29.3 pg/mL; n = 48) were significantly elevated ($p < 1 \times 10^{-14}$) compared to the controls (mean ± SD: 8.3 ± 3.9 pg/mL; n = 16) by two-sample t-test. Mean plasma increases over baseline were dose- and exposure-dependent and were found at the 800 and 1200/

Table 5. MedDRA SMQs[1] ("optic nerve disorders," "retinal disorders," and "peripheral neuropathy") by system organ class and preferred term.

	SAD			MAD							OLE				
SMQs	SAD-S1 (*n* = 9)	SAD-S2 (*n* = 8)	All SAD patients (*n* = 17)	MAD-M1 100 mg (*n* = 8)	MAD-M2 200 mg (*n* = 4)	MAD-M3 400 mg (*n* = 4)	MAD-M4 800 mg (*n* = 4)	MAD-M5 1200 mg (*n* = 4)	MAD pooled placebo (*n* = 6)	All MAD patients (*n* = 30)	OLE-M3 (*n* = 11)	OLE-M4 (*n* = 11)	OLE-M5 (*n* = 4)	OLE-M5a (*n* = 6)	All OLE patients (*n* = 32)
Optic nerve disorders	2 (22)	0	2 (12)	1 (12)	0	0	0	0	0	1 (3)	1 (9)	1 (9)	1 (25)	0	3 (9)
Retinal disorders	3 (33)	0	3 (18)	1 (12)	0	0	1 (25)	0	1 (16.7)	3 (10)	2 (18)	3 (27)	0	1 (17)	6 (19)
Peripheral neuropathy	1 (11)	0	1 (6)	0	0	0	0	1 (25)	0	1 (3)	3 (27)	7 (64)	1 (25)	3 (50)	14 (44)
System organ class preferred term															
Eye disorders															
Visual acuity reduced	2 (22)	0	2 (12)												
Eye disorder	1 (11)	0	1 (6)												
Maculopathy	1 (11)	0	1 (6)												
Vision blurred	1 (11)	0	1 (6)	0	0	0	1 (25)	0	0	1 (3)	0	2 (18)	0	0	2 (6)
Vitreous detachment	1 (11)	0	1 (6)												
Photophobia				0	0	0	0	0	1 (17)	1 (3)					
Retinal disorder				0	0	0	1 (25)	0	0	1 (3)	1 (9)	2 (18)	0	0	3 (9)
Optic disc disorder											0	1 (9)	0	0	1 (3)
Optic ischemic neuropathy											0	0	1 (25)	0	1 (3)
Optic nerve disorder											1 (9)	0	0	0	1 (3)
Retinal hemorrhage											0	0	0	1 (17)	1 (3)
General disorders and administration site conditions															
Gait disturbance	1 (11)	0	1 (6)	0	0	0	0	1 (25)	0	1 (3)					
Nervous system disorders															
Paresthesia	1 (11)	0	1 (6)								1 (9)	4 (36)	0	2 (33)	7 (22)
Visual field defect				1 (12)	0	0	0	0	0	1 (3)	1 (9)	0	0	0	1 (3)
Burning sensation				0	0	0	0	1 (25)	0	1 (3)	1 (9)	3 (27)	1 (25)	2 (33)	7 (22)
Hypoesthesia											1 (9)	1 (9)	0	0	2 (6)
Dysesthesia											0	1 (9)	0	0	1 (3)
Muscular and connective tissue disorders															
Muscular weakness				0	0	0	0	1 (25)	0	1 (3)	1 (9)	2 (18)	0	1 (17)	4 (12)
Skin and subcutaneous tissue disorders															
Skin burning sensation											0	1 (9)	0	0	1 (3)

Data are no. of patients (%). Percentages are rounded to the nearest integer. SMQs, standardized MedDRA queries; SAD, single ascending dose; MAD, multiple ascending does; OLE, open-label safety expansion.

[1]MedDRA (v. 23.0) system organ classes and preferred terms were combined under the following SMQs: optic nerve disorders: eye disorders (visual acuity reduced, optic disc disorder, optic ischemic neuropathy, optic nerve disorder), nervous system disorders (visual field defect).

Retinal disorders: eye disorders (photophobia, retinal disorder, visual acuity reduced, eye disorder, maculopathy, vision blurred, vitreous detachment, retinal hemorrhage), nervous system disorders (visual field defect).

Peripheral neuropathy: general disorders and administration site conditions (gait disturbance), nervous system disorders (burning sensation, paresthesia, hypoesthesia, dysesthesia), musculoskeletal and connective tissue disorders (muscular weakness), skin and subcutaneous tissue disorders (skin burning sensation).

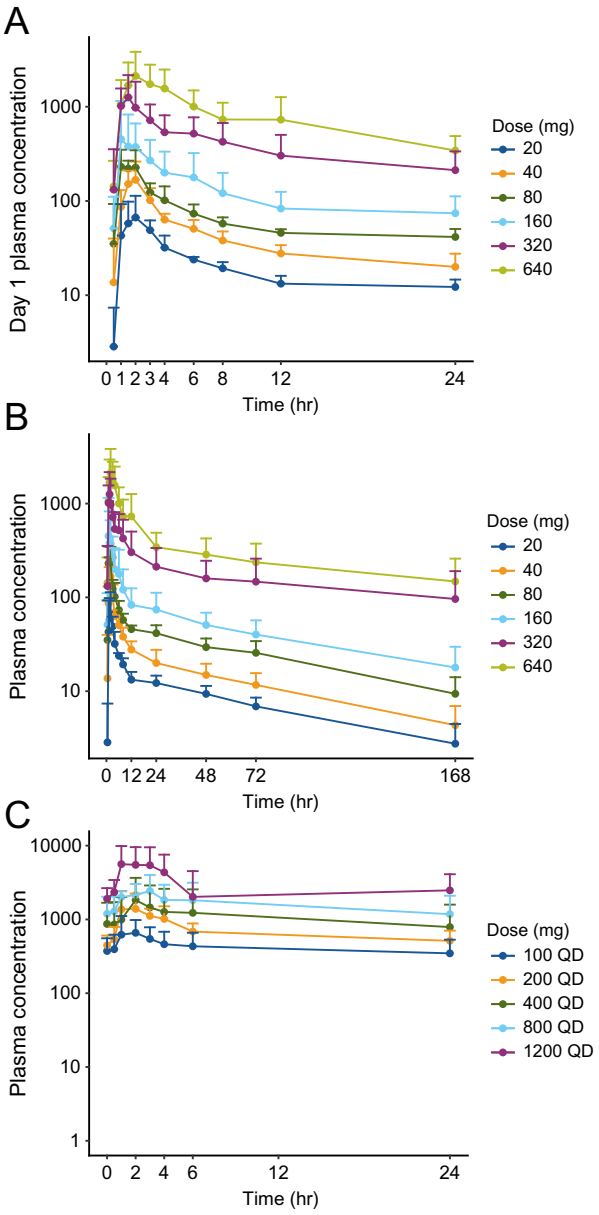


Figure 2. GDC-0134 pharmacokinetics. Mean (+SD) plasma concentration-time profiles of GDC-0134 over (A) 24 h and (B) 168 h following a single dose of GDC-0134 in the SAD stage. (C) Mean (+SD) steady-state plasma concentration-time profiles of GDC-0134 following multiple doses of GDC-0134 in the MAD stage. SAD, single ascending dose; MAD, multiple ascending does; SD, standard deviation; QD, once daily.

600 mg BID dose levels following 2 or more weeks of treatment (Fig. 3A and B). NFL changes were reversible or trended towards baseline with reduction of dose or discontinuation of GDC-0134 ($n = 9$).

In preclinical testing, we also examined the effect of DLK deletion on circulating NFL levels in DLK cKO mice.[10] Plasma NFL increased 1.7-fold ($p < 0.05$; $n = 8$) in DLK heterozygous (−/+) cKO mice with reduced DLK expression, and increased 2.6-fold ($p < 0.001$, $n = 8$) in DLK homozygous (−/−) cKO mice at 9 weeks of age, both relative to wild-type littermate controls ($n = 5$) (Fig. 3C).

Discussion

This Phase 1 trial met its objectives of characterizing safety and PK of GDC-0134 in ALS patients. It also provided several meaningful insights that may shape future research of DLK, as well as our understanding of NFL as an emerging biomarker. After dose adjustments in the OLE, most patients were exposed to the highest dose of GDC-0134 (600 mg BID), enabling characterization of safety and tolerability beyond the 28 day MAD. This approach optimized the primary objective of assessing PK and safety, and ultimately revealed safety concerns that would otherwise have appeared in a Phase 2 study, after exposure of many more patients. While we could not determine the effect on disease progression, the safety-centric design enabled critical clinical development decisions.

The safety profiles in the SAD and the 28 day MAD were unremarkable. However, longer drug exposure during the OLE was associated with frequent sensory complaints, including burning sensations and paresthesias, which contributed to dose reductions and a high rate of treatment discontinuation (28%), the majority (15/23) of which were reported after at least 28 days of treatment. Together, these findings raised concerns about the tolerability of GDC-0134 as a longer-term treatment option, and highlighted the importance of observing safety during extended dosing periods.

One patient in the OLE developed NAION during the third month of dosing. NAION is the most common cause of optic neuropathy in older adults,[28] and the pathogenesis is poorly understood.[29] Prior reports have suggested that NAION can be caused by drug exposure, in particular with PDE-5 inhibitors and cyclosporine.[30–33] This study was conducted with a specific focus on ocular findings due to peripapillary retinal abnormalities observed in earlier NHP studies of GDC-0134, although these abnormalities did not lead to observable visual defects and appeared to be clinicopathologically distinct from NAION on examination (Genentech, data on file). The ocular assessments included in this Phase 1 trial, including the longitudinal safety assessments throughout the OLE, also provide a dataset for analyzing the ocular safety profile of GDC-0134. A deeper analysis of these data is required to evaluate translation of the nonclinical toxicology findings to humans. Still, the irreversible nature of NAION, its uncertain cause, the lack

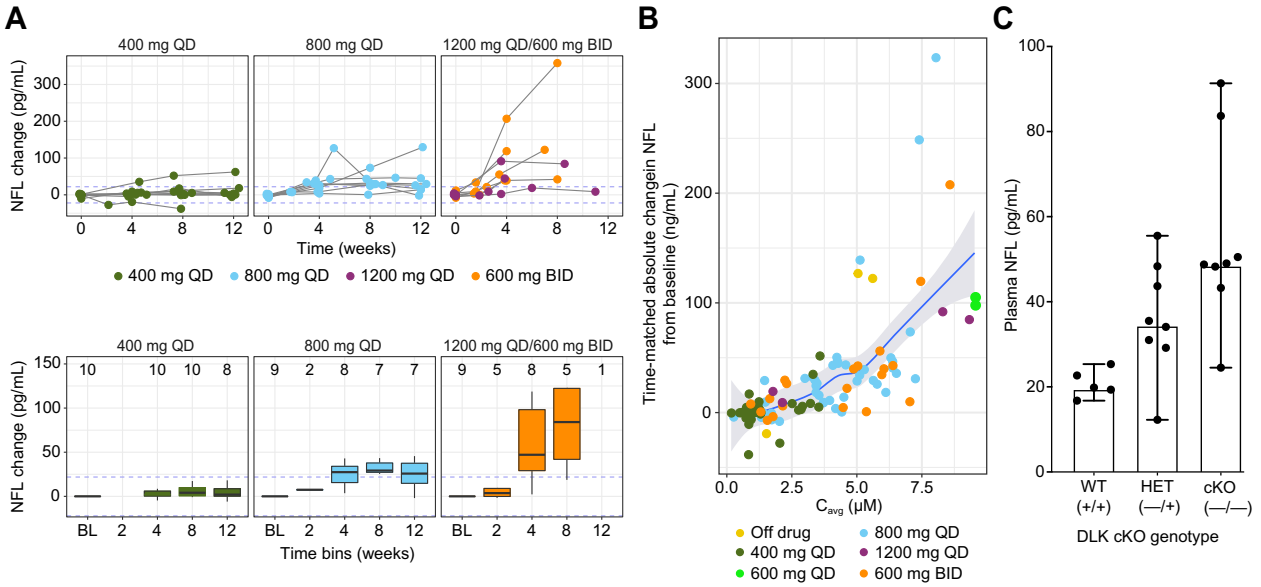


Figure 3. NFL. (A) Plasma NFL changes from baseline in the first 12 weeks of the OLE by dose level and grouped by patient (top panels) or binned by treatment duration (bottom panels). (B) Data points from panel (A) plotted versus average GDC-0134 concentration (μmol/L) at the time of NFL observation. All available data over the first 12 weeks of OLE (Day 85) until dose reduction or discontinuation are shown. MAD data are included for patients who entered the OLE without an interruption in GDC-0134 treatment. In (A), dotted lines represent the approximate 99% CI of variance attributable to technical and biological factors. CIs were calculated from longitudinal analysis of timepoints in the SAD/MAD when patients were not receiving study drug, and the influence of treatment was believed to be negligible. Baseline is an average of both screening and Day 1 predose samples. Boxplots represent the median and IQRs; whiskers represent the range to a maximum of 1.5× the IQR. The number of patients evaluated at each timepoint is indicated above the boxplots. In (B), gray shading represents the 95% CI of the Lowess curve. (C) Plasma NFL levels (median +/− IQR) in WT (+/+) mice compared to DLK cKO heterozygous (−/+) and DLK cKO homozygous (−/−) mice. NFL, neurofilament light; CI, confidence interval; OLE, open-label safety expansion; MAD, multiple ascending does; SAD, single ascending dose; IQR, interquartile range; DLK, dual leucine zipper kinase; cKO, conditional knock out; WT, wild-type; HET, heterozygotes; QD, once daily; BID, twice daily.

of clear premonitory signs, and the additional non-ocular AEs indicate that GDC-0134 has an unacceptable safety profile. These results, combined with the factors of a long half-life and a highly variable dose-exposure relationship, led to the discontinuation of GDC-0134 development.

CSF and plasma levels of NFL are robustly elevated in ALS and other neurodegenerative diseases and this biomarker has emerged as a candidate pharmacodynamic biomarker for use in clinical trials.[34,35] In this trial, GDC-0134 induced dose- and time- dependent increases in NFL. In DLK cKO mice, plasma NFL was increased in mice lacking DLK, suggesting that the increase of NFL induced by GDC-0134 in patients may be an on-target effect of DLK inhibition. DLK cKO mice do not exhibit observable neurodegeneration or motor or cognitive deficits.[10,16] Thus, although the molecular pathway from DLK to NFL is not known, the cKO studies suggest that decreasing DLK activity may lead to increases in NFL that are not driven by nerve injury. Regardless, the observed GDC-0134 induced increases in NFL in the current study are of concern.

The OLE explored a variety of dose levels in 28 patients with heterogeneous disease duration and rates of calculated pre-study decline. This limits our ability to interpret the role of GDC-0134 on ALSFRS-R change. While the mean rate of progression based on ALSFRS-R measurements over the OLE treatment period was higher than the estimated slope prior to OLE entry, the variability of these measures as well as the imprecision of pre-study calculations preclude a statistical comparison. There are also inherent challenges to comparing pre-study and post-study progression rates, in general, and analyses of large data sets from ALS clinical trials have found steeper on-study decline compared to pre-study.[36] Since this Phase 1 study was not designed to enable a conclusive assessment of ALS disease progression, a larger, placebo-controlled trial with stable exposure to GDC-0134 would be needed to understand any effect on disease progression; however, this is unlikely to be pursued due to safety concerns.

In conclusion, the trial met its objectives of characterizing safety and PK and highlighted a potential complexity of interpreting NFL as a biomarker. Variable PK and

safety concerns resulted in a therapeutic window that was too narrow to enable further development of GDC-0134 in ALS. The decision to use an OLE highlights the value of collecting longer-term safety data early in the development of a new drug. Given the enthusiasm for the therapeutic potential of this target, the safety events warrant a careful evaluation of the NHP ocular findings, and the clinical ophthalmic data collected in this trial. The relationship between NFL dynamics and DLK inhibition can also be investigated further. Preclinical studies clarifying how DLK inhibition and plasma NFL elevations alter measures of neuroprotection, neurodegeneration, ocular toxicity, and peripheral sensation may help direct development of future DLK inhibitors and advance understanding of the utility of NFL as a marker of treatment response in ALS.

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Author Contributions

JSK, JDR, MEC, AG, ABH, CC, GAK, FLY, ASG, SC, MER, FB, and KRS contributed to the conception and design of the study. JSK, AG, BO, ABH, CC, JG, BLB, WC, FLY, SC, LB, LH, JAC, MER, FB, KRS, LvdB, JDB, and JDG contributed to the data acquisition and analysis. JSK, ABH, CC, JG, BLB, SC, LB, LH, and JDG drafted and revised a significant portion of the manuscript and figures. All authors contributed to critical review of manuscript for content.

Conflicts of Interest

JSK: Nothing to disclose. JDR: Nothing to disclose. MEC: Consultant for Avexis, consultant for Orion, consultant for Lilly, consultant for Biohaven, consultant for MT Pharma, consultant for Revalasio, consultant for Pontifex, consultant for Denali, consultant for Biogen, consultant for Pharm NEXT, consultant for Treeway, consultant for Takeda, consultant for Aclipse, consultant for Sunovian, consultant for Anelixis, consultant for Cytokinetics, consultant for Disarm, consultant for ALS Pharma, consultant for RRD, consultant for Immunity Pharma, consultant for Helixsmith, consultant for Wave, consultant for Transposon, consultant for Quralis. AG: Other from Quralis, other from Mitsubishi Tanabe Pharma America, other from Sanofi Genzyme, other from AL-S Pharma, other from Biogen, other from Novartis, other from Uniqure, other from Affinia, other from Apellis, other from Avenix, other from Alexion, other from Wave Life Sciences, other from Roche, other from Cytokinetics, other from Orion, other from Medtronic, other from Anaelixis, other from Viela, other from ArgenX, other from BMS, other from Sanofi Genzyme, other from Grifols, other from Ionis, other from Lily, other from Machinova, other from Novartis, other from Orphazyme, other from Pfizer, other from Ra Pharmaseuticals, other from Teva, other from UCB, during the conduct of the study. BO: Grants from Genentech, A Member of the Roche Group, during the conduct of the study; grants and personal fees from Biogen, grants from Eisai, grants and personal fees from Mitsubishi Tanabe, grants and personal fees from Medicinova, grants from AZ Therapeutics, personal fees from Biohaven, personal fees from Tsumura, outside the submitted work. ABH: Employee of Genentech, Inc. and shareholder in F. Hoffmann-La Roche, Ltd. CC: Employee of Genentech, Inc. and shareholder in F. Hoffmann-La Roche, Ltd. JG: Employee of Genentech, Inc. and shareholder in F. Hoffmann-La Roche, Ltd. BLB: Employee of Genentech, Inc. and shareholder in F. Hoffmann-La Roche, Ltd. WC: Employee of Genentech, Inc. and shareholder in F. Hoffmann-La Roche, Ltd. GAK: Employee of F. Hoffmann-La Roche, Ltd. FLY: Employee of Genentech, Inc. and shareholder in F. Hoffmann-La Roche, Ltd. ASG: Employee of Genentech, Inc. and shareholder in F. Hoffmann-La Roche, Ltd. SC: Employee of Genentech, Inc. and shareholder in F. Hoffmann-La Roche, Ltd. LB: Employee of Genentech, Inc. and shareholder in F. Hoffmann-La Roche, Ltd. LH: Employee of Genentech, Inc. and shareholder in F. Hoffmann-La Roche, Ltd. JAC: Employee of Genentech, Inc. and shareholder in F. Hoffmann-La Roche, Ltd. MER: Employee of Genentech, Inc. and shareholder in F. Hoffmann-La Roche, Ltd. FB: Employee of Genentech, Inc. and shareholder in F. Hoffmann-La Roche, Ltd. KRS: Nothing to disclose. LvdB: Nothing to disclose. JDB: Grants from Genentech, during the conduct of the study; personal fees from Biogen, personal fees from Clene Nanomedicine, grants from Alexion, grants from Biogen, grants from MT Pharma of America, grants from Anelixis Therapeutics, grants from Brainstorm Cell Therapeutics, grants from nQ Medical, grants from NINDS, grants from Muscular Dystrophy Association, grants from ALS One, grants from Amylyx Therapeutics, personal fees from MT Pharma Holdings of America, grants from ALS Association, grants from ALS Finding A Cure, outside the submitted work. JDG: Emory University was a clinical trial site during the conduct of the study.

Data Availability

Qualified researchers may request access to individual patient level data through the clinical study data request

platform (https://vivli.org/). Further details on Roche's criteria for eligible studies are available here (https://vivli.org/members/ourmembers/). For further details on Roche's Global Policy on the Sharing of Clinical Information and how to request access to related clinical study documents, see here (https://go.gene.com/datasharing).

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