In vivo topical gene therapy for recessive dystrophic epidermolysis bullosa: a phase 1 and 2 trial

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Recessive dystrophic epidermolysis bullosa (RDEB) is a lifelong genodermatosis associated with blistering, wounding, and scarring caused by mutations in COL7A1, the gene encoding collagen VII (C7). Here, we evaluated beremagene geperpavec (B-VEC), an engineered, non-replicating COL7A1 containing herpes simplex virus type 1 (HSV-1) vector, to treat RDEB skin. B-VEC restored C7 expression in RDEB keratinocytes, fibroblasts, RDEB mice and human RDEB xenografts. Subsequently, a randomized, placebo-controlled, phase 1 and 2 clinical trial (NCT03536143) evaluated matched wounds from nine RDEB patients receiving topical B-VEC or placebo repeatedly over 12 weeks. No grade 2 or above B-VEC-related adverse events or vector shedding or tissue-bound skin immunoreactants were noted. HSV-1 and C7 antibodies sometimes presented at baseline or increased after B-VEC treatment without an apparent impact on safety or efficacy. Primary and secondary objectives of C7 expression, anchoring fibril assembly, wound surface area reduction, duration of wound closure, and time to wound closure following B-VEC treatment were met. A patient-reported pain–severity secondary outcome was not assessed given the small proportion of wounds treated. A global assessment secondary endpoint was not pursued due to redundancy with regard to other endpoints. These studies show that B-VEC is an easily administered, safely tolerated, topical molecular corrective therapy promoting wound healing in patients with RDEB.
modifications\(^1\). HSV-1 viruses, in contrast, have transgene payload capacities exceeding 30 kb. Because they are non-integrating and episomal, HSV-1 vectors do not pose any insertional mutagenesis risk. In total, these properties make HSV-1 vectors particularly suitable for in vivo direct gene transfer.

We describe here the development and clinical translation of a topical gene therapy treatment for RDEB that can be repeatedly applied without serious adverse events. Beremagen giperpavec (B-VEC), a replication-defective HSV-1 vector containing two copies of the COL7A1 coding sequence, efficiently restored C7 expression in RDEB keratinocytes and fibroblasts in vitro. Typical B-VEC promoted skin integrity and robust C7 expression, followed by its assembly into basement membrane-associated AFs in vivo in C7-deficient mice and primary human RDEB skin xenografts. With preclinical data providing the scientific rationale, we evaluated the clinical translation to humans in a phase 1 and 2 exploratory study, that is, the first-ever clinical trial of topical gene therapy. Taken together, we demonstrate here a novel, easy-to-administer, and highly accessible gene therapy capable of reversing genetic disease through repeated application directly to patient skin wounds.

**Results**

**Restoration of C7 expression in RDEB patient cell culture.** To initially evaluate B-VEC as a cutaneous gene delivery vector, we examined its ability to promote C7 expression in primary skin cells of patients with RDEB in vitro. As seen in Fig. 1a, C7-null primary RDEB patient keratinocyte and fibroblast cultures demonstrated C7 expression 48 hours after B-VEC treatment. Dose-dependent increases in transduction efficiency were also demonstrated, targeting up to 100% of cells at a multiplicity of infection (MOI) of 1, 3 and 10 (Fig. 1abc), with a slowing of proliferation observed at an MOI of 10 after 48 hours. Western blot analysis of primary RDEB keratinocyte and fibroblast cell lysates identified a dose-dependent increase in the expression of full-length C7 (Fig. 1d). Demonstrating that C7 expression is attributable to B-VEC, the expression of the HSV-1 early protein, ICP0, closely correlated with C7 expression in vitro (Extended Data Fig. 1). These data demonstrate that B-VEC is capable of gene delivery and expression in the specific C7-contributing cell types in patient skin.

**Molecular correction of C7 expression in vivo.** To evaluate in vivo B-VEC-mediated COL7A1 gene delivery, intradermal B-VEC injections were administered to C7-deficient mice, which closely recapitulate the RDEB skin phenotype\(^2\). We performed three sets of experiments with the RDEB mice cohort, using three mice per cohort. Each mouse received three injections of B-VEC and one saline injection (n=27 B-VEC, n=9 saline). Some mice received a second injection on day 3 at the same four sites. At day 3 after B-VEC treatment (dose of 4.6\(\times\)10\(^6\) p.f.u. per 50\(\mu\)l per injection), C7 protein was localized in a linear pattern (Fig. 1e) at the dermal-epidermal junctions of the epidermis and in hair follicles. At day 7, widespread continuous linear C7 distribution through a larger area of skin was detected (as shown by joining multiple overlapping microscopy fields by tile imaging; Fig. 1f). COL7A1 transgene delivery and expression was detected using quantitative polymerase chain reaction with or without reverse transcription (RT–qPCR or qPCR) at two time points, at 3 and 7 days, after a single or two injections (Fig. 1g,h). Evaluation of C7 expression kinetics (day 3, day 5, day 7) using indirect immunofluorescence microscopy (IDIF) demonstrated C7 expression in treated skin at both high doses (4.6\(\times\)10\(^6\) p.f.u. per 50\(\mu\)l per injection) and low doses (4.6\(\times\)10\(^5\) p.f.u. per 50\(\mu\)l per injection) of the virus (Fig. 1i). Because homozygous RDEB mice are too fragile to tolerate a wounding and topical application assessment, heterozygous RDEB mouse skin was evaluated following topical application of B-VEC on wounded skin. Linear C7 expression, as detected using human-specific C7 antibodies, was observed 14 days after treatment (Fig. 1j).

To further evaluate the in vivo effect of B-VEC, we studied primary regenerated human C7-null RDEB skin xenografted onto immuno-deficient mice, a model generally regarded as the closest preclinical approximation to human RDEB skin. Xenograft experiments were conducted in two rounds, using 10 mice in each round. Eight mice in each round were treated with B-VEC, and two mice in each round were treated with topical saline control (total grafts, n=16 B-VEC, n=4 control). Control grafts (n=4) had multiple areas of dermal-epidermal separation (Fig. 1k) not observed in xenografts following application of topical B-VEC (n=16). These results are in agreement with a previous study that also reported dermal-epidermal...
Molecular correction and treatment effect in patients with RDEB. **Patients and treatment.** Preclinical in vitro and in vivo data provided the scientific rationale for the initiation of a randomized, open-label, placebo-controlled phase 1 and 2 trial of topical B-VEC for treatment of RDEB conducted in an outpatient setting at Stanford University. The phase 1 and 2 study enrolled nine adult and pediatric patients (Fig. 2, Extended Data Fig. 3 and Extended Data Table 1) who had a clinical phenotype consistent with generalized RDEB, confirmed COL7A1 gene mutations, reduced C7 NC1, and absent C7 NC2 protein expression and absent AFs as assessed with immunofluorescence and immunoelectron microscopy. Baseline wound surface area ranged from 0.89 to 65.29 cm² (Extended Data Table 2).

**Safety.** No deaths, serious or significant adverse events were reported (Extended Data Table 3). Of 129 topical B-VEC doses given in the trial, 21 adverse events were reported. One adverse event was moderate and was deemed unlikely to be related to the investigational product by the principal investigator. The remaining adverse events were mild: 13 adverse events were deemed unrelated to the investigational product and nine were related (fever, urinary tract infection, rash, itching, and arthralgia).

**Findings following intervention.** Images of all wounds studied in patients 1–11 are shown in Fig. 3, both at baseline and at 3 months after treatment with B-VEC or placebo. Closure in the B-VEC group was achieved in all wounds after 3 months, with the exception of a chronic (5 year) dorsal foot wound in patient 3. This wound had partial closure within 1 month of B-VEC treatment (Fig. 3; patient 3, wound 3), and complete closure upon re-treatment (Fig. 3; patient 11, wound 3). This wound remained healed throughout monitoring (8 months), highlighting the continued B-VEC effectiveness following repeated application. All other B-VEC-treated study wounds closed and remained healed for at least 3 months, while placebo wounds demonstrated a fluctuation of healing and re-blostering (Fig. 3). No healing rate differences were noted with respect to patient age or sex, B-VEC dosage or rates of application.

Wound closure analyses are given in Table 1 and Extended Data Table 4. Results and analysis reported are based upon observed data without imputed values for missing data. B-VEC-treated wounds were statistically significantly different from placebo wounds (P = 0.0026) based upon wound closure responder analysis; however, on both time to and duration of wound closure, the trend was numerically favorable towards B-VEC (Table 1). This observed treatment effect in B-VEC-treated wounds compared with the placebo control is suggestive of improved wound healing. Taken together with the evidence for molecular correction demonstrated in Fig. 4, this observed treatment effect suggests that, attributable to the expression of full-length C7,
topical application of B-VEC improves RDEB wound healing, specifically, in the clinically meaningful terms of complete wound closure, time to wound closure and durability of wound closure.

To further support these results, the mean percent change from baseline in wound surface area was analyzed (Fig. 3c). Comparisons used the Wilcoxon rank-sum test to demonstrate that the treatment
effect of reduction in wound area is statistically significant (P < 0.025) at weeks 8, 10 and 12.

To validate wound closure assessments, 90 day wound images were evaluated by two blinded independent reviewers. Comparison of the unblinded principal investigator image assessments to this blinded review demonstrated 100% concordance.

Table 1 presents the analysis of time to and duration of wound closure using the Kaplan–Meier method. Time to wound closure was defined as the time from the first treatment to complete wound closure (defined as a ≥95% reduction in wound surface from baseline for 2 consecutive weeks). Duration of wound closure was defined as the time from complete closure to the first re-opening. Compared with placebo, B-VEC-treated wounds demonstrated a numerical trend of shorter time to wound closure and longer wound closure duration.

Characteristic of RDEB wounds, placebo-treated wounds fluctuated between natural open and healed states21, while B-VEC-treated wounds remained durably and consistently closed (Fig. 3). This wound closure durability is supportive of the effectiveness of B-VEC in promoting dermal–epidermal cohesion. Resistance to blister extension into a B-VEC-treated wound area is shown in Supplementary Videos 1 and 2, supportive of the effectiveness of B-VEC in promoting dermal–epidermal cohesion.

Patient 12 was a 10-year-old boy who enrolled late in the study with large right and left lateral chronic (4 years) chest wounds that were much larger than those observed in the other patients. For that reason, his wound analysis was completed separately from the other patients (Extended Data Figs. 5, 6 and Extended Data Table 2). He had reduced by only 34% (Extended Data Fig. 6).

In a subset of seven patients (Supplementary Tables 3 and 4), biopsies from intact B-VEC-treated skin were evaluated for percent C7 NC1/NC2 expression compared with unaffected human skin (non-RDEB) using immunofluorescence and for AFs using immunoelectron microscopy, as previously described22,23. Some C7 NC1 and NC2 expression variability was noted in these post-treatment biopsies (Supplementary Table 3). However, most healed samples from this subset of seven patients had positive linear deposition indicative of full-length C7 expression as seen in representative images from patients 9 (day 15) and 10 (days 15 and 97) (Fig. 4a). NC2 expression in patient 10 on treatment day 15 was further analyzed by tiling multiple fields together to demonstrate continuous linear expression across the entire tissue section (Fig. 4b).

All patients who had biopsies prior to treatment had a lack of AFs at baseline (Table 2). Following B-VEC treatment, immunoelectron microscopy analysis23 showed C7 NC1 and NC2 expression and mature AFs (Fig. 4c and Supplementary Table 4) in patients whose specimens were amenable to immunoelectron microscopy analysis (n = 3). As shown in Fig. 4c, patient 10 lacked AFs and C7 NC2 staining, and had reduced C7 NC1 expression at baseline. Healed skin at treatment day 97 demonstrated increased C7 NC1 lamina densa localization and prominent C7 NC2 localization approximately 300 nm below the lamina densa (Fig. 4c), consistent with localization in normal skin24. These patient immunofluorescence and immunoelectron microscopy data suggest that B-VEC-mediated COL7A1 gene delivery targets the proper cells and directs the expression of functional, full-length C7 (inclusive of both NC1 and NC2) at the correct location in the dermal–epidermal basement membrane. Providing even further evidence for expression of functional C7 and molecular correction, B-VEC-mediated COL7A1 gene delivery promoted assembly of mature AFs.

Discussion

Direct topical in vivo gene transfer with B-VEC fulfills a long-standing goal of direct in vivo gene therapy to the skin. The ability of B-VEC to be shipped off the shelf, applied topically in a local outpatient setting and repeatedly dosed on demand offers a number of advantages over earlier ex vivo approaches. These include eliminating the need for long-distance travel by fragile-skinned RDEB patients to specialized medical centers, making gene therapy
Fig. 4 | C7 expression and AF formation after B-VEC topical therapy. a, Indirect immunofluorescence analysis of C7 NC1 and NC2 expression in topical B-VEC-treated patient skin. In a subset of seven patients (Supplementary Tables 3 and 4), biopsies from intact B-VEC-treated skin were evaluated for C7 NC1 and NC2 expression. Representative images are shown from patients (P) 9 and 10 (collected on the indicated days (D)). These were analyzed using dual label immunofluorescence for expression of the C7 NC1 and NC2 domains using anti-NC1 antisera (red) and an anti-NC2 monoclonal antibody, LH24 (green), and counterstained with nuclear stain (blue). The arrows indicate the dermal–epidermal junction. b, Extended examination of C7 NC2 expression across an entire tissue section of topical B-VEC-treated patient skin. Multiple sections of a skin biopsy taken from a healed wound area 15 days after treatment with topical B-VEC were analyzed using immunofluorescence and tiled together to show the results across the entire tissue section. c, Immunoelectron microscopy of C7 NC1 and NC2 expression and AFs in B-VEC-treated patient skin. Representative images are shown from patient 10 (collected at the indicated times) and were analyzed with immunoelectron microscopy using antibodies to the C7 NC1 domain (NP185) and C7 NC2 domain (LH24). (C7 NC1 and NC2 expression was assessed in patients whose specimens were amenable to immunoelectron microscopy analysis (n = 3; Supplementary Table 4.) The arrow in the upper panel and center panel shows positive immuno-gold staining for the NC1 domain in the lamina densa region. Arrows in the lower two panels show the presence of mature banded AFs associated with immuno-gold staining for the NC2 domain approximately 300 nm from the lamina densa. Scale bars, 500 nm.
accessible to patients who do not have access to specialized medical facilities, such as those living in underdeveloped countries. B-VEC therapy does not require patient biopsies for autologous cell engineering, nor does it require anesthesia or hospitalization. Instead, in vivo B-VEC topical gene therapy can be applied during routine dressing changes, minimizing any additional trauma and maximizing convenience to the patient.

Inflammation following in vivo treatment with gene therapy vectors has been a longstanding problem in the gene therapy field. The most well-known example of this involved a patient fatality following the use of an adenoviral vector for the treatment of ornithine transcarbamylase deficiency, in which the adenoviral vector activated innate immune responses and caused the acute release of inflammatory cytokines. The development of less immunogenic vectors such as adeno-associated virus and lentivirus has decreased but not eliminated inflammation, and repeated application of these vectors in vivo still usually requires concomitant systemic steroids.

Table 2 | Baseline characteristics of the study patients

| Patient | Age (years) | Sex | Mutation 1   | Mutation 2   | NC1 | NC2 | AF  | Collagen VII antibodies | Clinical diagnosis   |
|---------|-------------|-----|--------------|--------------|-----|-----|-----|-------------------------|----------------------|
| 1       | 36          | Male | c.6527dupC   | c.7485+5G>A   | -   | -   | -   | -                       | Generalized RDEB     |
| 2       | 28          | Male | c.90delC     | c.5048_5051dupGAAA | -   | -   | -   | +                       | Generalized RDEB     |
| 3       | 21          | Male | c.1837C>T    | c.5047C>T     | +   | -   | -   | -                       | Generalized RDEB     |
| 4       | 18          | Male | c.1637-1G>A  | c.5047C>T     | +   | -   | -   | -                       | Generalized RDEB     |
| 5       | 13          | Female | c.4478delA   | c.5047C>T     | +   | -   | -   | -                       | Generalized RDEB     |
| 6       | 14          | Male | p.G2233RfsX57| p.Q2488X      | -   | -   | -   | -                       | Generalized RDEB     |
| 7       | 15          | Male | p.G2233RfsX57| p.Q2488X      | -   | -   | -   | -                       | Generalized RDEB     |
| 8       | 14          | Female | c.4478delA   | c.5047C>T     | +   | -   | -   | -                       | Generalized RDEB     |
| 9       | 21          | Male | p.G2177AfsX29| p.G2177 AfsX29| +   | -   | -   | -                       | Generalized RDEB     |
| 10      | 33          | Female | c.6501G>A    | c.5048_5051dupGAAA | +   | -   | -   | -                       | Generalized RDEB     |
| 11      | 22          | Male | c.1837C>T    | c.5047C>T     | +   | -   | -   | -                       | Generalized RDEB     |
| 12      | 10          | Male | c.5009G>A    | c.5132_5133insS | +   | -   | -   | -                       | Generalized RDEB     |

B-VEC therapy may reduce cancer risk via mechanisms independent of insertional oncogenesis. Lethal squamous cell carcinoma, a devastating RDEB complication, arises from chronic wounding, inflammation and fibrosis. Any C7 replacement therapy, such as topical B-VEC, if implemented early in the wounding process, has the potential to halt wound chronicity and fibrosis, which in turn could reduce lethal squamous cell carcinoma development, one of the most devastating complications in severe RDEB patients. Thus B-VEC therapy may not only improve but also prolong the lives of patients with RDEB. Further studies are needed to address this.

B-VEC treatment promoted molecular correction of RDEB skin concurrent with durable wound closure for 3 months or longer. In this study, wound healing durability and resistance to blistering was potentially aided by the long half-life of C7 in human skin. Given that new trauma to other areas of skin can cause new blisters outside of previously treated wounds, it is anticipated that periodic B-VEC dosing of new wounds outside of previously treated wound areas will be necessary.

Whereas ectopic suprabasal C7 expression has been noted as a side effect in previous C7 gene replacement studies, in the current study C7 was correctly localized solely to the dermal–epidermal junction. Many previous RDEB skin studies examined C7 NC1 expression but not the C7 NC2 domain. Because the NC2 domain of C7 is essential for AF assembly and function, NC1 expression by itself has not always been found to correlate with AF formation in previous clinical studies. In contrast, this study examined both NC1 and NC2 expression as an indication of full-length functional C7 expression, both by immunofluorescence and immunoelectron microscopy. Although some variability was observed in NC1 and NC2 expression in this study, as has been noted in previous studies of COL7A1 gene replacement, when areas of C7 expression were noted, they were linear and continuous.

Keratinocytes and fibroblasts each contribute C7 to the dermal–epidermal basement membrane, and a recent study has suggested that expression of C7 by both cell types may be required for optimal formation of normal AFs. Although other COL7A1 gene replacement approaches have targeted either keratinocytes or fibroblasts, the ability of B-VEC to induce robust C7 expression in both cell types may offer a distinct advantage and may have contributed to the AF formation and wound healing seen in this study.

Limitations of this phase 1 and 2 trial include treatment of only open RDEB wounds with topical B-VEC as opposed to intact skin. B-VEC would not be expected to induce basal epidermal C7 expression when topically applied to intact skin because this vector has not
been shown to efficiently penetrate intact skin in animal models. When possible, wounds of similar location and size were selected. However, due to the random nature of patient wounding and limited patient numbers, some wounds were not fully matched in location and size. Another limitation was the inability to correlate wound healing with patient-reported outcomes of severity and pain. The reasons for this we believe are twofold, in that patients on the study continued to use their regular pain medications, and the wounds treated in this early exploratory trial represented only a small subset of the total wounding and disability burden experienced by the patients. The secondary objective of investigator wound image assessment was redundant given that the primary objective of complete wound healing assessed by the investigator involved evaluation of both remote and on-site images taken at weeks 8, 10 and 12, and hence it was not re-analyzed.

In this exploratory phase 1 and 2 trial involving patients with RDEB, repeat topical B-VEC applications were associated with durable wound closure and full-length cutaneous C7 expression and AF assembly with minimal adverse events. Future therapeutic directions deserving to be investigated include topical delivery to mucosal surfaces affected in RDEB such as oropharynx, esophagus, or eyes, and HSV-1 transgene delivery for other genetic diseases. In total, the preliminary conclusions and treatment advances described here have far-reaching implications with the potential to broadly transform the gene therapy field. A phase 3 study of B-VEC is underway (ClinicalTrials.gov: NCT04491604).

Online content
Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at https://doi.org/10.1038/s41591-022-01737-y.

Received: 26 April 2021; Accepted: 8 February 2022; Published online: 28 March 2022

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Methods

Study design. This completed study was a single-center open-label, intra-patient, randomized, phase 1 and 2 placebo-controlled trial conducted at Stanford University (first patient enrolled on 6 May 2018, last patient enrolled on 3 September 2019). Eligible male and female patients were at least 6 years old and had been diagnosed with generalized RDEB using clinical guidelines. RDEB diagnosis was molecularly confirmed and C7 serology was determined for each patient, as previously described.1,33

Patients had at least two skin areas (0.09-65.29 cm²) with at least one wound in each area at the time of enrollment. Of nine patients screened, all were enrolled in this trial (Fig. 2). One patient withdrew at day 30 due to travel inability. Three patients enrolled into a later trial phase following a 3 month wash-out period and were considered independent for wound efficacy analyses. The principal investigator selected at least two and up to three target wounds meeting the inclusion criteria.

A complete randomized-block design, with each patient designated as a block to receive all conditions, was used to assign treatment. Investigator-assigned wound numbers were randomized using a pre-generated randomization schedule. Randomization schemes were generated via a customized program prior to the study, and randomization assignments for the treatment sites at each study patient were provided in sealed envelopes. Once the wound pairs were identified and labeled, the randomization envelope was opened and the assigned dose was then given.

One wound was treated with placebo and the other(s) treated with B-VEC (Extended Data Table 2). Following the safety board review of initial results, pediatric patients were included, and total dosing was increased to 2–8 × 10⁶ pfu per wound per day.

The study was conducted in accordance with the 1996 Health Insurance Portability and Accountability Act and approved by the FDA Center for Biologics Evaluation and Research, and Stanford's institutional review board (NCT03366413, registered 20 April 2018). Written informed consent was obtained from patients or from the patients’ legally authorized representatives for participation in the study, as well as for publication of all recorded wounds observed during the study. Patients received compensation to cover travel costs and meals for the day of the visit. A safety review committee conducted periodic assessments. Data were collected by the principal investigator and staff. Krystal Biotech prepared the statistical analysis plan and performed the statistical analysis. Version 9.1.3 of the SAS statistical software package was used to generate summaries, listings, graphs and statistical analyses. The principal investigator prepared the first manuscript draft. All authors interpreted the data, collaborated in preparing the manuscript, and vouch for data accuracy, completeness, and fidelity of the trial description to the protocol and the reporting of adverse events presented within the full text of this article. All authors and their institutions had confidentiality agreements with the sponsor.

Study objectives. Phase 1. The primary objectives were to preliminarily assess the safety of topical B-VEC compared with placebo and to demonstrate evidence of molecular correction, that is, functional collagen VII (C7) expression and Aβ1, associated with B-VEC after treatment. The secondary objective was to preliminarily assess the effect of B-VEC on wound closure compared with placebo (wound surface area reduction, duration of wound closure). Other safety-related objectives were to assess changes in laboratory values and the condition of the patients (HSV and C7 antibodies, vital signs, physical examination).

Phase 2. The primary objective of the phase 2 trial was the further assessment of the effect of B-VEC on wound closure compared with placebo (wound surface area reduction, duration of wound closure, time to wound closure). An additional objective was to demonstrate evidence of molecular correction (functional C7 expression) associated with B-VEC after treatment.

Note that two additional secondary objectives were not reported in the manuscript. Patient-reported outcomes for pain and severity were not amenable to interpretation for two reasons: the patients on the study continued to use their regular pain medications, and the wounds treated in this early exploratory trial represented only a small subset of the total wounds and disability burden experienced by the patients. The investigator's global assessment analysis was not reported due to redundancy relative to the primary objective. The investigator evaluated both remote and on-site images taken at weeks 8, 10 and 12 for wound healing, and these images were not re-analyzed.

Inclusion criteria. To be eligible for inclusion, each patient fulfilled each of the following criteria: clinical diagnosis of the recessive form of dystrophic epidermolysis bullosa; age (initial three patients: 18 years or older; subsequent three patients: 5 years or older); willingness and ability to give consent or assent; confirmation of RDEB diagnosis on genetic testing, immunofluorescence and electron microscopy; LH24 antibody staining negativity and NC1 positivity (this criterion is applicable to the first two adults on the study; subsequent patients could be NC1 positive or negative); confirmed RDEB COL7A1 mutations; at least one wound between 5 and 10 cm² in wound area; and ability and willingness (in the opinion of the investigator) to understand the study, cooperate with the study procedures and to return to the clinic for all of the required follow-up visits.

Exclusion criteria. Patients were excluded from the study if any of the following criteria were met: medical instability limiting ability to travel to the investigative center; the presence of medical illness expected to complicate participation and compromise the safety of the treatment, such as active infection with HIV, hepatitis B or hepatitis C (as determined by hepatitis B surface antigen screening, detection of hepatitis C antibodies, or positive result of hepatitis C polymerase chain reaction (PCR) analysis); serum antibodies to type VII collagen demonstrated on ELISA, indirect immunofluorescence microscopy or Western blot, or cell-mediated immunity to ELISPOT (patients with negative results within 12 months of screening are eligible); active infection in the area that will undergo injection; evidence of systemic infection; known allergy to any of the constituents of the product; current evidence or a history of squamous cell carcinoma in the area that will undergo treatment; active drug or alcohol addiction; hypersensitivity to local anesthesia (lidocaine or prilocaine cream); receipt of a chemical or biologic study product for the treatment of RDEB in the past 3 months; specific wounds that have previously been treated with investigational gene or cell therapy; the use of systemic antibiotics in the past 7 days; positive pregnancy test or current breast-feeding; clinically significant abnormalities (grade 2 or higher on the National Cancer Institute (NCI) toxicity scale) on laboratory tests before treatment (except for the following specific exclusionary laboratory threshold results, subject to fair approval or epidermolysis bullosa physician: albumin <1.7 g dl⁻¹, leukocytes >20,000 μl⁻¹, hemoglobin <7.5 g dl⁻¹ (low hemoglobin is treated at the discretion of the investigators and the epidermolysis bullosa physician) and any additional exceptions made at the discretion of the investigators and the epidermolysis bullosa physician). All patients enrolled into a later trial phase following a 3 month wash-out period of the product; current evidence or a history of squamous cell carcinoma in the past 12 months of screening are eligible; active infection in the area that will undergo injection; evidence of systemic infection; known allergy to any of the constituents of the product; current evidence or a history of squamous cell carcinoma in the area that will undergo treatment; active drug or alcohol addiction; hypersensitivity to local anesthesia (lidocaine or prilocaine cream); receipt of a chemical or biologic study product for the treatment of RDEB in the past 3 months; specific wounds that have previously been treated with investigational gene or cell therapy; the use of systemic antibiotics in the past 7 days; positive pregnancy test or current breast-feeding; clinically significant abnormalities (grade 2 or higher on the NCI toxicity scale) identified through medical history and physical examination at day 0 (with the following exceptions: anorexia, can enroll up to grade 4 (inclusive); constipation, can enroll up to grade 2 (inclusive); dysphagia, can enroll up to grade 4 (inclusive); keratitis, can enroll up to grade 4 (inclusive); bone pain, can enroll up to grade 2 (inclusive); and additional exceptions made at the discretion of the investigators and the epidermolysis bullosa physician).

Clinical study treatment. The topical B-VEC gel or the placebo gel (produced according to Good Manufacturing Practice, GMP) was given dropwise by pipette uniformly across the surface area of the wound, without touching the wound itself, and not extending to normal skin surrounding the wound. Once B-VEC was given, it was applied to the wound, a non-adhesive bandage (Tegaderm, 3M Health Care) was placed over the treated area, creating a uniform spread of a thin layer of gel across the wound surface, which was then covered by a secondary bandage for padding (Mepilex, Mölnlycke), and self-clinging gauze (Kerlix) was used to hold the dressings in place. The patients were advised to leave the dressings in place for 24–48 h. After bandage change, the bandage materials in direct contact with the treated wound were disposed of in a biohazard bag.

The vehicle was a heat sterilized 3% METHOCEL™ aqueous gel, formulated in water, and manufactured to GMP standards by Veleco Pharmaceuticals. Several viscosities of this composition (ranging from 2% to 4%) were tested prior to the addition of 0.07% of the gel. In addition, other excipients including poloxamer 407 and F127 were evaluated at multiple concentrations. The 3% METHOCEL™ formulation had optimal compatibility with B-VEC, based on the maximal stability of the investigational product (B-VEC+gel) at multiple temperatures (2–8°C, ambient, 33°C) and the in vivo safety and efficacy in animal models. A volume of ~0.2–0.4 ml, a 1:1 ratio of B-VEC:gel and excipient gel was used for all B-VEC wounds ≥20 cm². Placebo-treated wounds received the same volume of excipient diluted in 0.9% saline gel.

Vector detection (blood and urine). Blood and urine samples were collected before treatment and on all scheduled visits, as often as amenable (blood and urine sampling is uncomfortable for patients with epidermolysis bullosa) and assessed for the presence of B-VEC DNA using a validated quantitative PCR assay.

Evaluation of immune response to HSV. Sera samples collected before treatment and during specific visits were evaluated for anti-drug antibodies against HSV with a neutralization plaque reduction test (PRNT) that included pool B-VEC. The PRNT assay determines the percent reduction in B-VEC-mediated plaque production in the presence of different dilutions of patient sera and is reported as PRNT₅₀, which is the serum dilution at which a 50% reduction in plaques is observed. An increase in PRNT₅₀ over time is suggestive of an increase in the presence of anti-HSV antibodies in the sera.

Evaluation of immune response to collagen VII. Sera samples, collected before treatment and at intervals during the study, were evaluated with an anti-collagen VII ELISA (EA 1947–4001 G, Euroimmun), which determines the levels of human collagen VII immunoglobulin G (a level ≥20 relative units (RU) ml⁻¹ is considered to be positive).

Wound imaging. Images were taken using an iPhone camera system with the WoundMatrix application loaded on the iPhone device. WoundMatrix is a complete mobile wound management and telehealth solution for the secure
capture, measurement and upload of images and data elements at the investigator-patient point of care. The WoundMatrix application requires a wound image with a predefined calibration marker (ruler in cm) placed on the same surface as the wound. The software allows the investigator or user to draw a line on the calibration marker that represents 1 cm. The software uses this line to count the number of pixels that the 1 cm represents. After the calibration process is complete, the software allows the user to trace the wound to determine its shape. Once the user completes the tracing, the software calculates the number of pixels (using predefined algorithms) for the irregular traced shape. The software uses this information to calculate the surface area using the number of pixels, the calibration line and the number of pixels in the irregular wound shape. All measurements are stored in the application and the investigator can create progress graphs to see the change in area, width and length over time. The application allows the investigator to create wound reports on specific patients to monitor the wound progression. To avoid overlap in wound areas, the investigator used a 1 cm predefined reference calibration. If a wound was completely closed, no tracing is required, and the image would be saved for documentation. If the image is captured off-site, the patient or their caregiver captured an image using the WoundMatrix application on their personal device. The clinical site used a mobile device to download the software at the first visit and trained the patient or their caregiver in how to capture images. They were sent home with imaging guidance provided by WoundMatrix to assist them when they were off-site. The patient had limited access to and no control regarding the measurement of the images. When off-site the patient made sure that the lighting was similar to the on-site lighting and cleaned and dried the wounds before imaging. Once an image was captured using the predefined calibration marker (ruler in cm) the patient or their caregiver used the descriptive image given on the device, created by the investigator, noting which region to capture and select before imaging (that is, planar (bottom) of the right foot). The image was uploaded to the investigator and they traced the wound through the application interface remotely to determine the surface area.

Evaluation of wound areas. Post hoc statistical analysis was performed on the wound measurement data obtained from patients in the phase 1 and 2 clinical study. Data from randomized wounds were pooled for wound closure efficacy analysis of B-VEC compared with placebo wounds. Wound data from patients 1-12 were not included in the analysis because the wound was a great deal larger than the others studied and the intent was to observe the impact of B-VEC on a large chronic wound.

Main protocol changes. Three key protocol amendments corresponding to the study phases were made: phase 1 (v1.0, 19Apr2018) phase 2a (v2.2, 08Oct2018), phase 2b (v3.1, 12Mar2019) and phase 2c (v4.0, 01Aug2019) (Extended Data Table 1). The protocol versions represent intended design that was amended in consultation and with approval from oversight committees and regulatory authorities.

After two patients were enrolled into phase 1, the protocol was amended to increase the frequency and p.f.u. level of doses and to include patients aged 5 years and older (to v2.2). After four patients were enrolled into phase 2a, the protocol was amended to administer B-VEC every 2-3 days to correspond with bandage changes and to increase the total number of doses. The dose level was set at 2×10^6 p.f.u. per wound per treatment.

Phase 2b enrolled five patients (Extended Data Fig. 3 and Extended Data Table 1). Three patients who participated in phase 2a also participated in phase 2b (Extended Data Fig. 3 and Extended Data Tables 1, 2). New, untreated wounds were selected for the patients who rolled over into the phase 2b portion of the protocol except for one chronic wound in patient 3 who became patient 11. For the re-enrolled patients, a wash-out period of 3 months passed between treatments in phases 2a and 2b. These patients were enrolled in phase 2a as patients 3, 5 and 6 and in phase 2b as patients 11, 8 and 7, respectively (Extended Data Fig. 3). (Note that the patient identification numbers run from 1 to 12 because although not all enrolled, there were 12 enrolled in total with unique identification numbers.) The primary differences between phase 2a and phase 2b were frequency and dose level. Phase 2b allowed for treatment at bandage changes, every 1-3 days, and the dose level was set at 2×10^6 p.f.u. per wound per treatment (Extended Data Table 1). Following v3.2, the protocol was amended to administer B-VEC in two cycles. The age of inclusion was lowered to 2 years old. Wound areas were increased to up to 50 cm², and with the increase in area the dose was increased to 6×10^6 p.f.u. per wound per treatment (see protocol appendix for further details).

Phase 2c enrolled one pediatric male patient (Extended Data Fig. 3 and Extended Data Table 1). One large, chronic wound larger than 60 cm² was treated with B-VEC. A similar wound, contralaterally located, was selected as the placebo wound. Due to the large surface area, 8×10^6 p.f.u. B-VEC was topically administered to the wound per dose. The placebo wound was treated with vehicle control. The wounds underwent two cycles of topical treatment: cycle 1 was 25 days and consisted of 20 treatments, and cycle 2 was 24 days and consisted of 21 treatments.

Statistical analysis. Two wound types were assessed: a more common variant, recurrent wounds, which repeatedly close and re-blister; and a less common variant, chronic wounds, of 12 weeks or more duration. In this study, patients could have either or both wound types. Considering the diverse wound nature and the local topical B-VEC treatment effect without systemic exposure, efficacy assessments and analyses were performed on wounds randomized to either B-VEC or placebo for each patient. Target wounds were imaged at trial visits and at home using the WoundMatrix phone application. Wound images were assessed by the blinded principal investigator and by two independent blinded physician-dermatologists with bullous disease expertise. Biopsies from intact B-VEC-treated skin were evaluated for percent C7 NC1 and NC2 expression compared with normal skin (non-RDEB) using immunofluorescence, and for Aβ4 using immunoelectron microscopy, as previously described. Safety assessments were conducted with a single systemic patient response as the independent outcome. Adverse events and concomitant medications were assessed at each visit. Blood and urine were assessed for B-VEC DNA using a validated qPCR assay, and blood was assayed for C7 and HSV-1 antibodies (Extended Data Fig. 4 and Supplementary Tables 1, 2).

Because this was a first-in-human topical gene therapy exploratory study in an ultra-rare disease, a formal sample size calculation was not performed. A priori, the study was powered to detect a 60% difference in the incidence of complete wound closure between B-VEC-treated wounds and placebo-treated wounds was performed on observed data (no imputed values). This expectation informed the timepoint selection for analysis.

After consultation with the regulatory agencies and upon further consideration, the original statistical analysis approach using the Cochran-Mantel-Haenszel test and the Breslow–Day test was modified considering the paired binary nature of endpoint measurements. To account for within-pair or within-patient correlations and prevent bias of treatment effect estimation and statistical inference, McNemar's test was used for the primary analysis of efficacy data (responder analysis) reported in Table 1.

Given the 2 month cutaneous half-life of C7 and the 4–8 week epidermis turnover rate, the durability of B-VEC wound closure is anticipated to last around 12 weeks. This expectation informed the timepoint selection for analysis (landmark analysis). Based on a responder definition of complete wound closure (>95% reduction in wound surface area from baseline) for at least 2 consecutive weeks (weeks 8 and 10, or weeks 10 and 12), responder analysis comparison of the incidence of complete wound closure between B-VEC-treated wounds and placebo-treated wounds was performed on observed data (no imputed values). This responder analysis included only patients for whom data points for either the week
8 and 10 timepoint, and/or the week 10 and 12 timepoint were collected (patients 3 and 5–10). The phase 1 protocol did not include collection of wound measurements at weeks 8, 10 and 12 (patients 1 and 2). Patient 4 dropped out of the study after initial treatment and was lost to follow-up (the patient was visited every two weeks) at weeks 8 and 10 due to an inability to remove the bandaging from the wound without causing significant discomfort to the patient. Patient 12’s wounds were outside of the size range of other wounds in the analysis and were excluded from the analysis. In Fig. 3c, in which the mean percent change in wound area was assessed by treatment and timepoint, all observed wound closure data for patients 1–11 were analyzed and are presented with P values based on the Wilcoxon rank-sum test.

Vector structure and description. B-VEC is a vector derived from wild-type HSV-1 through the deletion of two copies of the viral immediate-early gene ICP4, rendering the vector replication incompetent; in addition, ICP22 was also deleted to reduce the cytotoxic effect (the vector diagram is given in Supplementary Fig. 1). Two full-length copies of the human COL7A1 gene, each with their own expression control elements, were then independently inserted into each ICP4 locus.

Vector production. B-VEC was produced in a GMP-certified engineered cell bank infected with the same E7 virus seed stock. Virus stocks were stored at −80 °C until just before use. The vehicle used was Dulbecco's phosphate-buffered saline + 10% glycerol.

Cell culture. Cells were previously isolated from skin biopsies taken as part of routine surgical or diagnostic procedures. All cells were cultured at 37 °C in 5% CO2. RDEB and normal human fibroblasts were grown in DMEM (Corning) supplemented with 10% FBS (PEAK Serum, cat. no. PS-FB1). RDEB and normal human keratinocytes were cultured in a 1:1 mix of defined keratinocyte serum-free medium (SMF; Life Technologies) and Medium 154 (Cascade Biologies) at 37 °C in a humidified 5% CO2 incubator.

Virus infection. Viral aliquots were stored at −80 °C and thawed 10 min before infection. Multiplicity of infection was calculated from the virus titre and target cell number and the appropriate volume of virus stock was diluted in DMEM and incubated with the target cells for 2 h at 37 °C. Virus was then removed, inactivated with acetic acid and discarded, and fresh media was supplied to target cells after washing twice with pre-warmed media.

Western blot analysis. The cell lysate was prepared as follows: 8 × 105 fibroblasts or keratinocytes were plated in a 100 mm dish to achieve 70–80% confluence the following day. At 48 h after infection, cells were lysed by lysozyme-riboimmunoprecipitation assay buffer. Lysate was centrifuged at 13,500 g for 5 min at 4 °C, and the supernatant was mixed with a 6x Laemmli loading buffer. Before loading onto the SDS–PAGE gel, the samples were heated for 5 min at 95 °C. For C7 detection, 5–30 μg protein was loaded onto a 6% acrylamide gel. The primary antibodies used were C7 polyclonal rabbit antibody (Sigma prestige Ab, cat. no. HPA042420), GAPDH and β-actin (Santa Cruz Biotechnology). Resolved proteins were transferred onto nitrocellulose membrane with a BioRad Trans-Blot-Turbo (BioRad), blocked in PBS and 0.1% Tween with 5% milk or 5% BSA according to the requirements of the primary antibody, and incubated overnight with the primary antibody. After incubation with HRP-conjugated secondary antibody (Santa Cruz Biotechnology), the membrane was incubated with Pierce ECL western blotting substrate (ThermoFisher Scientific) and exposed to CL-XPose X-ray film (ThermoFisher Scientific). C7 was quantified by densitometry (ImageJ v1.52), using a known concentration of purified recombinant C7 (donated by Krystal Biotech) for comparison.

Protein quantification. Total cell lysates were quantified with the Pierce BCA (bicinchoninic acid) Protein Assay Kit (ThermoFisher Scientific) and proteins were loaded onto an SDS–PAGE gel. The Western blot signal was quantified with ImageJ v1.52. Type VII collagen was quantified relative to the non-treated control, GAPDH, and to purified recombinant type VII collagen supplied by Krystal Biotech.

qRT–PCR. RNA was isolated using the RNeasy Mini Kit (Qiagen) according to the manufacturer’s instructions. RNA extractions were quantified using a NanoDrop Spectrophotometer (ThermoFisher Scientific), and 1.5 μg RNA was used for complementary DNA synthesis with the SuperScript III First-Strand Synthesis System (ThermoFisher Scientific). For detection of COL7A1 expression the following primers were used: forward primer, GCCGCTCAATCCTCGTACG; reverse primer, CTGTGAGGCAACTCGCTTCA. For ACTB amplification the reverse primer, CTGTGAGGCAACTCGCTTCA. For ACTB amplification the forward primer, CTGTGAGGCAACTCGCTTCA. For ACTB amplification the reverse primer, CTGTGAGGCAACTCGCTTCA. For qPCR, the SYBR Select Master mix (Life Technologies) was used and cDNA samples were diluted 1:25 to serve as the template. Experiments were performed in triplicate.

Animals. A colony of homozygous Col7a1tm1mm mice, which are strain expressing only 10% of the amount of murine type VII collagen found in normal mouse skin, was used. These mice have a phenotype similar to severe generalized human RDEB21. These were housed under a 12 hr light-dark cycle, temperature of 22–23 °C and 40–60% humidity.

Preclinical B-VEC viral vector application. Before and during the test article treatment, mice were maintained under inhalation anesthesia using 2% isoflurane. Eye ointment (Paralube Vet) was applied on the eyes to prevent dryness. The back and flank area was shaved using an electrical pet clipper and the area was wiped with an alcohol wipe. The viral vector was kept on dry ice until 10–15 min before injection, thawed at room temperature, and used within 15–30 min of thawing.

For intradermal injections the animal’s back was shaved and the mouse was injected at four sites on the back, consisting of one control vehicle injection and three viral vector injections. Introducers were performed using the Mantoux technique with a 31 G BD SafetyGlede 0.5 ml insulin syringe, ensuring the delivery of a superficial wheal at each site. Up to four intradermal injections were given to the back of each mouse at the doses specified in the Results section. The edges of the wheal were marked with a permanent marker. All mice received a dose of 4.6 × 10−4 p.f.u. per 50 μl per injection site by intradermal injection. In some experiments intradermal injection of the DME injection was made at day 3. For RDEB mouse topical applications, a full-thickness 8-mm-diameter wound on the animal’s mid-back was locally anesthetized and covered with Tegaderm (3M Health Care), and the virus was injected into the space between the Tegaderm and wound surface. The Tegaderm was preserved on a mouse until tissue collection (2, 7, 10, 14 days). For xenograft topical applications the virus was injected into the Tefla non-adherent pad directly in contact with the xenograft surface.

3D xenograft preparation. Primary fibroblasts and keratinocytes cultured from a skin biopsy of patient with RDEB lacking C7 NC1 and NC2 expression were used to seed on porcine dermalized dermis. To produce a composite skin graft preparation, porcine dermalized dermis was cut into 2 × 2 cm square pieces and left to dry under aseptic conditions at the bottom of the 6-well tissue culture plate for 24 h. Fibroblasts were seeded from the bottom of the dermis and grown for 3 days. At day 4, 5 × 104 keratinocytes were resuspended in a maximal volume of 150 ml of 50/50 V and placed in the middle of the dermis to avoid spillage of the cells onto the plate surface. The dermis squares were placed on special frames, to allow nutrients access to both sides of the graft. After another 2 h, wells were filled with 2.5 ml keratinocyte growth medium and allowed to grow further for at least 5 days. Next, the composite skin graft was removed from the plate with forceps touching only one side of the graft and used immediately for transplantation as described in the following section.

Graft transplantation. Mouse grafting was performed as previously described. In brief, 6–8-week-old mice were anesthetized with inhalation of 1.5–2% isoflurane. After shaving the hair from the mouse back, a rectangular region of mouse skin (~1.6 × 1.4 cm) was removed with scissors and the composite graft was sutured to the mouse skin using an interrupted stitch technique. Both epidermal and epidermal components were kept moist with sterile culture medium throughout the transplantation procedure. Non-adherent dressing (Telfa; Tyco Healthcare/ Kendall) was cut into 2 × 2 cm squares and placed on top of the graft. Next, Tegaderm dressing (3M Health Care) was wrapped around the mouse and then covered with a Celpak sterile dressing (BSN Medical). Last, a double layer of CoFlex (Andover Healthcare) was wrapped around the mouse. The mouse was subcutaneously injected with carprofen analgesic in the vicinity of the graft. The dressing was removed 10–14 days after grafting and the grafts were then further characterized.

Tissue collection. Before tissue collection, animals were euthanized by CO2 inhalation followed by cervical dislocation. The back area was shaved, as needed to identify the marked injection sites or topical application areas, and the treated sites were biopsied using sharp scissors. Approximately one-half was snap-frozen in liquid nitrogen for qPCR analysis and the other half was cryopreserved in optimal cutting temperature (OCT) compound.

Histologic analysis. The cryopreserved tissues in OCT compound were sectioned at a thickness of 5–8 μm and left to air dry for up to 1 h. The slices were dipped in 100% MeOH for 10 min at −20 °C and left to air dry. Methanol-fixed sections were rehydrated in PBS for 5 min at room temperature. The slices were analyzed using hematoxylin and eosin (H&E) staining or indirect immunofluorescence.
microscopy. H&E staining was performed using standard procedures with Weigert’s modified hematoxylin (HEXWHALE 100) and Eosin Y solution (HT110116). Indirect immunofluorescence microscopy was performed as previously described44. Primary antibodies were incubated for 16 h at 4 °C after 1 h of incubation at 20 °C with fluorescence (4',6-diamidino-2-phenylindole) for nuclei staining. The stained sections were mounted with mounting media (Fluoromount G, cat. no. 0100-01, Southern Biotech) and overlaid with a glass coverslip. Antibodies used for the staining are listed in Supplementary Table 5. All imaging and image processing (fluorescent and H&E), including tiling of the images, was done using AxiosVision SE64 v4.9.1 by Zeiss.

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Acknowledgements
The authors thank A. Ponakala, S. Boddu, P. Khau and T. Parry for their assistance with this study, and all of the patients who participated in this trial. This study was funded by Krystal Biotech through a sponsored research award administered through the Stanford University Office of Research Management. M.P.M. also received funding from the Office of Research and Development at the Palo Alto Veterans Affairs Medical Center.

Author contributions
M.P.M. had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Concept and design: M.P.M., A.P.S., S.M.K. Acquisition, analysis, or interpretation of data: M.P.M., I.G., K.S., V.K., V.K.Y., S.F.T., D.R.K., J.A.D. IS.B. Drafting of the manuscript: M.P.M. Critical revision of the manuscript for important intellectual content: all authors. Statistical analysis: M.P.M., S.M.K. Obtained funding: M.P.M. Administrative technical, or material support: S.M.K., P.A., P.P.Z., S.O., H.L., N.R., N.S., M.O., A.P.S., M.P. Supervision: M.P.M., S.M.K. Working in concert with the principal investigator (M.P.M.), the sponsor provided suggestions on the design and conduct of the study, reviewed the data and reviewed the manuscript. M.P.M. made the final decision to submit the manuscript to Nature Medicine for publication.

Competing interests
M.P.M. received funding from Krystal Biotech to conduct this study through a sponsored research award administered through the Stanford University Office of Research Management. M.P.M. is also an investigator for the following companies that are studying molecular corrective therapies for recessive dystrophic epidermolysis bullosa: Castle Creek Pharmaceuticals, Abeona Therapeutics, WINGS therapeutics and Phoenix Tissue Repair. A.P.S. owns stock in Krystal Biotech. P.A., PPZ. and S.O, as well as H.L., N.R., N.S., M.O. and S.M.K. are employees of Krystal Biotech. All other authors have no competing interests.

Additional information
Extended data are available for this paper at https://doi.org/10.1038/s41591-022-01737-y.
Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41591-022-01737-y.
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Peer review information Nature Medicine thanks David Schaffer and the other, anonymous, reviewer(s) for their contribution to the peer review of this work. Anna Maria Ranzoni was the primary editor on this article and managed its editorial process and peer review in collaboration with the rest of the editorial team.
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Extended Data Fig. 1 | Co-expression of C7 with B-VEC viral markers. RDEB fibroblasts and keratinocytes were infected in vitro with B-VEC vector (top 2 rows). Representative of 3 replicates for each condition. RDEB mice were injected intradermally with B-VEC vector (bottom row). Representative of 3 mice; 3 treated back areas per mouse. Co-expression of C7 (yellow) and ICP0 (pink) is shown in RDEB fibroblast culture (top panel), keratinocyte culture (middle panel), and in mouse skin (bottom panel). Scale bar is 100 μm. B-VEC, beremagene geperpavec; RDEB, recessive dystrophic epidermolysis bullosa.
Extended Data Fig. 2 | In vivo co-expression of B-VEC and keratinocyte, fibroblast-specific markers. Three days following B-VEC injection, RDEB mouse skin was biopsied and analyzed by dual label immunofluorescence microscopy using antibodies to the fibroblast marker vimentin (red, arrow upper panel), or antibodies to keratin 14 (yellow, arrow lower panel) combined with antibodies to ICP0. Scale bar is 50 μm. Representative of 3 mice; 3 treated back areas per mouse. B-VEC, beremagene geperpavec; RDEB, recessive dystrophic epidermolysis bullosa.
Extended Data Fig. 3 | Overview of Phase 1 and 2 study design. *Three (3) patients were enrolled into both Phase 2a and Phase 2b. These patients’ Phase 2b wounds were treatment-naïve except for chronic (5 year) dorsal foot wound on Pt 3. A wash-out period of 3 months passed between treatments in Phase 2a and 2b. Patients were enrolled in Phase 2a as Pts 3, 5, and 6, and in Phase 2b as Pts 11, 8, and 7, respectively. 1Phase 1, 2a, 2b, 2c; all interventions all wounds. 2Phase 1, 2a, 2b; randomized wounds (Pt 12, 2c considered separately for efficacy). 3Patients were enrolled in Phase 2a as Pts 3, 5, and 6 and in Phase 2b as Pts 11, 8, and 7, respectively. Pt, Patient.
Extended Data Fig. 4 | Patient antibody analysis. A. Quantification of collagen VII antibodies in patient sera. Patient sera was collected at baseline (day 0) or at indicated times following initiation of B-VEC treatment and analyzed for presence of collagen VII IgG antibodies using ELISA. A level greater than or equal to 20 reference units (RU) per mL is considered to be positive. B. Quantification of HSV-1 antibodies in patient sera. Patient sera was collected at baseline (day 0) or at indicated times following initiation of B-VEC treatment and analyzed for the presence of antibodies against HSV using a qualified Plaque Reduction Neutralization Test (PRNT), which utilizes B-VEC. *Patients 3, 5, and 6 subsequently re-enrolled as Patients 11, 8, and 7, respectively. B-VEC, beremagene geperpavec; ELISA, enzyme-linked immunosorbent assay; HSV-1, herpes simplex virus type 1; IgG, immunoglobulin G; RDEB, recessive dystrophic epidermolysis bullosa.
Extended Data Fig. 5 | B-VEC treatment of large chronic wound. Results of Patient 12 treatment after two cycles of topical B-VEC therapy. Left panel shows presence of chronic wound 1 year prior to treatment. Center panel shows baseline appearance. Right panel shows the wound following B-VEC treatment. B-VEC, beremagene geperpavec.
Extended Data Fig. 6 | Patient 12, % change from baseline over time. The percent change in wound surface area for both B-VEC and placebo wounds was calculated over the course of treatment of Patient 12 and was plotted as a bar graph (end of cycle, % change area of wound from baseline). B-VEC, beremagene geperpavec.
Extended Data Table 1 | Summary of protocol phases/versions and amendments submitted to FDA and oversight committees

| Trial Phase | Protocol [version/date] | Number of Patients Enrolled | Wound Size | Number of Wounds Treated Per Patient [topical] | B-VEC Dose | Dosing days |
|-------------|-------------------------|-----------------------------|------------|---------------------------------------------|------------|------------|
| 1           | v1.0, 19Apr2018         | Two (2) adults; Pts 1, 2    | Up to 10cm²| Two (2): one B-VEC, one placebo             | 1e8 PFU/wound/day | 0, 2, 14, 28, 30 |
|             |                         | Six (6) patients planned    |            |                                             |            |            |
|             |                         | Four (4) [2 adults, 2       |            | Three (3): two B-VEC, one placebo          | 3e8 PFU/wound/day | 1, 2, 3, 4, 5, 30, 60, 90 |
|             |                         | children aged 13 and 14],  |            | with the option to escalate to 6e8          |            |            |
|             |                         | Pts 3², 4, 5, 6²           |            | PFU/wound/day                               |            |            |
|             |                         | Four (4) patients planned  |            |                                             |            |            |
|             |                         | Five (5) [3 adults, 2      |            |                                             |            |            |
|             |                         | children aged 14 and 15],  |            |                                             |            |            |
|             |                         | Pts 7², 8², 9, 10, 11²    |            |                                             |            |            |
|             |                         | Up to 12 patients          |            |                                             |            |            |
|             |                         | planned, including phase   |            |                                             |            |            |
|             |                         | 1 and phase 2              |            |                                             |            |            |
|             |                         | One (1) child aged 10;    |            |                                             |            |            |
|             |                         | Pt 12                      |            |                                             |            |            |
|             |                         | Up to 14 patients          |            |                                             |            |            |
|             |                         | planned, including phase   |            |                                             |            |            |
|             |                         | 1 and phase 2              |            |                                             |            |            |
|             |                         | this version intends to    |            |                                             |            |            |
|             |                         | enroll up to 3 patients    |            |                                             |            |            |
|             |                         | Up to 50 cm²               |            |                                             |            |            |
|             |                         | Up to 3; two B-VEC, one    |            |                                             |            |            |
|             |                         | placebo                    |            |                                             |            |            |

B-VEC has been administered to nine (9) patients, in twelve (12) siloed participant treatment applications. Patients (IDs: Pts 1-12) enrolled separately onto one of 4 versions of the Phase 1 and 2 protocol. A summary of the protocols and patients enrolled are presented below. *Three patients were enrolled into both Phase 2a and Phase 2b. These patients’ Phase 2b wounds were treatment-naive except for a chronic (5 year) dorsal foot wound on Pt 3. A wash-out period of 3 months passed between treatments in Phase 2a and 2b. Patients were enrolled in Phase 2a as Pts 3, 5, and 6 and in Phase 2b as Pts 11, 8, and 7, respectively. Pts # refer to patient identification numbers 1-12; nine total patients enrolled; three enrolled in both 2a and 2b with unique identification number. Protocol versions represent intended design which was amended in consultation and with approval from oversight committees and regulatory authorities; protocol amendments correspond to Phases (1, 2a, 2b, 2c). *v3.2 clarified the long-term follow-up. B-VEC, beremagene geperpavec; Pt, Patient.
## Extended Data Table 2 | Patient wound disposition and treatment

| Pt | Wound | Size (cm²) | Wound Location               | Therapy | Dose | Study Days in Which Dosing Occurred |
|----|-------|-----------|------------------------------|---------|------|-----------------------------------|
| 1  | 1     | 5         | Anterior upper right thigh   | B-VEC   | 1e8  | 1, 3, 29, 31                      |
|    | 2     | 5.86      | Anterior upper right thigh   | Placebo |      |                                   |
| 2  | 1     | 6.61      | Posterior right forearm      | Placebo | 1e8  | 1, 3, 15, 29, 31, 43              |
|    | 2     | 2.68      | Anterior left forearm        | B-VEC   |      |                                   |
| 3  | 1     | 10.1      | Lateral left upper arm       | B-VEC   | 3e8  | 1, 2, 3, 4, 5, 16, 34             |
|    | 2     | 3.1       | Medial left upper arm        | Placebo |      |                                   |
|    | 3     | 6         | Medial right foot            | B-VEC   |      |                                   |
| 4  | 1     | 12.5      | Posterior right elbow        | B-VEC   | 6e8  | 1, 2, 3, 4, 5                     |
|    | 2     | 5         | Posterior left elbow         | B-VEC   |      |                                   |
|    | 3     | 2.3       | Anterior left wrist          | Placebo |      |                                   |
| 5  | 1     | 3.6       | Anterior right knee          | Placebo | 3e8  | 1, 2, 3, 4, 5, 36                 |
|    | 2     | 15.6      | Medial lower right leg       | B-VEC   |      |                                   |
|    | 3     | 14.9      | Dorsum right foot            | B-VEC   |      |                                   |
| 6  | 1     | 9.7       | Anterior right upper arm     | B-VEC   | 3e8  | 1, 2, 3, 4, 5, 34, 41             |
|    | 2     | 6.2       | Lateral left breast          | B-VEC   |      |                                   |
|    | 3     | 15.2      | Scapular right back          | Placebo |      |                                   |
| 7  | 1     | 1.31      | Anterolateral right arm      | B-VEC   | 2e8  |                                   |
|    | 2     | 1.95      | Anteromedial right arm       | B-VEC   |      |                                   |
|    | 3     | 0.89      | Right underarm               | Placebo |      | 1, 4, 6, 8, 11, 13, 15, 18, 29, 71, 96 |
| 8  | 1     | 3.81      | Lateral right ankle          | Placebo | 2e8  |                                   |
|    | 2     | 2         | Anterior right leg           | B-VEC   |      |                                   |
|    | 3     | 12.91     | Posterior right ankle        | B-VEC   |      | 1, 3, 5, 9, 12, 15, 17, 29, 64, 89 |
| 9  | 1     | 4.65      | Lateral right thigh          | Placebo | 2e8  |                                   |
|    | 2     | 3.16      | Lateral right thigh          | B-VEC   |      |                                   |
|    | 3     | 9.21      | Lateral left shoulder        | B-VEC   |      | 1, 2, 5, 8, 10, 12, 15, 33, 61, 92 |
| 10 | 1     | 7.02      | Anterior right forearm       | B-VEC   | 2e8  | 1, 2, 5, 8, 10, 12, 15, 33, 64, 97 |
|    | 2     | 16.06     | Anterior left underarm       | Placebo |      |                                   |
|    | 3     | 3.27      | Anterior right forearm       | Placebo |      |                                   |
| 11 | 1     | 2.2       | Posterior right upper arm    | B-VEC   | 2e8  | 1, 2, 5, 8, 10, 12, 15, 33, 37, 38, 60, 92 |
|    | 2     | 1.36      | Posterior right upper arm    | Placebo |      |                                   |
|    | 3     | 3.94      | Medial right foot            | B-VEC   |      |                                   |
| 12 | 1     | 65.29     | Left infrascapular and lumbar| B-VEC   | 8e8  | Cycle 1: 1, 2, 5, 7, 8, 10, 11, 12, 14, 15, 16, 17, 18, 19, 21, 22, 23, 24, 25, 26 Cycle 2: 38, 39, 40, 41, 42, 43, 44, 46, 47, 48, 50, 51, 52, 53, 54, 55, 57, 58, 59, 60, 61 |
|    | 2     | 36.17     | Right infrascapular and lumbar| Placebo |      |                                   |

Note: Patient 3 in Phase 2a was enrolled in Phase 2b as Patient 11. Patient 5 in Phase 2a was enrolled in Phase 2b as Patient 8. Patient 6 in Phase 2a was enrolled in Phase 2b as Patient 7. *Patient 4 enrolled in Phase 2a and dropped out after initial dosing.
| Patient | Adverse Event               | Start Date | End Date  | IP Related | Placebo Related | Severity | Outcome | Treatment                      |
|---------|----------------------------|------------|-----------|------------|----------------|----------|---------|--------------------------------|
| 3       | diarrhea                   | 12/6/18    | 12/9/18   | unrelated   | unrelated       | mild     | resolved| none                           |
| 4       | nausea                     | 12/19/18   | 12/20/18  | unrelated   | unrelated       | mild     | resolved| none                           |
| 5       | streptococcal pharyngitis  | 2/7/19     | 2/16/19   | unlikely   | unrelated       | mild     | resolved| concomitant medication         |
| 7       | bacterial skin infection   | 4/24/19    | 5/6/19    | unrelated   | unrelated       | mild     | resolved| concomitant medication         |
| 7       | penicillin allergy         | 5/3/19     | 5/6/19    | unrelated   | unrelated       | mild     | resolved| other                          |
| 8       | bacterial skin infection   | 8/26/19    | 9/5/19    | unrelated   | unrelated       | mild     | resolved| concomitant medication         |
| 8       | application site discharge | 5/22/19    | 5/22/19   | probable   | unrelated       | mild     | resolved| non-drug treatment             |
| 8       | application site discharge | 5/22/19    | 5/22/19   | probable   | unrelated       | mild     | resolved| none                           |
| 8       | respiratory congestion     | 6/17/19    | 6/17/19   | unrelated   | unrelated       | mild     | resolved| concomitant medication         |
| 9       | itching: facial redness    | 8/16/19    | 8/18/19   | unlikely   | unrelated       | moderate | resolved| concomitant medication         |
| 9       | fever                      | 6/18/19    | 6/29/19   | possible    | unrelated       | mild     | resolved| concomitant medication         |
| 10      | peculiar taste             | 6/17/19    | 6/17/19   | possible    | unrelated       | mild     | resolved| none                           |
| 10      | application site rash      | 6/18/19    | 6/18/19   | possible    | unrelated       | mild     | resolved| none                           |
| 10      | generalized rash           | 8/19/19    | 8/19/19   | unrelated   | unrelated       | mild     | resolved| concomitant medication, other  |
| 10      | bacterial vaginosis        | 9/9/19     | 9/20/19   | unrelated   | unrelated       | mild     | resolved| none                           |
| 10      | generalized rash           | 9/20/19    | 9/20/19   | unrelated   | unrelated       | mild     | resolved| none                           |
| 11      | application site itching   | 6/21/19    | 6/21/19   | possible    | unrelated       | mild     | resolved| none                           |
| 11      | application site bruising  | 6/24/19    | 6/24/19   | unrelated   | unrelated       | mild     | resolved| non-drug treatment, other      |
| 11      | Pseudomonas skin infection | 7/19/19    | 7/19/19   | unrelated   | unrelated       | mild     | resolved| non-drug treatment, other      |
| 12      | nasogastric tube insertion | 10/14/19   | 10/14/19  | unrelated   | unrelated       | mild     | resolved| non-drug treatment             |
| 12      | generalized rash           | 10/27/19   | 10/28/19  | unrelated   | unrelated       | mild     | resolved| non-drug treatment, other      |
Extended Data Table 4 | Supplemental analysis of reduction in wound surface area

| Week | B-VEC | Placebo | Treatment Difference |
|------|-------|---------|----------------------|
|      |       |         |                      |
| **CBL Wound Area ≥ 90%** |
| 8    | 14/17 (82.4%) | 0/8 (0%) | 82.4%                |
| 10   | 12/16 (75%)  | 2/6 (33.3%) | 41.7%                |
| 12   | 12/14 (85.7%) | 1/7 (14.3%) | 71.4%                |
| **CBL Wound Area = 100%** |
| 8    | 14/17 (82.4%) | 0/8 (0%) | 82.4%                |
| 10   | 12/16 (75%)  | 1/6 (16.7%) | 58.3%                |
| 12   | 9/14 (64.3%) | 1/7 (14.3%) | 50.0%                |
| **CBL Wound Area ≥ 75%** |
| 8    | 14/17 (82.4%) | 2/8 (25%) | 57.4%                |
| 10   | 14/16 (87.5%) | 2/6 (33.3%) | 54.2%                |
| 12   | 12/14 (85.7%) | 1/7 (14.3%) | 71.4%                |

CBL, change from baseline.
Reporting Summary

Nature Portfolio wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Portfolio policies, see our Editorial Policies and the Editorial Policy Checklist.

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

- The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
- A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
- The statistical test(s) used AND whether they are one- or two-sided
  
  *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
- A description of all covariates tested
- A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
- A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
- For null hypothesis testing, the test statistic (e.g. F, t, r) with confidence intervals, effect sizes, degrees of freedom and P value noted
  
  *Give P values as exact values whenever suitable.*
- For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
- For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
- Estimates of effect sizes (e.g. Cohen’s d, Pearson’s r), indicating how they were calculated

*Our web collection on statistics for biologists contains articles on many of the points above.*

Software and code

Policy information about availability of computer code

Data collection

Data was collected using case report forms and data was entered into an electronic data capture system. All imaging and image processing (fluorescent and H&E), including tile images was done using AxioVision SE64 Rel. 4.9.1 Software by Zeiss. Densitometry of Western Blots was performed using ImageJ software v1.52. Images were taken using an iPhone camera system with the Wound Matrix application that was loaded on the iPhone device.

Data analysis

Version 9.1.3 of SAS statistical software package was used to generate summaries, listings, graphs and statistical analyses. Figure 1 data plots, including error bars and p-values, were generated using GraphPad Prism software v8.3.0.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. Github). See the Nature Portfolio guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

All requests for data will be reviewed by the leading clinical site, Program in Epithelial Biology and Department of Dermatology, Stanford University School of Medicine, and the study sponsor, Krystal Biotech, to verify whether the request is subject to any intellectual property or confidentiality obligations. Requests for
Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

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For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-list.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size
This was an exploratory study to evaluate the safety and dose evaluation of B-VEC in DEB patients and hence no formal sample calculation was done. The pharmacology studies in diseased DEB animal models clearly demonstrated B-VEC was able to produce functional COL7 that is needed to treat the patient’s underlying disease condition. The main objectives of the Phase 1/2 study in DEB patients were to evaluate safety, molecular correction and preliminary efficacy in 10 to 12 subjects. Results from completed study in this small number of patients clearly demonstrated safety, molecular correction and preliminary efficacy. Also, post hoc responder analysis of the clinical data from the Phase 1/2 study was sufficient for the calculation of an effect size that helped in determining the sample size for the pivotal Phase 3 study without the need for more additional patients in the Phase 1/2 study.

Data exclusions
Data from two subjects was excluded from the efficacy analysis. The pooled primary clinical efficacy data set consisted of wounds from 11 subjects. Three subjects rolled over from phase 2b to phase 2c and were then re-counted as new subjects. The two patients were included in the responder analysis of the observed data because as per FDA guidance for efficacy wound healing had to be demonstrated at two consecutive time points weeks 8 and 10 or weeks 10 and 12. One of the patient dropped out of the study after 30 days after the initial dosing due to an inability to travel and had missing data that could not be imputed. The other subject was part of an exploratory evaluation for treatment of large chronic wound and was pre-established to be excluded from the responder analysis.

Replication
Three subjects rolled over from Phase 2a to 2b portion of the study however different wounds within the subjects were treated and evaluated except for one wound of a subject that did not close during the 2a phase was re-treated in phase 2b.

Replicates:
Figure 1
a–d: n=3 (wells for each condition);
e, f, g, h bottom: Representative images of 3 injections (3 mice) of B-VEC and 3 injection sites of placebo (PBS); i: Representative images of 3 injections (3 mice) of B-VEC with 2 injections (doses) each;
j: Representative images of 4 injection sites (4 mice) of B-VEC for each dose, high and low;
k: Representative of 8 grafts treated with B-VEC (mice) and 2 placebo;
l: Representative of 8 grafts treated with B-VEC and 2 grafts treated with placebo.

Randomization
The study has a complete randomized-block design in which each subject serves as a block to receive all of the treatment conditions.

Blinding
The study was an open label randomized study in which complete closure of the treated wounds were evaluated by the investigator at weeks 8, 10 and 12. In addition, complete wound closure evaluation at the 8, 10 and 12 week time points were conducted by two blinded evaluators. The blinded evaluation results showed similar trends in efficacy. For preclinical studies, investigators evaluating immunofluorescence microscopy and electron microscopy images were blinded. For in vitro western blot analysis experiments, investigators evaluating densitometry were blinded.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

**Materials & experimental systems**

- n/a
- Involved in the study
- Antibodies
- Eukaryotic cell lines
- Palaeontology and archaeology
- Animals and other organisms
- Human research participants
- Clinical data
- Dual use research of concern

**Methods**

- n/a
- Involved in the study
- ChiP-seq
- Flow cytometry
- MRI-based neuroimaging
Antibodies

Antibodies used

Table S5 WITH additional Ab and dilution column

| Target molecule | Origin/isotype/type | Primary/Secondary | source | CAT#      | dilution |
|-----------------|---------------------|-------------------|--------|-----------|----------|
| Human type VII collagen | Rabbit, monoclonal, IgG | Primary           | Sigma  | HP6042420 | 1:250    |
| Integrin alpha 6 (clone 4G10) | Rat, IgG | Primary           | BD Biosciences | 552734 | 1:500    |
| Anti-human type VII collagen NC1 domain antibody (clone NP185) | Mouse monoclonal IgG | Primary           | Lab    | Reference 3 | 1:100    |
| Anti-human type VII collagen NC2 domain antibody (clone U16A) | Mouse monoclonal IgM | Primary           | Lab    | Reference 2 | 1:5      |
| Anti-laminin 332 antisera (okal) | Rabbit polyclonal IgG | Primary           | Lab    | Reference 3 | 1:300    |
| Anti-Rabbit AF647 | Goat anti-Rabbit IgG | Secondary         | Invitrogen | A21243 | 1:400    |
| Anti-Rat AF594 | Goat anti-Rat IgG | Secondary         | Invitrogen | A11007 | 1:400    |
| Anti-mouse M488 Mouse IgM | Goat anti-Mouse IgM | Secondary         | Invitrogen | A-21042 | 1:400    |
| Anti-mouse IgG-AF594 | Goat Anti Mouse | Secondary         | Invitrogen | A11032 | 1:400    |
| Anti-Rb-594 | Goat anti-Rabbit | Secondary         | Invitrogen | A32740 | 1:400    |
| GAPDH | GADPHSG-91 mouse monoclonal IgG | Primary | Santa Cruz | SC-355960-2020 | 1:200    |

Validation

Antibodies were validated in previous publications or from manufacturer data sheets.

Animals and other organisms

Policy information about studies involving animals: ARRIVE guidelines recommended for reporting animal research

Laboratory animals

B6C3F1 mice were obtained from The Jackson Laboratory. For xenografting, NOD/SCID mice were used (NOD.CB17-PrkdcSCID/J mice; stock 001303; The Jackson Laboratory).

Col7a1f1Neo mice were established from a breeding pair donated by Dr. Leena Bruckner-Tuderman (Freiberg, Germany). Mice 6 to 8 weeks old were used. Animals were housed with the following conditions: 14 hour light/10 hour dark cycle, temperature of 18-23 degrees C, 40-60% humidity.

Wild animals

No wild animals were used.

Field-collected samples

No field-collected samples were used.

Ethics oversight

Stanford APLAC committee approved all animal studies.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Human research participants

Policy information about studies involving human research participants

Population characteristics

DEB is a ultra rare genetic disease where the patients have a mutation in the COL7A1 gene and are unable to produce functional COL7 protein. The population selection for the study was based on confirmation of the genetic defect in the COL7A1 gene. 9 males and 3 females with generalized recessive dystrophic epidermolysis bullosa, ages ranging from 10-36 years, were included in the study.

Recruitment

Patients were recruited out of Dr. Marinkovich’s Bullous Disease Clinic at Stanford University. Patients were recruited based on confirmation of a genetic mutation in the COL7A1 gene; there was no selection bias in recruiting or enrollment of patients in the Phase 1/2 trial.

Ethics oversight

The study protocol, all its amendments, and the patient information sheet(s) were reviewed and approved by the appropriate independent Stanford Universities ethics committees. Written informed consent was obtained from patients or patients’ legally authorized representatives. Patients received compensation to cover travel costs and meals for the day of the visit.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Clinical data

Policy information about clinical studies

All manuscripts should comply with the ICMJE guidelines for publication of clinical research and a completed CONSORT checklist must be included with all submissions.

Clinical trial registration

[NCT03536143] May 24th 2018
Study protocol
A Phase 1/11 Study of B-VEC, a Non-Integrating, Replication-Incompetent HSV Vector Expressing the Human Collagen VII Protein, for the Treatment of Dystrophic Epidermolysis Bullosa (DEB).

Data collection
Data was captured using case report forms and data was entered into an electronic data management system. Patients were recruited from Stanford Epidermolysis Bullosa clinic, starting from May 1, 2018 until Sept 1, 2019. Data collection was from May 3, 2018, until November 25, 2019.

Outcomes
Since the Phase 1/2 trial of B-VEC was a first-in-man gene therapy trial, safety of B-VEC was the primary objective and was defined as the primary end point. B-VEC is a gene replacement corrective therapy hence evaluation of molecular correction and durability of wound closure associated with molecular correction were the secondary end points.

To evaluate safety and tolerability of repeat B-VEC use, adverse events (AEs), and changes in vitals, physical exam, and laboratory results, including anti-COL7 and anti-HSV-1 antibodies were assessed. Molecular correction was established by taking biopsies of the healed skin of the treated area and showing presence of functional COL7 protein by Immunofluorescence (IF) and formation of anchoring fibrils (AF) by immunoelectron microscopy (IEM). Wound healing was captured using a validated imaging device.