ORIGINAL ARTICLE

CRISPR-Cas9 In Vivo Gene Editing of *KLKB1* for Hereditary Angioedema

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ABSTRACT

BACKGROUND

Hereditary angioedema is a rare genetic disease that leads to severe and unpredictable swelling attacks. NTLA-2002 is an in vivo gene-editing therapy based on clustered regularly interspaced short palindromic repeats (CRISPR)–CRISPR-associated protein 9. NTLA-2002 targets the gene encoding kallikrein B1 (*KLKB1*), with the goal of lifelong control of angioedema attacks after a single dose.

METHODS

In this phase 1 dose-escalation portion of a combined phase 1–2 trial of NTLA-2002 in adults with hereditary angioedema, we administered NTLA-2002 at a single dose of 25 mg, 50 mg, or 75 mg. The primary end points were the safety and side-effect profile of NTLA-2002 therapy. Secondary and exploratory end points included pharmacokinetics, pharmacodynamics, and clinical efficacy determined on the basis of investigator-confirmed angioedema attacks.

RESULTS

Three patients received 25 mg of NTLA-2002, four received 50 mg, and three received 75 mg. At all dose levels, the most common adverse events were infusion-related reactions and fatigue. No dose-limiting toxic effects, serious adverse events, grade 3 or higher adverse events, or clinically important laboratory findings were observed after the administration of NTLA-2002. Dose-dependent reductions in the total plasma kallikrein protein level were observed between baseline and the latest assessment, with a mean percentage change of –67% in the 25-mg group, –84% in the 50-mg group, and –95% in the 75-mg group. The mean percentage change in the number of angioedema attacks per month between baseline and weeks 1 through 16 (primary observation period) was –91% in the 25-mg group, –97% in the 50-mg group, and –80% in the 75-mg group. Among all the patients, the mean percentage change in the number of angioedema attacks per month from baseline through the latest assessment was –95%.

CONCLUSIONS

In this small study, a single dose of NTLA-2002 led to robust, dose-dependent, and durable reductions in total plasma kallikrein levels, and no severe adverse events were observed. In exploratory analyses, reductions in the number of angioedema attacks per month were observed at all dose levels. (Funded by Intellia Therapeutics; ClinicalTrials.gov number, NCT05120830.)

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N Engl J Med 2024;390:432-41.
DOI: 10.1056/NEJMoa2309149

Hereditary angioedema is a rare, autosomal dominant genetic disorder that is characterized by painful, unpredictable, and potentially fatal attacks of swelling primarily in the gastrointestinal tract and cutaneous and submucosal tissues of the body. Symptoms often first appear during childhood, with attacks occurring in patients throughout their lifetime. Angioedema attacks may occur as frequently as every several days[1] and can last from a few hours to several days[2]; laryngeal edema can be life-threatening because it may result in airway obstruction and death by asphyxiation.[3,4]

The symptoms of hereditary angioedema result from the dysregulation of the contact activation pathway. C1 esterase inhibitor (C1-INH) is a key regulator of the pathway and attenuates the production of bradykinin, a peptide that leads to increased vascular permeability and subsequent tissue swelling, by means of the inhibition of the proteases factor XIIa and plasma kallikrein. In the most common types of hereditary angioedema, C1-INH deficiency (in type 1 disease) and C1-INH dysfunction (in type 2 disease) lead to increased bradykinin production and angioedema attacks.[3,4]

Plasma kallikrein is directly responsible for the cleavage of bradykinin from high-molecular-weight kininogen and has been clinically validated as a therapeutic target for the treatment and prevention of angioedema attacks.[5] Inhibitors of plasma kallikrein activity are now approved for long-term prophylaxis. More recently, RNA-silencing therapeutic approaches that reduce the total levels of plasma kallikrein (plasma kallikrein and its zymogen precursor, plasma prekallikrein) have shown promise in early trials.[6,7] Although these and other therapies have substantially improved the management of hereditary angioedema in recent years, lifelong administration of these treatments is necessary.[5,8-10]

NTLA-2002 is an in vivo gene-editing therapy that is based on clustered regularly interspaced short palindromic repeats (CRISPR)–CRISPR-associated protein 9 (Cas9). It is designed to be a systemically delivered one-time treatment to reduce the total plasma kallikrein protein level by permanently editing the gene responsible for the production of plasma prekallikrein (*KLKB1*). NTLA-2002 uses lipid nanoparticle (LNP) technology[11] with liver tropism to encapsulate two genome-editing components: messenger RNA (mRNA) encoding the Cas9 endonuclease and single guide RNA (sgRNA) with a 20-nucleotide sequence targeting *KLKB1*, which is primarily expressed in the liver.[12] The LNP is rapidly taken up by hepatocytes by means of endocytosis mediated by low-density lipoprotein receptors, followed by translation of Cas9 mRNA in the cytoplasm and formation of a ribonucleoprotein complex between the Cas9 endonuclease and the sgRNA (Fig. S1 in the Supplementary Appendix, available with the full text of this article at NEJM.org). The complex translocates into the nucleus and is directed to the *KLKB1* locus (because of its sequence complementarity with the sgRNA), where it makes precise double-strand breaks in *KLKB1*. Repair of double-strand breaks by the nonhomologous end-joining pathway introduces small insertions and deletions into *KLKB1*, which disrupts the production of plasma prekallikrein and leads to a reduced total plasma kallikrein level.

The intended effect of treatment with NTLA-2002 is to permanently rebalance the disease pathway by decreasing the production of bradykinin, which results in the prevention of angioedema attacks. Here, we report data from the phase 1 portion of a phase 1–2 trial to evaluate the safety, pharmacodynamics, pharmacokinetics, and preliminary efficacy of NTLA-2002 for the treatment of patients with hereditary angioedema.

METHODS

STUDY DESIGN AND OVERSIGHT

The phase 1 portion of this phase 1–2 trial is an ongoing multicenter, open-label, dose-escalation study with up to 30 patients that is assessing the safety, side-effect profile, pharmacodynamics, pharmacokinetics, and preliminary efficacy of NTLA-2002 therapy in patients with hereditary angioedema. The study does not have a placebo control. Phase 1 was designed to include up to three dose-escalation cohorts and two optional dose-reduction cohorts, with three to six evaluable patients per cohort for the assessment of dose-limiting toxic effects. Patients were enrolled at three sites in New Zealand, the Netherlands, and the United Kingdom.

This study was approved by the institutional review board or ethics committee at each participating study site. All the patients provided written informed consent. The study was performed in

accordance with the principles of the Declaration of Helsinki and with the current Good Clinical Practice guideline of the International Council for Harmonisation. An independent data monitoring committee provided study oversight. The authors vouch for the accuracy and completeness of the data and for the adherence of the study to the protocol, available at NEJM.org.

PATIENTS

Eligible patients were 18 years of age or older, had received a diagnosis of type 1 or type 2 hereditary angioedema, and had had at least three investigator-confirmed attacks during the 90 days before screening (the historical attack period). Patients were excluded if they had a concurrent diagnosis of any other type of recurrent angioedema or if they were known to have had an adverse reaction or hypersensitivity to any LNP component. Full eligibility criteria are listed in the protocol.

TREATMENT

NTLA-2002 was administered as a single intravenous infusion over a minimum of 2 hours. To mitigate the risk of infusion-related reactions, patients received a pretreatment regimen consisting of a dose of a glucocorticoid administered between 8 hours and 24 hours before the infusion of NTLA-2002 and one dose each of a glucocorticoid, an H_1 blocker, and an H_2 blocker administered approximately 1 hour before the infusion. C1-INH therapy was required to be at the bedside during the infusion of NTLA-2002. All the patients could continue to use previously prescribed long-term prophylaxis and were permitted to use on-demand therapy for angioedema attacks at any time during the study.

The primary observation period was defined as the first 16 weeks after the receipt of NTLA-2002 treatment. This was followed by a long-term observation period that lasted 88 weeks (104 total weeks of observation). Details about the starting dose, including the justification for its selection, and dose escalation are provided in the Supplementary Appendix.

CLINICAL ASSESSMENTS

Each patient recorded angioedema symptoms, attacks, and treatments in an electronic diary. The investigator reviewed the diary at each study visit to confirm whether an event represented an attack due to angioedema.

Samples for pharmacodynamic and pharmacokinetic analyses were obtained at each study visit in accordance with the schedule of activities provided in the protocol. Vital signs were measured, clinical history was obtained, and physical examinations, laboratory tests, and electrocardiography were performed before the infusion and at each visit. Adverse events were graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events, version 5.0.

STUDY END POINTS

The primary end points of the phase 1 study were the safety and side-effect profile of NTLA-2002 as assessed on the basis of adverse events and the incidence of dose-limiting toxic effects. The secondary end points included the change from baseline in the total plasma kallikrein protein level and the change from baseline in the plasma concentrations of the components of NTLA-2002, which included exogenous lipids (LP000001 and DMG-PEG 2000), Cas9 mRNA, and sgRNA. Details about the LNP components and the bioanalytic assays that were used in the end-point assessments are provided in the Supplementary Appendix. Exploratory efficacy end points were the number of all angioedema attacks per month (defined as 28 days) during weeks 1 to 16 and weeks 5 to 16 and the number of angioedema attacks leading to on-demand therapy per month during weeks 1 to 16 and weeks 5 to 16.

An adverse event was defined as any event that started or worsened after the administration of NTLA-2002. For the analysis of end points related to angioedema attacks, the number of attacks per month at baseline was based on attacks that occurred during the screening period (≤42 days before the infusion of NTLA-2002).

STATISTICAL ANALYSIS

The percentage of patients with dose-limiting toxic effects was calculated in the evaluable population, defined as all the patients who received at least a partial dose of NTLA-2002 and had at least one dose-limiting toxic effect and all the patients who received the full assigned dose without a dose-limiting toxic effect and were assessed on day 29. Safety analyses included all the patients who received at least a partial dose

of NTLA-2002 (safety population). Pharmacodynamic analyses included all the patients in the safety population in whom the total plasma kallikrein protein level was measured at least once before and at least once after the administration of NTLA-2002. Pharmacokinetic analyses included patients in the safety population who had at least one blood sample in which concentrations of any of the four plasma analytes (the two exogenous lipid components and the two RNA components) of NTLA-2002 could be evaluated.

The exploratory analysis of angioedema attacks included all the patients in the safety population. For the number of all angioedema attacks per month and the number of all angioedema attacks leading to on-demand therapy per month, the observed values and the percentage changes from baseline to weeks 1 through 16 and from baseline to weeks 5 through 16 were summarized descriptively. Because no minimum number of attacks was required during screening, the percentage change from baseline in the number of angioedema attacks per month was calculated only for the patients who had attacks before the infusion of NTLA-2002 (during the screening period). We also calculated the percentage change in the number of angioedema attacks per month overall between baseline and the period from the administration of NTLA-2002 through the latest assessment (defined as the last assessment as of the data-cutoff date). Details about the prespecified analyses are included in the statistical analysis plan, which is available with the protocol.

RESULTS

PATIENTS

We screened 11 patients, of whom 10 received NTLA-2002 between December 10, 2021, and August 30, 2022. One eligible patient withdrew voluntarily during the screening period, before the study treatment was administered. The data-cutoff date was February 17, 2023, and the median follow-up was 13.1 months in the 25-mg group, 5.7 months in the 50-mg group, and 9.3 months in the 75-mg group. The median age of the patients was 51 years (range, 26 to 73), and 6 patients were men (Table 1). Six patients had type 1 hereditary angioedema, and 4 had type 2 hereditary angioedema. Nine patients had received at least one type of long-term prophylaxis before enrollment. The median number of angioedema attacks during the 90 days before screening (historical attack period) was 6 (range, 3 to 54). The study population was generally representative of patients with hereditary angioedema (Table S1).

SAFETY

All the patients received the intended dose of NTLA-2002. No dose-limiting toxic effects were observed. The most common adverse events were infusion-related reactions (in 70% of the patients) and fatigue (in 60%) (Table 2 and Table S2). Most of the symptoms that were related to infusion-related reactions were assessed to be grade 1 in severity and resolved on the day when NTLA-2002 was administered, without sequelae (Table S3). One grade 2 infusion-related reaction — back pain — resulted in the temporary interruption of the NTLA-2002 infusion. No grade 3 or higher adverse events or serious adverse events occurred after the administration of NTLA-2002.

Increases in the alanine aminotransferase level, the aspartate aminotransferase level, or both were reported in 60% of the patients (in two patients at each dose level); all increases were grade 1 in severity, were transient, and resolved between 1 day and 8 weeks after onset. Values peaked between day 1 and day 15 (Figs. S2 and S3). Transient, dose-dependent increases in D-dimer levels were observed, which typically returned to baseline levels by day 4. No patients had a prolonged activated partial thromboplastin time.

Immunogenicity assessments showed that transient antidrug antibodies to the LNP component of NTLA-2002 developed in one patient (Table S4). As we expected, transient anti-Cas9 protein antibodies developed in all the patients. Anti-Cas9 protein antibody was detected in one patient before the infusion of NTLA-2002; this response was probably the result of an environmental exposure to Cas9 (Table S5). We did not detect discernible effects of the anti-Cas9 protein antibodies on pharmacokinetics, pharmacodynamics, or safety.

Before the initiation of this phase 1 study, the potential off-target editing activity of NTLA-2002 was assessed in order to understand the potential long-term safety risks of the treatment. Preclinical studies did not identify off-target editing activity at clinically relevant exposures of NTLA-2002 (see the Supplementary Methods,

Table 1. Characteristics of the Patients at Baseline.*

Characteristic	NTLA-2002, 25 mg (N=3)	NTLA-2002, 50 mg (N=4)	NTLA-2002, 75 mg (N=3)	All Patients (N=10)
Median age (range) — yr	30 (26–52)	65 (52–73)	45 (27–49)	51 (26–73)
Male sex — no. (%)	3 (100)	1 (25)	2 (67)	6 (60)
White race — no. (%)†	3 (100)	4 (100)	3 (100)	10 (100)
Median weight (range) — kg	83 (78–135)	86 (74–107)	72 (64–84)	83 (64–135)
Type of hereditary angioedema — no. (%)				
Type I	2 (67)	2 (50)	2 (67)	6 (60)
Type II	1 (33)	2 (50)	1 (33)	4 (40)
Previous receipt of long-term prophylaxis — no. (%)	2 (67)	4 (100)	3 (100)	9 (90)
Concomitant receipt of long-term prophylaxis — no. (%)‡	2 (67)	3 (75)	1 (33)	6 (60)
Typical severity of angioedema attacks — no. (%)				
Mild	1 (33)	2 (50)	1 (33)	4 (40)
Moderate	1 (33)	2 (50)	1 (33)	4 (40)
Severe	1 (33)	0	1 (33)	2 (20)
Previous occurrence of laryngeal attacks — no. (%)	1 (33)	4 (100)	3 (100)	8 (80)
Median no. of angioedema attacks during the historical attack period (range)§	7 (6–45)	3 (3–6)	14 (6–54)	6 (3–54)
Type of angioedema attack during the historical attack period — no. (%)§				
Peripheral	3 (100)	1 (25)	1 (33)	5 (50)
Abdominal	2 (67)	1 (25)	3 (100)	6 (60)
Laryngeal	1 (33)	0	2 (67)	3 (30)

* Percentages may not total 100 because of rounding.
† Race was reported by the patient.
‡ Concomitant prophylaxis is defined as prophylaxis that was ongoing at the time of the administration of NTLA-2002.
§ The historical attack period is defined as the 90 days before the screening period.

Tables S6 and S7, and Figs. S4 through S6). Although we plan to follow all the patients enrolled in this study in a separate study for up to 15 years for the long-term assessment of safety, the small sample size and limited duration of follow-up at this time precludes our assessment of the long-term safety profile of NTLA-2002 here.

PHARMACODYNAMICS

A dose-dependent reduction in the total plasma kallikrein protein level was observed, with a mean percentage change from baseline to the latest assessment of −67% in the 25-mg group, −84% in the 50-mg group, and −95% in the 75-mg group (Fig. 1). Observed pharmacodynamic responses were sustained for the duration of follow-up. A dose-dependent reduction in the plasma kallikrein activity was also observed (Fig. S7), a finding that correlated strongly with the reduction in the total plasma kallikrein protein level (R^2=0.82) (Fig. S8).

PHARMACOKINETICS

The concentration–time profile of NTLA-2002 showed that exposure was dose-dependent, as assessed by the analysis of the ionizable lipid LP000001, an analyte representative of the LNP (Fig. S9). The LP000001 concentrations in each dose cohort decreased rapidly from peak levels, had a secondary peak 4 to 8 hours after the infusion, and then entered a log-linear elimination phase with a half-life ranging from 15.9 to 25.4 hours. After the infusion of NTLA-2002, LP000001 was quantifiable in plasma (lower limit of quantification, 10 ng per milliliter) for up to 15 days in the 75-mg group and for up to 8 days in the 25-mg and 50-mg groups. In all the patients,

Table 2. Adverse Events after the Administration of NTLA-2002.*

Event	NTLA-2002, 25 mg (N=3)	NTLA-2002, 50 mg (N=4)	NTLA-2002, 75 mg (N=3)	All Patients (N=10)
		number of patients (percent)		
Any event	3 (100)	3 (75)	3 (100)	9 (90)
Infusion-related reaction	2 (67)	2 (50)	3 (100)	7 (70)
Fatigue	1 (33)	3 (75)	2 (67)	6 (60)
Coronavirus disease 2019	3 (100)	1 (25)	1 (33)	5 (50)
Upper respiratory tract infection	1 (33)	1 (25)	2 (67)	4 (40)
Oropharyngeal pain	2 (67)	0	1 (33)	3 (30)
Abdominal pain	1 (33)	0	1 (33)	2 (20)
Headache	0	0	2 (67)	2 (20)
Viral upper respiratory tract infection	0	0	2 (67)	2 (20)

* Shown are adverse events, coded according to preferred terms in the *Medical Dictionary for Regulatory Activities*, version 24.0, that occurred in at least two patients after the administration of NTLA-2002. No adverse events after the administration of NTLA-2002 were assessed to be grade 3 or higher in severity.

concentrations of both RNA components decreased to below the lower limit of quantification (0.08 ng per milliliter) by day 29 after the infusion.

ANGIOEDEMA ATTACKS

During the 16-week primary observation period, the mean percentage change from baseline in the number of angioedema attacks per month was –91% in the 25-mg group, –97% in the 50-mg group, and –80% in the 75-mg group (Table 3 and Fig. S10). Among all 10 patients, the mean percentage change in the number of angioedema attacks per month between baseline and the latest assessment was –95% for all attacks and –93% for attacks that led to the use of on-demand therapy (Table 3 and Table S8). Because the screening period was shorter for some patients than for others, we performed a supportive analysis in which the baseline data included attacks that occurred during the 90-day historical attack period and attacks that occurred during the screening period, and the percentage change was similar to that when the baseline data included only attacks that occurred during the screening period (Table S9).

In the six patients who were receiving concomitant long-term prophylaxis (defined as prophylaxis that was ongoing at the time of the administration of NTLA-2002) at study entry, in whom the number of attacks per month ranged from 0.9 to 14.0 during the historical attack period, prophylaxis was withdrawn between 2.6 months and 5.4 months after the administration of NTLA-2002 (Fig. 2 and Table S10). The decision to withdraw long-term prophylaxis was made at the discretion of the investigator and the patient. These patients did not report any attacks after the withdrawal of long-term prophylaxis. The median

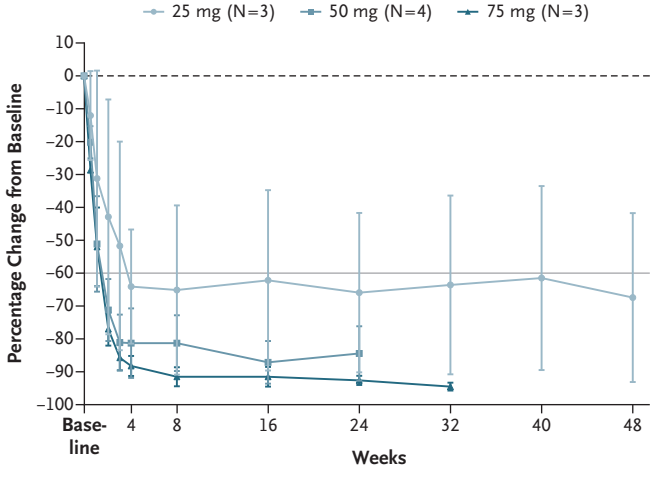

Figure 1. Change from Baseline in the Total Plasma Kallikrein Protein Level According to NTLA-2002 Dose.

Shown is the mean percentage change from baseline in the total plasma kallikrein protein level in each dose cohort. I bars indicate the standard deviation.

Table 3. Change from Baseline in the Number of Investigator-Confirmed Angioedema Attacks per Month.*

Variable	NTLA-2002, 25 mg (N=3)	NTLA-2002, 50 mg (N=4)	NTLA-2002, 75 mg (N=3)	All Patients (N=10)
No. of patients with attacks during the screening period (%)	3 (100)	3 (75)	3 (100)	9 (90)
Percentage change from baseline in no. of attacks per mo†				
To wk 1–16	−91±16	−97±5	−80±30	−89±19
To wk 5–16	−89±19	−100±0	−87±23	−92±16
To wk 1–24	−94±11	−98±3	−86±20	−93±13
Through the latest assessment	−95±4	−98±3	−93±11	−95±6

* Plus–minus values denote the means ±SD. Baseline was defined as the screening period (≤42 days before infusion) before the administration of NTLA-2002.

† The percentage change from baseline was calculated only for patients with angioedema attacks during the screening period. The latest assessment was defined as the last assessment as of the data-cutoff date.

number of angioedema attacks per month between the start of the infusion of NTLA-2002 and the latest assessment (in four patients) and between the withdrawal of concomitant long-term prophylaxis and the latest assessment (in six patients) was 0.0 (range, 0.0 to 1.3), as compared with 3.5 (range, 0.0 to 7.2) at baseline (Fig. S11). In all the patients, NTLA-2002 treatment led to a high degree of control of angioedema attacks.

DISCUSSION

In 10 patients with hereditary angioedema, a single dose of NTLA-2002 resulted in no apparent safety concerns. The safety profile of NTLA-2002 was substantially similar to that of NTLA-2001, which is not surprising because the two drug products differ only with respect to the 20-nucleotide targeting sequence at the 5′ end of the sgRNA.[13] We therefore propose that the short-term safety events associated with the use of each product are caused by the LNP or the expression of Cas9 protein, rather than by the editing activity of the treatment.

From baseline through the latest assessment, a rapid, robust, dose-dependent, and durable reduction of 67 to 95% in the total plasma kallikrein level and a mean reduction of 95% in the number of angioedema attacks per month were observed in all the patients. Patients who discontinued concomitant long-term prophylaxis continued to have well-controlled disease. Although our study was small and had no placebo control, these findings are consistent with those from a recent double-blind, randomized, placebo-controlled, phase 2 trial, in which a mean reduction from baseline of 60% in the total plasma kallikrein protein level was associated with nearly complete control of angioedema attacks across the entire cohort.[7] On the basis of the results of an earlier trial,[5] a threshold of 60% in the reduction from baseline in the total plasma kallikrein level was prespecified in the protocol as a criterion for the selection of the NTLA-2002 dose in the phase 2 trial. All dose levels in the current study met this criterion and showed preliminary evidence of having led to a reduction in the number of angioedema attacks per month. There was no dose-response relationship with respect to the number of attacks per month after baseline (Table 3). Therefore, the 25-mg and 50-mg doses were selected for investigation in the double-blind, randomized, placebo-controlled phase 2 portion of this phase 1–2 trial.

Preclinical data in animal models suggest that gene editing by NTLA-2002 is durable and that the edits are passed to daughter cells during cell division in the liver.[14,15] NTLA-2002 therapy permanently alters hepatocyte DNA and thus has the potential for long-term control of hereditary angioedema after a single dose, unlike the treatments for hereditary angioedema that temporar-

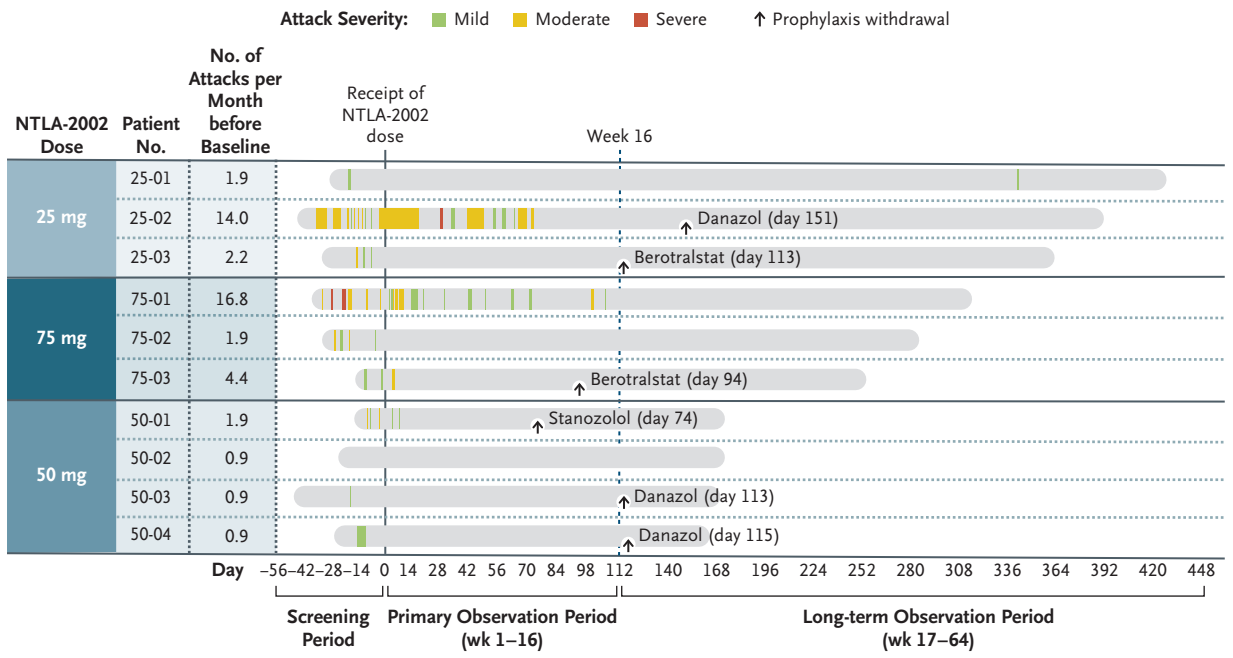

Figure 2. Angioedema Attacks before and after the Infusion of NTLA-2002.

Each row shows individual patient data on angioedema attacks; the number of attacks per month in each patient during the historical attack period (defined as the 90-day period before the screening period) is also shown. Patient data are listed from top to bottom according to the length of follow-up. The length of the gray bars indicates the interval from the start of the screening period to the last assessment as of the data-cutoff date. The length of the colored bars indicates the duration of the attack. Arrows indicate the time of withdrawal of any concomitant long-term prophylaxis. Patients without arrows were not receiving concomitant long-term prophylaxis. Patient 25-01 had mild swelling of the hand precipitated by a sports injury on day 343; the swelling did not lead to a medical intervention or to the use of on-demand therapy, and it resolved within 2 days.

ily disrupt protein expression or function and are administered on a long-term basis. NTLA-2002 treatment was safe in this small study, but longer follow-up will be needed for a robust assessment of safety and efficacy outcomes in patients with hereditary angioedema.

All the patients enrolled in this phase 1 study had varying numbers of angioedema attacks per month during the historical attack period. Some patients had more severe disease than other patients at baseline (Fig. 2), and the time needed to control the angioedema attacks was longer in these patients. Nine of the 10 patients remained free of angioedema attacks from the end of the primary observation period through the latest assessment.

Because treatment with NTLA-2002 resulted in a substantial reduction in the total plasma kallikrein level, a comparison may be drawn between the patients in our study and patients with congenital total plasma prekallikrein deficiency (Fletcher factor deficiency), a rare, autosomal-recessive disorder.[16] Patients with congenital total plasma prekallikrein deficiency have been reported to be overtly healthy, and the only well-established hallmark of prekallikrein deficiency is a prolonged activated partial thromboplastin time without apparent clinical consequence.[17] Despite this abnormal laboratory finding, the prevalence of major bleeding events was found to be very low among these patients and was similar to that in the general population.[17] None of the patients in the current study had a prolonged activated partial thromboplastin time or any thromboembolic or bleeding events.

Limitations of the study include its small sample size and limited duration. The study was a single-group study without a placebo control and permitted the concomitant use of long-term prophylaxis after NTLA-2002, which potentially makes it difficult to isolate the treatment effect of NTLA-2002. However, none of the six patients

who were receiving long-term prophylaxis had an angioedema attack once the long-term prophylaxis was withdrawn, a finding that is consistent with the clinical benefit having been derived from NTLA-2002 therapy alone. The ongoing randomized phase 2 trial requires washout of long-term prophylaxis before the administration of NTLA-2002 therapy in order to more precisely define its treatment effect. In addition, the number of attacks during the historical attack period was reported by the patients, whereas angioedema attacks during the screening period and throughout follow-up were adjudicated by the investigators. It is possible that some of the patient-reported attacks had an alternate cause (such as gastrointestinal illness) or were due to other underlying conditions, which may have inflated the historical attack rate. Although the screening period was shorter for some patients than for others, the reduction in the number of investigator-confirmed attacks per month from baseline through the latest assessment was similar in the dose cohorts (Table 3), and the reduction remained similar in the dose cohorts when the baseline data included attacks that occurred during the historical attack period and attacks that occurred during the screening period.

CRISPR-Cas9–mediated gene editing involves a risk of unintended genetic changes. The NTLA-2002 sgRNA targeting *KLKB1* underwent a rigorous assessment as described previously[13] in order for the risk to be characterized more fully before we administered this therapy to patients in this study (see the Supplementary Appendix). No physiological consequences of unintended genetic changes were identified in preclinical studies. However, understanding the long-term safety profile of NTLA-2002 will require longer follow-up.

Together with previously reported results from an ongoing phase 1 study of NTLA-2001 in patients with transthyretin amyloidosis,[13] these results from our study of NTLA-2002 in patients with hereditary angioedema support the modularity of CRISPR-Cas9–based in vivo gene editing as a therapeutic platform with the potential for broad application in the treatment of genetic diseases. None of the patients who received NTLA-2002 had serious adverse events, and treatment with the drug elicited a dose-dependent reduction in the total plasma kallikrein level. After the receipt of NTLA-2002 therapy, patients had a decreased number of angioedema attacks per month. These results support the continued investigation of NTLA-2002 as a new therapy for the treatment of hereditary angioedema.

Supported by Intellia Therapeutics.

Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

A data sharing statement provided by the authors is available with the full text of this article at NEJM.org.

We thank the patients and their caregivers; study-site coordinators and support staff; the staff of Simbec-Orion for assistance with study management and operations support; Daniel O'Connell, Nishit Patel, Sucharitha Parthasarathy, Joseph Rissman, Ruta Shinde, Kellie Kolb, Palak Sharma, and Zachary Dymek (all of Intellia Therapeutics) for contributions to preclinical data generation, analysis, and interpretation; and the members of the independent data monitoring committee (James Lewis [chair], Daniel Gillen, F. Ida Hsu, and Alvin Schmaier).

APPENDIX

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