Review Article

CRISPR-Based Gene Editing Therapy for Epidermolysis Bullosa Simplex: Molecular Targets and Therapeutic Strategies

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ABSTRACT

Epidermolysis Bullosa (EB) is a genetic skin condition characterized by extensive skin fragility with blistering and open sores following mild mechanical stress or injury. Among several subtypes, Epidermolysis Bullosa Simplex (EBS) is the most common type, usually occurring through mutations in the KRT5, KRT14, and PLEC genes that weaken the integrity of keratinocytes in the basal epidermis. Current treatments are mainly supportive care and focus on making the symptoms more manageable through bandaging wounds, nutritional intervention, and surgical treatment. However, advances with gene-editing tools like CRISPR-Cas9 offer revolutionary opportunities for therapeutic strategies seeking a curative advantage. In this paper, we review the genetic basis of EBS, the molecular consequences of profound mutations, and the utility of CRISPR-based methods to correct these with high fidelity through homology-directed repair (HDR), base editing, and prime editing. Both *in vitro* and *in vivo* methods used to transplant gene-engineered keratinocytes and the emerging role played by *in vivo* delivery systems in targeting epidermal tissue by CRISPR are discussed. While current issues on delivery efficiency remain to be conquered, cell specificity and the long-term preservation of the edited phenotype are still matters to be addressed. However, CRISPR-based approaches offer a promising direction to correct EBS at the molecular level and offer hope for long-term therapeutic success.

Keywords: epidermolysis bullosa (EB); CRISPR-Cas9; KRT5; KRT14; PLEC

INTRODUCTION

Epidermolysis Bullosa (EB) is a rare but severely debilitating group of hereditary connective tissue disorders that features extremely fragile skin that is easily blistered and damaged by minor rubbing, thermal stress, or trauma (1) . Although disease severity varies among patients, EB often severely impairs quality of life from early childhood and may include chronic wounds, infection, disfiguring scarring, and possible premature death. It is important to view EB as not a solitary condition but as a broad category comprising several different forms, including EB simplex (EBS), dystrophic EB (DEB), junctional EB (JEB), and Kindler syndrome, each caused by mutation of genes

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Accepted September 4, 2025
https://doi.org/10.70251/HYJR2348.35103111

that encode proteins essential for the skin's strength and adhesive function (1, 2). EBS, recognized as the most common subtype of EB, is defined by internal epidermal blistering, with onset usually in infancy or early childhood. This specific subtype comprises roughly 75–85% of total cases of EB and is most often inherited in an autosomal dominant fashion (1).

Even with the extensive research carried out during recent decades, there is no definitive cure for EBS. The management consists of mainly supportive care, focusing on symptom relief, relieving pain, and wound healing. Often using measures including protective bandages, antiseptic wound care, and nutritional supplementation, especially diets with high protein and calorie content to enable tissue repair (3). When there is increased severity, surgical measures may be required to treat esophageal strictures, deformities of anatomical hands, or to place feeding or ventilation tubes. Physical therapy plays an important role in preventing contracture and maintaining mobility. These measures, however, do not alter the inherent genetic pathology, and there are recurrent complications for patients and a loss of quality of life (1, 3).

At the molecular level, EBS is primarily associated with mutations in the genes KRT5 and KRT14, which encode the proteins keratin 5 and keratin 14, respectively. These proteins form part of the keratin intermediate filament network in the basal keratinocytes of the epidermis, the outermost layer of skin. Together, keratin 5 and 14 heterodimerize to provide tensile strength and resilience to skin cells and tissue, allowing them to withstand mechanical stress (4, 5). Mutations in either gene weaken this structure, making basal cells prone to rupture. These ruptured cells lead to the formation of fluid-filled blisters that develop spontaneously or after minor skin trauma. The most frequent mutations are missense mutations that alter amino acids in critical regions of the protein, especially the helix boundary domains essential for filament assembly (5, 6).

The KRT5 gene on chromosome 12, with 9 exons, encodes for a 58 kDa protein specifically expressed by basal keratinocytes. KRT5 is approximately 40-70 kDa and is found in cells that make up the outer layer of the skin which are called keratinocytes. KRT5 mutations most often happen within the protein's rod domain to prevent keratin filament assembly, which results in cell fragility. KRT14 on chromosome 17 encodes for a 50 kDa protein with similar structural roles. KRT14 mutations, like KRT5 mutations, interfere with the cytoskeletal structure that keeps the cell intact (4). KRT5 and KRT14 are very conserved intermediate filament proteins with ~80–85% sequence homology within their rod domains. Such high conservation is essential to their function of forming stable keratin heterodimers, which is important for maintaining epidermal integrity. These mutations are of dominant-negative action, whereby a single non-functional gene copy destabilizes the entire keratin structure (5). In milder forms like EBS-localized, blistering is limited to hands and feet and often disappears with time. In more aggressive types, such as EBS-generalized or EBS Dowling-Meara, blisters are generalized, painful, and may be complicated by abnormalities such as dystrophy of nails, erosions of the oral cavity, and hyperkeratosis of palms and soles (palmoplantar keratoderma) (1).

Although most cases of EBS involve mutations in genes encoding keratins, 8% of EBS patients that involves mutations in the PLEC gene that encodes plectin, a massive (~500 kDa) cytolinker protein that binds keratin intermediate filaments onto hemidesmosomes, the anchoring junctions to which basal keratinocytes attach to the subjacent dermis (7, 8). Mutations in PLEC destabilize these junctions and result in a condition of EBS termed EBS with muscular dystrophy (EBS-MD). This form not only causes blistering but also progressive muscle weakness, typically presenting in childhood or adolescence (7, 8). Unlike KRT mutations, PLEC-related EBS generally has an autosomal recessive pattern of inheritance. Disease severity is mutation type-specific, frameshift and nonsense mutations cause severe phenotypes with early-onset muscular dystrophy, while missense mutations may cause milder skin manifestations without muscle disease (7, 9).

The urgency for therapeutic measures has led researchers to test gene-editing technologies, most prominently CRISPR-Cas9, as a promising means to correct for the underlying mutations responsible for EBS. With the ability to target specifically the mutated DNA of skin cells, CRISPR offers a potentially permanent solution that attacks at the cause of the disease, and not just the manifestations in symptoms alone (10, 11). Early research has already proven feasible applying CRISPR to restore normal keratin expression to keratinocytes derived from affected individuals and within animal models as well, and thereby paving the future for subsequent clinical applications (10, 11, 12).

CRISPR, which stands for Clustered Regularly Interspaced Short Palindromic Repeats, is gene-

editing technology that originated from the natural immune mechanisms of bacteria to allow researchers to make precise DNA amendments to a target gene (10). CRISPR functions are based on the activity of the CRISPR-associated protein (Cas9) enzyme, which is a double-stranded DNA endonuclease that is directed to a target genomic site by an RNA molecule known as the guide RNA or gRNA (10). The Cas9 enzyme will be preceded by a short sequence of DNA, called the protospacer adjacent motif (PAM) immediately downstream of the site to be targeted. The PAM sequence has become a requirement for the functioning of Cas9; in the absence of it, the enzyme cannot induce a double-strand break in the DNA. After DNA cleavage, the cell attempts to repair the break by using one of various repair pathways and then facilitates the repair of the target gene. Homology-directed repair (HDR), one of the pathways used for DNA repair, utilizes an external homologous DNA template to specifically correct double-strand breaks (DSBs) (2, 10). This approach is of important relevance to the treatment of mutations that are situated within the KRT5 and KRT14 genes and that are associated with several types of Epidermolysis Bullosa (EB) (2, 10). However, HDR is significantly more effective within dividing cells, thus limiting its use within post-mitotic keratinocytes for the focused delivery of both the CRISPR system and the donor DNA (2, 13). On the other hand, base editing is an advanced strategy that gets rid of the need for or danger in cleavage of DNA. This system uses an engineered variant of the Cas9 enzyme, more specifically its nickase or inactive variant (3, 11). This system has proven to be highly effective for correcting point mutations, such as single-nucleotide variants within KRT5 and KRT14, while also decreasing the risk of off-target genomic changes (3, 11). Moreover, an emerging new method known as prime editing is more convenient and accurate for modifying DNA without the need for a donor DNA template (4, 14). Prime editing utilizes a dual combination of Cas9 nickase and reverse transcriptase, plus a specific guide RNA known as prime editing guide RNA, to add or replace specific sequences of DNA at defined sites (4). The process reduces the risk of off-target effects yet permits precise sequence modification; however, optimization for use within living organisms, especially within keratinocytes, still needs to occur (4, 14).

Several challenges remain in base and prime editing applied to keratinocytes; therefore, these advantages are somewhat limited. One of the main challenges is that editing efficiencies in post-mitotic skin cells are usually lower than those in dividing cells, thus limiting robust correction (15, 16). Then comes the matter of delivery: engineering the packaging of these base and prime editors into viral vectors such as AAV is a big challenge due to their large size, while non-viral delivery approaches such as lipid nanoparticles or micro-needle arrays remain experimental for epidermal delivery (17, 18). Further, base-editing allows only for two particular types of nucleotide transition events (C→T and A→G), whereas prime-editing has a wider base of applications but needs careful optimization to represent enough stability to work in skin tissue (10, 12). Lastly, there is the targeting of epidermal stem cell populations adequately for long-term correction in spite of natural skin turnover (11, 16). The realization of these stumbling blocks continues to offer clear reasons as to why the delivery systems and editor variants need further improvement before they could be applied as a routine in EBS therapy (19).

Overall, these CRISPR techniques hold great promise for epidermolysis bullosa (EB) management using ex vivo gene editing, where the corrected keratinocytes, manipulated to correct mutations within KRT5, KRT14, or PLEC, are expanded to produce epidermal sheets that are then transplanted to patients to regenerate skin function (2, 5, 9).

The aim of this research is to study the genetic processes linked with mutations of KRT5, KRT14, and PLEC in the framework of Epidermolysis Bullosa Simplex, and to evaluate the potential for CRISPR-based therapeutic technologies for gene correction of these mutations. While examining the molecular basis for EBS and an overview of the research into genome-editing technologies, this investigation will address the practicability, risks involved, and potential future for creating an effective genetic therapy for this debilitating dermatological disorder.

CRISPR-CAS9 TO TARGET KRT14

Mutations in a number of genes have been linked to Epidermolysis Bullosa Simplex (EBS), but KRT14 has been of particular importance given that it encodes Keratin 14, a protein that forms part of intermediate type I filaments responsible for maintaining the structural integrity of basal keratinocytes. Mutations in KRT14 cause keratin filament assembly defects, which contribute to increased mechanical fragility of skin cells and a greater tendency for intraepidermal

blistering in response to minimal stress (11, 20).

In vitro experiments have determined roles for mutations in KRT14 in cellular instability and inflammatory signaling in patient keratinocytes with EBS. In a 2013 paper by Wally *et al*., patient keratinocytes carrying the p.Arg125Pro mutation in KRT14 had elevated IL-1β levels and aberrant keratin filament networks, leading to increased fragility of the cells (20). IL-1β inhibition with diacerein led to decreased JNK phosphorylation and restoration of keratin filament orientation, but not of the underlying causal mutation. These findings confirm the crucial role for KRT14 in cytoskeletal strength and reveal an anti-inflammatory pathway for which diacerein improves structural derangement in mutant cells.

Even though diacerein directly targets downstream inflammatory symptoms and not the genetic mutation itself, the resulting phenotypic rescue is consistent with that anticipated from ex vivo gene repair by CRISPR-Cas9 for genetically correcting such KRT14 mutations as being of long-term mutation-specific therapy potential. (6, 20). This would include autologous keratinocytes gene repair, culture, and replanting to the patient epidermis. As autologous cells are from the patient, immune rejection is prevented and a special, long-term therapy that can specifically address the genetic flaw and not just its downstream impact is made possible (6, 20). However, while promising, this approach is plagued by challenges such as the stability of long-term gene expression and the complete elimination of off-target effects, issues that must be addressed before CRISPR treatments can be used safely and reliably in the clinic (6).

Besides the laboratory observation, *in vivo* studies using knockout models in people also shed more light on the function of KRT14. Using CRISPR gene editing methods, a study published in 2023 knocked out Krt14 from the basal cells of mice, demonstrating the dual function: KRT14 not only serves as a structural scaffold, but also determines cell fate decisions within regenerating tissue (21). The absence of Krt14 also hampered the development of advanced epidermal lineages from basal cells, pointing out its vital function to maintain normal stratification. Krt14-deficient cells did, however, show an improved clonal proliferation capacity, implying that deletion of this cytoskeleton component disrupts a regulatory check point related to stem-like proliferation. Krt14-KO basal cells exhibited higher colony forming efficiency and more EdU incorporation at day 7 of culture, indicating elevated clonal proliferation (21). Clinical evidence also supports the pathogenic role of mutations in KRT14. A study on patients with severe epidermolysis bullosa (EBS-gen sev) reported a number of patients who harbor disease-causing mutations specifically within KRT14, viz., c.368A>G (p.Asn123Ser), c.416T>C (p.Met119Thr), c.355A>G (p.Met119Val), and c.1131dupT in exon 6, all of which are associated with severe early-onset features like skin blistering within days after birth (11, 13). These recurring mutations highlight the mutation hotspot regions in exon 1 and exon 6, thus making them ideal candidates for CRISPR-Cas9 therapy approaches. (13)

This dual function description provides important insight into the regenerative defects seen for EBS and related keratinopathies, which indicates that therapeutic interventions need to target not only the repair of structural integrity but also the modulation or the reprogramming of proliferation and differentiation capacity of epidermal stem cells (13, 21). The congruence between results from mouse knockouts and mutation mapping from patient information supports the use of CRISPR-Cas9 technology for correction of pathogenic mutations in KRT14, specifically in exon 1, constituting a targeted strategy aimed at restoring keratin filament stability and reducing clinical severity in those suffering from EBS (13).

Together, the Krt14 knockout model provided empirical proof regarding the effect of this gene on both mechanical stability and developmental pathways of basal cells, creating a solid basis for the design of gene therapies, either by reintroduction of functionally normal KRT14 or by the deliberate modification of compensatory pathways (19). Consequently, the CRISPR-Cas9 system would be targeted to the specific recurring mutations in exon 1 and exon 6 of the KRT14 gene in keratinocytes isolated from EBS patients, thus enabling precise correction of the defective genetic sequence, long-lasting restoration of cytoskeletal integrity, and autologous transplantation to the skin, making it a very targeted and mutation-focused approach aligned with the overall therapeutic goals of this study.

RNA TRANS-SPLICING TO TARGET KRT5

The KRT5 gene plays a critical role in maintaining epidermal integrity. KRT5 codes for the protein Keratin 5, which, together with Keratin 14, forms critical heterodimers that allow the formation of the

intermediate filament network supporting keratinocytes in the basal layer of the epidermis. KRT5 mutations undermine this cytoskeletal framework, leading to skin vulnerability and the formation of blisters under minimal mechanical stress. While mutations in both KRT5 and KRT14 disrupt keratin filament networks and lead to EBS, KRT14 mutations more frequently cause the severe Dowling-Meara subtype, characterized by generalized blistering that starts early in life, whereas KRT5 mutations tend to produce milder, local presentations of EBS such as Weber-Cockayne (6, 7). In an effort to correct these defects at the RNA level, scientists have investigated gene correction using RNA trans-splicing, a novel strategy that corrects defective mRNA without altering the DNA template itself. In a landmark *in vitro* study, scientists created synthetic pre-trans-splicing molecules (PTMs) with specificity for KRT5 pre-mRNA, thus replacing the mutated exon 1 with a properly functioning counterpart. This strategy relies on the ability to circumvent the need for treating individual mutations unique to each patient and targets instead a universally affected area (exon 1) involved in a variety of disease-associated variants (6).

Following transfection of patients' keratinocytes, the use of PTMs successfully altered splicing processes, leading to the production of chimeric mRNA containing corrected sequences, which, in turn, contributed to the reconstitution of normal-functioning keratin 5 protein. In a groundbreaking study by Wally *et al*. (2008), approximately 20–25% of the total KRT5 mRNA in the corrected cells was shown to be the repaired, chimeric transcript, thereby resulting in partial but significant restoration of levels of K5 protein. Importantly, immunostaining procedures confirmed the reappearance of coherent networks of filaments within the cytoplasmic compartment, thus indicating not just the correction of the genetic message but also its phenotypic rescue as keratinocyte integrity and mechanical stress resistance (20) . This confirmation holds important implications for therapeutic treatment of those who have EBS due to various or yet to be recognized KRT5 mutations. Additionally, given that RNA-based therapies do not involve permanent changes to the genetic code, they are perhaps a relatively safer therapeutic avenue, especially against the background of potential genotoxicity, off-target modification, or immune stimulation that can arise with DNA-level genome editing (22). Therefore, the demonstrated therapeutic effect of RNA trans-splicing in the correction of KRT5 mutations highlights the potential of post-transcriptional repair strategies to reestablish epidermal function. Not only do these findings refine the pathophysiological insight into EBS but they also justify the universal applicability of mRNA-based approaches to the treatment of genetically heterogeneous skin disorders without the necessity for precise matching of mutations (22).

CRISPR-CAS9 TO TARGET PLEC

In additional to KRT14 and KRT5, which code for structural keratins, PLEC codes for plectin, a large cytolinker protein that mediates interaction of the intermediate filaments and hemidesmosomal proteins and thus supports the adhesion of the keratinocytes to the basement membrane (15). PLEC gene mutations may cause severe Epidermolysis Bullosa Simplex manifestations, often involving multiple systems including muscular dystrophy and gastrointestinal complications, particularly in EBS with muscular dystrophy (8). For therapeutic applications, CRISPR-Cas9 gene editing was conducted *in vitro* in patient-derived keratinocytes with PLEC mutations responsible for EBS. Unlike research on keratin genes like KRT5 and KRT14, functional studies for PLEC are comparatively limited. Still, initial findings from initial experiments conducted by Chamcheu *et al*. (2020) were promising: using the repair capability of CRISPR, gene-edited patient-derived cells expressed approximately 30–40% plectin protein restoration, as demonstrated through Western blotting. This molecular repair was also accompanied by incomplete reorganization of the cytoskeleton and increased hemidesmosomal adhesion in vitro, key structural features that help in stable anchoring of keratinocytes to the basement membrane (23). Such changes establish that even a partial gene repair might result in clinically important improvement in cell stability. While these findings are encouraging, editing efficiency was clone-variable and the study emphasized the need for ongoing optimization of fidelity improvement, more frequent repair, and safety prior to such approaches being realized on autologous cell therapy in EBS patients (23).

In vivo murine models deficient in plectin have been crucial for investigating the widespread pathophysiologic consequences of PLEC mutations linked to epidermolysis bullosa simplex (EBS). Mice with a targeted deletion of exon 31 of the PLEC gene display typical features of EBS-MD (EBS with muscular dystrophy) that include extreme dermal blistering, impaired hemidesmosome assembly, progressive

muscular atrophy, and neonatal mortality, most of these mice die between 3 to 5 days post-natally owing to the impaired integrity of the epidermal and muscular systems (24, 25). The phenotypic manifestations seen closely mimic the multisystemic presentations of severe human EBS, thus providing a valuable model not only to decipher the pathology of the disease but also to evaluate the efficacy of gene therapy interventions in systemic physiological conditions.

The CRISPR-Cas9 method has been used *in vivo* in PLEC-deficient mouse models to assess treatment effectiveness. In research, the CRISPR method was applied to correct a deletion of one exon of PLEC by homology-directed repair (HDR) in zygotes, leading to mice with restored expression of plectin in dermal and muscular tissues (26). Genetically restored mice had increased resilience of the skin and overall survival rate compared to untreated controls; however, the rate of correction was rather low, only around 10–15% of alleles were altered at the target site (26). The results suggest that even the partial correction can result in considerable phenotypic improvements, which notably corresponds to the systemic actions of plectin in various tissues.

Apart from the restoration of structural integrity, the *in vivo* studies further clarified additional benefits related to mechanical stress responses and intracellular signaling pathways. More precisely, the CRISPR-mediated restoration led to the increased recruitment of integrin-linked kinase (ILK) to hemidesmosomes, along with the restoration of mechanical signaling pathways abrogated upon plectin deficiency (25). These findings highlight the central importance of targeting both the mechanical and biochemical functions of PLEC, and thus suggest the inclusion of multifaceted correction strategies in the gene therapies for EBS-PLEC. Moreover, the improvement in *in vivo* CRISPR delivery methodologies and specificity will be central to advancing these therapy efforts toward the clinic. An effective correction of the Plec mutations in mouse models will closely reflect the outcome in therapeutic processes in humans since both the gene and the disease phenotype conserve highly across species.

DISCUSSION

Epidermolysis Bullosa Simplex (EBS) is the result of mutations in keratin genes (such as KRT14 and KRT5) and structural proteins such as plectin, leading to mechanical fragility of basal keratinocytes. This paper suggests direct targeting of these mutations at a genomic level using CRISPR-Cas9 gene editing. CRISPR can correct the existing deficiencies in keratinocyte cultures or *in vivo* using an innovative delivery method by designing mutation-specific guide RNAs. This gene-editing has potential to aid in treating EBS as it could restore skin integrity by determining cytoskeletal integrity and minimizing skin blisters. Gene editing with CRISPR-Cas9, for instance, has been shown *in vitro* to correct causative mutations in keratinocytes, rescuing cytoskeletal architecture and inhibiting blistering phenotypes. The newest gene editing technologies have now offered novel therapeutic hope, but this DNA-level approach is still not clinically available.

Other technologies are in the pipeline that can perhaps supplement or even temporarily supplant CRISPR gene editing, particularly where permanent genomic modification is risky or premature (10, 22, 27). Concurrent use of RNA-based methods that target KRT14 represents a complex and flexible strategy that acts post-transcriptionally without the need for modification to the genomic framework. Antisense oligonucleotides (ASOs) are designed to hybridize specifically to KRT14 pre-mRNA precursors to correct aberrantly splicing mutations or to induce degradation of abnormal RNA isoforms (3, 10, 27). Reducing the expression of KRT14 is used to block the accumulation of dominant-negative keratin proteins that disrupt assembly and destabilize cell integrity (3). While ASOs only temporarily address a therapeutic need compared to CRISPR, they are a potential clinical option whenever considerations regarding permanent genetic modification or off-targeting create safety concerns. However, ASO has not been used in clinical trials yet and remains an experimental approach.

LIMITATIONS

While promising, RNA-based and CRISPR therapies are faced with enormous challenges. For CRISPR, some of the greatest hurdles include off-target cleavage potential, immune responses to Cas9 protein, and the need for effective and safe delivery into cells within the skin (11, 16, 19). Additionally, while ex vivo gene correction and autologous keratinocyte transplantation has proven to work in associated skin diseases such as dystrophic and junctional forms of epidermolysis bullosa, where patients received skin grafts, these processes might not be practical for all

patients due to the pain and invasiveness of biopsies and grafting procedures (15, 21). Long-term stability of gene correction is also an issue, particularly in regard to skin renewal and turnover (16, 28). RNA-based treatments, being less dangerous in negatively impacting genomic integrity, are plagued by requiring frequent administration due to the transient nature of mRNA correction (22). Their clinical application will hinge critically on the ability to reliably dose without inducing immune activation or toxicity (10, 22).

ETHICAL AND REGULATORY OVERSIGHT

The translational potential of gene editing for EBS is quite constrained by the ethical and regulatory considerations. As most EBS cases come to the fore during childhood, the interventions would mostly be given to pediatric populations, so one begins to question informed consent processes, long-term monitoring options, and intergenerational concerns (6, 21). In theory, germline modifications could be included, which introduces concerns about its possibility, however remote, whereas in somatic therapies such concerns are not raised. Hence a strong sterilization framework should be implemented to prevent passage to subsequent generations (29). Apart from the biological considerations, the regulatory authorities such as the FDA and EMA insist that preclinical tests should comprehensively demonstrate durability along with being clear on any off-target effects and immune responses before trials are permitted to go ahead (17, 18). Because of these concerns, technical merit alone will not suffice to bring CRISPR-based therapies for EBS into success but require ethical oversight and regulatory frameworks to ensure patient safety and public trust.

FUTURE WORK

Specificity Enhancements

Further research and development of CRISPR systems are still required in order to decrease off-target editing. SpCas9-HF1 and eSpCas9 are newly-derived high-fidelity Cas9 enzymes, as is the gRNA design (where specificity is only now being studied). These enzymes have also got certain point mutations in them, which minimizes the non-specific binding of DNA leading to a huge reduction in the off-target activities without any compromise in on-target activity (16). Meanwhile, algorithms with in silico computational prediction of off-target potential, like CRISPOR and GUIDE-seq, are being developed and perfected as a pre-screen of potential off-target before *in vivo* (16).

Delivery Methods

Delivery systems must be very strong to favor successful *in vivo* gene editing. Today science is examining topics of viral vectors and non-virally delivered materials such as: lipid nanoparticles and polymeric nanocarriers (10, 16). Recently mRNA vaccines that employ lipid nanoparticles (LNPs) clinically-tested in the COVID-19 pandemic are being applied to CRISPR and ASO topical and intradermal administration through such a route (22). As an example, Wu *et al*. employed ribonucleoprotein delivery employing LNPs to target skin stem cell-based gene editing, efficiently correcting a mouse model of recessive dystrophic EB, and providing strong *in vivo* editing (16). The use of injection strategies, with microneedle arrays, and intradermal injection (or hydrogel assisted delivery technology) is also under investigation to provide local delivery with reduced invasiveness (10, 16, 28). Similar LNP-based and hydrogel platforms have been evaluated as vehicles by gene editing of skin diseases such as pachyonychia congenita and Netherton syndrome to achieve localized and repeated administration without strong systemic exposure (10, 30).

Clinical Trials

Lastly, these therapies will require careful clinical studies to translate their efficiency and practicality to human therapy in terms of safety, editing, immune tolerance, and long-term advantage. The planned pilot clinical studies in other skin disorders that clinically resemble EBS including dystrophic epidermolysis bullosa (DEB) provides a useful proof-of-concept of CRISPR approaches in EBS (10, 15). As an example, ex vivo CRISPR-Cas9 correction of COL7A1 in recessive dystrophic EB (RDEB) patients showed long-term engraftment of corrected stem cells and functional protein expression with long-term maintenance of type VII collagen expression in grafted skin *in vivo* (15). Nevertheless, since EBS is associated with several mutations of various keratin and structural protein genes, clinical trials of the future will need to be mutation-based and there must be strong patient stratification so as to achieve efficacy (10, 17). In the meantime, the open clinical trials like the B-VEC topical gene therapy (NCT03536143 and NCT04491604) and the QR-313

antisense oligonucleotide study (NCT03605069) serve as models of testing localized approaches of gene targeting in EB skin diseases (10, 30).

In essence, CRISPR-based genome editing enables treatment of Epidermolysis Bullosa Simplex through an unremitting and specific delivery of therapeutic outcomes by mending the genetic mutations in KRT5, KRT14, and PLEC. Despite the limited technical barriers, such as delivery efficiency, off-target effects, and patient genetics variability, continuous research in base editing, prime editing, and treatment topical delivery systems and RNA-based treatments still extend the clinical horizon. Notably, preclinical successes and early stage clinical trials in related EB subtypes have proved that a lasting molecular and phenotypic correction can be done. With the further development of this area, the emergence of mutation-specific approaches under the influence of innovative platforms of delivery and editing has a potential of making precision, curative treatment a reality in the case of EBS patients. Finally, the effectiveness of these strategies will depend on interdisciplinary research that spans, among other spectrums, genetics, dermatology, and molecular therapeutics in bringing in a new era of skin diseases treatment.

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