The potential of CRISPR guided therapies in the dermatology clinic

Prashant Bhat, Lilit Garibyan

PII: S2667-0267(22)00009-1
DOI: https://doi.org/10.1016/j.xjidi.2022.100103
Reference: XJIDI 100103

To appear in: JID Innovations

Received Date: 10 November 2021
Revised Date: 3 January 2022
Accepted Date: 10 January 2022

Please cite this article as: Bhat P, Garibyan L, The potential of CRISPR guided therapies in the dermatology clinic, JID Innovations (2022), doi: https://doi.org/10.1016/j.xjidi.2022.100103.

This is a PDF file of an article that has undergone enhancements after acceptance, such as the addition of a cover page and metadata, and formatting for readability, but it is not yet the definitive version of record. This version will undergo additional copyediting, typesetting and review before it is published in its final form, but we are providing this version to give early visibility of the article. Please note that, during the production process, errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

© 2022 Published by Elsevier Inc. on behalf of the Society for Investigative Dermatology.
REVIEW

The potential of CRISPR guided therapies in the dermatology clinic

Prashant Bhat¹,² and Lilit Garibyan³†

(1) David Geffen School of Medicine, University of California, Los Angeles, Los Angeles, CA 90095, USA
(2) Division of Biology and Biological Engineering, California Institute of Technology, Pasadena, CA 91125, USA
(3) Department of Dermatology, Harvard Medical School, Wellman Center for Photomedicine, Massachusetts General Hospital, Boston, MA 02114, USA.

Corresponding author:

*Lilit Garibyan, Wellman Center for Photomedicine, Massachusetts General Hospital, 50 Blossom Street-Thier 2, Boston, MA 02114, Email: lgaribyan@mgh.harvard.edu

ORCiDs
Prashant Bhat: https://orcid.org/0000-0003-3832-4871
Lilit Garibyan: https://orcid.org/0000-0002-9266-0887

Keywords: CRSPR, gene editing, genodermatoses, inflammatory disorder, skin infection, Cas9, RNA

Abbreviations: CRISPR, Clustered Regularly Interspaced Short Palindromic Repeats; sgRNA, short guide RNA; Cas, CRISPR associated; RDEB, recessive dystrophic epidermolysis bullosa; AD, atopic dermatitis; PAM, Protospacer Adjacent Motif; MAZ, microscopic ablation zones

Corresponding Author Twitter Account: @DrLilitGaribyan
ABSTRACT

Over the past decade, CRISPR has rapidly made its way from the bench to the bedside – providing a newfound therapeutic avenue to not only treat genetic diseases, but to permanently cure them. While there are several clinical trials in early stages, there are so far no CRISPR-based clinical trials for cutaneous disease. In this review, we describe multiple cutaneous diseases that represent ideal targets for CRISPR based therapeutics due to known single-gene-causing-mutations. We also explore the potential of CRISPR nucleases to treat inflammatory disorders such as eczema and psoriasis, which are not classically categorized as genodermatoses. We describe therapeutic solutions for these diseases that are guided by various CRISPR-associated (Cas) effector proteins – for example, using Cas9 to permanently edit the DNA of somatic cells, Cas3 to target foreign DNA to combat viral/bacterial skin infections, and Cas13 to edit mutated RNA transcripts in diseases where permanent DNA editing is untenable. Furthermore, we discuss various drug delivery modalities for CRISPR therapeutics – including transdermal patches and microneedles – that are uniquely suited for dermatological disease. In sum, we highlight the potential of CRISPR-based therapeutics to revolutionize the treatment of cutaneous disease with a goal of being accessible to the practicing dermatologist.
INTRODUCTION

The standard of care for the majority of cutaneous diseases – including genodermatoses, inflammatory disorders, and bacterial skin infections – largely involve treatment of symptoms rather than the underlying cause of disease. Although dermatologists have a repertoire of pharmacotherapy available to prescribe to patients (e.g., topical steroids, anti-inflammatory biologics, or antibiotics), these treatment options are often short-term solutions for long-term chronic problems and come with undesirable side effects. For example, corticosteroids for atopic dermatitis can result in stretch marks, thinning and darkening of the skin or chronic use of antibiotics for acne vulgaris can lead to resistance and poor outcomes. In addition, although biologics have revolutionized the way severe inflammatory skin diseases are treated, a major drawback is that patients typically need to take the medication for life. Hence, there is an urgent need for treatment modalities to target the underlying cause of disease rather than focus on symptomatic management.

Recent advances in genetics and molecular biology have revealed that many cutaneous diseases stem from changes in DNA – either DNA mutations in the host (genodermatoses (Ko et al. 2019) and inflammatory disorders (Bowcock and Cookson 2004)) or pathogenic DNA in viruses (de Buhr and Lebbink 2018) and bacteria (Greene 2018; Pursey et al. 2018; Viertel et al. 2014) (skin infections) – opening a new avenue to target these diseases. Specifically, the discovery of Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) and corresponding CRISPR-associated (Cas) nucleases has enabled the editing of precise molecular targets (e.g., DNA and RNA sequences) in a variety of clinically applicable contexts (Doudna and Charpentier 2014; Fellmann et al. 2017). While several clinical trials using CRISPR nucleases – including Cas9 and Cas3 – are in progress for blood disorders (Frangoul et al. 2021; Frangoul 2020), cancers (Lacey and Fraietta 2020; Lu et al. 2020; Stadtmauer et al. 2020), eye diseases, chronic infections (Lenneman et al. 2021), and protein-folding disorders, there are so far no CRISPR-based clinical trials for dermatologic diseases.

To that end, CRISPR-based therapies have enormous implications for three classes of cutaneous disorders: genodermatoses, inflammatory disorders, and bacterial infections (overview of strategy presented in (Figure 1)). Specifically, many genodermatoses are monogenic – that is, associated with a mutation in a single gene (for example, recessive dystrophic epidermolysis bullosa (RDEB),
congenital ichthyosis) and present ideal candidates for CRISPR targeting. Beyond monogenic disorders, other therapeutic targets for CRISPR include inflammatory disorders, such as atopic dermatitis, in which certain disease-causing mutations are well known (Wan et al. 2021). Furthermore, other Cas nucleases such as CRISPR-Cas3 have recently been the focus of clinical trials for treating bacterial urinary tract infections (Lenneman et al. 2021) – this opens another avenue for using CRISPR nucleases to treat antibiotic resistant or latent bacterial (Pursey et al. 2018; Viertel et al. 2014) (or viral (de Buhr and Lebbink 2018)) skin infections.

Recently, several informative review articles in dermatology journals have focused on ex vivo DNA/gene-editing therapies for rare genodermatoses (Jayarajan et al. 2021; March et al. 2018; De Rosa et al. 2020). Here, we include both ex vivo and in vivo gene editing applications in dermatology and furthermore, focus our attention on incorporating all classes of CRISPR nucleases – DNA and RNA targeting – into the repertoire of clinical tools available to the dermatologist. Lastly, given the unique structural barriers of the epidermis for drug delivery, we discuss advances in delivering CRISPR-based therapies to the skin and challenges that will need to be overcome to bring CRISPR into the clinic. In sum, we highlight the potential of CRISPR-based therapeutics to revolutionize the treatment of cutaneous disease with a goal of being accessible to the practicing dermatologist.

2 | TARGETED TREATMENT OF GENODERMATOSES AND ATOPIC DERMATITIS

Treatment of genetic diseases that affect the skin – including keratinization disorders (e.g., ichthyoses, Darier disease), blistering disorders (e.g., epidermolysis bullosa), or subtypes of inflammatory dermatitis (e.g., eczema and psoriasis caused by various CARD mutations) – has traditionally consisted of medications that treat symptoms rather than the underlying cause of disease. These treatment options generally have included potent topical and systemic steroids, immune suppressive agents, and emollients which provide short term relief and thereby limit long
term improvement in patient satisfaction. In addition, cases refractory to treatment are at risk of breaking the protective stratum corneum layer and thus are at higher risk of developing secondary bacterial infections. Despite the urgent need, there are no cures for genodermatoses and treatment options for atopic dermatitis are limited. Newer IL-4 inhibitor-based therapies for AD (e.g., Dupixent (Beck et al. 2014) show promising clinical benefit but patients must remain on the medication for life and often times experience significant side effects. Recently, genetic mutations associated with atopic dermatitis (Wan et al. 2021) and mutations underlying several monogenic genodermatoses (Kocher et al. 2017; Shinkuma et al. 2016) have been the focus of CRISPR-Cas9 mediated therapy in mouse and cellular models (Benati et al. 2018; Hainzl et al. 2017; Shinkuma et al. 2016; Wan et al. 2021; Webber et al. 2016). Although much of this work is still in the early stages, there is now precedence for Cas9 mediated therapies in non-dermatological conditions (Frangou et al. 2021) and non-CRISPR gene therapy for dermatological indications (reviewed elsewhere (Ain et al. 2021)) to treat patients and improve outcomes. In this section, we discuss recent progress on utilizing CRISPR-Cas9 to successfully repair DNA mutations in genetic cutaneous diseases.

While Cas9 has not made it into the dermatology clinical trials, alternative gene therapy methods repairing damaged DNA in cutaneous disease have largely focused on ex vivo therapies, namely, extracting and modifying cells from patients before re-engraftment back to the body. For example, current clinical trials are underway using gene replacement strategies for recessive dystrophic epidermolysis bullosa (NCT04186650), Netherton syndrome (NCT01545323), and congenital ichthyosis (NCT04047732). Many of these clinical trials have been successful in treating these skin disorders by way of taking a biopsy and expanding keratinocytes, correcting the gene mutation responsible for the disease, and re-grafting skin equivalents back into patients (NCT04186650). Similar ex vivo delivery of CRISPR would be indicated for genodermatoses in which there is severe skin involvement. While CRISPR for treating genodermatoses has been explored in animal and cellular models, virtually all progress has been focused on one severe skin blistering disease: epidermolysis bullosa. Specifically, researchers have used Cas9 to successfully restore enough gene function (of full length type VII collagen, COL7A1) in rodent and cellular models of EB that are considered sufficient for a scarless phenotype after engraftment onto a human body (Benati et al. 2018; Bonafont et al. 2019; Izmiryan et al. 2018; Jacków et al. 2019; Kocher et al. 2020). Nevertheless, it remains to be seen how CRISPR-Cas9 based therapy of EB compares to existing
gene therapies. Future work will determine the safety and efficacy of Cas9 mediated therapy for EB and eventually other genodermatoses such as Netherton syndrome, congenital ichthyosis, and other monogenic skin disorders.

Beyond monogenic skin disorders, there is growing interest in understanding the molecular genetics underlying inflammatory skin diseases, particularly eczema and psoriasis (Bieber 2008; Weidinger and Novak 2016), to research new ways to target these mutations for therapy. Many subtypes of psoriasis harbor gene mutations in CARD14 which results in the upregulation of inflammatory cytokines (Capon 2017) and similarly, eczema carries similar causative mutations in CARD11 (Ma et al. 2017). In contrast to cases carrying gene mutations in CARD, one of the most common associations of genetic mutation with atopic dermatitis is in the FLG gene encoding filaggrin (Irvine et al. 2011; O’Regan and Irvine 2008). Lastly, another genetic similarity between eczema and psoriasis is in an inflammasome called NLRP3 which when activated leads to a greater inflammatory response and more resistance to glucocorticoid therapy. It was recently shown that co-delivering Cas9 targeting NLRP3 with dexamethasone in mouse models alleviated symptoms – reduction in skin edema, reduced infiltration of mast cells, and overall improvement in inflammatory activity – in comparison to the Cas9-NLRP3 treatment alone or the dexamethasone treatment alone (Wan et al. 2021).

Furthermore, the advent of RNA-editing using CRISPR-Cas13 (Abudayyeh et al. 2017) may enable further treatment options for genetic diseases where permanently editing the DNA might not be tenable or dangerous due to unintended off-target effects. Taking the example above, knocking out the NLRP3 gene through Cas9 may be effective, but may also cause unintended off target effects that would be permanent for the duration of the cell lifetime. In contrast, editing the mRNA product prior to translation would obviate the need to target the genome directly and instead prevent the expression of the pro-inflammatory protein. This type of precision and targeted therapy would be useful in cases where systemic corticosteroid therapy is untenable due to adverse side effects or resistance. In summary, targeted treatment via Cas9 of genodermatoses and atopic dermatitis have been incredibly promising in animal and cellular models of disease and poses a hopeful opportunity to translate these findings into humans.
3 | ANTIBIOTIC-FREE TREATMENT OF BACTERIAL SKIN INFECTIONS

Needless to say, mainstay treatment of bacterial infections – from acne or skin infections caused by bacteria– has centered around prescribing antibiotics. Though effective in many cases, antibiotic resistance is a significant problem and alternative, innovative options for treating infections are needed. While antibiotics typically target bacterial cell machinery involved in essential growth processes (e.g., protein synthesis, transcription of RNA, etc), an alternative approach is to specifically target bacterial genome sequences to disable the pathogen, prevent replication, and treat infection. Recently, researchers have repurposed a CRISPR-Cas system (Cas3) to target bacterial infections (Lenneman et al. 2021; Selle et al. 2020) by delivering CRISPR machinery packaged within viral vectors (bacteriophage) that exclusively infect bacterial cells and not human cells. Rather than using CRISPR to modify or edit host DNA, the central tenet of the CRISPR-Cas3 strategy is to make thousands of cuts in bacterial DNA and leave human DNA unmodified. In contrast to Cas9 which makes a single cut in DNA (Jinek et al. 2012), Cas3 is a processive nuclease and helicase that uses ATP to unwind DNA and successively degrade long segments of DNA (Hochstrasser et al. 2014; Redding et al. 2015). As a result, the bacteria cannot replicate with its genetic code disabled.

CRISPR-Cas complexes with sgRNAs complementary in sequence to pathogenic bacterial sequences can be repurposed to target and kill specifical bacterial species. For example, Cas9 was recently used to exclusively target one of two strains of *E. coli* in a mouse model (Lam et al. 2020) and Cas3 was recently used to target *C. difficile* (Selle et al. 2020) in vivo; furthermore, Cas3 is currently in clinical trials for *E. coli* urinary tract infections (e.g., NCT04191148). Although the latter study has confirmed the safety and efficacy of this strategy of antimicrobial targeting, challenges in delivery remain to be optimized. Namely, the current Cas3 clinical trial is testing administration of the phage treatment directly into patients’ bladders (via catheterization) with an immediate next goal of intravenous or intramuscular delivery – all of which largely require treatment by medical professionals.

Nevertheless, the Cas3 clinical trial sets an important precedent for considering CRISPR-Cas treatment for bacterial skin infections. Specifically, applications in dermatology where CRISPR-Cas mediated antimicrobial treatment would be beneficial include skin infections like *S. aureus* (including MRSA) or *P. aeruginosa* folliculitis. Instances in which a patient cannot tolerate the
standard antibiotic due to an allergy or is refractory to treatment due to antibiotic resistance would be indicators for using CRISPR-mediated therapy. Whereas antibiotics (even relatively selective ones) may kill both “bad” bacteria as well as normal flora that exists on the skin, the advantage of the CRISPR-Cas strategy is that normal flora can be spared by programming the CRISPR machinery to target bacterial genes conserved within a strain or even a specific species. Furthermore, acne has a wide range of treatment options, ranging from oral contraceptives to topical creams to antibiotics to isotretinoin. Some patients either do not respond to treatment or are concerned about significant side effects (e.g., birth defects, liver failure, etc.) and therefore would be good candidates for incorporating CRISPR mediated treatment for acne exacerbated by bacteria. Since the cause of acne is multifactorial, CRISPR will not be a monotherapy and will be administered with already existing topical acne therapies which are often combined with antimicrobial therapy.

In sum, CRISPR guided targeting of bacterial infections adds another layer of antimicrobial strategies for managing infections. In practice, a combination of approaches may be beneficial for treatment – for example, targeting bacterial replication through CRISPR targeting of the DNA in addition to mild topical creams and facial cleansing solutions that can work synergistically.

4 | DELIVERING CRISPR TO THE SKIN

The success of any treatment depends on the efficiency of uptake and downstream bioavailability of the drug. Delivery of CRISPR to treat cutaneous disease can be divided into two large categories: (i) *ex vivo*, in which primary cells are treated outside the body and reintroduced to patients upon gene correction and (ii) *in vivo*, in which the CRISPR components are directly delivered to patients (*Figure 2*). Further, viral vectors (Kimura et al. 2019) and non-viral delivery systems (e.g., lipid-based (Buck et al. 2019) and polymeric nanoparticles (Malloggi et al. 2015), electroporation (Labala et al. 2017), ultrasound (Lifshiz Zimon et al. 2018; Pereira et al. 2017), and microneedles (Dul et al. 2017)) have been utilized to deliver gene therapies and similarly hold promise for CRISPR-based therapeutics. In this section, we discuss emerging technologies undergoing development to improve drug delivery for dermatological disease and how this can potentially be applied for delivery of CRISPR in the skin.
The skin presents unique advantages due to the accessibility of the epidermis as well as unique challenges due to the relatively impermeable barrier of the stratum corneum and the size of this organ. Topical creams spread over the surface of the skin rely on diffusion to penetrate the skin barrier, resulting in 1-5% bioavailability (Surber and Davis 2002). Another strategy is transdermal drug delivery, which still must overcome the resilient barrier of the epidermis (Alkilani et al. 2015; Jeong et al. 2021; Prausnitz and Langer 2008). Physical delivery methods via hypodermic needles are direct approaches to improve bioavailability but require penetrance into the dermis layer and is particularly painful, thereby reducing patient compliance. Laser assisted drug delivery creates microscopic ablation zones (MAZs) with vertical channels penetrating past the stratum corneum and accessing deep into the dermis (Haedersdal et al. 2016). Though this strategy has largely focused on delivery of drugs like methotrexate (Lee et al. 2008) and methyl aminolevulinate (Haedersdal et al. 2014), it remains to be demonstrated if laser-assisted drug delivery can be repurposed for delivering gene therapy vehicles like CRISPR-Cas9.

Recent work has been done in delivering CRISPR based therapeutics in animal models as well as patients through clinical trials. Viral vector based methods such as adeno-associated viral vectors (AAVs) in theory could deliver CRISPR-Cas9, guide RNA, and a donor template (with the corrected mutation) into cells (Yang et al. 2016), but require more than one vector to simultaneously deliver the components, thereby reducing the efficiency of targeting. Another viral based method for delivering CRISPR is phage therapy (Lam et al. 2020; Lenneman et al. 2021; Selle et al. 2020) for treatment of bacterial infections. The central tenet here is that bacteriophage selectively infect bacterial cells and can target a specific bacterial species DNA when packaged with CRISPR-Cas3 as has been successfully done in a recent clinical trial for the treatment of lower urinary tract infections caused by *E. coli* (NCT04191148).

Non-viral methods for CRISPR delivery have also gained traction. For example, intradermal injections followed by electroporation of CRISPR-Cas9 complexes targeting collagen VII facilitated the transfection of skin stem cells and improved skin adhesion in a mouse model of EB (Wu et al. 2017). Hypodermic needles or intradermal injections (Jacków et al. 2019) are physical methods to directly deliver CRISPR therapeutics past the epidermis but administration to patients can be painful and dampen clinical utility. Relatively new and innovative methods include microneedle technology (Dul et al. 2017; Wan et al. 2021) which utilize hollow and dissolvable microneedles to create small pores in the epidermis and successfully deliver drug into the dermal
layer. Specifically, CRISPR-Cas9 complexes targeting a pro-inflammatory gene NLPR3 in combination with dexamethasone was delivered via microneedle patch in a mouse model of atopic dermatitis [REF]. In addition, to facilitate delivery, the microneedle was loaded with a polymer-encapsulated Cas9 and a dexamethasone-containing polymeric nanoparticle (Wan et al. 2021). Further, this approach comes with markedly less pain because the microneedle does not encounter nocireceptors in the dermis layer. Future work will be needed to demonstrate the safety and efficacy of this delivery strategy in patients and also the feasibility of extending this approach to whole body administration. Further, though many of these studies have been studied within the context of in vitro and animal models, it is our view that continued progress in this area will enable these therapeutics to reach human patients.

5 | LIMITATIONS OF CRISPR

Several concerns exist for the implementation of CRISPR for gene editing, which we briefly list here. First, there are concerns of off-target effects; while some studies have shown that there are insignificant or even undetectable levels of off target effects (Long et al. 2016), others have clear documentation of large insertions or deletions that may result in unintended consequences (Shin et al. 2017b). Second, immunogenicity against CRISPR proteins would need to be monitored given observations of pre-existing serum antibodies to Cas9 in some donors (Charlesworth et al. 2019) or prevalence of Cas9-reactive T cells in some patients (Wagner et al. 2019). The development of CRISPR inhibitors offer a solution to immunogenicity by disabling the gene editing enzymes after DNA cleavage (Shin et al. 2017a). Lastly, most CRISPR-mediated gene therapy has focused on ex vivo treatment – namely, editing of stem cells outside the body and subsequent reintroduction of corrected cells back into patients. Though this strategy has been done to edit human epidermal stem cells or induced pluripotent stem cells from patients (Jayarajan et al. 2021) and successfully re-graft corrected cells into mice, in vivo editing directly on the skin has been limited to a modicum of studies. Further, gene editing of stem cells in vivo would target both stem and differentiated cells – this is likely not a limitation since stem cells will persist and the differentiated skin cells will eventually die and slough off via the natural course of skin cell maturation. Another challenge includes treating wide areas of skin where whole body involvement and treatment by CRISPR might not be tenable. Future work will need to be done to determine how efficiently emerging
technologies in dermatology (i.e., microneedle drug delivery) will improve in vivo gene editing capabilities in human tissue.

6 Conclusion

Treating the underlying cause of certain skin diseases via CRISPR-Cas gene editing in combination of symptomatic management will be crucial for better long term patient outcomes. To that end, we are proposing that CRISPR will not altogether replace current therapies but rather enable dermatologists to provide higher level of care to patients. For example, phototherapy is life changing for patients with severe atopic dermatitis (Rodenbeck et al. 2016), but they still may be at risk for bacterial skin infections and chronic use of antibiotics might not be tenable, thereby requiring local administration of CRISPR therapy to treat skin infection. Nevertheless, the burgeoning field of CRISPR has only been around for a decade with many open questions and room for improvement. While CRISPR has tangible progress for localized administration in dermatological disease, further work will be needed to address delivery strategies for disorders in which the whole body is affected (including multi-organ involvement). Furthermore, gene editing efficiency is not 100%, thereby generating a heterogeneous mixture of cells that contain repaired DNA and the original, mutated DNA. Ongoing work will be needed to investigate what level of efficiency is “enough” to significantly improve long-term patient outcomes. We expect that recent advances in increasing editing efficiency and decreasing undesirable off target effects (Donohoue et al. 2021) will bring us closer to incorporating CRISPR mediated therapies in the dermatological setting.
ORCiDs
Prashant Bhat: https://orcid.org/0000-0003-3832-4871
Lilit Garibyan: https://orcid.org/0000-0002-9266-0887

ACKNOWLEDGMENTS
PB is supported by the University of California, Los Angeles (UCLA)-Caltech Medical Scientist Training Program (MSTP), National Institutes of Health (NIH) F30CA247447, a Chen Graduate Innovator Grant, and the Josephine de Kármán Fellowship Trust. The figures were created using Biorender.

AUTHOR CONTRIBUTIONS
Conceptualization: PB, LG; Writing – original draft: PB; Writing – review & editing: PB, LG

CONFLICT OF INTEREST
LG: Received gift/grant support from AID (Advancing Innovation in Dermatology) and from LEO Pharma for the Virtual Magic Wand program. “Magic Wand” is a service mark of The General Hospital Corporation, owner of Massachusetts General Hospital. No other relevant COI with the subject matter.
REFERENCES


Doudna JA, Charpentier E. Genome editing. The new frontier of genome engineering with


Frangoul H. 4 Safety and Efficacy of CTX001 in Patients with Transfusion-Dependent β-Thalassemia and Sickle Cell Disease: Early Results from the Climb THAL-111 and Climb SCD-121 Studies of Autologous CRISPR-CAS9–Modified CD34+ Hematopoietic Stem and Progenitor Cells. Am. Soc. Hematol. 2020;


FIGURE LEGENDS

FIGURE 1: Overview of CRISPR-based treatment strategies for cutaneous disease. Strategy for targeted treatment of (a) inherited cutaneous disorders by CRISPR-Cas9 or (b) bacterial infections by CRISPR-Cas3. Cas9 loaded with a short guide RNA (sgRNA) recognizes the Protospacer Adjacent Motif sequence (PAM), hybridizes at a specific genomic locus, and generates a double stranded break in DNA. At this point, there are two options for editing: (left) disrupting a gene of interest through non-homologous end joining or (right) correcting a gene of interest through homology-directed repair via integration of a donor DNA template carrying the correct sequence. Whereas Cas9 is a single protein that has both DNA targeting and cutting activity, CRISPR-Cas3 involves a complex of multiple proteins called the Cascade complex and recruits a trans-nuclease-helicase called Cas3 to make the initial cut in DNA. After making a cut, Cas3 can use ATP to processively degrade DNA making it useful for cleavage of long segments of bacterial DNA. Delivery strategies for components are discussed in Figure 2.

FIGURE 2: CRISPR delivery strategies to the skin. (a) Ex vivo delivery strategy involves deriving patient skin stem cells, treating with CRISPR Cas + sgRNA against the targeted gene, and reintegrating corrected skin stem cells back into patients. (b) Effective in vivo delivery strategies for CRISPR may include hollow, dissolvable microneedles that penetrate the epidermis, hypodermic needles, and phage delivery (for bacterial infection applications).
CRISPR-Cas9 Gene Editing

**Applications:**
- RDEB
- Ichthyoses

**Degraded bacterial DNA**

**Applications for skin infections:**
- Acne vulgaris
- Propionibacterium acnes
- S. aureus (including MRSA)
- P. aeruginosa folliculitis

CRISPR-Cas3 Gene Degradation

**Applications:**
- Non-homologous end joining
  - Disrupts gene of interest
- Homology-directed repair
  - Corrects gene of interest

**Double-stranded break**

**Donor DNA**

**Repaired DNA**

**Nucleotide deletion**

**Nucleotide addition**

Figure 1
In vivo delivery strategies

Microneedles and transdermal patches

Injection

Phage therapy

Ex vivo delivery strategy

Patient-derived skin stem cells

CRISPR + sgRNA

Corrected skin stem cells

Figure 2