ABSTRACT

Objective: Epidermolysis bullosa (EB) is an orphan disease that affects about half a million people worldwide, but may not be familiar to all clinicians. The authors' goal was to present a short description of this condition and current research in the form of a narrative review. **Methods:** The authors reviewed the literature on epidermolysis bullosa in order to describe the condition and current genetic research. **Results:** There are at least 31 subtypes of EB, including junctional EB, dystrophic EB, and Kindler syndrome. Genetic research is crucial in finding strategies to manage and possibly cure EB, which is often undiagnosed or misdiagnosed. EB may present in newborns and may persist over the course of a lifetime. Serious complications can occur with EB, including chronic blisters, wounds, ulcers, pruritus, clubbing of hands and feet, and amputations. Pain is frequently reported. About 80 percent of patients with recessive dystrophic EB will succumb to squamous cell carcinoma by age 55. Promising directions for future research include genome editing, gene therapy, and cell-based therapies. **Conclusion:** Our growing understanding of genetics and cell therapies may lead to promising therapeutic advances to treat this challenging condition.

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REVIEW

Raising Awareness Among Healthcare Providers about Epidermolysis Bullosa and Advancing Toward a Cure

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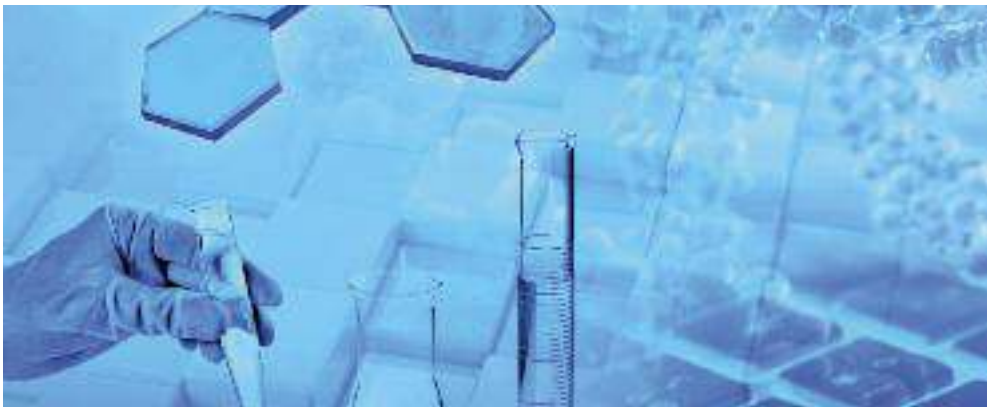

CONSIDERED AN ORPHAN disease, the clinical variants of epidermolysis bullosa (EB) affect about half a million people worldwide, many of whom are children.[1] This rare, incurable, and genetically heterogeneous group of disorders is characterized by skin fragility and blistering that can result in painful wounds, scarring, infection, and functional impairment.

EB affects all racial and ethnic groups with no clear sex predominance.[2] Signs typically appear at or near birth and persist over a lifetime. However, the onset of lesions in some individuals may not appear until adolescence or early adult life, and in some variants, blistering improves with increasing age. Blisters may be tense or flaccid and can rupture and form erosions and crusts. While blisters and eruptions may occur anywhere on the body, typical sites for these blisters are the areas of normal mechanical

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trauma, including the palms, soles, limbs, face, and diaper area. Mucous membranes (mouth, nasopharynx, ocular region, gastrointestinal tract, and genitalia) may become involved and blisters may lead to scarring.[3] The skin condition can be disfiguring and may necessitate frequent and extremely painful dressing changes. Patients suffering from EB may experience fusion of their digits leading to clubbed hands and feet and may undergo amputation.

Despite the devastation caused by this disease, it remains relatively unknown by the general public and infrequently encountered by healthcare professionals. In a study using semi-structured interviews with 11 families of children with EB, areas of concern for parents and caregivers were the lack of knowledge among healthcare professionals about EB and, specifically, how to care for their children.[4] This same lack of awareness makes children with EB targets of bullying by other children and of hurtful, intrusive comments by unthinking adults.[5] However, EB is more than just a cosmetic skin condition; it can be a severe, life-altering disease associated with significant morbidity, early death, excessive hardships for caregivers, increased healthcare utilization and costs, and pain for the afflicted, which can at times be severe.

There is an urgent unmet medical need to better diagnose, treat, and possibly one day cure and eradicate this devastating disease. Our increasing understanding of genetics, novel sequencing techniques, genome editing, protein replacement, autologous and allogeneic stem cell therapies, breakthroughs in our understanding of cancer biology, revertant mosaicism, and induced pluripotent stem cell techniques all hold tremendous promise.[1] The purpose of this narrative review is to raise awareness about this condition and advocate for channeling adequate resources toward finding a cure for EB.

TYPES OF EB

EB is not one disease and attempts to simplify it as one set of conditions fail. There are at least 31 subtypes of EB and it is possible that future research will add to this list. The classification of EB is based upon the location of the cleavage plane in the skin: simplex or epidermolytic with blistering within the epidermis, junctional with blisters in the epidermal-dermal junction, dystrophic or dermolytic with blistering in the dermis, and Kindler syndrome with multiple splits in the skin.[6,7]

Epidermolysis bullosa simplex. Epidermolysis bullosa simplex (EBS) represents both the largest patient population of EB patients (about 70%) and usually the mildest form of the disease, although some generalized subtypes can be severe particularly in early infancy.[3] Basal EBS, in which blisters form within the basal cell layer of the epidermis accounts for the majority of cases. Although blisters generally heal quickly without scarring, it is possible that scarring, nail dystrophy, and hypo- and/or hyperpigmentation may occur.

Mutated autosomal dominant genes are responsible for EBS although in about 25 percent of cases, recessively inherited EBS may occur[8] and *de novo* mutations have also been reported.[9] Many gene mutations have been associated with EBS, including keratin-5 (KRT5) and KRT14. To date, nine EBS genes have been implicated in several autosomal recessive subtypes: KRT14, PLEC, DST-e, PKP1, DSP, JUP, TGM5, and EXPH5.[10,11] The manifestation of EB in a given patient may be explained by the topographic location of the expression of the mutant gene(s) within the basement membrane zone (BMZ), which types and combinations of mutations are involved, and how these mutations have been affected by the environment (i.e., trauma).[7]

Junctional epidermolysis bullosa. Junctional epidermolysis bullosa (JEB) is most always recessively inherited and caused by mutations in the laminin-332 ($\alpha3A\beta3\gamma2$) gene.[12] Specific mutations that affect the N-terminus of the αa3A-chain are associated with a non-blistering cutaneous condition of altered granulation tissue response.[12] JEB is the rarest form of EB and is associated with blistering of the epidermal-dermal junction and the upper portion of the dermis with variable scarring and nail dystrophy. Severe mucocutaneous disease with involvement of the oral region and nasopharynx as well as the gastrointestinal tract may be associated with poor weight gain, secondary infection, and death early in infancy.[3] Mutations in genes responsible for the formation of laminin-32 in the BMZ are most commonly associated with JEB. A recessive lethal generalized severe

JEB was identified in a small population of Hungarian Roma, determined to be associated with an unconventional intronic splice site mutation (LAMB3).[13] A recent report of an autosomal dominant form of JEB with a somewhat milder clinical presentation, expands its genotypic heterogeneity.[14] Further research is warranted.

Dystrophic epidermolysis bullosa. Dystrophic epidermolysis bullosa (DEB) presents variably from mild clinical forms (usually autosomal dominant) that improve with age and more severe variants (usually autosomal recessive) associated with severe scarring of the skin and mucous membranes.[15] About a quarter of all EB cases are DEB.[3] The number of cases may actually be higher than reported, since mildly affected patients may not be diagnosed. Type VII collagen (C7) homopolymerizes at the junction of the dermis and epidermis and, in this way, forms the main component of anchoring fibrils that interlace with interstitial collagen fibers, allowing the basement membrane to attach to the underlying papillary dermis.[16] DEB is caused by any of several possible mutations in the gene encoding C7, that is, COL7A1, located on chromosome 3p21.3, which results in absent or abnormally formed anchoring fibrils. By the time DEB is diagnosed, a cascade of changes has already occurred in other mucocutaneous cellular complexes along with local and systemic inflammation and fibrosis.[16] Thus, even if COL7A1 might be corrected, it may not reverse the disease in that maladaptive changes in the DEB phenotype can occur in a patient over time. For that reason, any curative approach to DEB must accommodate not only gene-correcting techniques, but also gene-regulating approaches.[16]

Kindler syndrome. Kindler syndrome is a rare autosomal recessive disorder associated with mutations of the FERMT1 gene.[17] It is considered distinct from EBS, but is likewise characterized by skin fragility,[18] and also poikiloderma, photosensitivity (more often in childhood than adulthood), nail dystrophy, digital webbing, and can involve the oral cavity (causing dysphagia, tooth decay, gingivitis, periodontal disease), constipation, and anal fissures.[17,19]

Although it goes beyond the scope of this article, it is worth mentioning here that there is also EB acquisita (EBA), which is caused by the production of auto-antibodies directed against COL7. EBA is not an heritable condition, but rather an autoimmune disorder (e.g., associated with systemic lupus), which presents with findings similar to DEB.[3,20–22]

GENETIC IMPLICATIONS

Genetic research is crucial to finding strategies to manage and possibly cure the many types of EB. Research into specific mutations within families and studies involving both affected and unaffected family members will help better elucidate the complex inheritance patterns associated with EB. The mutations themselves may affect inheritance patterns. For example, mutations in KRT14 typically result in autosomal dominant forms of EB, but recently there have been reports of autosomal recessive inheritance of KRT14 mutations.[7] Revertant mosaicism describes the co-existence of cells that carry disease-causing mutations alongside cells in which the mutation has been genetically corrected by some spontaneous event. Revertant mosaicism has been observed in EB along with a number of other genodermatoses.[25] Revertant mosaicism may be observed in some EB patients in whom normal-looking skin appears adjacent to blistered and severely affected skin. It remains to be elucidated how spontaneous genetic correction occurs and what repair mechanisms are involved.

DIAGNOSIS OF EB

Because of its rarity, EB may be undiagnosed or misdiagnosed. The many types of EB also make diagnosis challenging. A family history of any of the forms of EB or blistering in areas of mechanical trauma is suggestive, but a differential diagnosis must exclude other blistering conditions, such as bullous pemphigoid, pemphigus vulgaris, friction blisters, and insect bites. When EB is highly likely clinically, a skin biopsy may not be necessary to confirm the diagnosis, but rather genetic testing may be indicated instead. Diagnostic methodology is evolving rapidly with greater reliability of genetic testing, whole exome sequencing, and the ability to evaluate known mutations in an "EB panel." When the diagnosis is less clear, a skin

biopsy may help to confirm the clinical diagnosis of EB. Diagnosis should be based on the patient's clinical history, signs and symptoms, histology, direct and indirect immunofluorescence microscopy, and genetic testing.[26]

Early and accurate diagnosis can aid in proper treatment and counseling regarding the prognosis and long-term management of the condition. Serologic diagnosis of EBA relies on the detection of circulating auto-antibodies to C7, but current technology is limited by its relatively low sensitivity.[27] Kindler syndrome is diagnosed based on molecular genetic testing (identification of either biallelic FERMT1 pathogenic variants), but can sometimes be diagnosed by other clinical and histological findings.[17]

Fine et al[8] have proposed an updated classification scheme for EB that they describe as "onion skinning," which involves a sequential review of the major EB type present (based on the level of skin cleavage), phenotypic characteristics (including distribution and severity of the disease, extracutaneous features, if any), mode of inheritance, targeted protein and its relative expression in the skin, gene involved, type(s) of mutation present, and the specific mutation(s) and location(s) if this can be ascertained.[8] Note that the inability to fill in all of these blanks does not preclude a preliminary diagnosis, since the "onion skin" model allows clinicians to be incomplete and describe only the layers that they can describe.

THE BURDEN OF EB

The burden of EB includes a disfiguring and extremely painful cutaneous condition, the need for special lifestyle modifications, frequent and potentially excruciatingly painful dressing changes, ongoing care and support, and frequent, lifelong monitoring for squamous cell carcinoma (SCC). Using semi-structured interviews, the main problems experienced by pediatric EB patients were itchy skin, pain, not being able to participate in things with other children, being misunderstood, and feeling different. Children with mild EB were more affected by concerns for their appearance (teasing, staring, rude comments) than children with severe EB.[5]

Although rare, EB presents a substantial burden to the healthcare system in terms of healthcare-related costs, non-healthcare-related costs, and lost productivity. The incidence of all types of DEB in the United States is 6.5 per million live births,[28] but some type of EB occurs in roughly 1 out of every 50,000 live births.[29] It is estimated that around the world, about half a million people are living with EB.[30] A survey in Europe (n=204) estimated that in 2012, the average annual cost per patient was estimated to be about 31,390 Euros (roughly US$36,000) of which 18.0 percent was direct healthcare costs, 74.8 percent direct non-healthcare costs, and 7.2 percent lost productivity.[31]

TREATMENT OF EB

Many forms of EB, especially the severe variants, can be challenging and difficult-to-manage conditions, which may affect a patient over the course of a lifetime. A multidisciplinary team is key to the effective management of EB patients. The team might include a dermatologist, pediatrician and/or neonatologist or internist, anesthetist, pathologist, medical geneticist, pain specialist, specialized nurses, and psychiatrist or psychologist.[17,32] For a more comprehensive and more severe forms of EB, this team might expand to include an ophthalmologist, gastroenterologist, dentist, otolaryngologist, and endocrinologist. Some EB patients may require specialists in surgery, plastic surgery, radiology, dietetics, and speech therapy.[32]

The severe variants of EB are rare so that no large randomized trials have been conducted. For that reason, recommendations for treatment come largely from expert consensus, literature review, and observations.[32]

AREAS OF RESEARCH

There are currently no systemic pharmacological treatment options for EB. An oral green tea extract, epigallocatechin-3-gallate (EGCG) was tested in DEB patients in a randomized, double-blind controlled trial (n=17), but showed no benefit over placebo at four months.[33]

Research efforts are ongoing to find better treatments and curative approaches.[34] Protein replacement therapy, being studied by research scientists at the University of Southern California and Stanford University, are trying to replace or boost defective and/or missing

Table 1. EB patients may experience many different types of pain, which can be moderate to severe or very severe[33]

TYPE OF PAIN	CAUSE	NOTES
Acute	New-onset mucosal lesions	These may occur in the eye, mouth, esophagus, trachea, or anus
Chronic	Inflammation Neuropathy Bone pain	These maladaptive forms of pain can be more difficult to treat; symptoms may be diffuse
Incidental	Pain associated with bathing, dressing changes	Analgesia may be required
Post-procedural pain	A form of acute pain associated with an operation or procedure	Post-procedural pain should be discussed with patient and caregiver in advance
Cancer	Related to development of squamous cell	

proteins in certain EB patients using localized injections, micro-needle devices, and systemic therapies.[35] A commercial company is working on protein replacement therapy for DEB. Protein replacement therapy would not represent a cure but it would allow for better disease management.[35]

Cell therapy, in which stem cells, fibroblasts, or gene-corrected cells can be locally or systemically injected, represents another approach to EB that is being evaluated in a clinical trial at the University of Minnesota.[35] Research efforts at Kings College in the United Kingdom have had promising results by injecting fibroblasts into the skin of EB patients.[35]

Gene therapy uses methods to carry the correct gene into the patient's cell with the goal that the correct gene will cause the cell to manufacture the correct protein and is currently being evaluated in EB.[36] In a study at Stanford University, small amounts of skin are taken from a person with recessive dystrophic epidermolysis bullosa (RDEB), skin is then cultured and grown, and a virus is used to carry the correct gene into the cells.[35] The gene-correct skin cells are then grafted back onto the patient's body. A similar study is ongoing in Europe for JEB patients.[35,36]

Stem cell therapy could represent a curative approach to EB. Stem cell therapy allows the cells of an individual with EB to be "gene-edited" so that the gene that causes EB can be targeted and replaced. The corrected cells then are reintroduced into the body.[35,36]

PAIN ASSOCIATED WITH EB

The first evidence-based care guidelines for pain control for EB patients were published in 2014.[37] The guidelines recognize that pain can be persistent and severe in EB patients. Selected EB patients require round-the-clock analgesia, which may be initiated with acetaminophen, but advance over time to stronger analgesics, including opioids. Morphine or other opioids may be appropriate to be administered on an as-needed basis to control pain associated with dressing changes.[38] The types of pain an EB patient may experience are described in Table 1.

Anesthesia can be challenging for EB patients, although there are no contraindications to inhaled anesthetics or regional anesthesia (such as spinal or epidural).[39] The surgical team must minimize skin and airway trauma to the patient. Thick padding and lubrication should be used to reduce potential friction and shearing. For example, ECG gel pads should be used, blood pressure cuffs should be used with thick cotton dressings, and intravascular catheters are better anchored with sutures than tape. As much as possible, upper airway devices should not be used owing to the possibility of friction in the airways, which can cause trauma,

obstruct breathing, and result in bleeding. When intubation is necessary, the laryngoscope and tracheal tubes should be well-lubricated so as to reduce friction as much as possible.[35] It may be beneficial for EB patients to have dental work done under general anesthesia.[36] In some patients, dressing changes may require anesthesia.

EB patients who develop SCC may experience pain associated with the primary tumor, its spread, distant metastases, and pain associated with oncology care. At end-of-life, opioids may be administered to such patients without concern about tolerance or addiction. Topical morphine hydrogel can be applied directly to a painful malignant wound and replaced with each change of dressing.[37] Opioids may also be administered orally, parenterally,[38] or using a transdermal system providing that it can be removed with a medical adhesive-removal spray in such a way to protect the patient's fragile skin.[39]

COMPLICATIONS OF EB

Cutaneous complications associated with EB include chronic blisters, wounds/ulcers, pruritus, palmoplantar keratoderma, EB nevi, scarring and contractures and SCC.[28] When cleansing a wound, a mild antiseptic product, such as chlorhexidine 0.1%, can be used.[40,41] Some wounds may require topical antibiotics and, at times, a course of oral antibiotics. Prescribing decisions for antibiotic therapy should be based on a wound culture.[3] Wound debridement can accelerate healing and prevent infection, but must be used with caution since aggressive debridement may result in new blisters and erosion.[46]

Pruritus. Pruritus is a frequently reported complication and should be addressed quickly to minimize the risk of new lesions caused by scratching. Antihistamines may reduce pruritus and the frequent use of emollients can further reduce skin irritation and sensitivity. Recalcitrant pruritus may require treatment with a short course of oral and/or topical steroids.[3] Many EB patients complain of hyperhidrosis, which can exacerbate pruritus.[32] This may be addressed with topical corn starch or other powders and the cautious use of topical aluminum chloride or oral low-dose sodium glycopyrrolate.[47]

Blisters. Although sites of blister formation vary with various EB types, blisters may occur at any cutaneous site. Blisters in the anal region can cause severe pain and result in constipation. Blisters in the mouth or nasopharyngeal region can limit food intake and result in scarring with stricture formation. Ocular involvement can result in corneal erosions, blepharitis, conjunctival granulation tissue, scarring with significant vision impairment, including blindness.[32] Meibomian gland dysfunction (MGD) occurs in many pediatric EB patients. In a prospective study of 105 children with different forms of EB, 88 percent exhibited one or more features of MGD.[48] In a large cohort study of adult patients with different forms of EB (n=181), ocular problems were found in 12 percent of EBS and 40 percent of JEB patients and 51 percent of the RDEB patients had eye problems, with corneal problems being the most common.[49] The National Epidermolysis Bullosa Registry reported ocular problems varied by type of EB, occurring in 74 percent of all patients with RDEB, 48 percent of all patients with JEB (including 6% who reported blindness). The cumulative risks of non-scarring or scarring corneal lesions in junctional EB Herlitz (JEB=H) patients at age 5 are 83 percent and 27 percent, respectively; at age 25, these risks are 83 percent and 72 percent, respectively.[50]

Gastrointestinal (GI) strictures or webs. GI strictures or webs involving primarily the esophagus may develop and require surgical intervention. Upper respiratory tract complications, musculoskeletal symptoms, and cardiovascular complications, such as dilated cardiomyopathy, have been reported.[32] Infants with EB may suffer from failure to thrive and may require high-calorie formulas and/or nutritional supplementation. DEB may result in endocrine system complications, which, in a child, may result in delayed onset of puberty and amenorrhea.

Deformities of the feet. Deformities of the feet may occur as a result of scarring and contractures of toes; equinus and cavus deformities may also appear. In a retrospective analysis of 13 surgical procedures in six patients to correct foot deformities caused by EB, 85.7 percent of those with extension contracture of the toes were asymptomatic at follow-up, but 42.9 percent developed hammertoe.[51]

Squamous cell carcinoma. The most potentially dangerous complication of EB is SCC. It is by far the greatest cause for mortality in recessive DEB patients with 80 percent succumbing to SCC by age 55.[52] Unfortunately, SCC is often invasive and metastasizes rapidly in patients with EB. Moreover, there is no clear consensus regarding the best treatment strategies.[42] Research is underway to determine if restoration of COL7 by cell therapy or gene therapy might be helpful to reduce or even prevent cancer.[53,54]

It is often difficult to identify an SCC in an EB with chronic ulcers and granulation tissue. Clinicians are advised to look for atypical wounds, such as those that take longer to heal than expected, wounds that grow rapidly (particularly those with exuberant granulation), deep and hollowed-out ulcers with raised edges, hyperkeratotic areas surrounded by raised skin, and wounds with altered sensations (for example, tingling).[42] Suspicious areas should be biopsied early. If SCC is diagnosed, a multidisciplinary approach should be taken, including an oncologist, histopathologist, dermatologist, and plastic surgeon, among others.

Surgery is the primary course of treatment, but frequent metastases and relapses may necessitate radiotherapy and/or chemotherapy and/or targeted therapy.[55] Tumor excision may be performed with wide local excision (the preferred treatment option for EB patients), Mohs micrographic surgery, or amputation. When minimally invasive surgical techniques can be used, Mohs surgery, rush paraffin sections ("slow Mohs"), and chemosurgery have been described.[42] Wound closure may require partial- or full-thickness skin grafting, including the use of allografts (or artificial skin equivalents).

A new treatment option for localized treatment of SCC in DEB patients is electrochemotherapy (ECT), which combines low-dose systemic or intralesional cytotoxic agents (such as bleomycin or cisplatin) combined with high-intensity electrical pulses that are designed to temporarily enhance skin-cell permeability and thus allow for better drug delivery into tumor cells.[56] ECT has been shown effective in the treatment of cutaneous and subcutaneous metastasis and nonmelanoma skin cancer (NMSC) and SCC.[57]

PSYCHOLOGICAL SUPPORT OF EB PATIENTS AND THEIR CAREGIVERS

The rarity of the severe variants can make patients and their families feel isolated and misunderstood. The fact that the condition has nso cure and may well persist over a lifetime can become a major psychological burden.[32] As such, EB takes a terrible toll on the patient, family, friends, and caregivers.[3] EB can give rise to feelings of intense guilt, extreme stress, and hyper-responsibility in some parents. Family members can become fearful about handling a child with EB and may even avoid being around the child. EB can be disruptive to the family structure, in that non-affected siblings may feel that the EB child diverts attention and parental care away from them. Parents may find their primary relationship strained in caring for a child with EB. In some cases, one parent may refuse to participate in the child's care or even deny that the child has EB. In such situations, the child with EB can feel isolated, depressed, or estranged in the family setting.[32] Psychological and social support for EB may be part of a holistic, multidisciplinary approach. There is an urgent need for psychological support if the patient is diagnosed with SCC or is fearful of such a diagnosis.[42] Longer-term psychological support may also be needed. Interpretive phenomenological analysis found six patients with severe forms of EB reported their main issues as coping, pain, perceptions, emotional impact, social impact, and support network.[58]

PROMISING DIRECTIONS IN TREATMENT

Our increasing understanding of genetics and cell therapy along with novel technologies hold great promise for conquering EB. Gene therapies in preclinical or clinical trials include *ex vivo* keratinocyte therapy, direct gene application (the "gene gun"), genome repair, trans-splicing of genes, and gene silencing (siRNA).[59] Protein-replacement therapy is also being evaluated in preclinical studies with C7 for recessive DEB and laminin-332 for JEB.[59] Molecular therapies including gene editing and the repurposing of small molecules, such as aminoglycosides and angiotensin receptor blockers, are being evaluated in preclinical studies.[60] Next-generation

sequencing (NGS) of deoxyribonucleic acid (DNA), including whole-exome sequencing, may develop into an important tool for EB diagnostics.[61] Bone marrow transplants continue to be of interest for treating EB. A short summary highlighting some recent work from the literature appears below. Future merging of biotechnology and biomedicine will help meet the challenge of EB, but thus far outcome data are limited.

Genome editing. Genome engineering, which seeks to manipulate DNA, historically commenced using tools such as DNA-sequence-complementary oligonucleotides and self-splicing introns. Genome editing can be carried out using older, conventional gene replacement therapy with viral or nonviral transfection of cDNA or it can use site-specific endonucleases. New genetic engineering tools, such as meganucleases, allow clinicians to target a precise genomic location within a primary cell with limited genotoxicity.[62] Gene editing with zinc finger nucleases or other nucleases can mediate genome modification.[16] For that reason, gene editing offers a new approach to gene therapy for human genodermatoses, including but not limited to EB. Beyond gene editing, customized nucleases may be able to help regulate disease-modifying factors.[16]

Virally mediated transgenesis is used today, with site-specific DNA-protein recognition of nucleases. These nucleases encompass zinc finger nuclease, transcription activator-like effector nuclease (TALEN),[16] clustered regularly interspaced palindromic repeats (CRISPR/CRISPR-associated [Cas] system), and meganculeases.[62–64] These so-called "designer nucleases" have become a major scientific breakthrough in many fields of medicine in that they allow genetic engineers to reprogram genetic information—which, in turn, has transformed the treatment of human disease. Genes are no longer deterministic and unyielding, but rather are tools that can be modified to promote better health.[16] As a relevant example, single-chain LAGLIDAG-homing endonucleases (LHEs) can restore COL7 expression in unselected human primary DEB cells.[16] Homology-directed repair is required to correct genes, so a donor DNA sequence complementary to the targeted region ("wild type repair template") was co-transduced with increasing doses of LHE meganucleases.[16]

Using patient-derived COL7A1-corrected epithelial keratinocyte sheets, autologous grafting can be used with sequential reprogramming and adenovirus-associated viral genome editing to create corrected induced pluripotent stem cells (iPSCs).[64] Keratinocytes derived from these iPSCs can be produced, which, in turn, secrete wild-type COL7. This work has been carried out *in vitro* with organotypic cultures and *in vivo* in mice.

Genome editing using engineered site-specific endonucleases requires nonhomologous end-joining, which, in turn, disrupts the reading frame. For dominant negative disorders, the mutant allele can be knocked out and the normal allele left intact. The literature reports investigators using genome editing with TALENs and CRISPR/Cas9 targeting the mutation (c.8068_8084delinsGA) to treat DEB. Cas9 was then cotransfected and guide RNA expression vectors expressed with GFP and DsRed, respectively, into induced iPSCs. The iPSCs were generated from DEB fibroblasts. After sorting, most of the iPSCs were edited and four of those gene-edited lines were chosen for further investigation. These iPSCs differentiated themselves into keratinocytes and fibroblasts secreting COL7. These gene-edited COL7 with frameshift mutations degraded at the protein level and could not associate with normal COL7 or undergo triple helix formation. Thus, it has been shown to be feasible to perform site-specific genome editing in disorders where the mutant allele can be knocked out and the normal allele left intact.[65]

Gene therapy. Clonal gene therapy protocols require manipulation and molecular editing of epidermal stem cells.[66] *Ex vivo* gene therapy clinical trials have been conducted for COL7A1 gene mutations associated with recessive DEB, but other approaches are limited as surgical interventions are extremely dangerous for patients with such fragile skin.[67] In gene therapy, multiple accessory sequences that allow for plasmid DNA manufacture and antibiotic resistance sequences must be removed[68] and a therapeutic gene must be constructed in a minicircle (MC) design.

Investigators hypothesized that a

polymeric gene vector with a minimized nonintegrating DNA construct could be injected into the skin (or perhaps applied topically), which would reduce the expression of C7 to human skin in patients with recessive DEB. In a murine study with grafted human recessive DEB skin, animals received one intradermal injection of HPAE-MCC7 polyplexes. By Day 5, only one mouse was positive for the recombinant protein. However, when the mice received two or three injections, all skin grafts tested positive for recombinant protein. Topical application also worked well; five days after a single topical application, recombinant protein could be visualized along the BMZ. Thus, the expression of C7 was visible at 10 weeks after topical applications along the length of the human skin graft at the BMZ.[67] Episomal transgenes may be an important topical product to use in the rapidly growing keratinocyte population because they avoid mutagenicity. Complexing MCCY with a polymeric vector may facilitate large-area applications with a cost-efficient simple formula.[67] Topically delivered KGF-1 DNA plasmid can increase epithelial thickness and strength, demonstrating potential for this approach to restore compromised skin.[69]

Cell-based therapies. A wide range of cell-based clinical approaches to EB are in clinical trial or preclinical development and include fibroblast therapy for recessive DEB, mesenchymal stem cell application, induced pluripotent stem cells, and bone marrow transplant.[59] Cell-based therapies, such as allogeneic fibroblasts to the skin, are under exploration, with the putative concept being that allogeneic fibroblast injection will cause cytokine expression, including the expression of heparin-binding-EGF-like growth factor (HB-EGF), which can up-regulate the expression of patient-specific COL7A1 allele.[70]

Bone marrow transplants have been used to treat EB. A preclinical study suggested that the SDF-1α/CXCR4 signaling axis might induce transplanted bone marrow-derived circulating PDGFRα(+) mesenchymal cells to supply function C7 to regenerate the skin in recessive DEB.[71] Another preclinical study found that wild-type congenic bone marrow cells homed to damaged skin produced C7 protein and associated anchoring fibrils, which, in turn, reduced skin fragility.[72] Indeed, allogeneic blood and marrow transplants can improve the integrity of cutaneous tissue and mucous membranes.[73]

It remains unclear which bone marrow cells are the most useful for skin recovery and production of C7.[74] In a randomized controlled trial of 14 patients with recessive DEB, patients received non-hematopoietic bone marrow stem cells (NHBMSC) under the hypothesis that these stem cells would develop into fibroblasts and be able to synthesize C7.[75] Seven of the patients received cyclosporine after the NHBMSC infusion; seven did not. Pre- and post-treatment skin biopsies under electron microscopy revealed significant reduction in the number of new blisters and significantly faster healing of blisters in both groups (no significant difference between groups). No major side effects were reported in any patient at one-year follow-up.[75]

In a study of six pediatric patients with recessive DEB, all patients were treated with immune-myeloablative chemotherapy and allogeneic stem-cell transplantation.[76] Using immunofluorescence staining, C7 expression was evaluated and electron microscopy was used to visualize anchoring fibrils. All patients exhibited improved wound healing and had fewer blisters 30 to 130 days post-treatment; five patients had increased C7 deposition at the dermal-epidermal junction, but did not have normalized anchoring fibril formation. Five patients survived to 130 to 799 days post-transplantation; one died at 183 days post-treatment as a result of graft rejection and infection. All six patients had substantial proportions of donor cells in their skin and none had detectable anti-C7 antibodies.[76]

Neo-cells. Pioneering work in "neo-cells" holds great promise. These so-called "new cells" derive their name because they had not existed in nature previously. Neo cells can be designed with customizable nucleases to target and regulate specific disease pathways, such as DEB.[16] Technological advancements permit the engineering of highly specialized neo-cells designed to target the polyfunctional regulation of disease pathways.[77]

RNA trans-splicing. RNA trans-splicing can correct genetic

mutations at the RNA level. Human mRNA is a good target for this type of RNA splicing as only a portion of the transcript needs to be delivered into target cells. Using a gene-gun delivery system, this method was evaluated in the skin of wild-type mice, which restored COL7 expression to treat DEB.[78]

CONCLUSION

EB in its many forms continues to afflict children and adults around the world—modern medicine is just beginning to address long-term treatment strategies. There is finally a reason to be optimistic about these diseases because of our growing understanding of genetics and cell therapies along with new technologies that may unlock the secret to these grave genetic disorders. EB is a group of diseases that afflicts about half a million people around the world. It is a devastating and painful condition and finding effective treatment options is an urgent and unmet medical need.

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