Progress toward Treatment and Cure of Epidermolysis Bullosa: Summary of the DEBRA International Research Symposium EB2015

Jouni Uitto[1], Leena Bruckner-Tuderman[2], Angela M. Christiano[3], John A. McGrath[4], Cristina Has[2], Andrew P. South[1], Brett Kopelan[5] and E. Clare Robinson[6]

Epidermolysis bullosa (EB), a group of complex heritable blistering diseases, is the topic of triennial research meetings organized by DEBRA International, Vienna, Austria, the network of national EB patient advocacy organizations. The DEBRA 2015 Research Conference, held in May 2015, brought together investigators and clinicians from around the world working at the forefront of EB research. Discussing the state-of-the-art approaches from a wide range of disciplines, there was a palpable excitement at this conference brought about by the optimism about applying new sequencing techniques, genome editing, protein replacement, autologous and allogeneic stem cell therapy, innovations in cancer biology, revertant mosaicism, and induced pluripotent stem cell techniques, all of which are aimed at developing new therapies for EB. Many in the field who have participated in EB research for many years were especially enthusiastic and felt that, possibly for the first time, the field seems uniquely poised to bring these new tools to effectively tackle EB. Multiple complementary approaches are currently in motion toward improved quality of life and eventually a cure for patients suffering from EB, a currently intractable disease.

Journal of Investigative Dermatology (2016) **136,** 352–358; doi:10.1016/j.jid.2015.10.050

INTRODUCTION

Epidermolysis bullosa (EB), a group of heritable blistering disorders, consists of four main subtypes primarily distinguished by the level of blistering within the cutaneous basement membrane zone (Table 1). Each of these subtypes can display a spectrum of phenotypic severity reflecting the types and combinations of mutations in different genes, together with modifying environmental factors. The types of mutations also determine the mode of inheritance, either autosomal dominant or autosomal recessive. Currently 18 genes have been shown to be associated with the different subtypes of EB (Table 1). In spite of the tremendous progress made in understanding the molecular basis of different forms of EB, there is no cure for this disease.

DEBRA International, Vienna, Austria, an organization advocating on behalf of the patients with EB and their families, sponsors Triennial Research Conferences. The latest one in this series, organized by DEBRA of America, New York, in Braselton, GA, in May 2015, was attended by more than 100 researchers, physician-scientists, trainees, and patient support group representatives (Figure 1). This meeting report summarizes the presentations and discussions that took place in this conference.

MODEL SYSTEMS FOR EB

Animal models

In addition to many naturally occurring EB forms in animals reviewed previously (Bruckner-Tuderman et al., 2010, 2013; Uitto et al., 2010), a variety of model systems have been generated.

Novel murine models. Some recently developed animal models have revealed unexpected consequences and improved our understanding of phenotypic variability. For example, careful analysis of mouse models for junctional EB (JEB) identified the first major genetic modifier of JEB phenotype due to a laminin-γ2 mutation by collagen XVII, in particular molecular variations in its NC4 domain (Sproule et al., 2014). Also, a recently reported knock-in mouse model for JEB that displays alternative splicing of the *Lamb3* gene will aid in defining further genetic modifiers of JEB phenotypes (Hammersen et al., 2015).

Another interesting finding relating to junctional skin blistering was revealed by the deletion of the linker extracellular domain of transmembrane collagen XVII in mice. This led to alternative shedding of the ectodomain, but not to JEB. Instead, induction of autoimmune blistering and itching were observed, and the phenotype of the mice mirrored signs of bullous pemphigoid, including perilesional eosinophilic infiltrations, blood eosinophilia, and elevated serum IgE levels (Hurskainen et al., 2015). Future work will be aimed at discerning mutations and disease mechanisms predisposing to mechanobullous versus inflammatory blistering phenotypes in both humans and mice.

Because of the multiorgan involvement, the severity of the phenotypes, and significant unmet medical need, the

[1]*Department of Dermatology and Cutaneous Biology, Thomas Jefferson University, Philadelphia, Pennsylvania, USA;* [2]*Department of Dermatology, Medical Center, University of Freiburg, Freiburg, Germany;* [3]*Department of Dermatology, Columbia University, New York, New York, USA;* [4]*Genetic Skin Disease Group, St. John's Institute of Dermatology, King's College London (Guy's Campus), London, UK;* [5]*DEBRA of America, New York, New York, USA; and* [6]*DEBRA International, Vienna, Austria*

Correspondence: Jouni Uitto, Department of Dermatology and Cutaneous Biology, The Sidney Kimmel Medical College at Thomas Jefferson University, 233 S. 10th Street, Suite 450 BLSB, Philadelphia, Pennsylvania 19107, USA. E-mail: Jouni.Uitto@jefferson.edu

Abbreviations: BM, bone marrow; BMT, bone marrow transplantation; DEB, dystrophic EB; EB, epidermolysis bullosa; HMGB1, high mobility group box 1; iPSCs, inducible pluripotent stem cells; JEB, junctional EB; MSC, mesenchymal stromal/stem cell; RDEB, recessive DEB; SCC, squamous cell carcinoma

Table 1. Molecular heterogeneity of different forms of EB

Disease	Gene	Cytogenetic location	Inheritance	Proportion of EB attributed to mutations in this gene
Simplex epidermolysis bullosa (EBS)	*KRT5*	12q13.13	AD	75% of EBS-AD cases;
	KRT14	17q21.2	AR, AD	15 cases of EBS-AR have been reported with KRT14 mutations
	TGM5	15q15.2	AR	Up to 10% cases of EBS
	DSP	6p24.3	AR	
	PKP1	1q32.1	AR	
	JUP	17q21.2	AR, AD	
	EXPH5	11q11.3	AR	
	PLEC	8q24.3	AR, AD	
	DST	6p12.1	AR	
	ITGB4	17q25.1	AR	
	COL17A1	10q24.3-q25.1	AR	
Junctional epidermolysis bullosa (JEB)	*LAMA3*	18q11.2	AR	9% of all JEB cases; specific mutations in the LOC (Shabir) syndrome
	LAMB3	1q32.2	AR	70% of all JEB cases
	LAMC2	1q25.3	AR	9% of all JEB cases
	COL17A1	10q24.3-q25.1	AR	10% of all JEB cases
	ITGA6	2q31.1	AR	A few cases reported
	ITGB4	17q25.1	AR	Many cases reported
	ITGA3	17q21.33	AR	A few cases reported
Dystrophic epidermolysis bullosa (DEB)	*COL7A1*	3p21.31	AR, AD	100% of all DEB cases
Kindler syndrome (KS)	*FERMT1*	20p12.3	AR	100% of all KS cases

Abbreviations: AD, autosomal dominant; AR, autosomal recessive; LOC, laryngo-onycho-cutaneous syndrome.

dystrophic forms of EB (DEB) have been the focus of many investigations often using previously developed collagen VII knockout or hypomorphic mice (Fritsch et al., 2008; Heinonen et al., 1999). In addition, a rat model for dominant DEB, which exhibits a gene dosage effect, offers a possibility of evaluating the influence of modifier genes on DEB phenotype (Nyström et al., 2013).

Zebrafish and drosophila. Interesting alternative animal models to study EB have recently been reported, including zebrafish and *drosophila*. Several of the EB-relevant genes are expressed in zebrafish, and therefore, this model system has been used to generate skin-blistering phenotypes reflecting features of EB, such as morpholino-mediated knockdown of collagen XVII gene expression (Kim et al., 2010; Li and Uitto, 2014). Recent work has used the keratin-free tissue environment in *drosophila* to investigate the formation of keratin networks and to define mechanisms by which mutated keratins cause cellular pathology (Bohnekamp et al., 2015). Human keratins 5 and 14, when expressed in *drosophila* epithelia, formed well-organized keratin networks thus validating the fly as a novel genetic model system for keratin physiology and pathology. Inclusion of a mutated keratin 14 in the networks caused semilethality, wing blisters, and perturbed cellular integrity. This *drosophila* model of EBS will be valuable for further investigation of the effects of different keratin mutations, their cellular consequences, and possibilities for therapeutic interventions.

Organotypic cultures

Yet another model to investigate disease mechanisms and test therapeutic approaches is the 3D skin equivalent organotypic cultures. One study treated grafted human recessive DEB (RDEB) equivalents topically with recombinant human collagen VII and showed that the therapeutic collagen restored

Figure 1. Participants in the EB2015 Research Symposium held in Braselton, GA, in May 2015.

anchoring fibrils and promoted dermal-epidermal adhesion (Wang et al., 2013). Another investigation combined gene corrected epithelial stem cell clones for the epidermal compartment and fibroblasts in the dermal compartment and used the equivalents to test the structure and stability of the corrected skin (Duarte et al., 2014).

SQUAMOUS CELL CARCINOMA IN RDEB

Clinical challenge

Squamous cell carcinoma (SCC) remains the biggest cause of mortality in patients with RDEB with more than 80% succumbing by age 55 years (Fine et al., 2009). These tumors, while histologically often well differentiated, demonstrate aggressively invasive behavior with development of multifocal lesions and rapid metastasis. Clinically, there is still no consensus on the best way to tackle this formidable clinical problem, and new and effective therapies are in urgent need (Mellerio et al., 2015).

Therapy development

It is clear that lack of type VII collagen in RDEB has a significant impact on overall dermal architecture (Küttner et al., 2013; Nyström et al., 2013) that has been shown to promote tumor progression (Ng et al., 2012). One plausible strategy for therapy would be to target this altered, fibrotic microenvironment. For example, targeting the cancer-associated fibroblasts with the Janus Kinase (JAK) inhibitor ruxolitinib has been shown to prevent in vitro invasion of SCC tumors driven by the contractility of activated, surrounding fibroblasts (Albrengues et al., 2014). Given the demonstrable role of fibroblasts in RDEB-associated SCC (Ng et al., 2012), this small molecular inhibitor may well provide a viable SCC treatment option. With respect to targeting the tumor keratinocytes, screening of polo-like kinase inhibitors has identified a lead compound that showed good preclinical data in targeting tumor over normal cells, and this compound is currently in phase II/III trial for other malignancies. As such, there is hope that direct translation of this screen will be possible within a short timeframe.

SCC genomics

Genetically, RDEB-associated SCCs remain poorly characterized. Although a high burden of driver mutations has been highlighted in a handful of RDEB tumors as compared with UV-induced SCCs by traditional Sanger sequencing (Arbiser et al., 2004; Pourreyron et al., 2007; Wang et al., 2011), comprehensive analysis of all protein coding genes and overall understanding of tumor burden and spectrum of mutations in these tumors are lacking. In the light of a recent demonstration that non-RDEB UV-exposed skin harbors a huge burden of mutations (Martincorena et al., 2015), one might speculate that the altered microenvironment in RDEB is unable to suppress alterations in proliferation and differentiation as a result of mutations in genes such as *TP53* or *NOTCH1*. Efforts to collect and sequence RDEB-associated SCCs are in progress (Ray Cho, personal communication).

Roles of infection and inflammation

Recent work has identified a possible link with microbial infection, inflammation, and tumor development in RDEB (Hoste et al., 2015). Although inflammation has long been suspected to play a major role in tumor development in RDEB, documented evidence is lacking. Recent work using a mouse model has shown that tumors formed after wounding are accelerated by the addition of bacterial flagellin. Antibiotic administration reduced tumor burden, and tumor formation in this model was dependent on leukocytes, Myd88, and TLR5-driven NFκB signaling (Hoste et al., 2015). This evidence raises the possibility that a preventative measure might be to reduce bacterial load in RDEB skin.

THERAPY DEVELOPMENT

A number of novel approaches toward the treatment of EB have been recently developed, and in particular, significant progress has been made in cell-based therapies, in gene replacement and repair technologies, and in direct protein replacement therapy (Table 2). Many of these approaches have reached a milestone that allows them to move to early clinical trials; the currently approved clinical trials on EB are listed in Supplementary Table S1 online.

Table 2. Molecular and pharmacological approaches for the treatment of EB

Approach	Strategies	Current status[1]
Cell-based therapies	• Injection of allogeneic fibroblasts	CT
	• Systemic or perilesional administration of mesenchymal stem cells	CT
	• Autologous application of revertant mosaic cells	CT
	• Use of cord blood stem cells	PC
Bone marrow transplantation	• BMT after complete myeloablation	CT
	• Nonmyeloablative conditioning	CT
	• Autologous inducible pluripotent stem cells	PC
Gene therapy/mRNA editing	• Autologous keratinocyte and/or fibroblast therapy	CT
	• CRISPR/cas editing	PC
	• RNA *trans*-splicing	PC
	• PTC read-through and NMD antagonists	PC
Protein replacement therapy	• Delivery of recombinant type VII collagen in recessive dystrophic epidermolysis bullosa (RDEB)	PC
Novel and repurposed drug treatments	• Anti-itch medications	PS
	• Antifibrotic molecules (losartan and ruxolitinib)	PC
	• Anti-inflammatory therapies	PS
	• Enhanced wound healing (cathelicidin, Zorblisa, Keragel)	PS

Abbreviations: CT, clinical trials initiated, ongoing or recently completed; PC, these approaches are tested in preclinical studies, often utilizing appropriate mouse models of EB; PS, testing of these drugs is at the planning stages; BMT, bone marrow transplantation; PTC, premature termination codon; NMD, nonsense-mediated mRNA decay.

[1]For details on ongoing clinical trials; see Supplementary Table S1.

Cell therapy

Fibroblasts. Therapeutic application of autologous or allogeneic cells is now being explored in clinical trials in different forms of EB. Two proof-of-concept studies in RDEB subjects demonstrated that a single intradermal injection of allogeneic fibroblasts increased *COL7A1* gene expression in most individuals (Nagy et al., 2011; Wong et al., 2008). These trials also demonstrated the low immunogenicity of allogeneic fibroblasts and lack of host response. Two subsequent randomized double-blind studies then assessed the impact of allogeneic fibroblasts on wound healing in RDEB: one found no differences in the extent or rate of re-epithelization of chronic erosions (Venugopal et al., 2013), whereas the other showed that a single injection could speed up wound healing for up to 28 days compared with vehicle (Petrof et al., 2013).

Mesenchymal stromal/stem cells. The use of intradermal mesenchymal stromal cell (MSC) therapy was first reported in two patients with RDEB in 2010, and recently, clinical trials using intravenous BM-derived MSCs from unrelated donors into subjects with RDEB have been performed; clinical improvements in wound healing were observed in most subjects for 4–6 months (Conget et al., 2010; El-Darouti et al., 2015). Furthermore, an early phase clinical trial of intravenously administered allogeneic MSCs in 10 children with RDEB has recently been published (Petrof et al., 2015). In the latter study, although no significant safety concerns were raised, skin biopsies did not reveal an increase in collagen VII or new anchoring fibrils. There were, however, indications of reduced skin inflammation and better wound healing, as well as less skin pain and itching. Thus, although further assessment, including placebo-controlled studies, will be necessary, the anti-inflammatory effects of allogeneic MSCs appear to offer a rationale for their use in clinical care (Petrof et al., 2015). The above observation is in line with preclinical studies showing that a critical number of MSCs has to be reached in skin for significant restoration of collagen VII expression (Kuehl et al., 2015). By virtue of the cells' low engraftment in peripheral tissues with current treatment regimens this cell number cannot be achieved in skin and oral mucosa by systemic delivery; however, steps are being taken to improve engraftment rates (see below).

A clinical trial of intradermal MSCs to improve wound healing in adults with RDEB is also currently being conducted in Japan (Katsuto Tamai, personal communication, June 2015), with preliminary evidence for improved and sustained wound healing for more than 12 months after a single injection of MSCs into wound margins. Additional studies have demonstrated that preconditioning of MSCs with growth factors or cytokines to augment collagen VII production might have clinical relevance (Perdoni et al., 2014).

Bone marrow transplantation. Results of an early clinical trial of whole bone marrow transplantation (BMT) in children with RDEB have been reported (Wagner et al., 2010). In this study, seven patients entered the initial trial and six underwent BMT. All individuals had some clinical improvement and five of the six showed increased collagen VII at the dermal-epidermal junction. No individual has been cured after BMT, but several have shown a marked reduction in blister formation and major improvement in quality of life (Jakub Tolar, personal communication). Nevertheless, toxicity relating to complete myeloablation has been a concern, especially because two of the seven patients enrolled in the initial study died from complications of this procedure. Consequently, BMT protocols have been refined to introduce reduced intensity conditioning with decreasing mortality rates while maintaining clinical improvement. Specifically, to overcome the challenges of complete myeloablation, several studies are underway using nonmyeloablative conditioning to determine the safety and efficacy of this approach using a less toxic conditioning regimen, with early results being reported as having reduced complications and mortality (Geyer et al., 2015; Jakub Tolar, personal communication).

Mechanisms of BMT. To explore possible mechanisms of BMT, a mouse model was used to demonstrate that BM-derived keratinocytes represent a specific subpopulation of lineage-negative, platelet-derived growth factor receptor alpha-positive cells, still a somewhat heterogeneous collection of cells (Tamai et al., 2011). The study proposed that skin grafts (and blister roofs in RDEB) act as hypoxic bioreactors, rapidly releasing high mobility group box 1 (HMGB1). After skin grafting, HMGB1 levels in serum increased and HMGB1 was shown to mobilize the lineage-negative, platelet-derived growth factor receptor alpha-positive cells from the BM and recruit these cells along a concentration gradient to the area of hypoxic keratinocytes. Differentiation of these cells into keratinocytes and the capacity to generate new collagen VII in the skin were clearly demonstrated (Tamai et al., 2011). Subsequent work has identified the importance of a stromal derived factor 1-alpha – C-X-C chemokine receptor type 4 signaling pathway in the recruitment of the key regenerative cells (Iinuma et al., 2015). These data pave the way for clinical translation, with recombinant HMBG1 peptides to mobilize BM progenitors, and cell therapy approaches using specific subpopulations of MSCs are likely to enter clinical trials in the near future.

Alternative sources of stem cells. Cord blood and other compartments of the umbilical cord, such as Wharton's jelly or tissues associated with the placenta, are rich sources of stem cells. In addition to hematopoietic stem cells, cord blood is an important source of other progenitor cells, as well as MSCs, very small embryonic/epiblast I-like stem cells and unrestricted somatic stem cells, which may have individual or collective value in regenerative medicine. Nevertheless, a comparison of umbilical cord cells versus BM stem cells in individuals with RDEB has shown better skin engraftment with a BM-derived population (Tolar et al., 2012), and therefore, the clinical utility of cord cells in EB or other skin disorders remains to be determined in future clinical trials. Other populations, such as human cord blood-derived unrestricted somatic stem cells, are also being explored, in the EB mouse models, in preparation for clinical application (Liao et al., 2014, 2015).

Revertant mosaicism and inducible pluripotent stem cells

One striking observation in the skin of patients with EB is that some patches of skin can undergo spontaneous correction of the genetic defect, a phenomenon known as revertant mosaicism or "natural gene therapy" (Jonkman et al., 1997). The predominant mechanisms of gene correction include back mutation, gene conversion, intragenic recombination, and second-site mutation (Kiritsi et al., 2014; Pasmooij et al., 2012). The genetic correction appears to be limited to keratinocytes, but the opportunity to expand keratinocytes derived from a patch of revertant mosaicism in culture,

followed by application of a graft to the affected skin, creates a translational opportunity for personalized revertant cell therapy. The first attempt at revertant cell therapy was reported in an individual with generalized intermediate junctional EB, which yielded inconclusive results because the revertant keratinocyte population dropped from 30% to 3% in culture and no clinical benefits were noted after grafting (Gostynski et al., 2009). An alternative approach using pinch/punch grafting of skin from the revertant patches, however, has been used successfully to heal chronic erosions in a patient with a similar form of EB with mutations in *LAMB3* (Gostynski et al., 2014).

One potentially exciting future therapeutic approach may be to combine the natural phenomenon of revertant mosaicism with recent stem cell biology techniques, specifically in creating inducible pluripotent stem cells (iPSCs). Spontaneously corrected cells for iPSC generation that are derived from revertant keratinocytes would avoid the need for further genetic correction or gene editing. With regard to skin, iPSCs have recently been generated from keratinocytes and fibroblasts derived from individuals with EB (Sebastiano et al., 2014; Wenzel et al., 2014) and also from revertant keratinocytes (Tolar et al., 2014; Umegaki-Arao et al., 2014). Although no therapeutic use of iPSCs in dermatology has been achieved yet, it is clearly poised to undergo rapid translation in the future as the entire iPSC field moves into clinical applications.

Gene correction technologies

Over the years, EB investigators' efforts have revolved around gene replacement, and indeed, several clinical trials are now in progress based mainly on ex-vivo culture of EB keratinocytes, transduction with viral vectors containing genes of interest, and regrafting back onto the patient's skin (for active clinical trials in EB, see Supplementary Table S1). Other innovative genome editing techniques are emerging, including antisense-mediated exon skipping to restore the open reading frame of nonsense-bearing mRNA transcripts, spliceosome-mediated RNA *trans*-splicing, and premature termination codon read-through coupled with antagonists of nonsense-mediated mRNA decay (Bidou et al., 2012; Koller et al., 2015; Turczynski et al., 2012). Finally, the advent of CRISPR/cas gene editing techniques is also poised to transform the combined approach of mutation correction with iPSC technologies.

Protein replacement therapy

The consequences of mutations in different genes in EB are varied, but in some cases, such as nonsense and premature termination codon mutations, there is a complete absence of the corresponding protein. The potential for protein replacement by the introduction of recombinant type VII collagen was initially tested in wound healing models in wild-type and *Col7a1* knockout mice. Recombinant type VII collagen, when injected intradermally to the mice or applied topically, incorporated into the dermal-epidermal junction followed by formation of anchoring fibrils with correction of the EB phenotype, as demonstrated by decreased skin fragility, reduced new blister formation, and markedly prolonged survival (Hou et al., 2015; Remington et al., 2009; Woodley et al., 2013).

Novel treatments in the pipeline

Treatment of itch. It has become increasingly clear that there is an immediate demand for so-called symptom-relief therapies to ameliorate the disease symptoms with improved quality of life for the patients. Recent surveys of patients with EB have identified intractable itch and pain as one of the main issues for the daily management from the patient's perspective (Snauwaert et al., 2014). In this regard, investigators with extensive background knowledge of itch have now initiated programs to address itch and its mechanisms in EB, with the hope that it can be effectively counteracted by pharmacological means. Critical for this is understanding the similarities and differences that itch in patients with EB may have in comparison to itch mechanisms as previously delineated in other dermatologic conditions.

Antifibrotic therapies. One of the major complications of EB, particularly the RDEB subtype, is extensive scarring and fibrosis that can result in functional limitations of movement when affecting the joints, and in extensive fusion of the digits in the hands leading to mitten deformities with compromised dexterity. Animal studies using the hypomorphic mouse model have suggested that the fibrosis is driven by transforming growth factor-β, as reflected by transition of dermal fibroblasts to myofibroblasts with capacity for extensive extracellular matrix production. Losartan, an angiotensin II type 1 receptor antagonist that is FDA/EMA approved for hypertension, has been shown to reduce transforming growth factor-β—mediated fibrosis in some connective tissue disorders although its effects are context and disease specific (Nyström et al., 2015). Treatment of hypomorphic DEB mice with Losartan clearly ameliorated disease signs by reducing fibrosis and inflammation, counteracting formation of mitten deformities (Nyström et al., 2015). These observations suggest that clinical trials of Losartan in patients with RDEB are indicated.

Other examples of repurposing FDA/EMA-approved drugs is ruxolitinib known to reduce JAK/STAT-mediated fibrosis (Albrengues et al., 2014). Other potential FDA-approved drugs for counteracting interstitial fibrosis, pirfenidone and nintedanib, could be repurposed for EB-associated fibrosis. Finally, 4-phenylbutyrate, a molecule known to untangle pathological protein aggregates, has been tested in plectin-deficient mice (Winter et al., 2014).

Anti-inflammatory therapies. Some of the new pharmacologic approaches attempt to target the inflammatory phenotype of EB. One such study has utilized topical application of diacerein, a prodrug of the IL-1 converting enzyme inhibitor, rhein, which has been approved for systemic treatment of osteoarthritis (Wally et al., 2013). Topical application of this molecule in patients with EBS reduced blistering that remained significantly below the initial level after randomized withdrawal. The application of diacerein was found to be safe, and it apparently prevents blistering by down-regulating the activated stress-signal cascade, including IL-1β-induced c-Jun N-terminal kinase pathway.

Enhanced wound healing. One of the major goals in EB is to enhance wound healing processes, and a novel therapeutic approach has been suggested to be the use of antimicrobial peptides that control

pathogenic infections and activate the adaptive immune system. One of such peptides is cathelicidin that not only has the capability of augmenting host defense but also appears to play a role in tissue repair and wound closure. Preliminary studies have indicated low expression levels of cathelicidin mRNA in RDEB keratinocyte cultures, suggesting that upregulation of cathelicidin could improve wound healing in RDEB. In this regard, vitamin D3 analog calcipotriol was shown to upregulate cathelicidin expression in a dose-dependent manner (Hüttner et al., 2012; Moniaga et al., 2013).

PATIENT PERSPECTIVES AND THE ROLE OF ADVOCACY ORGANIZATIONS

The ultimate beneficiaries of the ongoing research will be people with EB. It is, therefore, critical to involve the patients and their advocacy organizations, such as DEBRA International, Vienna, Austria, in the process of identifying the most important issues presented by this disease, as perceived by the patients themselves. In this regard, it has become increasingly evident that problems such as intractable itch and excruciating pain need to be addressed to improve quality of life for these patients. To this aim, DEBRA International has initiated the creation of clinical best practice guidelines for major aspects of EB care, including oral health care, wound care, and pain management, which are already freely available to clinicians (www.debra-international.org/med-professionals/clinical-practice-guidelines-cpgs/forEB.html); other guidelines on cancer management and nutrition are under development. In addition, although DEBRA International provides a route for coordinating information to patients and clinicians about research and clinical trials on EB, it conversely provides information about patients with EB and their priorities to those planning clinical trials. In this meeting, a presentation by DEBRA Ireland noted the importance of investing time in informing patients, and considering patients as participants in the process, and not just as trial subjects. Thus, it is critically important to solicit the patients' participation with meaningful involvement, and to inform the patient community of clinical trial outcomes.

The participants of the EB2015 included not only researchers but also patients and their family members. In fact, the President of DEBRA International, Rainer Riedl, and the Director of DEBRA of America, New York, Brett Kopelan, are also fathers of children with RDEB. In the closing, they shared the impressions of the patients and the advocacy organizations that EB research is speeding up dramatically and that a striking number of new clinical trials and new medical products can be expected in the very near future. In this regard, this meeting has provided inspiration not only to those working on understanding the disease and developing novel treatments, but also to the patients and the parents whose perspective has been increasingly heard.

CONFLICT OF INTEREST

The authors state no conflict of interest.

ACKNOWLEDGMENTS

The EB2015 symposium was supported in part by the National Institutes of Health Grant R13AR068235 from the National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS) and the National Center for Advancing Translation Sciences (NCATS). Support from the following companies is gratefully acknowledged: Scioderm, Fibrocell Science, Keraplast Technologies, and Shire.

SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at www.jidonline.org, and at http://dx.doi.org/10.1016/j.jid.2015.10.050.

REFERENCES

Albrengues J, Bourget I, Pons C, Butet V, Hofman P, Tartare-Deckert S, et al. LIF mediates proinvasive activation of stromal fibroblasts in cancer. Cell Rep 2014;7:1664–78.

Arbiser JL, Fan CY, Su X, Van Emburgh BO, Cerimele F, Miller MS, et al. Involvement of p53 and p16 tumor suppressor genes in recessive dystrophic epidermolysis bullosa-associated squamous cell carcinoma. J Invest Dermatol 2004;123:788–90.

Bidou L, Allamand V, Rousset JP. Namy O Sense from nonsense: therapies for premature stop codon diseases. Trends Mol Med 2012;18:679–88.

Bohnekamp J, Cryderman DE, Paululat A, Baccam GC, Wallrath LL, Magin TM. A drosophila model of epidermolysis bullosa simplex. J Invest Dermatol 2015;135:2031–9.

Bruckner-Tuderman L, McGrath JA, Robinson EC, Uitto J. Animal models of epidermolysis bullosa: update 2010. J Invest Dermatol 2010;130:1485–8.

Bruckner-Tuderman L, McGrath JA, Robinson EC, Uitto J. Progress in epidermolysis bullosa research: Summary of DEBRA International Research Conference 2012. J Invest Dermatol 2013;133:2121–6.

Conget P, Rodriguez F, Kramer S, Allers C, Simon V, Palisson F, et al. Replenishment of type VII collagen and re-epithelialization of chronically ulcerated skin after intradermal administration of allogeneic mesenchymal stromal cells in two patients with recessive dystrophic epidermolysis bullosa. Cytotherapy 2010;12:429–31.

Duarte B, Miselli F, Murillas R, Espinosa-Hevia L, Cigudosa JC, Recchia A, et al. Long-term skin regeneration from a gene-targeted human epidermal stem cell clone. Mol Ther 2014;22:1878–80.

El-Darouti M, Fawzy M, Amin I, Abdel Hay R, Hegazy R, Gabr H, et al. Treatment of dystrophic epidermolysis bullosa with bone marrow non-hematopoeitic stem cells: a randomized controlled trial. Dermatol Ther 2015; doi: 10.1111/dth.12305.

Fine JD, Johnson LB, Weiner M, Li KP, Suchindran C. Epidermolysis bullosa and the risk of life-threatening cancers: the National EB Registry experience, 1986-2006. J Am Acad Dermatol 2009;60:203–11.

Fritsch A, Loeckermann S, Kern JS, Braun A, Bosl MR, Bley TA, et al. A hypomorphic mouse model of dystrophic epidermolysis bullosa reveals mechanisms of disease and response to fibroblast therapy. J Clin Invest 2008;118:1669–79.

Geyer MB, Radhakrishnan K, Giller R, Umegaki N, Harel S, Kiuru M, et al. Reduced toxicity conditioning and allogeneic hematopoietic progenitor cell transplantation for recessive dystrophic epidermolysis bullosa. J Pediatr 2015;167:765–9.

Gostynski A, Deviaene FC, Pasmooij AM, Pas HH, Jonkman MF. Adhesive stripping to remove epidermis in junctional epidermolysis bullosa for revertant cell therapy. Br J Dermatol 2009;161:444–7.

Gostynski A, Pasmooij AM, Jonkman MF. Successful therapeutic transplantation of revertant skin in epidermolysis bullosa. J Am Acad Dermatol 2014;70:98–101.

Hammersen J, Hou J, Wünsche S, Brenner S, Winkler T, Schneider H. A new mouse model of junctional epidermolysis bullosa: the LAMB3 628G>A knockin mouse. J Invest Dermatol 2015;135:921–4.

Heinonen S, Männikkö M, Klement JF, Whitaker-Menezes D, Murphy GF, Uitto J. Targeted inactivation of the type VII collagen gene (Col7a1) in mice results in severe blistering phenotype: a model for recessive dystrophic epidermolysis bullosa. J Cell Sci 1999;112:3641–8.

Hoste E, Arwert EN, Lal R, South AP, Salas-Alanis JC, Murrell DF, et al. Innate sensing of microbial products promotes wound-induced skin cancer. Nat Commun 2015;6:5932.

Hou Y, Guey LT, Wu T, Gao R, Cogan J, Wang X, et al. Intravenously administered recombinant human type VII collagen derived from Chinese hamster ovary cells reverses the disease phenotype in recessive dystrophic epidermolysis bullosa mice. J Invest Dermatol 2015;135:3060–7.

Hurskainen T, Kokkonen N, Sormunen R, Jackow J, Loffek S, Soininen R, et al. Deletion of the major bullous pemphigoid epitope region of collagen XVII induces blistering, autoimmunization, and itching in mice. J Invest Dermatol 2015;135:1303–10.

Hüttner C, Gruber C, Lang R, Breitenbach J, Hintner H, Bauer JW. Expression and regulation of cathelicidin in keratinocytes from patients with epidermolysis bullosa. J Invest Dermatol 2012;132:S7–13.

Iinuma S, Aikawa E, Tamai K, Fujita R, Kikuchi Y, Chino T, et al. Transplanted bone marrow-derived circulating PDGFRalpha+ cells restore type VII collagen in recessive dystrophic epidermolysis bullosa mouse skin graft. J Immunol 2015;194:1996–2003.

Jonkman MF, Scheffer H, Stulp R, Pas HH, Nijenhuis M, Heeres K, et al. Revertant mosaicism in epidermolysis bullosa caused by mitotic gene conversion. Cell 1997;88:543–51.

Kim SH, Choi HY, So JH, Kim CH, Ho SY, Frank M, et al. Zebrafish type XVII collagen: gene structures, expression profiles, and morpholino "knock-down" phenotypes. Matrix Biol 2010;29:629–37.

Kiritsi D, Garcia M, Brander R, Has C, Meijer R, Jose Escamez M, et al. Mechanisms of natural gene therapy in dystrophic epidermolysis bullosa. J Invest Dermatol 2014;134:2097–104.

Koller U, Hainzl S, Kocher T, Huttner C, Klausegger A, Gruber C, et al. Trans-splicing improvement by the combined application of antisense strategies. Int J Mol Sci 2015;16:1179–91.

Kuehl TT, Mezger M, Hausser-Siller I, Handgretinger R, Bruckner-Tuderman L, Nyström A. High local concentrations of intradermal MSCs restore skin inegrity and facilitate wound healing in dystrophic epidermolysis bullosa. Mol Ther 2015;23:1368–79.

Küttner V, Mack C, Rigbolt KTG, Kern JS, Schilling O, Busch H, et al. Global remodeling of cellular microenvironment due to loss of collagen VII. Mol Syst Biol 2013. Online Publication 16.04.2013, doi:MSB2013.17.

Li Q, Uitto J. Zebrafish as a model system to study skin biology and pathology. J Invest Dermatol 2014;134:e21.

Liao Y, Itoh M, Yang A, Zhu H, Roberts S, Highet AM, et al. Human cord blood-derived unrestricted somatic stem cells promote wound healing and have therapeutic potential for patients with recessive dystrophic epidermolysis bullosa. Cell Transplant 2014;23:303–17.

Liao Y, Ivanova L, Zhu H, Yahr A, Ayello J, van de Ven C, et al. Rescue of the mucocutaneous manifestations by human cord blood derived nonhematopoietic stem cells in a mouse model of recessive dystrophic epidermolysis bullosa. Stem Cells 2015;33:1807–17.

Martincorena I, Roshan A, Gerstung M, Ellis P, Van Loo P, McLaren S, et al. Tumor evolution. High burden and pervasive positive selection of somatic mutations in normal human skin. Science 2015;348:880–6.

Mellerio JE, Robertson SJ, Bernardis C, Diem A, Fine JD, George R, et al. Management of cutaneous squamous cell carcinoma in patients with epidermolysis bullosa - best clinical practice guidelines. Br J Dermatol 2015; doi:10.1111/bjd.14104.

Moniaga CS, Egawa G, Miyachi Y, Kabashima K. Calcipotriol modulates IL-22 receptor expression and keratinocyte proliferation in IL-22-induced epidermal hyperplasia. J Dermatol Sci 2013;71:76–7.

Nagy N, Almaani N, Tanaka A, Lai-Cheong JE, Techanukul T, Mellerio JE, et al. HB-EGF induces COL7A1 expression in keratinocytes and fibroblasts: Possible mechanism underlying allogeneic fibroblast therapy in recessive dystrophic epidermolysis bullosa. J Invest Dermatol 2011;131:1771–4.

Ng YZ, Pourreyron C, Salas-Alanis JC, Dayal JH, Cepeda-Valdes R, Yan W, et al. Fibroblast-derived dermal matrix drives development of aggressive cutaneous squamous cell carcinoma in patients with recessive dystrophic epidermolysis bullosa. Cancer Res 2012;72:3522–34.

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.

Nyström A, Velati D, Mittapalli VR, Fritsch A, Kern JS. Bruckner-Tuderman L Collagen VII plays a dual role in wound healing. J Clin Invest 2013;123:3498–509.

Pasmooij AM, Jonkman MF, Uitto J. Revertant mosaicism in heritable skin diseases: mechanisms of natural gene therapy. Discov Med 2012;14:167–79.

Perdoni C, McGrath JA, Tolar J. Preconditioning of mesenchymal stem cells for improved transplantation efficacy in recessive dystrophic epidermolysis bullosa. Stem Cell Res Ther 2014;5:121.

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.

Petrof G, Martinez-Queipo M, Mellerio JE, Kemp P, McGrath JA. Fibroblast cell therapy enhances initial healing in recessive dystrophic epidermolysis bullosa wounds: results of a randomized, vehicle-controlled trial. Br J Dermatol 2013;169:1025–33.

Pourreyron C, Cox G, Mao X, Volz A, Baksh N, Wong T, et al. Patients with recessive dystrophic epidermolysis bullosa develop squamous-cell carcinoma regardless of type VII collagen expression. J Invest Dermatol 2007;127:2438–44.

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.

Sebastiano V, Zhen HH, Haddad B, Bashkirova E, Melo SP, Wang P, et al. Human COL7A1-corrected induced pluripotent stem cells for the treatment of recessive dystrophic epidermolysis bullosa. Sci Transl Med 2014;6:264ra163.

Snauwaert JJ, Yuen WY, Jonkman MF, Moons P, Naulaers G, Morren MA. Burden of itch in epidermolysis bullosa. Br J Dermatol 2014;171:73–8.

Sproule TJ, Bubier JA, Grandi FC, Sun VZ, Philip VM, McPhee CG, et al. Molecular identification of collagen17a1 as a major genetic modifier of laminin gamma 2 mutation-induced junctional epidermolysis bullosa in mice. PLoS Genet 2014;10:e1004068.

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.

Tolar J, McGrath J, Keene DR. Hematopoietic and mesenchymal cell transplantation after myeloablative and non-myeloablative conditioning for recessive dystrophic and junctional epidermolysis bullosa (RDEB, JEB). J Invest Dermatol 2012;132:A534 (Abstract).

Tolar J, McGrath JA, Xia L, Riddle MJ, Lees CJ, Eide C, et al. Patient-specific naturally gene-reverted induced pluripotent stem cells in recessive dystrophic epidermolysis bullosa. J Invest Dermatol 2014;134:1246–54.

Turczynski S, Titeux M, Pironon N, Hovnanian A. Antisense-mediated exon skipping to reframe transcripts. Methods Mol Biol 2012;867:221–38.

Uitto J, McGrath JA, Rodeck U, Bruckner-Tuderman L, Robinson EC. Progress in epidermolysis bullosa research: toward treatment and cure. J Invest Dermatol 2010;130:1778–84.

Umegaki-Arao N, Pasmooij AM, Itoh M, Cerise JE, Guo Z, Levy B, et al. Induced pluripotent stem cells from human revertant keratinocytes for the treatment of epidermolysis bullosa. Sci Transl Med 2014;6:264ra164.

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.e7.

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.

Wally V, Kitzmueller S, Lagler F, Moder A, Hitzl W, Wolkersdorfer M, et al. Topical diacerein for epidermolysis bullosa: a randomized controlled pilot study. Orphanet J Rare Dis 2013;8:69.

Wang NJ, Sanborn Z, Arnett KL, Bayston LJ, Liao W, Proby CM, et al. Loss-of-function mutations in Notch receptors in cutaneous and lung squamous cell carcinoma. Proc Natl Acad Sci USA 2011;108:17761–6.

Wang X, Ghasri P, Amir M, Hwang B, Hou Y, Khalili M, et al. Topical application of recombinant type VII collagen incorporates into the dermal-epidermal junction and promotes wound closure. Mol Ther 2013;21:1335–44.

Wenzel D, Bayerl J, Nystrom A, Bruckner-Tuderman L, Meixner A, Penninger JM. Genetically corrected iPSCs as cell therapy for recessive dystrophic epidermolysis bullosa. Sci Transl Med 2014;6:264ra165.

Winter L, Staszewska I, Mihailovska E, Fischer I, Goldmann WH, Schroder R, et al. Chemical chaperone ameliorates pathological protein aggregation in plectin-deficient muscle. J Clin Invest 2014;124:1144–57.

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.

Woodley DT, Wang X, Amir M, Hwang B, Remington J, Hou Y, et al. Intravenously injected recombinant human type VII collagen homes to skin wounds and restores skin integrity of dystrophic epidermolysis bullosa. J Invest Dermatol 2013;133:1910–3.