PRGN-2012 gene therapy in adults with recurrent respiratory papillomatosis: a pivotal phase 1/2 clinical trial

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Summary

Background Recurrent respiratory papillomatosis (RRP) is a rare debilitating condition caused by chronic infection with human papillomavirus (HPV) type 6 or 11. Papillomas develop in the aerodigestive tract, leading to significant voice disturbance and airway obstruction. No systemic treatment currently exists. We aimed to assess the safety and clinical activity of PRGN-2012 in adult patients with RRP treated at the recommended phase 2 dose.

Methods This was a single-centre, single-arm, phase 1/2 trial. Adult patients aged 18 years or older with RRP who required three or more interventions in the 1 year before treatment received adjuvant PRGN-2012 on day 1 following surgical debulking of disease, and on days 15, 43, and 85. Primary outcome measure was complete response rate, defined as the percentage of patients who did not require an intervention to control RRP in the 12 months after treatment. Safety outcomes included treatment-related adverse events. This study is registered ClinicalTrials.gov (NCT04724980).

Findings From March 16, 2021, to June 1, 2023, 38 patients were enrolled and received the 12-week treatment course. Among the 35 patients treated at the recommended phase 2 dose of $5x10^{11}$ particle units, 18 (51%) of 35 patients had a complete response (95% CI 34–69) with the median duration of complete response yet to be reached. Adverse events were mild and included grades 1–2 injection site reaction (34 [97%] of 35), fatigue (28 [80%] of 35), chills (25 [71%] of 35), and fever (24 [69%] of 35).

Interpretation PRGN-2012 treatment resulted in complete response in 51% of the patients treated and was safe. Based on these positive pivotal study results, a biologics license application to the US Food and Drug Administration (FDA) is planned, positioning PRGN-2012 to be an FDA-approved medical treatment for adult patients with RRP.

Funding National Institutes of Health.

Introduction

Recurrent respiratory papillomatosis (RRP) is a rare, neoplastic disorder caused by chronic infection with human papillomavirus (HPV) type 6 or 11. The incidence of juvenile-onset RRP (diagnosis in those younger than 13 years) is decreasing with increasing rates of preventative HPV vaccination in countries with sufficient vaccine access.[1,2] However, the incidence of RRP remains unchanged in unvaccinated adults and in countries without preventative vaccination programmes with an estimated worldwide prevalence of 20 000 cases.[3–5] RRP manifests as persistent papillomas throughout the aerodigestive tract, predominantly in the larynx, trachea, and lungs. Laryngotracheal papillomas can cause significant dysphonia and airway obstruction, and pulmonary papillomas can lead to recurrent post-obstructive pneumonias and death.[3] Additionally, there is a risk of dysplasia and malignant transformation in up to 7% of patients.[6,7] Along with substantial economic burden associated with treatment, studies investigating quality-of-life measures indicate higher levels of social anxiety, avoidance of social activities due to decreased voice quality, and decreased social mobility in individuals with RRP.[8]

There are no approved systemic medical therapies for RRP by a national or international regulatory authority. Current treatment consists of repeated laser ablation and surgical excision of papillomas to alleviate symptoms. These interventions do not address the underlying chronic HPV infection, resulting in papilloma recurrence with patients frequently requiring dozens to hundreds of clinically indicated interventions to maintain vocal function and a patent airway over time.[4] Adjuvant treatment strategies, such as cidofovir that treat the papilloma itself, but do not address the underlying HPV infection, have shown inconsistent clinical efficacy.[9] Off-label use of systemic bevacizumab has shown an ability to control disease burden and alleviate symptoms of RRP, but durability of response upon cessation of treatment is lacking because the underlying chronic HPV infection is not addressed and continuous dosing can lead to toxicity.[10]

Immunotherapy, aimed at activating HPV-specific T-cell responses, might result in control or elimination of papillomas and durable clinical benefit. Initial attempts to treat patients with RRP with adjuvant subcutaneous interferon failed to show durable control of papillomas.[11,12] PD-1 pathway immune checkpoint blockade reduced

Research in context

Evidence before this study

No approved systemic agents for the treatment of recurrent respiratory papillomatosis (RRP) currently exist. Widely accepted standard treatment consists of repeated laser ablation and surgical excision of papillomas to maintain voice and a patent airway that itself can cause irreversible airway damage. Publication review reveals that off-label use of systemic bevacizumab can result in significant disease control and reduced need for clinically indicated procedures. However, since bevacizumab does not address the underlying chronic human papillomavirus (HPV) infection that causes RRP, recurrence is common upon withdrawal of treatment.

Added value of this study

Our study shows safety and clinical activity of a novel HPV 6 or 11 gene therapy called PRGN-2012 that induces HPV-specific immunity to target papillomas. Following a single, 3-month treatment course, a majority (51%) of patients did not require an intervention in the 12 months following treatment in a population of patients that required a median of four [range 3–10] clinically indicated procedures in the 12 months before the trial. Importantly, the median duration of time-to-first intervention was not yet reached in patients with a complete response with a median follow up of 22 months (range 13–33), showing durability of response to this single treatment course.

Implications of all the available evidence

Based on these data, a biologics license application to the US Food and Drug Administration (FDA) is planned, positioning PRGN-2012 to be an FDA-approved medical treatment for adults with RRP.

papilloma disease burden and enhanced HPV-specific T-cell responses in some patients,[13,14] but short courses of treatment were not curative, and the potential for autoimmune toxicity limits treatment duration. Preventative HPV vaccination is highly effective in preventing infection but fails to consistently provide clinical benefit to those already infected and diagnosed with RRP.[2,15–17] Treatment strategies aimed at safely activating HPV-specific T-cell immunity in patients with RRP are needed.

PRGN-2012 is a gorilla adenovirus vector-based gene therapy capable of eliciting robust T-cell immunity specific to HPV 6 or 11.[18] In the phase 1 portion of the study, 15 patients were treated (three at dose level 1 and 12 at dose level 2). PRGN-2012 was safe, with no dose-limiting toxicities or grade 3 or greater treatment-related adverse events, and a complete response was observed in half of patients treated at dose level 2 of 5×10^{11} particle units per injection, which was chosen as the recommended phase 2 dose.[19] Based on these results, the US Food and Drug Administration designated PRGN-2012 Breakthrough Therapy Designation status for the treatment of adult patients with RRP. Here we report the safety and clinical results of the phase 1/2 pivotal trial of PRGN-2012 in adult patients with RRP.

Methods

Study design

This was a single-center, single-arm, phase 1/2 clinical trial (NCT04724980) conducted at the National Institutes of Health (NIH) in Bethesda, MD, USA. Two dose levels were tested (1×10^{11} and 5×10^{11} particle units). No dose-limiting toxicities occurred in three patients treated at dose level 1. An additional 12 patients were treated at dose level 2 in the phase 1 portion of the trial and there were no dose-limiting toxicities.[19] Due to the safety and clinical activity observed in the phase 1 portion of the trial, an additional 23 patients were treated at the recommended phase 2 dose (dose level 2) in the phase 2 portion of the study. All 35 patients treated at the recommended phase 2 dose were included in the analysis of primary and secondary outcome measures. All patients received four subcutaneous administrations of adjuvant PRGN-2012 with up to two optional debulking surgeries to maintain minimal residual disease if papilloma regrowth occurred during the 12-week treatment period. Briefly, patients underwent standard-of-care surgery before the first administration of PRGN-2012 subcutaneously. Patients received a second administration of PRGN-2012 at day 15. At days 43 and 85, patients who had visible papilloma regrowth underwent a surgery to maintain minimal residual disease throughout the treatment period, followed by a third and fourth administration of PRGN-2012, respectively. Patients without visible papilloma regrowth at days 43 and 85 received PRGN-2012 without an intervention. The initial standard-of-care clinical surgery and those needed to maintain minimal residual disease were performed at the NIH Clinical Center by a single surgeon. Interventions deemed to be clinically indicated in the 12 months before PRGN-2012 (pretreatment) and 12 months after the last dose of PRGN-2012 (post-treatment) were determined by the patient's home otolaryngologist, independent of the study team.

The protocol was approved by the National Cancer Institute's Institutional Review Board (approval number 21C0013) and was conducted in accordance with the principles of the Declaration of Helsinki and the International Conference on Harmonization Good Clinical Practice Guideline. An independent monitoring committee regularly reviewed safety data. All patients provided written informed consent. This study is registered ClinicalTrials.gov (NCT04724980).

Patients

Patients were aged 18 years or older with a clinical diagnosis of RRP, defined as a histological diagnosis

See Online for appendix

of papillomas, presence of laryngotracheal papillomas, and a history of three or more clinically indicated interventions in the 12 months before treatment with PRGN-2012. Clinically indicated interventions were defined as surgical resection of disease under general anaesthesia or laser ablation under local anaesthesia aimed at reducing voice and airway symptoms caused by the papilloma. Patients were required to have an Eastern Cooperative Oncology Group performance score of 0 or 1, a willingness to undergo endoscopic evaluation and operative interventions with biopsies in compliance with protocol requirements, not have a medical condition requiring systemic immunosuppressive medications, and to have not received systemic therapy for RRP in at least 3 half-lives from the previous drug.

Outcomes

The primary outcome measure was complete response rate, defined as the percentage of patients with no clinically indicated interventions during the 12 months after treatment. Secondary objectives included safety of PRGN-2012 at the recommended phase 2 dose; objective response rate defined as the percentage of patients with a complete response or partial response, defined as patients with at least a 50% decrease in the number of interventions in the 12 months after treatment compared with the 12 months before treatment; the percentage and absolute change in the number of interventions in the 12 months after treatment compared with the 12 months before treatment; time to first clinically indicated intervention from completion of PRGN-2012 treatment; the absolute and percentage change in the number of interventions in the 6 months after treatment compared with the 6 months before treatment; objective response rate of pulmonary lesions in participants with pulmonary disease; change in Derkay score that measures papillomatosis disease burden;[20] and change in Vocal Handicap Index 10 (VHI-10) that measures a patient's perception of vocal handicap.[21] Exploratory objectives include assessment of potential biomarkers of response and safety, including HPV type. An unplanned analysis of complete response rate comparing juvenile-onset versus adult-onset disease and the percentage of complete responders with complete visual resolution of disease was also done. Safety assessments were made based on the Common Terminology Criteria for Adverse Events version 5.0. Assessments of the number of clinically indicated interventions were based on documentation obtained from the patient's home care team. Assessments of Derkay and VHI-10 scores were made by nasopharyngolaryngoscopy and patient questionnaire, respectively. Assessment of pulmonary RRP response rate was made with CT scans and Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1. scoring criteria. The cutoff date for collecting data on post-treatment clinically indicated interventions was June 3, 2024. There were no protocol deviations that affected interpretation of the primary or secondary outcome measures. All primary and secondary outcome measures specified in the protocol are reported in the manuscript. All patient-level data is available in the appendix (pp 3–6). There are no dropouts or missing data.

Statistical analysis

A Fleming two-stage design was used to evaluate the primary outcome measure of complete response rate. The null hypothesis that the true complete response rate would be 10% was tested against a one-sided alternative of 30% or more. This design yielded a one-sided type 1 error rate of 0·025 and 80% power to detect a true response rate of 30% or more. If eight (22·9%) of 35 patients had a complete response, the lower one-sided 95% exact confidence limit would be 10·4%, which would show superiority of this regimen compared with the 10% threshold and the upper one-sided 95% CI of 40·1%. This would show consistency with a 30% or more complete response rate. The fraction of participants who are classified as having a complete response, the overall objective response rate, and the percentage of participants with any decrease in the number of clinical interventions in the 12 months after treatment compared with 12 months before treatment is reported along with 95% exact Pearson–Clopper confidence intervals. The number of clinical interventions in the 12-month period following PRGN-2012 treatment compared with the 12-month period before treatment used matched-pair (within participant change in number of interventions) Wilcoxon signed-rank to test for a significant change in number of interventions. A two-sided p value of 0·05 or less was considered statistically significant. RECIST criteria was used to determine overall response of pulmonary RRP. Safety analysis population includes all enrolled participants in the study (including phase 1 and 2) who received at least one dose of PRGN-2012. Participants evaluable for the primary outcome were those enrolled in either the phase 1 or 2 study who met eligibility criteria and received any administrations of the study drug at the recommended phase 2 dose. Patients evaluable for RECIST include participants in the efficacy-evaluable population who had concurrent pulmonary RRP present at baseline and had post-baseline RECIST assessment based on CT imaging.

Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. Authors employed by Precigen were given the opportunity to review and provide comments on the manuscript before publication.

Results

From March 16, 2021, to June 1, 2023, 38 patients were enrolled and received the 12-week treatment course (figure 1). For completeness, short-term follow-up data from patients treated on the phase 1 study,[19] now with

follow-up data (median 29 months [range 16–36]), are included. This includes the first three patients treated on dose level 1 and 12 patients treated on dose level 2 (the recommended phase 2 dose). In the phase 2 portion of the study, 23 patients were treated at the recommended phase 2 dose. We focused our analysis on the 35 patients treated at the recommended phase 2 dose. In these patients, the median age at RRP diagnosis was 35 years (range 1–68) and median age at the start of treatment was 49 years (range 20–88; table 1). 23 (66%) patients were diagnosed with adult-onset RRP and 12 (34%) with juvenile-onset disease. Most patients were male (20 [57%] of 35) and required up to hundreds of lifetime clinically indicated interventions to control RRP (median 40 [range 3 to >400. Four (11%) of 35 patients had pulmonary RRP evaluable by RECIST criteria. The median number of previous medical treatments for RRP was 2 (range 1–8). The median number of clinically indicated interventions in the 12 months before the study treatment was 4 (3–10). The median pretreatment anatomic Derkay score was 8 (3–31). The median pretreatment VHI-10 score was 24 (6–40), indicating severe dysphonia.[22]

Among the 35 patients from the phase 1 and 2 studies treated at the recommended phase 2 dose, the complete response rate was 51% (18 of 35; six of 12 complete responses from the phase 1 study and 12 of 23 from the phase 2 study; 95% CI [34–69]; figure 2; table 2), resulting in a positive pivotal study.[19] Complete responses were durable with 15 (83%) of 18 patients requiring no clinically indicated interventions beyond the 12 months after treatment with a median follow up of 22 months (range 13–33). Complete responders required a median of 1 (range 0–2) debulking surgeries during treatment to maintain minimal residual disease and non-complete responders required a median of 2 (range 1–2). Five complete responders did not require any debulking surgeries during treatment to maintain minimal residual disease. Five patients (one from phase 1 and four from phase 2) had a partial response, defined as a 50% or greater reduction in clinically indicated interventions after treatment compared with before treatment, leading to an objective response rate of 66% (23 of 35; 95% CI 48–81; table 2). Fewer clinically indicated interventions were observed in the 12 months after treatment compared with the 12 months before treatment in 30 (86%) of 35 (95% CI 70–95) patients. In the 12-month pretreatment period, complete responders required a median of 4 clinical interventions compared with non-complete responders who required a median of 3. Complete responders had a statistically significant reduction in the median number of interventions in the 12-months after treatment compared with non-complete responders (0 compared with 2; p<0·0001). The median surgery-free interval was not reached for complete responders and 6 months for non-complete responders. In addition, partial responders required a median of 1 clinical intervention after treatment compared with non-responders who required a median of 3 (table 2). In complete responders, the median number of clinically indicated interventions in the 6 months before treatment was 3 (range 2–5) and decreased to 0 (range

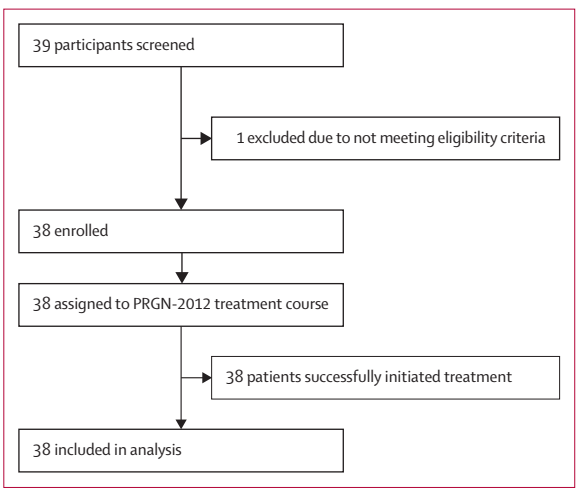

Figure 1: **Trial profile**

	PRGN-2012 (n=35)*
Age at start of PRGN-2012 treatment, years	49 (20–88)
18–37	8 (23%)
38–64	19 (54%)
≥65	8 (23%)
Age at diagnosis, years	35 (1–68; 4–47)
Juvenile-onset recurrent respiratory papillomatosis, age <13 years	12 (34%)
Adult-onset recurrent respiratory papillomatosis, age ≥13 years	23 (66%)
Sex	
Male	20 (57%)
Female	15 (43%)
Number of lifetime interventions for recurrent respiratory papillomatosis	40 (3 to >400; 10–100)
Tracheal recurrent respiratory papillomatosis	8 (23%)
HPV genotype 6 or 11	
HPV 6	24 (69%)
HPV 11	11 (31%)
Number of previous medical treatments for recurrent respiratory papillomatosis	2 (1–8; 2–3)
Previous administration of preventative HPV vaccine	
Yes	25 (79%)
No	10 (21%)
Number of clinically indicated interventions in the 12 months before start of PRGN-2012	4 (3–10; 3–5)
Baseline anatomic Derkay score	8 (3–31; 6–15)
Baseline Vocal Handicap Index-10 score	24 (6–40; 16–30)

Data are median (range), n (%), or median (range; IQR). HPV=human papillomavirus. *For completeness, patients with recurrent respiratory papillomatosis treated at the recommended phase 2 dose on the phase 1 portion are included.[19]

Table 1: **Baseline characteristics of the population**

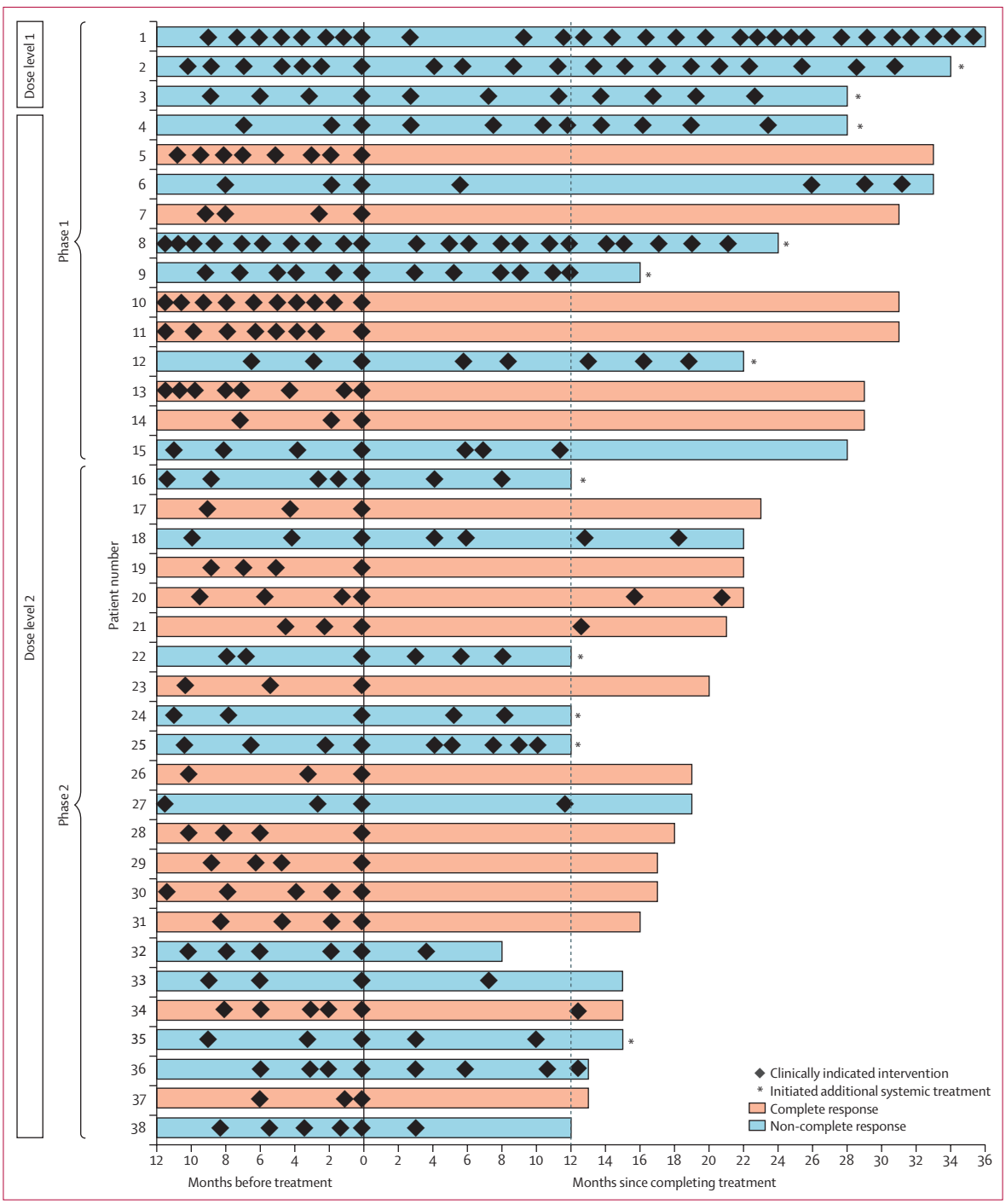

Figure 2: **Comparison of clinically indicated interventions before and after PRGN-2012 treatment**
Clinical benefit from PRGN-2012 treatment was determined by comparing the number of clinically indicated interventions in the 1 year following treatment to the 1 year before treatment for each patient. The solid vertical black line indicates the end of the 1-year pretreatment period and beginning of the post-treatment follow-up. The one-time 12-week treatment course, starting with the first administration of PRGN-2012 and ending with the last administration of PRGN-2012, is considered a single timepoint at month 0. The dotted vertical black line indicates the end of the 1-year post-treatment follow-up period. Interventions performed to maintain minimal residual disease are not indicated in the figure. For information on interventions done during the minimal residual disease period for each patient see the appendix (p 4).

0–0) in the 6 months following treatment. This is in comparison to non-complete responders in which the median number of clinically indicated interventions in the 6 months before treatment was 2 (range 1–5) and only decreased to 1 (range 0–3) in the 6 months following treatment.

Considering changes in Derkay and VHI-10 scores 6 months after PRGN-2012 treatment compared with before treatment, a median percent reduction in Derkay score of 90% (range 43–100) was observed in complete responders and 32% (range 0–100) in non-complete responders (tables 1, 2; appendix p 6). A median percent reduction in VHI-10 score of 95% (range 13–100) was observed in complete responders and 14% (range 0–97) in non-complete responders. In the four patients with evaluable pulmonary RRP as measured by RECIST, there was no significant change in measured index lung lesions following treatment with each patient having a best response of stable disease. Two of these four patients had laryngeal disease that responded to treatment (one partial response and one complete response). Taken together, these data indicate that PRGN-2012 treatment removed the need for clinically indicated interventions in more than half of treated patients by eliminating or reducing papilloma disease burden (table 2).

Treatment with PRGN-2012 was well tolerated. Adverse events in the entire study population did not differ from what was reported in the phase 1 portion of the study.[19] There were no serious adverse events, grade 3 or more treatment-related adverse events, or early treatment discontinuations (table 3). The most common adverse events were grade 1 injection-site reaction (34 [97%] of 35), fatigue (28 [80%] of 35), chills (25 [71%] of 35), fever (24 [69%] of 35), and myalgia (9 [26%] of 35). These events occurred more frequently following the first administration of PRGN-2012 and typically lasted 1–3 days. There was one death on study not related to PRGN-2012 treatment. Patient 32 was a man aged 88 years with a history of severe aortic stenosis, coronary artery disease, and chronic obstructive pulmonary disease. He received all four administrations of PRGN-2012. 7 months into the 12-month post-treatment period, he had a myocardial infarction. He subsequently developed cardiogenic shock and passed away on Oct 5, 2023. His death was attributed to a previous history of coronary artery disease and severe aortic stenosis.

In an exploratory analysis, a slightly higher response rate was seen in patients with HPV 6 (14 [58%] of 24) compared with HPV 11 (4 [36%] of 11), although the number of patients with HPV 11-driven disease was fewer (table 2). In a post-hoc analysis, the complete response rate was similar among patients with juvenile-onset disease (6 [50%] of 12) and adult-onset (12 [52%] of 23). In addition, long-term follow-up of patients treated on the phase 1 portion of the study revealed complete visual resolution of papillomatous disease in four (67%) of six complete responders with a median follow up of 30 months (range 26–32; appendix p 7). Complete visual resolution of disease at the 1-year post-treatment follow-up visit was also seen in five (42%) of 12 complete responders from the phase 2 portion of the study.

	PRGN-2012 (n=35)*
Objective response rate: complete or partial	23 (66%)
Type of response	
Complete	18 (51%)
Partial	5 (14%)
No response	12 (34%)
Complete response by onset of recurrent respiratory papillomatosis†	
Juvenile-onset recurrent respiratory papillomatosis, age <13 years	6/12 (50%)
Adult-onset recurrent respiratory papillomatosis, age ≥13 years	12/23 (52%)
Complete response by HPV type‡	
HPV type 6	14/24 (58%)
HPV type 11	4/11 (36%)
Number of clinically indicated interventions 12 months after treatment by response type	
Complete	0
Partial	1 (1–2; 1–1)
No response	3 (1–7; 2–4)§
Time to first intervention after treatment, months	
Partial response	6 (3–12; 3–7)
No response	3 (3–7; 3–5)
Post-treatment Derkay score¶	
Complete response	1 (0–5; 0–2)
Partial response	2 (1–15; 1–2)‖
No response	11 (0–21; 6–13)
Post-treatment Vocal Handicap Index-10 score¶	
Complete response	1 (0–30; 0–6)
Partial response	11 (1–20; 1–17)
No response	19 (7–27; 11–24)

Data are n (%), n/N (%), or median (range; IQR). HPV=human papillomavirus. *For completeness, patients with recurrent respiratory papillomatosis treated on the phase 1 study with the recommended phase 2 dose are included.[19] †Data are number of complete response by onset/number of patients with onset (%). ‡Data are number of complete response by HPV type/number of patients with HPV type (%). §Patient 32 was considered a non-responder. ¶Measurement at 24 weeks after PRGN-2012 treatment (closest timepoint to 24 weeks was used if not available). ‖Patient 38 did not have post-treatment Derkay scoring.

Table 2: **Response to treatment summary**

Discussion

The current standard of care for RRP is repeat laser ablation of papillomas, surgical resection of papillomas, or both. In addition to the immediate risks of multiple exposures to anaesthesia and post-operative complications, the cumulative effects of multiple interventions can include scarring of the larynx and trachea leading to irreversible vocal dysfunction and airway obstruction. In addition, financial hardship and psychological distress are common, especially in patients who require multiple clinical interventions per year. Therefore, the development of non-surgical treatments for RRP is a high unmet need. Our study shows that adjuvant PRGN-2012 for adult patients with RRP is safe and effective. Most patients (18 [51%] of 35; 95% CI 34–69) had clinical benefit, defined as the elimination of clinically indicated interventions to

	Grade 1	Grade 2	Grade ≥3
Adverse event			
Any	35 (100%)	6 (17%)	4 (11%)
Treatment related	35 (100%)	2 (6%)	0
Leading to discontinuation	0	0	0
Serious adverse event			
Any	0	0	1 (3%)
Treatment related	0	0	0
Treatment related adverse events*			
Injection site reaction	34 (97%)	0	0
Fatigue	28 (80%)	2 (6%)	0
Chills	25 (71%)	0	0
Fever	24 (69%)	0	0
Myalgia	9 (26%)	2 (6%)	0
Nausea	8 (23%)	0	0
Vomiting	2 (6%)	0	0
Hyperhidrosis	2 (6%)	0	0
Headache	2 (6%)	0	0
Dizziness	1 (3%)	0	0
Diarrhoea	1 (3%)	0	0
Dyspnea	1 (3%)	0	0
Blurred vision	1 (3%)	0	0
Injection site bruising	1 (3%)	0	0

Data are n (%). For completeness, patients treated at the recommended phase 2 dose on the phase 1 portion are included (n=35).[19] *Adverse events attributed to PRGN-2012.

Table 3: **Adverse events**

control RRP in the year following treatment. This clinical benefit was durable with 83% (15 of 18) of complete responses ongoing up to 33 months after completing the study treatment (median duration of response not yet reached). This is in contrast to patients with either partial or no response to treatment in which the median time to first intervention was 6 months and 3 months, respectively (table 2). These results occurred after a single course of adjuvant PRGN-2012. Scarce historical data from the Coordination of Rare Diseases at Sanford Registry Data estimate the rate of spontaneous disease remission in this patient population to be less than 5% (one of 22). A complete response rate above 50% shows the robust clinical activity of adjuvant treatment with PRGN-2012.

For the **Coordination of Rare Diseases at Sanford Registry Data** see https://rrpf.org/cords-global-patient-registry/

The underlying mechanism of PRGN-2012 is induction of T-cell immunity specific to HPV 6 or 11.[18,19] In the previously published phase 1 study, patients who had a complete response showed a greater expansion of peripheral HPV-specific T cells compared with non-responders.[19] Additionally, responders had increased HPV-specific T-cell responses detected in post-treatment papilloma infiltrating lymphocytes compared with before treatment. Patient five, who has had an ongoing complete response of 33 months, was found to have a papilloma-infiltrating clonally expanded HLA-B*55-restricted T-cell clone specific for the HPV 6 $E2_{165\text{-}173}$ antigen after treatment that was undetectable before treatment.[19] This suggests the ability of HPV-specific T-cell immune responses to control papillomatous disease in adults with RRP. Other therapeutic agents, including immune checkpoint blockade, have failed to consistently show the ability to induce HPV-specific immunity and durable papillomatous disease control.[13,23]

PRGN-2012 gene therapy enhances T-cell responses that are required to detect and potentially eliminate cells infected with HPV. Conversely, vaccination with a preventative vaccine such as Gardasil primarily induces humoral immunity and is highly effective at preventing HPV infection,[2] but fails to consistently result in clinical benefit when administered in the therapeutic setting.[15–17] PRGN-2012 monotherapy might lead to disease control and clinical benefit in a greater proportion of patients with RRP compared with patients with virally induced cancers that receive therapeutic vaccines because papillomatous lesions do not have mutations resulting in genetic immune escape that are often observed in malignancy.[6,24]

Most patients in this study had severe disease symptoms and required many clinically indicated interventions in the 12 months before study treatment. Many RRP patients require fewer than three clinically indicated interventions per year. Due to a favourable safety profile and ability to generate HPV 6 or 11-specific T-cell immunity,[19] clinical testing of PRGN-2012 in RRP patients with less severe disease is warranted. Additionally, clinical testing of PRGN-2012 in patients with other conditions that result from chronic infection with HPV 6 or 11 should also be considered.

Although 30 (86%) of 35 patients had a decrease in the requirement for clinically indicated interventions in the 12 months after treatment, some patients were considered non-responders. In the phase 1 portion of the study, reduced papilloma cell-specific HPV gene expression was associated with an inflammatory monocytic infiltration, greater expression of T-cell chemokines *CXCL9*, *CXCL10*, and *CXCL11*, and clinical response. Conversely, greater HPV gene expression was associated with greater expression of *CXCL8*, infiltration of immunosuppressive neutrophilic cells[25] and no clinical response. Despite all patients having chronic HPV infection, these results indicate that features of the papilloma microenvironment might contribute to an individual patient's clinical response. In this study, minimal residual disease was maintained during the 12-week PRGN-2012 treatment period to mitigate the effect of the immunosuppressive papilloma microenvironment and maximise the chance of clinical benefit. Consistent with clinical practice, repeat surgical intervention to maintain minimal residual disease alone did not generate durable disease control in PRGN-2012 non-responders.

The study design ensured that decisions regarding the need for clinically indicated interventions before and after PRGN-2012 treatment were made by the patient in consultation with their home physician, independent of the study team, thereby decreasing

potential bias. The single-arm, non-randomised study design was also important in reducing bias related to other factors. Although extensive heterogeneity of disease severity exists between patients, the requirement for clinically indicated interventions within a single adult RRP patient is relatively consistent. Therefore, a patient-specific comparison of the need for clinically indicated interventions before and after the study treatment provided a reliable gauge of clinical activity. This clinical outcome measure has been used in other RRP studies.[11–13,23,26] Using a stringent primary clinical outcome measure such as complete response compensates for mild fluctuations in disease severity that might exist within individual patients. The observed complete response rate of 51% (18 of 35; 95% CI 34–69) provides evidence of the drug's effect without the use of a placebo. Lastly, adult-onset RRP is rare with an incidence of 2 in 100 000 per year in the USA,[3] and randomised clinical trials have historically failed to complete accrual.[27–29]

There were some limitations in this study. Due to practical clinical considerations, it is not feasible to biopsy visibly normal laryngeal mucosa to determine whether HPV infection has been completely cleared from patients with complete responses. Therefore, it is unknown whether these patients have been cured. Longer follow-up will help determine whether the clinical course of RRP has been permanently affected by PRGN-2012 treatment. Limitations in trial design in this patient population also exist. Extensive inter-patient disease heterogeneity and rarity of the disease make randomisation and inclusion of a placebo-controlled group impractical. An additional confirmatory study designed similarly to this trial would help corroborate our findings of safety and clinical activity of PRGN-2012. An additional limitation is the lack of ethnicity data being reported.

In conclusion, data from this study shows that gene therapy with adjuvant PRGN-2012, which induces T-cell responses specific to HPV 6 or 11, is a safe and effective treatment for adults with RRP. Adjuvant PRGN-2012 represents a potentially new standard of care treatment for this devastating disease for which no approved medical therapies exist.

Contributors

SMN, JS, JLG, HS, DEB, and CTA contributed to the conception and design of the study. SMN and CTA drafted the manuscript. AL, JS, and JLG critically reviewed the manuscript. SMN, CTA, AL, HS, and DEB did the statistical analysis, interpreted the data, and verified the data. SMN, JV, SN, MK, EF, MW, LP-W, SLD, and CTA coordinated and performed clinical activities. SMN, AL, HS, DEB, and CTA supervised all aspects of the study. SMN, JS, JLG, and CTA were responsible for the decision to submit the manuscript. SMN is the principal investigator of the study. All authors had direct access to the raw data and read and approved the final version of the manuscript.

Declaration of interests

JS, JLG, and CTA are tenured senior investigators (equivalent to full professor) at the National Institutes of Health. AL, HS, and DEB are employees of Precigen and report stock or stock options in the company. AL reports support for attending meetings and travel by Precigen. DEB has a patent related to this work titled "Human papillomavirus vaccines and uses of the same for HPV associated diseases" with patent number WO2022/115470. HS reports other related patents planned, issued, or pending. JS reports that National Cancer Institute (NCI) has a Cooperative Research & Development Agreement with Precigen. All other authors declare no competing interests.

Data sharing

All of the de-identified patient-level data collected during the trial are available in the appendix (pp 3–6) and are immediately available to anyone who wishes to access the data for any purpose.

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