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We hope that making available the relevant information on Pachyonychia Congenita will be a means of furthering research to find effective therapies and a cure for PC.
First-in-human Mutation-targeted siRNA Phase Ib Trial of an Inherited Skin Disorder

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INTRODUCTION

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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Molecular Therapy
Preclinical studies have demonstrated that the TD101 siRNA is safe, as well as highly potent and specific.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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 \textit{in vivo} bioluminescence imaging\textsuperscript{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.\textsuperscript{13} Thus, preclinical studies in \textit{in vitro} and \textit{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>
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<th>Week</th>
<th>Dose no.</th>
<th>Days</th>
<th>Volume (ml)</th>
<th>Concentration of TD101 (mg/ml)</th>
<th>Total dose TD101 (mg)</th>
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<td>0.50</td>
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<td>22–28</td>
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<td>4.0</td>
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<td>2.0</td>
<td>8.5</td>
<td>17.0</td>
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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 cal- lus 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 res- olution, 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 intra- esional 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. 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 non- specific 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. 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.
escalating doses of TD101. Intradermal injections were given twice
patient, or other study personnel until the conclusion of the trial.
control or drug to the left or right foot. The decoded list of test agents was
assigned vehicle for intradermal injection to symmetric callus
manufactured by Agilent Technologies, Santa Clara, CA). The patient was
University of Utah Institutional Review Board and the Food and Drug
was enrolled in the phase Ib trial according to a protocol approved by the
sent, an adult participant with PC carrying a
KRT6A N171K mutation
Life Quality Index12 (permission for use, AY Finlay). To evaluate poten-
tive pain diary twice daily, and weekly completion of the Dermatology
standardized digital photography, callus and nail plate length and width mea-
surements during each clinic visit (carbon fiber composites digital caliper;
examination and clinical laboratory analyses. Clinical laboratory tests for
resulted in discontinuation of the study (see
or ulceration), or any adverse experience reported by the patient that
a grade 2 or higher injection site reaction (erosion, unacceptable pain,
maximum tolerated dose were defined on the basis of the patient having
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 ml/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 stan-
dardized digital photography, callus and nail plate length and width measures
during each clinic visit (carbon fiber composites digital caliper; Fisher Scientific, Pittsburgh, PA), an online, time and date stamped, sub-
jective pain diary twice daily, and weekly completion of the Dermatology
Life Quality Index15 (permission for use, AY Finlay). To evaluate poten-
tial 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.),

MATERIALS AND METHODS

Patient enrollment and study design. After providing informed con-
sent, 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 reg-
istration 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 cal-
usses on opposite feet. The test agents were packaged and labeled accord-
ing 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
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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
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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 stan-
dardized digital photography, callus and nail plate length and width measures
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Life Quality Index15 (permission for use, AY Finlay). To evaluate poten-
tial 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.**

**ACKNOWLEDGMENTS**

We are indebted to the pachyonychia congenita (PC) patient that made this study possible, as well as PC Project and the membership of the International Pachyonychia Congenita Consortium for their unfailing enthusiasm and support. We thank Manuel Flores for assistance with preparation of figures and Kristina Heintz for her nursing support. We also acknowledge financial sponsorship of the clinical trial by PC Project and FDA (US Food and Drug Administration) OOPD (Office of Orphan Products Development) grant 1-R01-FD-003553-01 (to S.A.L.). This work was further supported by NIH (National Institutes of Health) grant 1R43AR056559 (to R.L.K.), a fellowship grant from PC Project (to F.J.D.S.), and by grants to W.H.I.M. and F.J.D.S. from the Dystrophic Epidermolysis Bullosa Research Association, the UK Medical Research Council (G0700314), and the British Skin Foundation. S.A.L. accepts full responsibility for the data presented in this manuscript. R.L.K., R.P.H., F.J.D.S., and W.H.I.M. have filed patents relating to short-interfering RNA therapy for PC. We thank Huntsman Cancer Institute for the use of clinical facilities for this trial. This work was completed in Salt Lake City, UT.

**REFERENCES**


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