First-in-human Mutation-targeted siRNA Phase Ib Trial of an Inherited Skin Disorder

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The rare skin disorder pachyonychia congenita (PC) is an autosomal dominant syndrome that includes a disabling plantar keratoderma for which no satisfactory treatment is currently available. We have completed a phase Ib clinical trial for treatment of PC utilizing the first short-interfering RNA (siRNA)-based therapeutic for skin. This siRNA, called TD101, specifically and potently targets the keratin 6a (K6a) N171K mutant mRNA without affecting wild-type K6a mRNA. The safety and efficacy of TD101 was tested in a single-patient 17-week, prospective, double-blind, split-body, vehicle-controlled, dose-escalation trial. Randomly assigned solutions of TD101 or vehicle control were injected in symmetric plantar calluses on opposite feet. No adverse events occurred during the trial or in the 3-month washout period. Subjective patient assessment and physician clinical efficacy measures revealed regression of callus on the siRNA-treated, but not on the vehicle-treated foot. This trial represents the first time that siRNA has been used in a clinical setting to target a mutant gene or a genetic disorder, and the first use of siRNA in human skin. The callus regression seen on the patient's siRNA-treated foot appears sufficiently promising to warrant additional studies of siRNA in this and other dominant-negative skin diseases.

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INTRODUCTION

The emergence of short-interfering RNA (siRNA) as a powerful tool to reduce target gene expression *in vitro* and in animal model systems has led to recent therapeutic trials of siRNAs for macular degeneration, diabetic macular edema, solid tumors, respiratory syncytial virus, hepatitis B, and human immunodeficiency viral infections.[1–5] However, these trials have not yet been published. To date, the only published clinical trial reporting the use of a siRNA is a safety and tolerability study for respiratory syncytial virus.[6] This phase I siRNA trial for respiratory syncytial virus demonstrated few side effects and promising signs of efficacy. The siRNA trial reported herein is the first-in-man siRNA trial for a skin disorder as well as the first to target a mutated gene causing an inherited disease.

Pachyonychia congenita (PC) is an ultrarare (less than a few thousand cases worldwide[7]), highly disabling, autosomal dominant inherited disorder that affects the nails, skin, oral mucosae, hair, and teeth.[8,9] Manifestations are regionally variable but focally persistent and do not spontaneously resolve. The most disabling feature of PC is painful plantar calluses for which no satisfactory treatment is currently available. Although PC symptoms and pain levels vary between patients, many are unable to walk without the aid of crutches at least intermittently or must use a wheelchair. PC patients often walk on their knees while at home to avoid contact with the plantar calluses. Although it is not possible to effectively or safely remove PC calluses completely, patients routinely use pumice stones and razor blades to groom the calluses to help alleviate pain. These calluses are so exquisitely sensitive that patients rarely allow others, even family members, to touch their feet. PC patients could benefit enormously from an effective, locally administered therapy.

PC is caused by mutations in either keratin K6, K16, or K17 that act by a dominant-negative mechanism to cause the disease symptoms.[8,9] Selective depletion of the mutated keratin has the potential to directly target the molecular etiology of the disease, and there is compelling evidence from animal models, at least in the case of a similar keratin disorder, epidermolysis bullosa simplex (due to keratin 14), that even partial reduction of mutant keratin expression may have a beneficial clinical effect.[10] Further, substantial redundancy among PC-related keratins, as demonstrated in knockout mice,[11] reduces the likelihood of a severe adverse event if a single keratin is eliminated (*e.g.*, if both wild-type and mutant keratin 6a (K6a) were inhibited by the siRNA). Overall, the focal nature of PC, our understanding of the underlying molecular defects of the disorder, the lack of effective PC therapies, and the ability to visually observe changes during treatment make PC a particularly good human skin disease model for testing siRNA in a proof-of-concept trial for genetic disorders.

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Preclinical studies have demonstrated that the TD101 siRNA is safe, as well as highly potent and specific.[12,13] This siRNA has been shown to specifically target the cytosine-to-adenine single nucleotide K6a mutation (resulting in the amino-acid change N171K) in patient-derived immortalized keratinocytes.[13] It has also been shown to reverse the mutant phenotype of cells in a dominant-negative tissue culture model by restoring their ability to form a structurally intact keratin intermediate filament network.[12,13] Furthermore, the TD101 siRNA has been tested in a mouse model using bicistronic reporter constructs consisting of firefly luciferase linked to either wild-type or N171K mutant K6a. Co-delivery of these constructs and the mutation-specific siRNA resulted in potent inhibition of the mutant (but not the wild-type) version of the gene, as assayed by *in vivo* bioluminescence imaging[12] (and unpublished results). In a comparison of unmodified and modified siRNA, we found that some modifications eliminated the single nucleotide specificity, and no increase in efficacy was observed using modified versions (data not shown). Furthermore, we reasoned that if any unmodified siRNA were to enter the bloodstream, it would be quickly degraded, increasing the safety profile. For these reasons, unmodified TD101 was used in this trial. A mouse toxicity study demonstrated a lack of serious toxicity when the TD101 siRNA was delivered at high-dose levels by intradermal injection.[13] Thus, preclinical studies in *in vitro* and *in vivo* model systems demonstrated both safety and effective inhibition of N171K K6a by the TD101 siRNA.

The clinical efficacy and safety of TD101, administered by intralesional injection into a plantar callus, was evaluated in a single patient using a prospective, double-blind, split-body, vehicle-controlled, dose-escalation study design. Treatment was completed after 17 weeks of twice-weekly injections and was followed by a 3-month washout period (see **Table 1** for dosing schedule). Efficacy and safety measurements are detailed in the Materials and Methods section, as well as the **Supplementary Materials and Methods**.

RESULTS

At the conclusion of the washout period, the blinding code was broken and revealed that the right foot had received TD101 siRNA, whereas the left foot had received the vehicle-control solution. Both subjective patient data and physician-derived clinical data suggest that in similar symmetric calluses, there was a positive effect of the injection of TD101 in the right foot, but not in the vehicle control–injected left foot.

During the first 2 months of the trial, no dramatic differences (subjective or objective) between feet were noted by either the patient or physician. At this point in the trial, there were no visible responses in the calluses of either foot that would indicate either significant injury or efficacy from the drug. At approximately day 70 of the trial (dose = 2 ml; 3 mg/ml), the patient's subjective evaluation of the injected callus ("If you are receiving a study medication, evaluate if it is working and improving your PC symptoms." 0 = definitely working; 10 = definitely not working) began to indicate a marked difference in the right foot, but no change in the left foot (**Figure 1**). Measurements of the injected calluses also began to show a statistically significant decrease in length of the callus on the right foot only (**Figure 2**). On day 98 of the trial, after dose 28 (dose = 2 ml; 5.0 mg/ml), the callus at the site of injection on the right foot began to fall away and revealed healthy, pink skin. The underlying skin was remarkably nontender to palpation, whereas surrounding areas of callus retained sensitivity (**Figure 3a**). This type of behavior had never been observed by the patient previously (**Figure 1** and patient personal communication). This reduction in tenderness is the most dramatic clinical observation in the trial. By day 115, not only was the reduced length of the callus obvious,

Table 1 Dose-escalation schedule

Week	Dose no.	Days	Volume (ml)	Concentration of TD101 (mg/ml)	Total dose TD101 (mg)
1	1–2	1–7	0.1	1.0	0.10
2	3–4	8–14	0.25	1.0	0.25
3	5–6	15–21	0.50	1.0	0.50
4	7–8	22–28	1.0	1.0	1.0
5	9–10	29–35	1.5	1.0	1.5
6	11–12	36–42	2.0	1.0	2.0
7	13–14	43–49	2.0	1.5	3.0
8	15–16	50–56	2.0	2.0	4.0
9	17–18	57–63	2.0	2.5	5.0
10	19–20	64–70	2.0	3.0	6.0
11	21–22	71–77	2.0	3.5	7.0
12	23–24	78–84	2.0	4.0	8.0
13	25–26	85–91	2.0	4.5	9.0
14	27–28	92–98	2.0	5.0	10.0
15	29–30	99–105	2.0	6.0	12.0
16	31–32	106–112	2.0	7.0	14.0
17	33	113–119	2.0	8.5	17.0

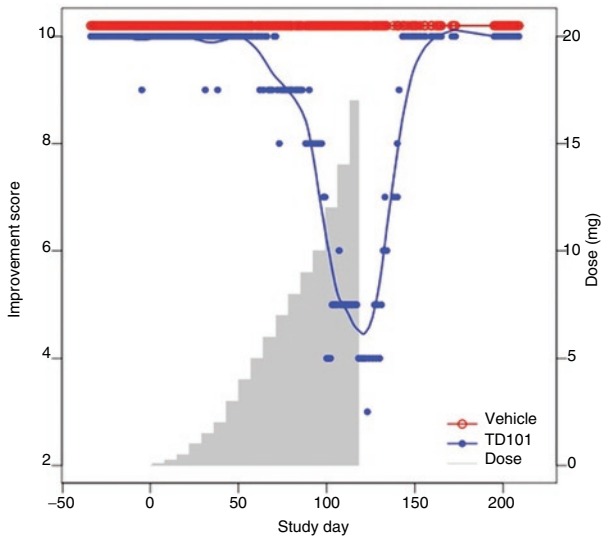

Figure 1 Patient assessment demonstrates subjective improvement in the right foot (TD101, blue) but not the left foot (vehicle, red) as determined by daily diary entries. Note that the scores for the left foot have been slightly offset so that the left and right foot scores do not overlap at baseline. The improvement scores and a curve fit through the scores are both plotted. The corresponding dose (gray) is indicated by a separate graph. An improvement score of 10 represents "definitely not working," whereas a score of 0 represents "definitely working." The improvement returns to baseline after treatment is completed.

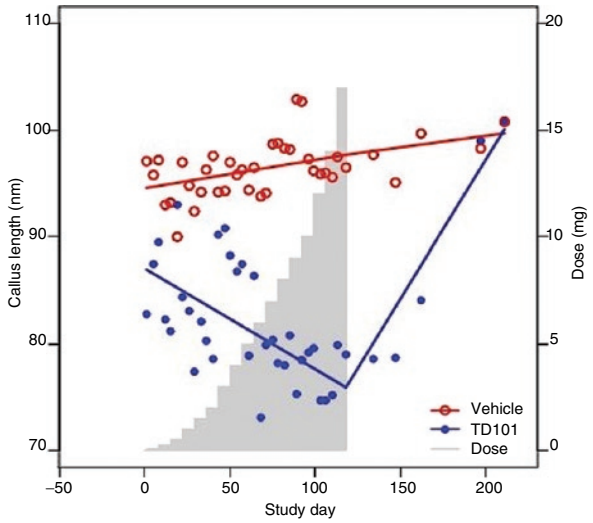

Figure 2 The length of the callus on the right foot (TD101, blue), but not the left foot (vehicle, red), decreased significantly during the dosing period (day 0 to day 118) (drug versus vehicle, $P = 0.004$). The measured callus length (dots and open circles) and a piecewise linear fit through the callus lengths are both plotted (see Materials and Methods). The fit has a change in slope at the end of the dosing period. The corresponding dose is indicated by a separate graph.

but there was also an area clinically free of hyperkeratosis in the center of the injection site (**Figure 3a**). Subjective and objective changes in the right foot began returning toward baseline after the drug was discontinued and reached baseline ~30–50 days after the last dose. **Figure 2** shows that the trend to shortening of the callus length occurred prior to day 98 when the callus began peeling away from the injection site.

Because of the dynamic nature of callus development and resolution, it is unclear at what dose the callus first began to regress. The cellular turnover and retention in PC calluses have never been measured, so it is difficult to surmise whether the lower doses of TD101 began to have a cumulative effect that was not observed until later or if the response at later time-points was due to an increased dose at that time. Future investigation with a single-dose level over a prolonged period of time may help to address these questions.

There were no clinical signs of a systemic response to the TD101 injections (*i.e.*, outside the injection site) in the skin, nails, or oral mucosa of the patient. Not unexpectedly (given the small treatment area), the patient reported no significant changes in overall quality of life during the treatment period ($P = 0.16$). There were no clinical signs of local or systemic toxicity at intralesional doses up to and including the highest TD101 dose administered (17 mg). Similarly, no laboratory values suggested toxicity (**Supplementary Tables S1–S4**).

DISCUSSION

On the basis of preclinical testing, and the dramatic and specific response of the patient's treated callus to TD101, we have every reason to believe, but cannot prove, that the mechanism of the clinical effect was through RNA interference. We were unable to obtain biopsy tissue for measurement of allele-specific mRNA levels because the protocol required repeated local administration of drug, and the safety evaluation arm of our protocol might have been compromised by repeated biopsies of the foot in an individual prone to blisters and infection. Recent data from animal studies of a siRNA for macular degeneration suggest that some of the clinical response may be a nonspecific reaction to the siRNA.[14] Once an animal model of PC is available, scrambled siRNAs and tissue biopsies will be important mechanistic controls for future studies. However, there is no evidence from our preclinical studies, where nonspecific siRNA controls were used at every stage, that TD101 is acting in a nonspecific manner unrelated to selective degradation of mutant K6a mRNA.[12,13,15]

The degree of pain experienced by the patient at the time of injection is a significant concern. Although pain related to the injection did not persist longer than a few hours after injection, the intense pain experienced at the time of injection will limit the utility of the drug by this delivery method. At the inception of this trial, the intradermal delivery route was selected to maximize the probability of observing an effect based on preclinical studies demonstrating reduced reporter gene expression after intradermal injection of specific siRNA in mice.[15,16] Future efforts must focus on improved delivery methods for TD101, such as pharmaceutical formulations for noninvasive topical delivery.

Despite our understanding of the molecular basis of PC,[17] current treatment is limited to mechanical removal of thick calluses, nonspecific topical keratolytics and oral retinoids, none of which alleviates blistering or plantar pain satisfactorily.[8] We believe the callus regression in this single-patient clinical trial of siRNA in PC is sufficiently promising to warrant additional studies of siRNA in this and other dominant-negative skin diseases.

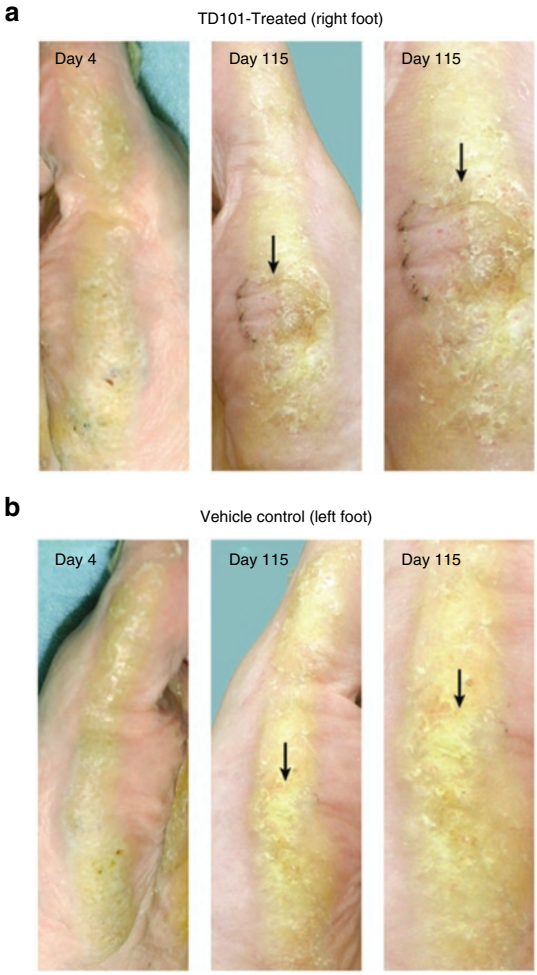

Figure 3 Improvement of pachyonychia congenita symptoms following TD101 administration. Callus regression is seen on the right foot at the site of injection of (**a**) TD101 (center arrow), but not at the site of injection of (**b**) vehicle on the left foot (arrows). Note that relative to the first injection, the callus on the right foot (but not on the left) developed a clearing of callus around the site of injection. The third photo in each panel is an enlargement of the site of injection from the day 115 photos. The callus on the right foot also shows some regression near the instep of the foot resulting in a shortening of the total callus length.

MATERIALS AND METHODS

Patient enrollment and study design. After providing informed consent, an adult participant with PC carrying a *KRT6A* N171K mutation was enrolled in the phase Ib trial according to a protocol approved by the University of Utah Institutional Review Board and the Food and Drug Administration (IRB no. 24013 and IND no. 77504; ClinicalTrials.gov registration no. NCT00716014; GMP (Good Manufacturing Practice) drug manufactured by Agilent Technologies, Santa Clara, CA). The patient was a 39-year-old female with no history of medical problems other than her PC symptoms. The patient served as her own control, with randomization of TD101 or vehicle control for intradermal injection to symmetric calluses on opposite feet. The test agents were packaged and labeled according to a computer-generated randomization list that assigned vehicle control or drug to the left or right foot. The decoded list of test agents was held in a secure place and not made available to the Principal Investigator, patient, or other study personnel until the conclusion of the trial.

The study evaluated safety and tolerance of multiple injections of escalating doses of TD101. Intradermal injections were given twice weekly over 17 consecutive weeks for a total of 33 injections in each foot (Table 1). Two symmetric calluses were selected for treatment—one on each foot. Test agent or vehicle-control solution was injected as indicated by the randomization list. The central region of each callus was marked and injected at the same site for each treatment; other locations on the calluses were never treated. The injection was performed using a 30-gauge needle, inserted with a single needle-stick to penetrate to the level of the superficial dermis. This depth of injection was determined by the dramatic decrease in resistance that occurred in the subepidermis. The 2 ml injections produced ~2 cm subepidermal blisters, which corresponds to the central region of response on the treated callus (Figure 3a).

Beginning on day 29, in response to intense injection-related pain, the patient was premedicated with 2.5 mg diazepam and 5.0/325 mg hydrocodone/acetaminophen prior to each treatment. In addition, beginning on day 32, the patient also received bilateral posterior tibial nerve blocks with 2% preservative-free lidocaine. The treatment sites were each evaluated for adverse reactions before injections.

TD101 was administered on a volume and dose-escalation schedule (Table 1). The optimal dosing schedule for unmodified siRNA in skin is not known. We based the frequency of our dosing on the stability of TD101 siRNA in skin as demonstrated in our preclinical studies.[15] We designed our treatment protocol to spread the injections out over a time period to optimize response and minimize the burden on the subject given the significant pain experienced by the subject during each injection. Initially, 0.1 ml of a 1.0 mg/ml solution of TD101 or vehicle alone (Dulbecco's phosphate-buffered saline without calcium or magnesium) was administered to symmetric calluses. Six rising dose-volumes were completed without an adverse reaction to the increases: 0.1, 0.25, 0.5, 1.0, 1.5, and 2.0 ml of a 1.0 mg/ml solution of TD101 solution per injection. As the highest planned volume (2.0 ml) was well tolerated, the concentration of TD101 was then increased each week from 1 mg/ml up to a final concentration of 8.5 mg/ml. The pH of the placebo and stock study drug (10 mg/ml) was identical (7.0); furthermore, saline dilutions of up to 40-fold had no effect on pH (data not shown). The patient was followed for 3 months after the final injection, at which point the study was unblinded to both the patient and the Principal Investigator.

Study end points for safety. The maximum tolerated volume and the maximum tolerated dose were defined on the basis of the patient having a grade 2 or higher injection site reaction (erosion, unacceptable pain, or ulceration), or any adverse experience reported by the patient that resulted in discontinuation of the study (see **Supplementary Materials and Methods** for details regarding clinical safety definitions). Safety evaluations included assessments of adverse experiences by targeted clinical examination and clinical laboratory analyses. Clinical laboratory tests for safety were performed before first dosing (baseline), and on days 1, 46, 92, 106, 114 (final injections), and 2 weeks after the final injections. These tests included hemogram, serum chemistry panel, antinuclear antibodies, C3a and Bb (complement split products), activated partial thromboplastin time, prothrombin time, dipstick urinalysis, and a urine pregnancy test (see **Supplementary Tables S1–S4** for study days and values of each test). No adverse clinical or laboratory events were noted during treatment or in the 3-month follow-up period.

Study end points for efficacy. Measures of efficacy included weekly standardized digital photography, callus and nail plate length and width measurements during each clinic visit (carbon fiber composites digital caliper; Fisher Scientific, Pittsburgh, PA), an online, time and date stamped, subjective pain diary twice daily, and weekly completion of the Dermatology Life Quality Index[12] (permission for use, AY Finlay). To evaluate potential systemic effects, an assessment of the degree of follicular keratoses on the forearm and oral leukokeratosis was made at each clinic visit (better, worse, or the same). In an effort to minimize any inter-rater variability, clinical measurements were obtained by a single investigator (S.A.L.),

with the exception of five measurements on days 15, 50, and 114, and two follow-up visits. The actual measure of the callus required the rater to visualize the point at which the callus ended and normal skin began. Because this change is gradual, the investigator had to carefully examine the skin, and then use their best judgment to determine where the callus actually began and ended.

Statistical analysis. All statistical analysis was performed using the R statistical software version 2.6.0 (The R Foundation for Statistical Computing, Vienna, Austria). Scatter plots for improvement scores were augmented with smooth curves produced by the "LOESS" function in R. The difference in callus length between the right and left foot was analyzed using an autoregressive model of order one, with a time-trend term. This model uses the previously occurring value as a predictor of the current value. A likelihood ratio test was used to determine statistical significance of the temporal trend during the treatment period. To plot temporal trends in callus length, we fit autoregressive models of order one to data from each foot separately. The models were piecewise linear, with a change in slope at the end of the dosing period, and were fit by maximum likelihood methods.

SUPPLEMENTARY MATERIAL

Table S1. Hemogram Values.
Table S2. Serum Chemistries.
Table S3. Urinalysis.
Table S4. Coagulation and Complement Parameters.
Materials and Methods.

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