ORIGINAL ARTICLE

CRISPR-Cas9 In Vivo Gene Editing for Transthyretin Amyloidosis

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ABSTRACT

BACKGROUND

Transthyretin amyloidosis, also called ATTR amyloidosis, is a life-threatening disease characterized by progressive accumulation of misfolded transthyretin (TTR) protein in tissues, predominantly the nerves and heart. NTLA-2001 is an in vivo gene-editing therapeutic agent that is designed to treat ATTR amyloidosis by reducing the concentration of TTR in serum. It is based on the clustered regularly interspaced short palindromic repeats and associated Cas9 endonuclease (CRISPR-Cas9) system and comprises a lipid nanoparticle encapsulating messenger RNA for Cas9 protein and a single guide RNA targeting *TTR*.

METHODS

After conducting preclinical in vitro and in vivo studies, we evaluated the safety and pharmacodynamic effects of single escalating doses of NTLA-2001 in six patients with hereditary ATTR amyloidosis with polyneuropathy, three in each of the two initial dose groups (0.1 mg per kilogram and 0.3 mg per kilogram), within an ongoing phase 1 clinical study.

RESULTS

Preclinical studies showed durable knockout of *TTR* after a single dose. Serial assessments of safety during the first 28 days after infusion in patients revealed few adverse events, and those that did occur were mild in grade. Dose-dependent pharmacodynamic effects were observed. At day 28, the mean reduction from baseline in serum TTR protein concentration was 52% (range, 47 to 56) in the group that received a dose of 0.1 mg per kilogram and was 87% (range, 80 to 96) in the group that received a dose of 0.3 mg per kilogram.

CONCLUSIONS

In a small group of patients with hereditary ATTR amyloidosis with polyneuropathy, administration of NTLA-2001 was associated with only mild adverse events and led to decreases in serum TTR protein concentrations through targeted knockout of *TTR*. (Funded by Intellia Therapeutics and Regeneron Pharmaceuticals; ClinicalTrials.gov number, NCT04601051.)

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This article was published on June 26, 2021, at NEJM.org.

DOI: 10.1056/NEJMoa2107454

Transthyretin amyloidosis, also called ATTR amyloidosis, is a progressive fatal disease characterized by accumulation in tissues of amyloid fibrils composed of misfolded transthyretin (TTR) protein.[1,2] ATTR amyloidosis may be acquired; referred to as wild-type ATTR amyloidosis, this form of ATTR amyloidosis is an increasingly recognized cause of cardiomyopathy and heart failure.[2] In rarer cases, ATTR amyloidosis is hereditary (known as variant or hereditary ATTR [hATTR] amyloidosis); hATTR amyloidosis can be triggered by more than 100 different pathogenic mutations in *TTR*.[3] The hereditary form of ATTR amyloidosis is thought to be present in approximately 50,000 persons worldwide[4,5]; it has an autosomal dominant pattern of inheritance and a clinical phenotype dominated by amyloid polyneuropathy or cardiomyopathy, with most patients having a combination of the two.[6] After the onset of symptoms, ATTR amyloidosis is progressive, culminating in death within a median of 2 to 6 years after diagnosis in patients with amyloid cardiomyopathy[7] and 4 to 17 years after symptom onset in patients with amyloid polyneuropathy in the absence of cardiomyopathy.[8]

Current therapeutic strategies for ATTR amyloidosis rely on reducing ongoing amyloid formation through stabilization of the tetrameric form of TTR (with diflunisal or tafamidis)[9,10] or through inhibition of TTR protein synthesis (with inotersen or patisiran) by means of degradation of *TTR* messenger RNA (mRNA).[11,12] Such treatments produce symptom relief and functional improvement and prolong survival[12-14] but are limited by the requirement for long-term administration to maintain TTR knockdown. In the case of patisiran, long-term treatment results in continued exposure to premedication with glucocorticoids and antihistamines.[15] In addition, patients receiving TTR-stabilizing agents have disease progression.[16] Inotersen is associated with serious side effects, including glomerulonephritis and decreased platelet counts.[17] More extensive TTR knockdown is associated with greater improvement in neuropathic end points in patients with hATTR polyneuropathy.[12] Enhancements in TTR reduction, including sustained knockdown, may translate into improved outcomes for patients with ATTR amyloidosis. A potential alternative to mRNA targeting-based gene silencing is use of the clustered regularly interspaced short palindromic repeats and associated Cas9 endonuclease (CRISPR-Cas9) system to achieve in vivo gene editing.[18-21] As a monogenic disease, ATTR amyloidosis represents an ideal target for the application of CRISPR-Cas9–mediated in vivo gene editing. The limited and specific normal function of TTR in thyroxine and vitamin A transport[22] means that knockdown has only limited additional physiological effects[23]; in addition, circulating TTR is produced almost entirely (>99%) within the liver,[2] for which established targeting systems such as lipid nanoparticles are available.

NTLA-2001 is a new CRISPR-Cas9–based in vivo gene-editing therapy, administered by intravenous infusion, that is intended to edit *TTR* in hepatocytes, leading to a decrease in the production of both wild-type and mutant TTR after a single administration. In mouse models and nonhuman primates (cynomolgus monkeys), single doses resulted in durable reductions in serum TTR protein of 95% or greater[24,25] and therefore provide potentially greater TTR knockdown than currently available therapies.

NTLA-2001 consists of a proprietary lipid nanoparticle (LNP) delivery system with liver tropism, carrying a single guide RNA (sgRNA) that targets human *TTR* and a human-codon–optimized mRNA sequence of *Streptococcus pyogenes* Cas9 protein (Fig. 1). LNPs have previously been used in vivo for liver-targeted delivery of a variety of therapeutic RNA cargoes, such as small interfering RNA and mRNA.[26,27] For NTLA-2001, the LNP formulation was developed for genome editing with regard to lipid composition and RNA cargo in order to enable efficient delivery to the liver in a variety of preclinical models. As described previously, on polyethylene glycol–lipid diffusion from this LNP, plasma apolipoprotein E binds to (opsonizes) the LNP surface in circulation; the LNP is then actively endocytosed by hepatocytes through the low-density lipoprotein receptor.[27] Given that the liver is the almost exclusive site of TTR manufacture,[2] this liver-targeting delivery system should maximize efficacy while minimizing systemic toxic effects.

Here, we report interim data from an ongoing clinical study evaluating single ascending doses of NTLA-2001 for *TTR* editing and knockout in the treatment of patients with hATTR amyloidosis with polyneuropathy.

METHODS

PRECLINICAL STUDIES

An sgRNA targeting the *TTR* sequence AAAGGCUGCUGAUGACACCU (human genome build hg38, chromosome 18: 31592987–31593007) was selected for efficient knockout and specificity after a comprehensive off-target characterization workflow that applied a combination of both computational modeling and empirical approaches. To select for a high therapeutic index (i.e., the ratio of on-target to off-target editing), we performed genomewide assays and targeted sequencing to identify and verify candidate sgRNA off-target sites. The in vitro dose–response and gene-editing potency of NTLA-2001 were assessed in primary cell cultures of human hepatocytes. Genomic loci with the potential for off-target editing were found with the use of complementary computational and laboratory-based approaches (Cas-OFFinder,[28] GUIDE-seq,[29] and SITE-Seq[30]). Candidate loci were validated for the detection of off-target insertions and deletions (indels) with the use of next-generation sequencing after NTLA-2001 treatment of primary human hepatocytes at concentrations up to 27 times as high as concentrations that achieved greater than 90% reduction in TTR protein (EC_{90}). Additional details of these studies are provided in the Supplementary Appendix, available with the full text of this article at NEJM.org.

Transgenic mouse models with either wild-type or mutant (p.V50M) human *TTR* were used for initial proof-of-mechanism studies of NTLA-2001 in which we evaluated *TTR* editing and reductions in circulating TTR protein levels.[24] The cynomolgus monkey was selected as a relevant nonclinical animal model for the evaluation of pharmacologic, toxicologic, pharmacokinetic, and metabolic properties, including *TTR* editing, reductions in circulating TTR protein levels, and serum concentrations of LNP and cargo components over time.[25]

CLINICAL STUDY OVERSIGHT

The clinical study is sponsored by Intellia Therapeutics and Regeneron Pharmaceuticals; the sponsors designed the protocol, available at NEJM.org. Study oversight was provided by an independent data and safety monitoring committee. Data were collected by study investigators in New Zealand and the United Kingdom and were analyzed by the sponsors. A medical writer prepared the first draft of the manuscript, under direction from the authors and with funding from the sponsors. The authors had access to the data and collaborated in the preparation and revisions of manuscript drafts before and after submission. The investigators and sponsors vouch for the accuracy and completeness of the data and for the fidelity of the study to the protocol. The protocol was reviewed by national and institutional ethics and regulatory bodies, including expert committees for the assessment of new studies of gene therapy, such as gene editing (Gene Therapy Advisory Committee of the Ministry of Health, New Zealand, and Gene Therapy Advisory Committee of the Medicines and Healthcare Products Regulatory Agency, United Kingdom). The study has been conducted in accordance with the Declaration of Helsinki and International Council for Harmonisation Good Clinical Practice guidelines, and all patients provided written informed consent.

CLINICAL STUDY DESIGN AND ELIGIBILITY

We report the results in two initial dose groups from part 1 of a two-part, global, phase 1, open-label, multicenter study. Patients were treated with a single dose of NTLA-2001 consisting of a total RNA dose of 0.1 mg per kilogram of body weight or 0.3 mg per kilogram of body weight administered intravenously between November 2020 and April 2021. Key eligibility criteria for part 1 of the study included an age of 18 to 80 years, a diagnosis of polyneuropathy due to hATTR amyloidosis (with or without cardiomyopathy), a body weight of 50 to 90 kg at the screening visit, and a lack of access to approved treatments for ATTR amyloidosis. Patients with non-ATTR amyloidosis, known leptomeningeal ATTR amyloidosis, or a history of receipt of RNA-silencing therapy were excluded. Previous use of TTR stabilizers was permitted with a washout period (3 days for diflunisal).

CLINICAL STUDY TREATMENT

Safety studies in cynomolgus monkeys were used to determine the no-observed-adverse-effect level (NOAEL), which was found to be a single administration of 3 mg per kilogram infused intravenously, equivalent to a dose of 1 mg per kilogram in humans. In accordance with allometric scaling based on total body-surface area and application

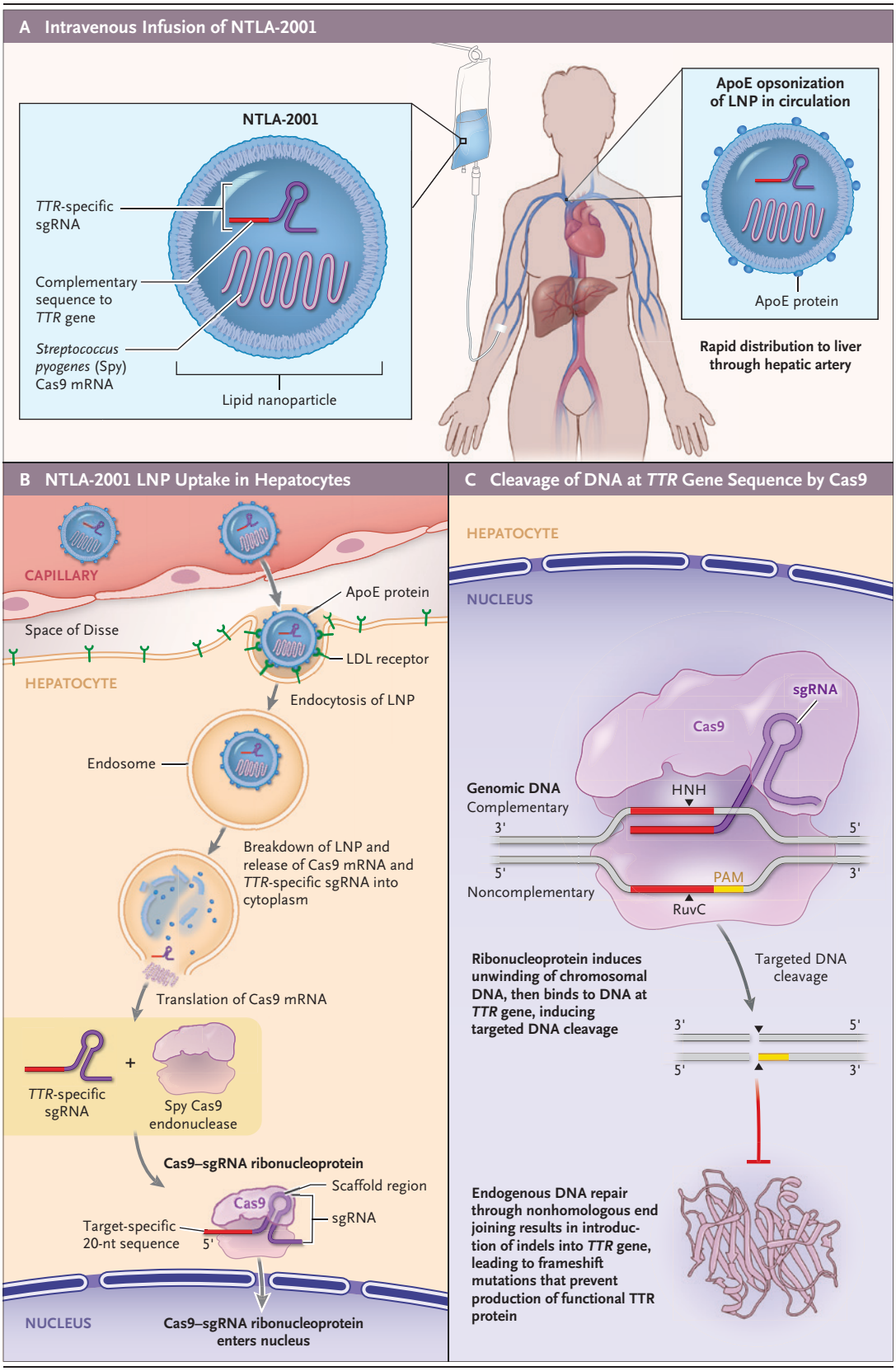


Figure 1 (facing page). Mechanism of Action of NTLA-2001.

Panel A shows the primary components of NTLA-2001. The carrier system for NTLA-2001 is a lipid nanoparticle (LNP). The LNP is based on a proprietary ionizable lipid, combined with a phospholipid, a pegylated lipid (molecular weight of polyethylene glycol, 2000 Da), and cholesterol, formulated in an aqueous buffer for intravenous administration. The active components of NTLA-2001 are a human-optimized messenger RNA (mRNA) molecule encoding *Streptococcus pyogenes* (Spy) Cas9 protein (an approximately 4400-nucleotide sequence with a molecular weight of approximately 1.5 MDa) and a single guide RNA (sgRNA) molecule (molecular weight of approximately 35 kDa) specific to the human gene encoding transthyretin (TTR). These components form the cargo of the LNP for drug administration. After intravenous administration of NTLA-2001 and entry into the circulation, the LNP is opsonized by apolipoprotein E (ApoE) and transported through the systemic circulation directly into the liver, where it is preferentially distributed. **Panel B** shows transport of the NTLA-2001 LNP into the capillaries of the hepatic sinusoids inside the liver. As with other clinically approved LNPs,[27] NTLA-2001 is then expected to undergo uptake by the low-density lipoprotein (LDL) receptor expressed on the surface of the hepatocytes, followed by endocytosis and endosome formation. After breakdown of the LNP and disruption of the endosomal membrane, the active components (the *TTR*-specific sgRNA and the mRNA encoding Cas9) are released into the cytoplasm. The Cas9 mRNA molecule is translated through the native ribosomal process, producing the Cas9 endonuclease enzyme. The *TTR*-specific sgRNA interacts with the Cas9 endonuclease, forming a clustered regularly interspaced short palindromic repeats (CRISPR)–Cas9 ribonucleoprotein complex. **Panel C** shows that the Cas9 ribonucleoprotein complex is targeted for nuclear import and enters the nucleus, where it recognizes the protospacer-adjacent motif (PAM) on the noncomplementary DNA strand in *TTR*. A target-specific 20-nucleotide sequence at the 5′ end of the sgRNA binds to the DNA double helix at the target site, allowing the CRISPR-Cas9 complex to unwind the helix and access the target gene. Cas9 undergoes a series of conformational changes and nuclease domain activation (HNH and RuvC domains), resulting in DNA cleavage that is precisely targeted to the *TTR* sequence, as defined by the sgRNA complementary sequence. Endogenous DNA-repair mechanisms ligate the ends of the cut, potentially introducing insertions or deletions of bases (indels). The generation of an indel may result in the reduction of functional target-gene mRNA levels as a result of missense or nonsense mutations decreasing the amount of full-length mRNA, ultimately resulting in decreased levels of the target protein. Indels that result in abrogated production of the target protein, in this case TTR, are termed knockout mutations.

of a safety factor of 10, the maximum recommended starting dose of NTLA-2001 for this study was 0.1 mg per kilogram. To mitigate against potential proinflammatory effects of intravenous LNP infusions, patients received glucocorticoid and histamine receptor type 1 and type 2 blockade before infusion.

ASSESSMENTS OF CLINICAL OUTCOMES

Patients were monitored for assessment of adverse events and laboratory findings. Serum samples were obtained at baseline and at weeks 1, 2, and 4 for analysis of TTR protein levels with a validated enzyme-linked immunosorbent assay. Evaluations of safety and therapeutic-activity outcomes are planned for 24 months after NTLA-2001 infusion. In accordance with the requirements of the regulatory authorities that approved the current protocol, longer-term safety follow-up is planned under a separate program currently in development.

STATISTICAL ANALYSIS

Descriptive analyses only were planned. Measurements of serum TTR protein levels at baseline were compared with those at day 28 and are presented as the mean percentage change and range.

RESULTS

PRECLINICAL STUDIES OF NTLA-2001

Preclinical development of NTLA-2001 (Fig. 1) included computational modeling and in vitro studies to ensure on-target gene editing (see the Supplementary Appendix). In primary human hepatocytes, NTLA-2001 was highly potent (EC_{50}, 0.05 to 0.15 nmol per liter; EC_{90}, 0.17 to 0.67 nmol per liter) and produced saturating levels of *TTR* editing (≥93.7%), resulting in 91% or greater reductions in *TTR* mRNA expression and 95% or greater reductions in TTR protein production (Fig. 2). Data from next-generation sequencing showed that NTLA-2001 induced knockout of *TTR*.

In off-target editing assays, among all the potential off-target loci identified by Cas-OFFinder, GUIDE-seq, and SITE-Seq, seven loci were identified as possible editing sites, all of which were located in noncoding regions (Fig. S1 in the Supplementary Appendix). For each of these sites, no evidence of off-target editing was found when primary human hepatocytes were treated with

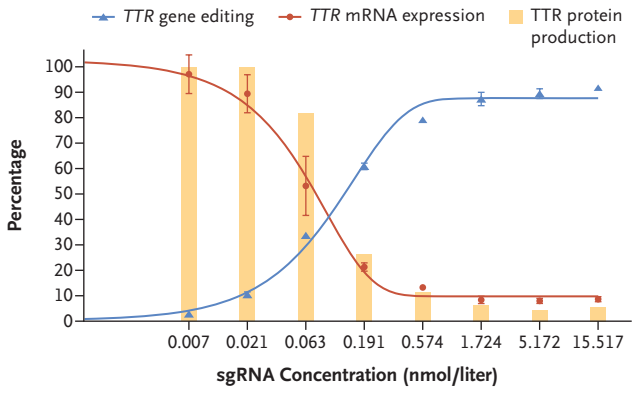


Figure 2. In Vitro Evaluations of the Potency of NTLA-2001.

Shown is the relationship between increasing concentrations of sgRNA and the consequent percentages of *TTR* editing, as well as *TTR* mRNA expression and TTR protein production in a single lot of primary human hepatocytes. The primary indel patterns were a single-nucleotide deletion or insertion at the cut site, inducing a frameshift mutation (data not shown).

concentrations of NTLA-2001 up to 3 times as high as the EC_{90} (Fig. S2).

Studies in transgenic mice revealed a dose-dependent and durable effect of NTLA-2001. Editing of *TTR* reduced circulating serum TTR protein levels, which reached a nadir by 4 weeks after receipt of the dose and were still maximally suppressed at 12 months of observation.[24] After resection of two thirds of the liver and subsequent full-liver regeneration, the gene-editing percentage and corresponding protein levels were unchanged, findings that supported the permanent nature of the edit (Fig. S7).

Studies in cynomolgus monkeys showed rapid initial distribution and clearance of the LNP components (Table S3 and Fig. S8). In addition, a single dose of Cyn-LNP (the nonhuman primate surrogate of NTLA-2001) at 3 or 6 mg per kilogram was associated with a maximum gene-editing percentage of 73% in whole liver and near-complete (>94%) reduction in serum TTR protein that was sustained over a period of 12 months (Fig. 3A). Editing of *TTR* was confirmed by next-generation sequencing analysis of hepatic tissue (Fig. 3B).[25]

Thus, preclinical studies in the mouse and cynomolgus monkey showed that a single dose of NTLA-2001 or its surrogate Cyn-LNP resulted in durable *TTR* editing and near-complete elimination of serum TTR protein expression at doses associated with no adverse effects. To evaluate the ability of NTLA-2001 to reduce serum expression of TTR protein after a single intravenous infusion in humans, we initiated an open-label, single-dose, proof-of-concept study involving patients who had hATTR amyloidosis with polyneuropathy.

PATIENTS

At one study site (Auckland, New Zealand), three patients underwent screening, of whom two were found to be eligible and were enrolled. One participant had a body weight that was above the upper limit allowed by the study protocol at that time. At the other study site (London, United Kingdom), four patients underwent screening, all of whom were found to be eligible and were enrolled. The patients were 46 to 64 years of age, and four of the six patients were men; the body weight ranged from 70 to 90 kg. Three patients had a p.T80A mutation, two a p.S97Y mutation, and one a p.H110D mutation. Three patients had received no previous therapy, and three had previously received diflunisal. All six patients had sensory polyneuropathy in the absence of motor symptoms (polyneuropathy disability score of 1) and a New York Heart Association heart failure class of I. The level of N-terminal pro–B-type natriuretic peptide ranged from 50 to 596 ng per liter.

SAFETY AND SIDE-EFFECT PROFILE

NTLA-2001 treatment was completed in all the patients without interruption of the infusion. No protocol-specified stopping events were observed. Adverse events that occurred during or after treatment, all of which were mild (grade 1) in severity, were reported in three of the six patients. One patient had an adverse event of special interest (a grade 1 infusion-related reaction; see Table S4). No serious adverse events were observed. Increased D-dimer levels were observed 4 to 24 hours after infusion in five of six patients; the elevations were lower than those observed at the NOAEL dose in nonhuman primates. The values returned to baseline in all six patients by day 7. Coagulation measures (activated partial thromboplastin time and prothrombin time) remained within 1.2 times the upper limit of the reference ranges, and fibrinogen levels and platelet counts remained above the lower limit of the reference ranges; liver-function measures (aspartate aminotransferase and alanine aminotransferase levels) remained within normal limits (Fig. S11).

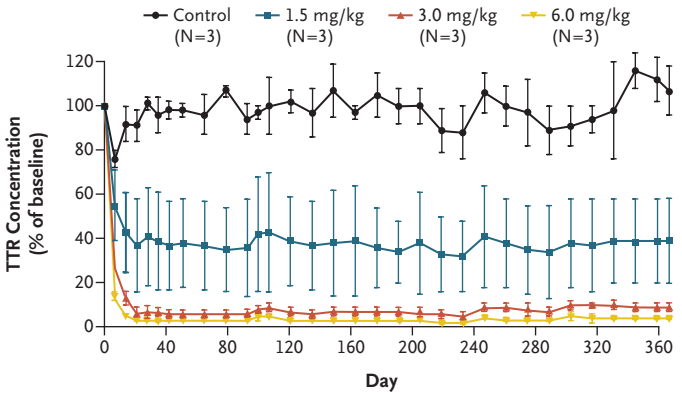
TTR PROTEIN REDUCTION

To determine the pharmacodynamic effects of NTLA-2001, concentrations of TTR in serum were evaluated. Reductions from baseline in the serum TTR protein concentration were observed by day 14 and deepened by day 28 (Fig. 4A and 4B). At day 28, NTLA-2001 was associated with mean TTR reductions of 52% in the group that received a dose of 0.1 mg per kilogram and 87% in the group that received 0.3 mg per kilogram (Fig. 4C). The effect was dose-dependent, with greater reductions in TTR concentration among patients who received a higher dose of NTLA-2001. In addition, the effect of NTLA-2001 was reproducible across patients at each dose level, with reductions at day 28 ranging from 47 to 56% in the lower-dose group and from 80 to 96% in the higher-dose group (Fig. 4A and 4B).

DISCUSSION

We report evidence of CRISPR-Cas9–based in vivo gene editing in humans. Systemic administration of NTLA-2001 to six patients with hATTR amyloidosis with polyneuropathy was associated in each case with sustained reductions in the serum TTR protein concentration. NTLA-2001 treatment was associated with a dose-dependent effect. At day 28, the time at which the drug effect had reached its permanent nadir in preclinical studies, the mean reduction from baseline in serum TTR protein concentration was 52% in the group that received the lower dose (0.1 mg per kilogram) and was 87% in the group that received the higher dose (0.3 mg per kilogram). NTLA-2001 treatment was associated with adverse events of only mild severity.

These data represent interim results from the first two dose groups in an ongoing dose-escalation study. The results closely follow the pattern observed in in vitro data from cell lines and in vivo data on potency in animals, which showed a deep and permanent reduction in serum TTR protein concentrations with NTLA-2001, thus providing evidence of the potential for in vivo gene editing as a therapeutic strategy for the treatment of hATTR amyloidosis. It is important to note that this study involves a very small number of patients with limited follow-up to date. Continued serial measurements of serum TTR concentration in the patients reported here are planned to confirm the durability of the effect.



B

Indel Size	Sequence	Indel Frequency %
Wild type	AGACACAAATACCAGTCCAGCGAGGCAGAG[G/A]AGGAGCAG	—
+1	AGACACAAATACCAGTCCAAGCGAGGCAGAG[G/A]AGGAGCAG	98.9
>+1	AGACACAAATACCAGTCCANGCGAGGCAGAG[G/A]AGGAGCAG	1.03

Figure 3. In Vivo Pharmacologic Properties of Cyn-LNP, the Nonhuman Primate Surrogate of NTLA-2001.

Panel A shows mean reductions in the serum TTR protein concentration as a percentage of the baseline concentration in cynomolgus monkeys (three per dose group) that received Cyn-LNP intravenously at doses of 1.5, 3.0, and 6.0 mg of total RNA per kilogram of body weight on day 0 and were followed up for 367 days. A control group that received no treatment is shown for comparison. I bars indicate standard deviations for the three animals in each group. Panel B shows the results of next-generation sequencing after administration of Cyn-LNP to cynomolgus monkeys. The sgRNA target sequence is indicated in blue next to the required PAM sequence, shown in red. [G/A] represents a naturally occurring single-nucleotide polymorphism in the cynomolgus monkeys used in the study. The nucleotide position of indels relative to the cynomolgus monkey genome (build mf5, chromosome 18) are +1: 50681549–50681550. The primary indel pattern was a single-nucleotide insertion at the cut site, inducing a frameshift mutation. An "N" at the insertion site indicates a multinucleotide insertion (e.g., AA or AGG), which in aggregate constituted 1.03% of all indels. The remaining fraction comprised deletions of various lengths.

Data from studies of RNA-targeting gene-silencing agents have shown that observed reductions in serum TTR protein translate into meaningful clinical benefits relative to placebo. These agents result in mean reductions from baseline in serum TTR concentrations of approximately 80% and show more favorable clinical effects in patients in whom lower concentrations of TTR protein are achieved. Maintenance of these reductions with RNA-targeting agents requires routine serial infusions. On the basis of data in animals, NTLA-2001 may be able to produce nearly com-

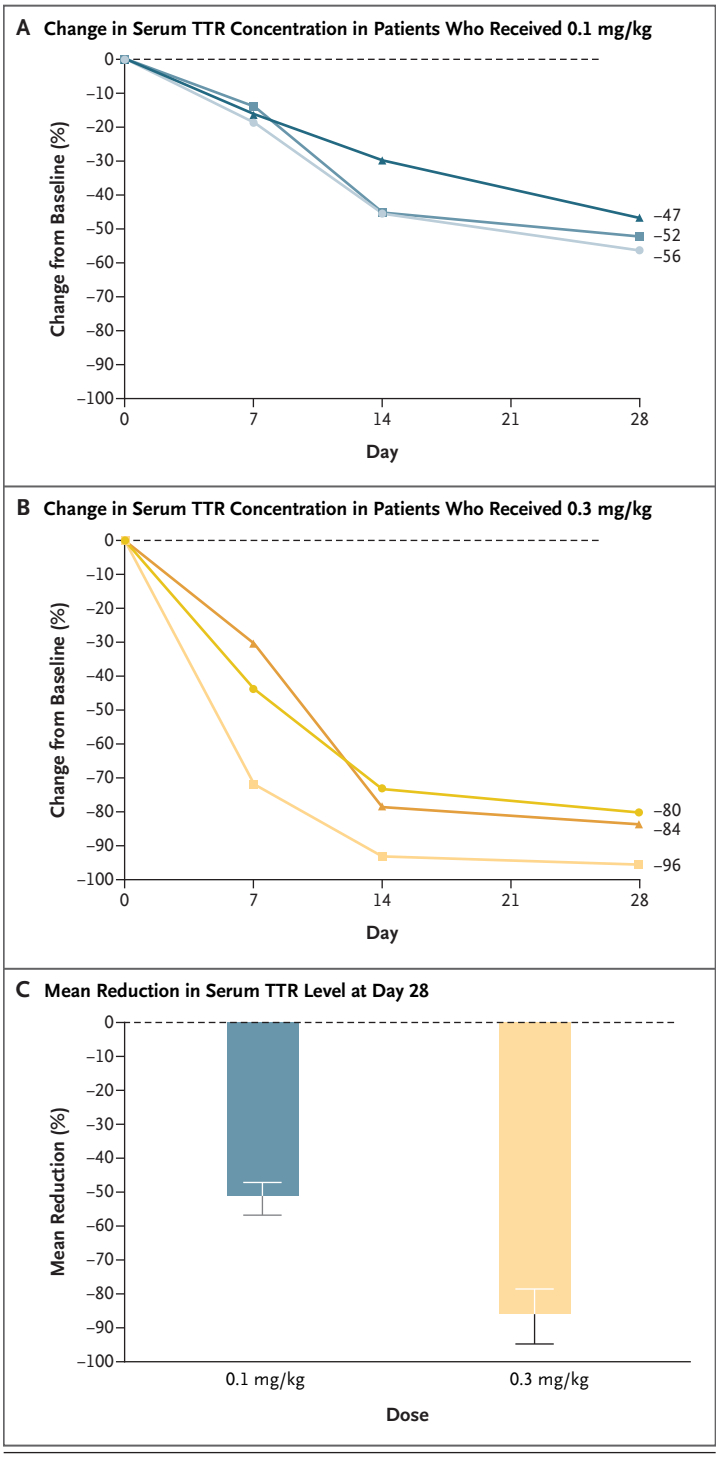


Figure 4. Reductions from Baseline in Serum TTR Protein Concentration after Infusion of NTLA-2001 in Humans.

Panel A shows the percentage change from baseline in total circulating serum TTR protein in the group of patients who received an NTLA-2001 dose of 0.1 mg per kilogram. TTR protein was quantified by a validated enzyme-linked immunosorbent assay method in accordance with regulatory guidelines for biomarker method validation. Serum samples were measured once, and each sample was tested in duplicate. In accordance with good laboratory practice, no retesting was conducted for successful assay runs. For each patient in the group that received a dose of 0.1 mg per kilogram, data are shown at postdose days 7, 14, and 28 for percentage reductions from the predose baseline value (mean concentration from three sampling time points). Panel B shows the percentage change from baseline in total circulating serum TTR protein concentrations in the group of patients who received an NTLA-2001 dose of 0.3 mg per kilogram. The methods and analysis were identical to those described in Panel A. Panel C shows the mean (three per group) percentage reduction from baseline in total circulating serum TTR protein at day 28 for both dose groups. The I bars indicate standard deviations.

plete and permanent knockdown of TTR expression with a single administration. As with current standard-of-care agents, patients will receive vitamin A supplementation in order to compensate for the loss of TTR, which has a normal physiological role in vitamin A transport.[31]

The potential for off-target gene editing with CRISPR-Cas systems has been raised as a concern with regard to the use of these therapies in humans.[32] In primary human hepatocytes, therapeutic concentrations of NTLA-2001 showed no evidence of previously described off-target mutagenesis mechanisms.[33,34] Computational modeling, biochemical cell-free assays, and in vitro cellular assays were used to identify the most likely sites in the genome outside of *TTR* into which NTLA-2001 might introduce off-target edits. Seven candidate sites for introduction of indels were confirmed in assays with cell lines in which supersaturating doses of NTLA-2001 were used. We assessed the therapeutic index for NTLA-2001 in primary human hepatocytes by determining the frequency of off-target edits at concentrations of sgRNA that caused high degrees of on-target edits and reductions in TTR protein production. In addition, we performed long-range sequencing to detect structural variants and to determine the risk of unintended genotoxicity or oncogenic transformation.[35,36] Indels and DNA structural variants are natural outcomes of double-stranded DNA break repair.[36] The DNA structural variants induced by CRISPR-Cas9 genome editing were not random but related to end-joining at the *TTR* on-target

site and are expected to be of low risk. The changes detected in the primary human hepatocytes could predict the DNA structural variants that may occur in vivo. Participants who volunteer to receive NTLA-2001 therapy will need to undergo long-term safety monitoring.

The CRISPR-Cas9 approach used for NTLA-2001 is modular and has the capacity to be adapted to treat other diseases with simple replacement of the sgRNA. Indeed, the in vivo gene-editing approach used in this study is currently being investigated for use in other diseases. Further clinical programs involving CRISPR-Cas9–based gene-editing strategies are planned by many investigators for a wide range of diseases; these programs may make use of the potential not only to knock out expression of harmful protein products but also to insert genes to produce functional proteins where mutations cause pathologic deficiencies.

The study reported here is ongoing. Dose escalation continues with a goal of producing greater reductions in serum TTR protein than are achieved with available therapies, with anticipated beneficial effects on disease progression, quality of life, and mortality. Data from the initial groups of patients in this study provide clinical proof of concept for in vivo CRISPR-Cas9–mediated gene editing as a therapeutic strategy.

Supported by Intellia Therapeutics and Regeneron Pharmaceuticals.

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 who participated in this study and their families; New Zealand Clinical Research and Richmond Pharmacology for contract research assistance; Altasciences, Charles River Laboratories, Precision for Medicine, PPD, and QPS for assistance with the studies in cynomolgus monkeys, enzyme-linked immunosorbent assay measurements of serum transthyretin, and pharmacokinetic and biomarker tests in the first-in-human study; Carri Boiselle, James Butler, David Cooke, Tracy DiMezzo, Richard Duncan, Eva Essig, Noah Gardner, Bo Han, Denise Hernandez, Tracy Jennings, Kellie Kolb, Rebecca Lescarbeau, Reynald Lescarbeau, Nishit Patel, Austin Ricker, Joseph Rissman, Matthew Roy, Philipp Schneggenburger, Palak Sharma, and Samantha Soukamneuth from Intellia Therapeutics for their dedicated investment in the development of NTLA-2001; and Ben Caldwell of Arc, a division of Spirit Medical Communications Group, for providing medical writing services.

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