

Newer Treatment Modalities in Epidermolysis Bullosa

Abstract

The term epidermolysis bullosa (EB) refers to a group of hereditary skin blistering diseases. The group is clinically and genetically heterogeneous, but all EB forms are associated with mechanically induced skin blistering and fragility. The causative gene mutations of most EB types are known. The current international consensus classification contains four main types: EB simplex (EBS), junctional EB (JEB), dystrophic EB (DEB), and Kindler syndrome (KS). The classification is based on the morphological level of blister formation. In EBS, the split is intra-epidermal, in JEB along the basement membrane and in DEB below the basement membrane. In Kindler syndrome, the dermal-epidermal junction is disorganized, and blisters can occur on all three levels. Each major EB type has further subtypes which may differ in terms of their genetic, biological or clinical characteristics. Traditionally, EB treatments have been symptomatic, but increasing understanding of disease etio-pathogenesis is facilitating development of novel evidence-based therapy approaches. First gene- and cell-based therapies are being tested at preclinical level and in clinical trials. New knowledge on secondary disease mechanisms has led to development and clinical testing of urgently needed symptom-relief therapies using small molecules and biologicals.

Keywords: Cell- and gene therapy, epidermolysis bullosa, skin fragility, symptom-relief therapies

Introduction

The EB family: A broad spectrum of clinical subtypes

EB is clinically heterogeneous. All EB types manifest with trauma-induced skin blistering and fragility, but the individual types vary in terms of severity and associated extracutaneous manifestations [Figure 1].^[1-3] Mutations in at least 20 different genes can cause EB [Table 1]. Molecular genetic diagnostics have led to revelation of the genetic heterogeneity of the EB, and modern high-throughput technologies, such as chip-based diagnostics or exome sequencing, will probably uncover a few more causative genes in ultra-rare EB forms.^[4-6] In recent years, the EBS group has expanded significantly, with very severe, but also several extremely mild subtypes that exhibit only minimal skin fragility [Table 1].^[4,6] The JEB group has grown with a syndromal subtype, which exhibits renal, lung, and skin involvement and is caused by mutations in the integrin alpha3 gene.^[7] As far as known today, all forms of dystrophic EB are caused by

mutations in the collagen VII gene, making DEB ideal subtype for the investigative studies to explore disease pathogenesis and targeted therapies.

Diagnosis and clinical management

Diagnostics and management of the various EB forms are complex and require interdisciplinary collaboration. Molecular diagnostics is strongly recommended for all the patients with EB, because a precise diagnosis and knowledge of the hereditary mode are important for prognosis, genetic counselling, prenatal diagnosis, and for the planning of personalized therapies. However, if molecular diagnostics are not available, a recently developed clinical diagnostic matrix is suitable for predicting the EB type and even subtypes.^[8] For molecular diagnostics, a two-step procedure has proven helpful. Traditionally, immunofluorescence mapping of a skin biopsy is performed first, followed by a mutation analysis of the candidate gene(s) that were determined on the basis on the immunofluorescence pattern. The introduction of next generation sequencing technologies has rendered immunofluorescence staining of a skin biopsy unnecessary as a first step. However,

Leena Bruckner-Tuderman

Department of Dermatology,
Medical Center -
University of Freiburg,
Hauptstrasse 7, Freiburg 79104,
Baden-Wuerttemberg, Germany

Address for correspondence:
Prof. Leena Bruckner-Tuderman,
Department of Dermatology,
Medical Center - University of
Freiburg, Hauptstrasse 7,
Freiburg 79104,
Baden-Wuerttemberg, Germany.
E-mail: bruckner-tuderman@
uniklinik-freiburg.de

Access this article online

Website: www.idoj.in

DOI: 10.4103/idoj.IDOJ_287_18

Quick Response Code:



How to cite this article: Bruckner-Tuderman L. Newer treatment modalities in Epidermolysis bullosa. Indian Dermatol Online J 2019;10:244-50.

Received: August, 2018. **Accepted:** October, 2018.

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

For reprints contact: reprints@medknow.com

Table 1: Major EB types and their molecular causes

| Major EB type | Gene | Affected protein | Mode of inheritance |
|---------------|--------------------|--------------------|---------------------|
| EBS | <i>DSP</i> | Desmoplakin | AR |
| JEB | <i>PKP1</i> | Plakophilin 1 | AR |
| | <i>JUP</i> | Plakoglobin | AR |
| | <i>KRT5</i> | Keratin 5 | AD |
| | <i>KRT14</i> | Keratin 14 | AD, AR |
| | <i>PLEC</i> | Plectin | AR |
| | <i>KLHL24</i> | Kelch-like protein | AD |
| | <i>DST</i> | BPAG1 | AR |
| | <i>EXPH 5</i> | Exophilin 5 | AR |
| | <i>CD151</i> | Tetraspanin 24 | AR |
| | <i>TGM5</i> | Transglutaminase 5 | AR |
| | <i>ITGB4</i> | a6b4 Integrin | AR |
| | <i>COL17A1</i> | Collagen XVII | AR |
| | <i>LAMA3 LAMB3</i> | Laminin-332 | AR |
| | <i>LAMC2</i> | Laminin-332 | AR |
| | <i>COL17A1</i> | Laminin-332 | AR |
| | DEB | <i>ITGA3</i> | Collagen XVII |
| <i>ITGA6</i> | | a3b1 Integrin | AR |
| <i>ITGB4</i> | | a6b4 Integrin | AR |
| <i>COL7A1</i> | | Collagen VII | AD |
| KS | <i>FERMT1</i> | Kindlin-1 | AR |

AR=Autosomal recessive; AD=Autosomal dominant



Figure 1: Clinical manifestations in epidermolysis bullosa. Upper panel: Junctional EB with mechanically induced blisters, wounds, and loss of nails in the right foot. Lower panel: Moderate dystrophic EB with mechanically induced skin fragility, inflammation, scarring, joint contractures and loss of nails in the left hand

it is often needed for validation of the genetic findings of uncertain significance.^[6] Similarly, although transmission

electron microscopy of a skin biopsy is nowadays rarely used for diagnostics any more, it can be of great value for determining the consequences of mutations in EB and for skin research.

The basics of topical treatment of EB include avoiding trauma, good skin care, disinfection, and careful wound management (<http://www.debra-international.org/clinical-guidelines>). However, since many EB forms represent a systemic disease rather than a sole skin disorder and since chronic skin fragility has a high personal, medical and socio-economic influence on the lives of patients and their families, modern clinical management of EB is based on interdisciplinary and multi-professional care^[9] (<http://www.debra-international.org/clinical-guidelines/complete-eb-guidelines.html>). Depending on the severity and the affection of different organ systems, including EB-associated skin cancer, care providers include dermatologists, pediatricians, dentists, ophthalmologists, gastroenterologists, surgeons, oncologists, and other medical specialists, primary care physicians and other relevant health professionals, including physical therapists, ergotherapists, nutritionists, psychologists, social workers, etc.

Expert centers on EB exist in many countries; they are networked internationally in the EB-Clinical Network “EB-Clinet” (www.EB-Clinet.org) and

collaborate closely with the patient advocacy groups (www.debra-international.org). The centers provide information, advice and an extensive range of diagnostic and clinical management services, including help for precise diagnosis, genetic counselling, interdisciplinary examinations and preparation of the management plan, which can then be implemented by physicians close to home.

Newer therapy approaches

The rapid scientific developments in recent years make various therapeutic strategies for the treatment of EB appear promising. The scientific and clinical EB communities work closely together in determining priorities. Several patient advocacy groups are remarkably active and successful in fund raising; they also fund research projects (www.debra-international.org). Obviously, severe forms of EB have the highest unmet medical need, and most research efforts focus on therapies for these forms. Both curative and symptom-relief therapies are being developed, as delineated below. A number of therapies are in preclinical development and the first ones have already reached clinical trial stage, either at Phase 1/2 or 3 [Table 2]. For

more details, the reader is referred to recent reviews on this topic.^[10-12]

Therapies with curative intention

Gene therapies: At the beginning of the “therapeutic era” for EB more than 10 years ago, the most obvious consideration was to replace a defective gene with gene therapy. An approach using retrovirus-mediated gene correction in keratinocytes and grafting has indeed been successful in individual cases of JEB,^[13-15] and a similar approach is being developed and tested for DEB^[16] [Table 2]. However, it turns out that this form of gene therapy brings with its complex issues and questions relating to technological development of gene vectors, oncogenic potential and future risk of malignancy, duration of therapeutic effects, etc. Current research on cure of EB focuses on different approaches including the use of gene-corrected patient’s own iPS cells, gene editing technologies, and polymer-mediated DNA delivery systems.^[17-21] It is important to note that no gene therapy approaches have been approved for EB; all these therapy developments are currently experimental and in *in vitro* or preclinical state, with a few exceptions that are tested in early clinical trials on individuals with EB.

Table 2: Currently recruiting clinical therapy trials for EB

| Therapy | Investigational Drug | EB Type | Trial identification Nr. |
|-----------------------------|---|--|--|
| Therapies with curative aim | | | |
| Gene therapy | Transplantation surgery of genetically corrected cultured epidermal autograft (ATMP) | JEB with <i>COL7A1</i> mutations | ClinicalTrials.gov Identifier: NCT03490331 |
| | Genetically corrected cultured epidermal autograft (ATMP) | RDEB* | ClinicalTrials.gov Identifier: NCT02984085 |
| | FCX-007, Genetically modified autologous human dermal fibroblasts | RDEB* | ClinicalTrials.gov Identifier: NCT02810951 |
| | KB103, a non-integrating, replication-incompetent herpes simplex virus vector expressing human collagen VII protein | DEB | ClinicalTrials.gov Identifier: NCT03536143 |
| Antisense oligonucleotide | QR-313, an antisense oligonucleotide (AON) | DEB with mutations in exon 73 of <i>COL7A1</i> | ClinicalTrials.gov Identifier: NCT03605069 |
| PTC read-through | Gentamicin, intravenous | RDEB | ClinicalTrials.gov Identifier: NCT03012191 |
| Cell therapy | Serial mesenchymal stem cell (MSC) infusions from a related donor | All EB types | ClinicalTrials.gov Identifier: NCT02582775 |
| | Allogeneic stem cell transplantation and “off-the-shelf” mesenchymal stem cells | All EB types | ClinicalTrials.gov Identifier: NCT01033552 |
| Symptom-relief therapies | | | |
| Anti-fibrotic | Losartan, systemic | RDEB* | EudraCT Number: 2015-003670-32 |
| Anti-inflammatory | Diacerein, topical | EBS | ClinicalTrials.gov Identifier: NCT03154333 |
| | Pharmacokinetics, safety of diacerein after maximum use | EBS | ClinicalTrials.gov Identifier: NCT03472287 |
| | Oleogel, topical | All EB types | ClinicalTrials.gov Identifier: NCT03068780 |
| | BPM31510 3.0% Cream, topical | All EB types | ClinicalTrials.gov Identifier: NCT02793960 |
| | Sirolimus, topical | EBS | ClinicalTrials.gov Identifier: NCT03016715 |

*RDEB=Recessive DEB

An interesting option is the so called “natural gene therapy” which is based on use of cells or tissue derived from revertant mosaic patches in the patient’s skin. Revertant mosaicism, a relatively common phenomenon in human genetic disorders, is based on the fact that in individual cells the inherited mutation is compensated by a second, somatic mutation that restores the expression of the lacking protein and reverts the disease phenotype in the cell. Revertant cells proliferate clonally, and mosaic skin patches can be found in all EB types. The genetic mechanisms of the reversion have been characterized,^[22] and a successful clinical application of small split thickness grafts derived from revertant skin was reported in an individual with JEB.^[23]

Cell therapies: have not only led more quickly to clinical studies, but also here the therapeutic context is more complex than initially anticipated. Intradermal injections of fibroblasts improved the dermal-epidermal adhesion in the DEB mouse model for several months,^[24] but pilot treatments of patients revealed this therapeutic modality to be very painful and not well tolerated^[25] – and not consistently efficacious. Indeed, one study found similar effects on wound healing with both fibroblast injections and vehicle injections.^[26] A systemic therapy for severe DEB by bone marrow transplantation improved symptoms in some patients, but did not cure DEB. The rate of adverse effects was high and some patients died from complications of bone marrow transplantation.^[27-29] Intravenous infusions with mesenchymal stromal stem cells (MSC) from the bone marrow relieved symptoms such as itch and improved the general well-being of children, but did not increase collagen VII in the skin.^[29,30] Current clinical trials are studying the effects of different kinds of MSC in adults with EB [Table 2]. All in all, the number of individuals treated with different cell therapy approaches is very low and, therefore, the percentage of patients with clinical benefits cannot be reliably estimated. More carefully designed and monitored studies will be needed before efficacy and cost-benefit relations can be determined.

Protein therapy: Protein replacement therapies have been successfully developed for a number of genetic diseases^[31] and might have potential for EB, too.^[32] A protein therapy approach using intravenous or intradermal recombinant collagen VII injections for treatment of DEB seemed promising at preclinical level,^[32,33] but further development and clinical assessment are still required before its suitability for treatment of patients can be determined.

Antisense oligonucleotides: The rationale here is that antisense oligonucleotide treatment of cells can lead to skipping of the mutated exons at the RNA level, and thus restore the synthesis of a nearly normal protein that lacks a small fragment encoded by the deleted exon.^[34] Diseases caused by collagen gene mutations are especially suitable candidates for this strategy, since most exons in these

genes are in-frame and their deletion does not cause major structural changes at the protein level. In the context of EB, the collagen VII gene and its disease, DEB, have been of particular interest. Preclinical testing in mice suggested that antisense oligonucleotide-based exon skipping can improve skin stability by partially functional collagen VII.^[35] A Phase 1/2 multicenter clinical trial tests this approach in DEB [Table 2].

PTC read-through: This approach is based on the fact that approximately 10% of genetic diseases are caused by mutations that generate premature termination codons (PTC) and lead to nonsense-mediated mRNA decay.^[36] If the PTC could be ignored by the transcription machinery, a full-length polypeptide with a minor modification and, hopefully, with adequate function, can be synthesized. Aminoglycoside antibiotics are known inducers of PTC read-through and have been tested for many genes. The efficiency of the read-through depends on the local nucleotide microenvironment of the mutations, and a careful selection of suitable mutations is an essential prerequisite for successful treatment. Gentamicins have demonstrated some efficacy for *COL7A1* and *LAMB3* mutations *in vitro*,^[37,38] and a pilot clinical study on five DEB patients treated with topical gentamicin showed increased collagen VII in the skin.^[39] Topical application may circumvent the renal and ototoxicity of gentamicin, although the capacity of aminoglycosides to induce contact dermatitis may pose a challenge. A minor gentamicin component, gentamicin B1, could be an alternative because it is a potent inducer of the PTC read-through and has low toxicity.^[40] Currently, a clinical trial is testing intravenous gentamicin for its efficacy in RDEB [Table 2]. Also, Amlexanox, an anti-inflammatory drug approved in some countries can induce PTC read-through and was shown to enhance collagen VII protein synthesis *in vitro* in DEB cells carrying nonsense mutations.^[41]

Symptom relief therapies

During the past few years, the complexity of issues relating to development and clinical implementation of curative therapies for EB has become obvious.^[10,11,29] The realization that such therapies will not enter the clinical routine until in years, combined with pressure from patient advocacy groups to finally obtain some efficacious treatments, has pushed the search for ways to ameliorate symptoms. Indeed, better understanding of molecular and cellular disease mechanisms in EB has identified novel therapeutic targets and allowed design of symptom relief therapies.^[11] Such treatments do not aim at a cure but at better functionality and improved quality of life of the affected individuals.

Topical therapies: Several different topical therapies have been tested in clinical trials for EB, all of them based on evidence from *in vitro* studies. Diacerein, an anti-inflammatory compound isolated from rhubarb root^[42] has been shown to increase skin stability in

EBS.^[43] In classical EBS, keratin 5 and 14 mutations cause aggregation of the intermediate filament cytoskeleton and cell fragility.^[1] This, in turn, leads to an inflammatory response, e.g., via interleukin-1 β signaling. *In vitro* testing indicated that diacerein can counteract the inflammatory process and stabilize the cells, and a subsequent, small, placebo-controlled phase 2/3 clinical study with diacerein cream demonstrated reduction of blister counts in >40% of the participants.^[43] A further, international multicenter study is currently ongoing [Table 2]. Another topical agent with anti-inflammatory properties is a betulin-based oleogel derived from birch bark. *In vitro* and *in vivo*, betulin enhanced keratinocyte differentiation.^[44] A small, open, blindly evaluated phase 2 pilot study in patients with DEB indicated that betulin-based oleogel promotes re-epithelialization and enhances wound healing.^[45] An ongoing international Phase 3 study tests the safety and efficacy of betulin-based oleogel in larger cohorts of all types of EB. An ongoing trial tests topical 2% sirolimus, an mTOR inhibitor, as treatment of plantar blistering in patients with EBS. The goal is to downregulate the expression of mutated keratins and thereby to improve blistering and hyperkeratosis [Table 2]. An already completed trial (ClinicalTrials.gov Identifier NCT01538862) used subcutaneous injections of granulocyte colony stimulating factor (GCSF) as therapy in seven individuals with DEB. Some improvement (approximately 30%) was observed relating to counts of active blisters and erosions, and to the surface area of non-healing erosions.

Systemic therapies: Evidence is accumulating that inflammation plays an important role in EB and aggravates the phenotypes. Recent publications have reported findings of increased cytokine levels in the skin and serum of individuals with EBS.^[46,47] Based on the high levels of Th17 cells/cytokines in lesional EBS skin, three patients received apremilast, which led to improvement of skin blistering.^[47] It would be interesting to extend the analyses to larger EBS cohorts and also to other EB types. A multicenter placebo-controlled clinical trial that tested oral Epigallocatechin 3 gallate (Polyphenon E), a proteinase inhibitor, as treatment for DEB in 17 individuals came to the conclusion that this compound was well tolerable but not more effective than placebo.^[48]

Since progressive soft tissue fibrosis is a major systemic feature in DEB^[1,49,50] and a prerequisite for development of secondary squamous cell carcinoma,^[3,51] small molecule drugs which reduce inflammation and fibrosis may be useful to postpone mitten deformities, joint contractures, and internal strictures.^[11] Losartan is an approved drug for treatment of hypertension, but it has also been shown to be beneficial in some rare diseases manifesting with secondary fibrosis.^[52,53] The rationale is that as an AT-1 receptor antagonist losartan counteracts TGF β signaling in a context- and disease-specific manner.^[54] In DEB, inflammation and TGF β - signaling are major drivers of

fibrosis^[49-51,55] and, therefore, losartan seems promising as a symptom-relief therapy. *In vitro*, it efficiently inhibited TGF β -signaling in DEB fibroblasts, and in the DEB mouse model losartan treatment reduced TGF β activity, inflammation and fibrosis in the skin *in vivo*. Macroscopically, this manifested in significant delay in the development of mitten deformities.^[56] As a clinical development, an ongoing Phase 1/2 trial (REFLECT) explores safety and tolerability of losartan in children with moderate-to-severe DEB. The trial also collects first information on the efficacy of losartan to improve specific clinical symptoms and quality of life (Table 2; D. Kiritsi, personal communication).

HMGB1: Tamai *et al.* took a different approach to reduce systemic fibrosis in DEB employing a HMGB1-derived peptide. Under physiological and pathological conditions HMGB1 (high mobility group box 1) has a number of different functions.^[57] In the context of EB it increases a specific bone marrow-derived mesenchymal stem cell population that migrates to the circulation, homes to damaged skin, and suppresses inflammation.^[58] At preclinical level, in the DEB mouse model, the HMGB1-derived peptide was effective against both skin fibrosis and gastrointestinal strictures.^[59] It will be intriguing to follow the progress of this therapeutic approach into clinical trials.

Conclusions and Outlook

Taken together, based on the above information, we can be optimistic that the repertoire of potential DEB therapies will continue to grow. Different evidence-based approaches are being considered and currently tested at preclinical and clinical levels for most severe EB types. Future research investigations must provide better understanding of disease mechanisms in all EB types and identify the most suitable therapeutic targets. It is unlikely, that there will be a “one-size-fits-all” therapy for each major EB type. Rather, the treatments will be based on individual mutations constellations, disease mechanisms, and phenotypic characteristics. Furthermore, it seems probable that combinations of different therapeutic principles, e.g., interval therapies using combined or alternating cell and drug therapies, to combat the causes and secondary disease features of EB will bring the best results.

Financial support and sponsorship

The author's work has been supported by Debra International and Debra Austria.

Conflicts of interest

There are no conflicts of interest.

References

1. Fine J-D, Eady RAJ, Bauer EA, Bauer JW, Bruckner-Tuderman L, Heagerty A, *et al.* The classification of inherited epidermolysis

- bullosa (EB): Report of the third international consensus meeting on diagnosis and classification of EB. *J Am Acad Dermatol* 2008;58:931-50.
2. Has C, Nyström A, Saeidian AH, Bruckner-Tuderman L, Uitto J. Epidermolysis bullosa: Molecular pathology of connective tissue components in the cutaneous basement membrane zone. *Matrix Biol* 2018;71-72:313-29.
 3. Montaudié H, Chiaverini C, Sbidian E, Charlesworth A, Lacour JP. Inherited epidermolysis bullosa and squamous cell carcinoma: A systematic review of 117 cases. *Orphanet J Rare Dis* 2016;11:117.
 4. McGrath JA. Recently identified forms of epidermolysis bullosa. *Ann Dermatol* 2015;27:658-66.
 5. Takeichi T, Nanda A, Liu L, Salam A, Campbell P, Fong K, *et al.* Impact of next generation sequencing on diagnostics in a genetic skin disease clinic. *Exp Dermatol* 2013;22:825-31.
 6. Has C, Fischer J. Inherited epidermolysis bullosa: New diagnostics and new clinical phenotypes. *Exp Dermatol* 2018. doi: 10.1111/exd.13668.
 7. Has C, Sparta G, Kiritsi D, Weibel L, Moeller A, Vega-Warner V, *et al.* Integrin $\alpha 3$ mutations with kidney, lung and skin disease. *N Engl J Med* 2012;366:1508-14.
 8. Yenamandra VK, Moss C, Sreenivas V, Khan M, Sivasubbu S, Sharma VK, *et al.* Development of a clinical diagnostic matrix for characterizing inherited epidermolysis bullosa. *Br J Dermatol* 2017;176:1624-32.
 9. Watkins J. Diagnosis, treatment and management of epidermolysis bullosa. *Br J Nurs* 2016;25:428-31.
 10. Uitto J, Bruckner-Tuderman L, McGrath J, Riedl R, Robinson C. Progress in epidermolysis bullosa research: Towards treatment and cure. *J Invest Dermatol* 2018;138:1010-6.
 11. Nyström A, Bernasconi R, Bornert O. Therapies for genetic extracellular matrix diseases of the skin. *Matrix Biol* 2018;71-72:330-47.
 12. Nyström A, Bruckner-Tuderman L. Injury- and inflammation-driven skin fibrosis: The paradigm of epidermolysis bullosa. *Matrix Biol* 2018;68-69:547-60.
 13. Mavilio F, Pellegrini G, Ferrari S, Di Nunzio F, Di Iorio E, Recchia A, *et al.* Correction of junctional epidermolysis bullosa by transplantation of genetically modified epidermal stem cells. *Nat Med* 2006;12:1397-402.
 14. Hirsch T, Rothoef T, Teig N, Bauer JW, Pellegrini G, De Rosa L, *et al.* Regeneration of the entire human epidermis using transgenic stem cells. *Nature* 2017;551:327-32.
 15. Bauer JW, Koller J, Muraier EM, De Rosa L, Enzo E, Carulli S, *et al.* Closure of a large chronic wound through transplantation of gene-corrected epidermal stem cells. *J Invest Dermatol* 2017;137:778-81.
 16. Siprashvili Z, Nguyen N, Gorell E, Loutit K, Dutt-Singh Y, Nazarov J, *et al.* Phase I/IIa clinical trial for recessive dystrophic epidermolysis bullosa using genetically corrected autologous keratinocytes. *J Invest Dermatol* 2017;137:S89 (abstract).
 17. Kogut I, McCarthy SM, Pavlova M, Astling DP, Chen X, Jakimenko A, *et al.* High-efficiency RNA-based reprogramming of human primary fibroblasts. *Nat Commun*. 2018;9:745.
 18. Shinkuma S, Guo Z, Christiano AM. Site-specific genome editing for correction of induced pluripotent stem cells derived from dominant dystrophic epidermolysis bullosa. *Proc Natl Acad Sci U S A* 2016;113:5676-81.
 19. Kocher T, Peking P, Klausegger A, Muraier EM, Hofbauer JP, Wally V, *et al.* Cut and paste: Efficient homology-directed repair of a dominant negative KRT14 mutation via CRISPR/Cas9 nickases. *Mol Ther* 2017;25:2585-98.
 20. Mencia Á, Chamorro C, Bonafont J, Duarte B, Holguin A, Illera N, *et al.* Deletion of a pathogenic mutation-containing exon of COL7A1 allows clonal gene editing correction of RDEB patient epidermal stem cells. *Mol Ther Nucleic Acids* 2018;11:68-78.
 21. Zhou D, Gao Y, Aied A, Cutlar L, Igoucheva O, Newland B, *et al.* Highly branched poly(β -amino ester)s for skin gene therapy. *J Control Release* 2016;244:336-46.
 22. Kiritsi D, He Y, Pasmooij AM, Onder M, Happel R, Jonkman MF, *et al.* Revertant mosaicism in a human skin fragility disorder results from slipped mispairing and mitotic recombination. *J Clin Invest* 2012;122:1742-6.
 23. Gostyński A, Pasmooij AM, Jonkman MF. Successful therapeutic transplantation of revertant skin in epidermolysis bullosa. *J Am Acad Dermatol* 2014;70:98-101.
 24. Kern JS, Loeckermann S, Fritsch A, Hausser I, Müller ML, Paul O, *et al.* Mechanisms of fibroblast cell therapy for dystrophic epidermolysis bullosa: High stability of collagen VII favors long-term skin integrity. *Mol Therapy* 2009;17:1605-15.
 25. Wong T, Gammon L, Liu L, Mellerio JE, Dopping-Hepenstal PJ, Pacy J, *et al.* Potential of fibroblast cell therapy for recessive dystrophic epidermolysis bullosa. *J Invest Dermatol* 2008;128:2179-89.
 26. Venugopal SS, Yan W, Frew JW, Cohn HI, Rhodes LM, Tran K, *et al.* A phase II randomized vehicle-controlled trial of intradermal allogeneic fibroblasts for recessive dystrophic epidermolysis bullosa. *J Am Acad Dermatol* 2013;69:898-908.
 27. Wagner JE, Ishida-Yamamoto A, McGrath JA, Hordinsky M, Keene DR, Woodley DT, *et al.* Bone marrow transplantation for recessive dystrophic epidermolysis bullosa. *N Engl J Med* 2010;363:629-39.
 28. Hammersen J, Has C, Naumann-Bartsch N, Stachel D, Kiritsi D, Söder S, *et al.* Genotype, clinical course and therapeutic decision-making in 76 infants with severe generalized junctional epidermolysis bullosa. *J Invest Dermatol* 2016;136:2150-7.
 29. Rashidghamat E, McGrath JA. Novel and emerging therapies in the treatment of recessive dystrophic epidermolysis bullosa. *Intractable Rare Dis Res* 2017;6:6-20.
 30. Petrof G, Lwin SM, Martinez-Queipo M, Abdul-Wahab A, Tso S, Mellerio JE, *et al.* Potential of systemic allogeneic mesenchymal stromal cell therapy for children with recessive dystrophic epidermolysis bullosa. *J Invest Dermatol* 2015;135:2319-21.
 31. Desnick RJ, Schuchman EH. Enzyme replacement therapy for lysosomal diseases: Lessons from 20 years of experience and remaining challenges. *Annu Rev Genomics Hum Genet* 2012;13:307-33.
 32. Nyström A, Bruckner-Tuderman L, Kern JS. Cell- and protein-based therapy approaches for epidermolysis bullosa. *Methods Mol Biol* 2013;961:425-40.
 33. Remington J, Wang X, Hou Y, Zhou H, Burnett J, Muirhead T, *et al.* Injection of recombinant human type VII collagen corrects the disease phenotype in a murine model of dystrophic epidermolysis bullosa. *Mol Ther* 2009;17:26-33.
 34. Turczynski S, Titeux M, Tonasso L, Décha A, Ishida-Yamamoto A, Hovnanian A. Targeted exon skipping restores type VII collagen expression and anchoring fibril formation in an *in vivo* RDEB model. *J Invest Dermatol* 2016;136:2387-95.
 35. Bornert O, Kühl T, Bremer J, van den Akker PC, Pasmooij AM, Nyström A. Analysis of the functional consequences of targeted exon deletion in COL7A1 reveals prospects for dystrophic epidermolysis bullosa therapy. *Mol Ther* 2016;24:1302-11.
 36. Mort M, Ivanov D, Cooper DN, Chuzhanova NA.

- A meta-analysis of nonsense mutations causing human genetic disease. *Hum Mutat* 2008;29:1037-47.
37. Cogan J, Weinstein J, Wang X, Hou Y, Martin S, South AP, *et al.* Aminoglycosides restore full-length type VII collagen by overcoming premature termination codons: Therapeutic implications for dystrophic epidermolysis bullosa. *Mol Ther* 2014;22:1741-52.
 38. Lincoln V, Cogan J, Hou Y, Hirsch M, Hao M, Alexeev V, *et al.* Gentamicin induces *LAMB3* nonsense mutation readthrough and restores functional laminin 332 in junctional epidermolysis bullosa. *Proc Natl Acad Sci U S A* 2018;115:E6536-45.
 39. Woodley DT, Cogan J, Hou Y, Lyu C, Marinkovich MP, Keene D, *et al.* Gentamicin induces functional type VII collagen in recessive dystrophic epidermolysis bullosa patients. *J Clin Invest* 2017;127:3028-38.
 40. Baradaran-Heravi A, Niesser J, Balgi AD, Choi K, Zimmerman C, South AP, *et al.* Gentamicin B1 is a minor gentamicin component with major nonsense mutation suppression activity. *Proc Natl Acad Sci U S A* 2017;114:3479-84.
 41. Atanasova VS, Jiang Q, Prisco M, Gruber C, Pinon Hofbauer J, Chen M, *et al.* Amlexanox induces premature termination codon read-through in *COL7A1* and expression of full length type VII collagen: Potential therapy for recessive dystrophic epidermolysis bullosa. *J Invest Dermatol* 2017;137:1842-9.
 42. Mohan GC, Zhang H, Bao L, Many B, Chan LS. Diacerein inhibits the pro-atherogenic & pro-inflammatory effects of IL-1 on human keratinocytes & endothelial cells. *PLoS One* 2017;12:e0173981.
 43. Wally V, Hovnanian A, Ly J, Buckova H, Brunner V, Lettner T, *et al.* Diacerein orphan drug development for epidermolysis bullosa simplex: A phase 2/3 randomized, placebo-controlled, double-blind clinical trial. *J Am Acad Dermatol* 2018;78:892-901.
 44. Woelfle U, Laszczyk MN, Kraus M, Leuner K, Kersten A, Simon-Haarhaus B, *et al.* Triterpenes promote keratinocyte differentiation in vitro, *ex vivo* and *in vivo*: A role for the transient receptor potential canonical (subtype) 6. *J Invest Dermatol* 2010;130:113-23.
 45. Schwieger-Briel A, Kiritsi D, Schempp C, Has C, Schumann H. Betulin-based oleogel to improve wound healing in dystrophic epidermolysis bullosa: A prospective controlled proof-of-concept study. *Dermatol Res Pract* 2017;5068969. doi: 10.1155/2017/5068969.
 46. Kumar V, Behr M, Kiritsi D, Scheffschick A, Grahert A, Homberg M, *et al.* Keratin-dependent TSLP expression suggests a link between skin blistering and atopic disease. *J Allergy Clin Immunol* 2016;138:1461-4.
 47. Castela E, Tulic MK, Rozières A, Bourrat E, Nicolas JF, Kanitakis J, *et al.* Epidermolysis bullosa simplex generalized severe induces a Th17 response and is improved by apremilast treatment. *Br J Dermatol* 2018 doi: 10.1111/bjd.16897.
 48. Chiaverini C, Roger C, Fontas E, Bourrat E, Bourdon-Lanoy E, Labreze C, *et al.* Oral epigallocatechin-3-gallate for treatment of dystrophic epidermolysis bullosa: A multicentre, randomized, crossover, double-blind, placebo-controlled clinical trial. *Orphanet J Rare Dis* 2016;11:31.
 49. Fritsch A, Loeckermann S, Kern JS, Braun A, Bösl MR, Bley T, *et al.* A hypomorphic mouse model for dystrophic epidermolysis bullosa reveals disease mechanisms and responds to fibroblast therapy. *J Clin Invest* 2008;118:1669-79.
 50. Nyström A, Velati D, Mittapalli VR, Kern JS, Fritsch A, Bruckner-Tuderman L. Collagen VII plays a dual role in wound healing. *J Clin Invest* 2013;123:3498-509.
 51. Mittapalli VR, Madl J, Löffek S, Kiritsi D, Kern JS, Römer W, *et al.* Injury-driven stiffening of the dermis expedites skin carcinoma progression. *Cancer Res* 2016;76:940-51.
 52. Akhurst RJ, Hata A. Targeting the TGFbeta signalling pathway in disease. *Nat Rev Drug Discovery* 2012;11:790-811.
 53. Dietz HC. TGF-beta in the pathogenesis and prevention of disease: A matter of aneurysmic proportions. *J Clin Invest* 2010;120:403-7.
 54. Ramirez F, Rifkin DB. Is losartan the drug for all seasons? *Curr Opin Pharmacol* 2012;12:223-4.
 55. Odorisio T, Di Salvio M, Orecchia A, Di Zenzo G, Piccinni E, Cianfarani F, *et al.* Monozygotic twins discordant for recessive dystrophic epidermolysis bullosa phenotype highlight the role of TGF-beta signalling in modifying disease severity. *Hum Mol Genet* 2014;23:3907-22.
 56. Nyström A, Thriene K, Mittapalli V, Kern JS, Kiritsi D, Dengjel J, *et al.* Losartan ameliorates dystrophic epidermolysis bullosa and uncovers new disease mechanisms. *EMBO Mol Med* 2015;7:1211-28.
 57. Tamai K, Yamazaki T, Chino T, Ishii M, Otsuru S, Kikuchi Y, *et al.* PDGFR alpha-positive cells in bone marrow are mobilized by high mobility group box 1 (HMGB1) to regenerate injured epithelia. *Proc Natl Acad Sci U S A* 2011;108:6609-14.
 58. Aikawa E, Fujita R, Kikuchi Y, Kaneda Y, Tamai K. Systemic high-mobility group box 1 administration suppresses skin inflammation by inducing an accumulation of PDGFRα(+) mesenchymal cells from bone marrow. *Sci Rep* 2015;5:11008.
 59. Shimbo S, Sasaki E, Kitayama T, Yamazaki S, Nishida M, Ouchi Y, *et al.* Systemic delivery of HMGB1 promotes tissue regeneration by activating PDGFRα cells in a mouse model of epidermolysis bullosa. *J Invest Dermatol* 2018;138:S143 (abstract).