BRIEF REPORT

Antisense Inhibition of Prekallikrein to Control Hereditary Angioedema

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SUMMARY

Hereditary angioedema is characterized by recurrent and unpredictable episodes of subcutaneous and mucosal swelling that can be life threatening. IONIS-PKK-L_{Rx} is a ligand-conjugated antisense oligonucleotide designed for receptor-mediated delivery to hepatocytes. In a compassionate-use pilot study, two patients with severe bradykinin-mediated angioedema were initially administered weekly subcutaneous injections of the unconjugated parent drug, IONIS-PKK_{Rx}, for 12 to 16 weeks, after which they received IONIS-PKK-L_{Rx} at a dose of 80 mg every 3 to 4 weeks for 7 to 8 months. Treatment was accompanied by a reduction in the angioedema attack rate. (Funded by Amsterdam UMC.)

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Hereditary angioedema is a rare autosomal dominant disease characterized by recurrent and unpredictable episodes of swelling, particularly of the skin and the gastric, oropharyngeal, and laryngeal mucosa, which can be life threatening.[1,2] The majority of cases of hereditary angioedema are caused by genetic mutations that lead to a deficiency (type I) or dysfunction (type II) of C1 esterase inhibitor (C1-INH), a serine protease inhibitor that regulates multiple pathways, including the kallikrein–kinin and contact system. In an especially rare third type of hereditary angioedema, patients have normal levels of functional C1-INH and often have a distinct clinical presentation, including a higher frequency of facial, pharyngeal, and tongue swelling.[3] The typical swelling in hereditary angioedema is caused by locally increased vascular permeability in response to excessive bradykinin formation, which results from inadequate control of the contact-system components factor XIIa and plasma kallikrein.[1]

For decades, C1-INH–replacement therapies have been the predominant option for the treatment of hereditary angioedema attacks.[4] Recently, inhibition of plasma kallikrein has also emerged as a promising strategy for the control of the condition. In 2018, lanadelumab (Takhzyro, Takeda), a monoclonal antibody targeting plasma kallikrein, was approved in the United States for prophylactic treatment of hereditary angioedema attacks.[5,6] In addition, treatment with the oral plasma kallikrein inhibitor berotralstat has been reported to result in a 44% reduction in attack rate in a phase 3 trial (Biocryst press release, May 21, 2019) that followed a successful dose-finding phase 2 trial.[7] However, injection-site reactions (with lanadelumab) and gastrointestinal side effects (with berotralstat) occurred in a substantial proportion of the patients.[4]

Targeting of plasma prekallikrein expression at the messenger RNA level could potentially reduce plasma kallikrein activity and concomitant bradykinin release. Proof of concept for this approach was shown in preclinical models with the use of antisense methods, followed by a phase 1 trial of a second-generation 2′-*O*-methoxyethyl (2′-MOE)–modified antisense oligonucleotide (ASO), IONIS-PKK$_{Rx}$, in healthy volunteers.[8-10] Recent advances in the antisense field have led to a new class of ASO designed for receptor-mediated delivery to liver hepatocytes through a triantennary *N*-acetylgalactosamine (GalNAc$_3$) moiety conjugated to the ASO. In humans, GalNAc$_3$-conjugated ASOs are as much as 30 times as potent as the parent 2′-MOE–modified ASO, and therefore similar pharmacologic activity is obtained with lower and less-frequent doses, which results in less systemic exposure than when the unconjugated parent ASO is used, and with fewer side effects.[11]

A phase 1, randomized, double-blind, placebo-controlled, dose-escalation trial was designed to assess the safety and side-effect profile, pharmacokinetics, and pharmacodynamics of monthly subcutaneous injections of IONIS-PKK-L$_{Rx}$ in healthy human volunteers (ClinicalTrials.gov number, NCT03263507). These data are provided in the Supplementary Appendix, available with the full text of this article at NEJM.org. (The protocol for the phase 1 trial is also available at NEJM.org.) Here, we report efficacy data from two patients with severe bradykinin-mediated forms of angioedema who were treated first with the unconjugated parent ASO, IONIS-PKK$_{Rx,}$ (hereafter, PKK$_{Rx,}$), and then with the ligand-conjugated ASO, IONIS-PKK-L$_{Rx}$ (PKK-L$_{Rx}$).

CASE REPORTS

Patient 1 was a 24-year-old woman with type 1 hereditary angioedema and frequent breakthrough attacks. The attacks started when she was 13 years of age, and the frequency of attacks increased after initiation of oral contraceptives. These had been prescribed because of dysmenorrhea and menorrhagia, for which other treatment options had been ineffective. Tranexamic acid had unacceptable side effects of abdominal discomfort. The abdominal and pharyngeal attacks led to the patient visiting the emergency department a mean of 1.2 times per month. Patient 1 was unable to self-administer C1-INH concentrate because of difficulties with venous access.

Patient 2 was a 27-year-old woman with a rare variant of bradykinin-mediated angioedema that has previously been reported in detail.[12] This patient had multiple, life-threatening breakthrough attacks despite having been treated with all available therapeutic options; these attacks led to recurrent admissions to the intensive care unit (ICU) because of imminent asphyxiation by laryngeal swelling. C1-INH levels and function were normal, and the results of sequencing, in a targeted fashion, of the genes encoding factor XII, plasminogen, and angiopoietin 1 and of whole-exome sequencing were unremarkable. Although the breakthrough attack rate decreased after initiation of weekly plasma exchange against an albumin–sodium chloride solution,[12] the patient's disease remained poorly controlled.

METHODS

OVERSIGHT

We selected the two patients with severe bradykinin-mediated angioedema for assessment of the clinical efficacy and side-effect profile of both PKK$_{Rx}$ and PKK-L$_{Rx}$. The Dutch Healthcare Inspectorate approved treatment with PKK$_{Rx}$ and PKK-L$_{Rx}$ on a compassionate-use basis. Written informed consent was obtained from both patients. The authors vouch for the accuracy and completeness of the data in this report.

TREATMENT AND ASSESSMENTS

Because the safety data for PKK-L$_{Rx}$ were pending at the start of the study, the patients were first treated with PKK$_{Rx}$ over a period of 12 to 16 weeks. They received initial subcutaneous doses of 200 mg once weekly, with an optional dose-loading schedule in the first 2 weeks. Predefined dose escalations to 300 mg after 6 weeks and to 400 mg after 12 weeks were permitted in the event of ongoing breakthrough attacks. The patients had a treatment-free interval of at least 6 weeks before administration of PKK-L$_{Rx}$. The planned subcutaneous dose of PKK-L$_{Rx}$ was 80 mg per month but was modified during the study to 80 mg every 3 weeks for Patient 2.

Breakthrough attacks were reported by the

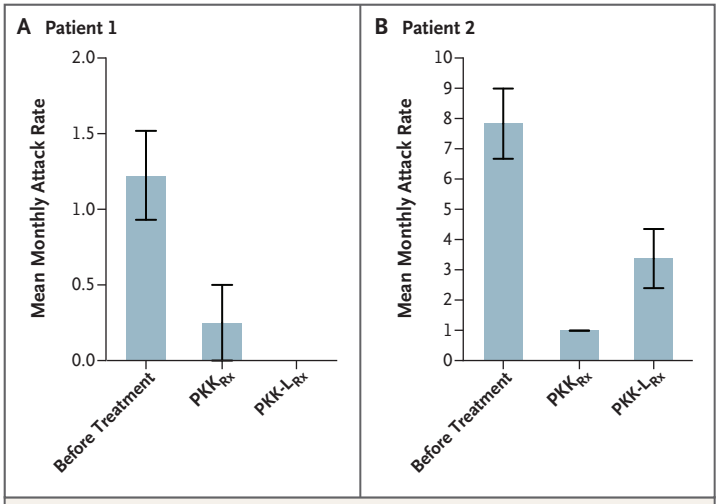

Figure 1. Mean Monthly Attack Rates.

Panel A shows the mean rates of breakthrough attacks in Patient 1 (who had type 1 hereditary angioedema) during the 30-week pretreatment period, the 12-week period of treatment with the parent antisense oligonucleotide (ASO), IONIS-PKK$_{Rx}$ (PKK$_{Rx}$), and the 30-week period of treatment with the conjugate ASO, IONIS-PKK-L$_{Rx}$ (PKK-L$_{Rx}$). Panel B shows the mean rates of breakthrough attacks in Patient 2 (who had a rare variant of bradykinin-mediated angioedema characterized by normal levels of C1 esterase inhibitor) during the 10-week pretreatment period, the 16-week PKK$_{Rx}$ treatment period, and the 34-week PKK-L$_{Rx}$ treatment period. I bars indicate the standard error.

patients with the use of a diary. Plasma prekallikrein activity was determined in a one-stage clotting assay with prekallikrein-depleted plasma (Technoclone) on a BCS XP Coagulation Analyzer, with Pathromtin SL (both from Siemens Healthcare Diagnostics) used as activator. Plasma prekallikrein values are expressed as percentages of the activity in pooled normal plasma.

RESULTS

PKK$_{Rx}$

During the course of treatment with PKK$_{Rx}$, the mean monthly attack rate decreased from 1.2 to 0.25 in Patient 1 and from 7.9 to 1.0 in Patient 2 (Fig. 1). Patient 1 received initial loading doses of 200 mg on days 1, 3, 8 and 10 and then received 200 mg weekly; she had a single breakthrough attack after 4 weeks. The dose was escalated to 300 mg weekly after 5 weeks, and the patient remained free of attacks during the remainder of treatment with the parent compound (a duration of 8 weeks). Plasma prekallikrein activity levels were suppressed (to <5%) from week 12 onward (Fig. 2A). Injection-site reactions occurred after all 16 administrations of PKK$_{Rx}$ (Table 1).

While Patient 2 was receiving weekly plasma exchange and before treatment with PKK$_{Rx}$ was initiated, she had 6 to 10 attacks per month. After the initial doses of 200 mg of PKK$_{Rx}$, the dose was escalated to 300 mg at 7 weeks and to 400 mg at 12 weeks; plasma exchange was able to be discontinued successfully at 8 weeks after the start of treatment. Plasma prekallikrein activity levels decreased from 85% before treatment to 14% after 16 weeks of treatment (Fig. 2B). Injection-site reactions occurred after 12 of 16 administrations of PKK$_{Rx}$.

PKK-L$_{Rx}$

During the PKK-L$_{Rx}$ treatment period of 7 and 8 months, the mean monthly attack rate was 0.0 in Patient 1 and 3.4 in Patient 2 (Fig. 1). During the 8-week interval between the last administration of PKK$_{Rx}$ and the first dose of PKK-L$_{Rx}$, Patient 1 had no recurrent breakthrough attacks. Plasma prekallikrein activity increased to a peak of 24% by the end of the 8-week interval between treatments and then decreased to 5% after 12 weeks of monthly dosing with PKK-L$_{Rx}$ (Fig. 2A). No adverse events were reported.

Patient 2 started to have new-onset breakthrough attacks 5 weeks after discontinuation of PKK$_{Rx}$ and was admitted to the ICU because of life-threatening laryngeal attacks at 7 weeks. PKK-L$_{Rx}$ was administered directly after emergency plasma exchange and administration of a cumulative dose of 5000 U of C1-INH concentrate (Cinryze, Takeda). Blood sampling for plasma prekallikrein activity before the first administration of PKK-L$_{Rx}$ was performed after plasma exchange. Hence, the plasma prekallikrein activity was estimated as 60% on the basis of our observation that plasma exchange reduced the plasma prekallikrein activity by a factor of 3 to 4 (Fig. 2B). After the first administration of PKK-L$_{Rx}$, the patient had four laryngeal attacks within 4 weeks, and therefore (weekly) plasma exchange was resumed. In the subsequent weeks, a series of severe attacks were reported after three non–angioedema-related hospitalizations. These hospitalizations were for pneumonia with respiratory failure (caused by *Haemophilus influenzae*), a central-venous-catheter–associated bloodstream

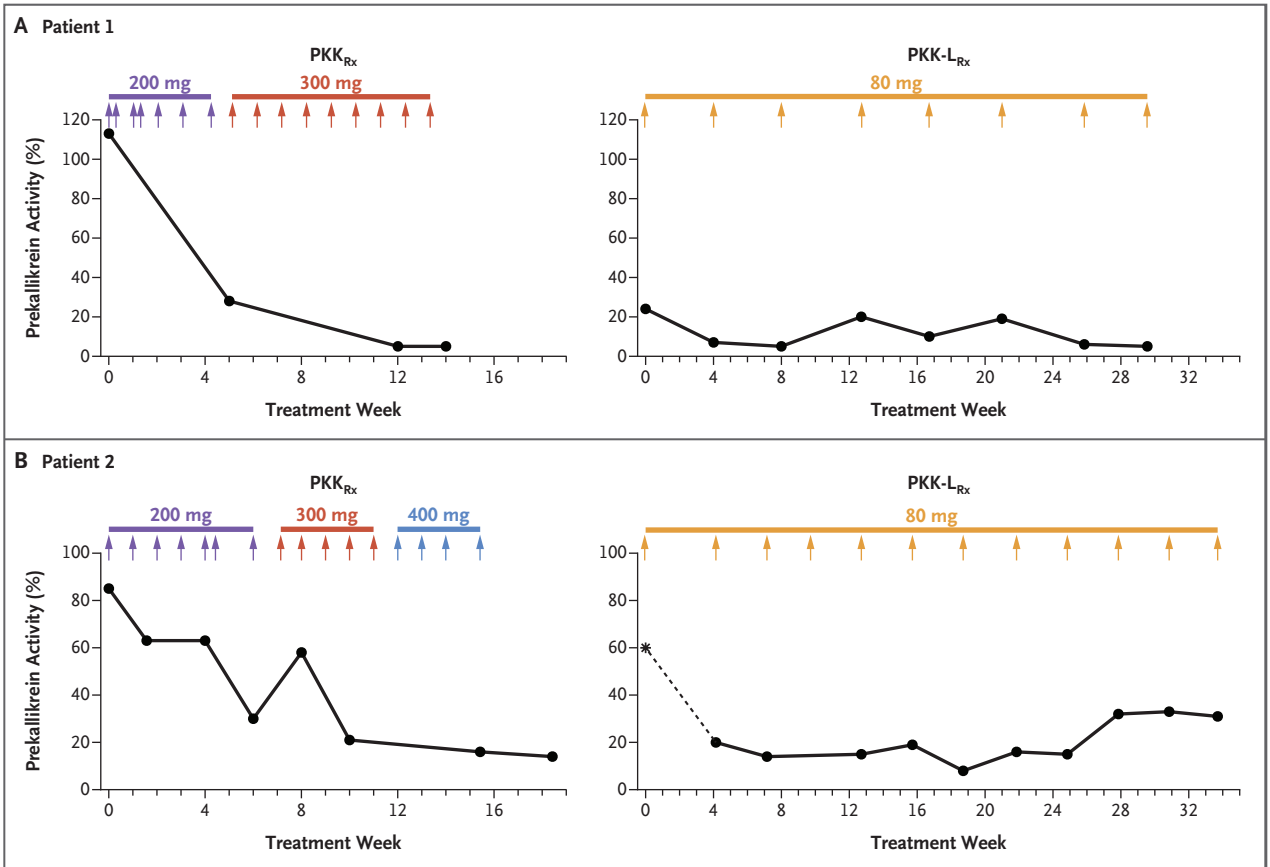

Figure 2. Prekallikrein Activity.

Panel A shows prekallikrein activity in Patient 1 during 12 weeks of treatment with PKK$_{Rx}$ (left) and 30 weeks of treatment with PKK-L$_{Rx}$ (right). Panel B shows prekallikrein activity in Patient 2 during 16 weeks of treatment with PKK$_{Rx}$ (left) and 34 weeks of treatment with PKK-L$_{Rx}$ (right). Arrows denote days on which treatment was administered, and colored horizontal lines denote the treatment periods for each dose. The dashed line in Panel B indicates estimated values; prekallikrein activity on day 1 in Patient 2 was estimated as 60% on the basis of the sampling after plasma exchange and the observation that this treatment reduced prekallikrein activity by a factor of 3 to 4.

infection, and surgery (insertion of a port and catheter [Port-a-Cath]). The dosing interval was decreased from once every 4 weeks to once every 3 weeks during the first hospitalization (which occurred at 7 weeks after the first PKK-L$_{Rx}$ dose), owing to relatively poor disease control (as compared with when the patient was receiving a weekly dose of 400 mg PKK$_{Rx}$ per week). Weekly plasma exchange was successfully discontinued 8 weeks after the most recent hospitalization, and during the final 2 months of the evaluation period, the monthly attack rate decreased to 1.0.

The activated partial thromboplastin time (aPTT) in Patient 1 remained at 21 seconds throughout the study (reference range, 22 to 29). In Patient 2, the aPTT, which had been 26 seconds at the start of treatment with PKK$_{Rx}$, increased to 29 seconds after 5 weeks of treatment with PKK-L$_{Rx}$ but did not exceed that level at any point during treatment thereafter.

Table 1. Adverse Events with PKK$_{Rx}$ and PKK-L$_{Rx}$.*

Patient No.	Events with PKK$_{Rx}$	Events with PKK-L$_{Rx}$
1	Injection-site reaction	None
2	Injection-site reaction	*Haemophilus influenzae* pneumonia, central-venous-catheter infection

* The patients were treated first with the unconjugated parent antisense oligonucleotide, IONIS-PKK$_{Rx}$, (PKK$_{Rx}$), and then with the ligand-conjugated antisense oligonucleotide, IONIS-PKK-L$_{Rx}$ (PKK-L$_{Rx}$).

DISCUSSION

This compassionate-use pilot study involving two patients with severe bradykinin-mediated angioedema supports antisense-mediated reduction in plasma prekallikrein levels as an experimental treatment. We observed a reduction in the number of attacks per month in both patients during treatment, including complete resolution in the patient with type 1 hereditary angioedema. In addition, a randomized, placebo-controlled, ascending-dose phase 1 study has shown that PKK-L_{Rx} reduces plasma prekallikrein activity in a dose-dependent manner. No serious adverse events were reported at the doses tested. These findings support continued development of the ligand-conjugated ASO inhibitor of hepatic prekallikrein expression for the treatment of patients with severe hereditary angioedema who have limited response to or unacceptable side effects from current therapies.

Previous preclinical and phase 1 clinical assessments have shown that ASO-mediated inhibition of plasma prekallikrein activity is not associated with increased bleeding, despite dose-dependent increases observed in aPTT as measured by an assay optimized for reduced levels of plasma prekallikrein.[10] These results are consistent with those in persons with genetic deficiencies in prekallikrein and factor XII, who have an increased aPTT but no increased risk of bleeding.[13,14] This phenomenon was also seen in Patient 2, who had an increase in aPTT of approximately 3 seconds. The aPTT in Patient 1 remained unchanged. These observations are also in line with findings from the Hereditary Angioedema Long-Term Prophylaxis (HELP) trial, in which the use of lanadelumab resulted in modest prolongation of aPTT without a concomitant increase in the risk of bleeding.[15]

In both patients in our study, PKK_{Rx} and PKK-L_{Rx} showed clinical efficacy in reducing the rate of breakthrough attacks. This response to treatment was concomitant with nadirs in activity of plasma prekallikrein of less than 5% in one patient and 16% in the other at the highest doses tested for the parent ASO and with a nadir of less than 10% with the $GalNAc_3$ conjugate, PKK-L_{Rx}, in both patients.

Patient 1 had a single breakthrough attack in the first month after initiation of treatment with the parent ASO. She is now receiving the $GalNAc_3$ conjugate at a lower dose and less frequently, resulting in 10 to 20 times less monthly exposure relative to that with the previous dose regimen with the parent ASO. While undergoing plasma exchange alone, Patient 2 had 6 to 10 angioedema attacks every 4 weeks; the attack rate was reduced by approximately 90% with PKK_{Rx} or PKK-L_{Rx} treatment. The large majority of attacks in Patient 2 occurred during three successive non–angioedema-related hospital admissions. The mean monthly attack rate decreased to less than 1.0 during treatment continuation beyond the initial evaluation period of 8 months, with only a single attack (which occurred during a suspected infection with severe acute respiratory syndrome coronavirus 2 [SARS-CoV-2]) occurring over a period of 4 months.

The antisense pharmacologic activity of both the parent drug and the $GalNAc_3$ conjugate reduced plasma kallikrein activity to a greater extent (i.e., to <60%) than was observed in trials of lanadelumab[5] and berotralstat.[7] However, with data from only two patients, we cannot discern whether this additional decrease in plasma kallikrein will affect clinical outcomes. A broader investigation is warranted.

We found that antisense-mediated reductions in plasma prekallikrein activity were accompanied by a decrease in attack rate in two patients, each of whom had a different type of bradykinin-mediated hereditary angioedema.

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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.

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