The DEBRA International Epidermolysis Bullosa Research Conference (EB 2009) was held in Vienna in September 2009 and was organized by the Epidermolysis Bullosa (EB) patient support organizations DEBRA Austria and DEBRA International. Attendance was by invitation only and limited to senior EB researchers and clinicians. About 70 specialists from 14 nations were discussing the latest achievements in care and research of EB.

In parallel with the conference, two of DEBRA’s Task Forces held closed satellite workshops: one on research into the aggressive squamous cell carcinomas associated with recessive dystrophic EB (RDEB), and the other reviewing the use of animal models in basic and clinical EB research.

EB is a group of mostly inherited, rare (orphan) diseases and affects approximately 500 individuals in Austria, about 30,000 in the European Union and around 500,000 worldwide. EB means, as the name implies, the blistering (‘bullous’) breakdown (‘lysis’) of the outer skin layer (‘epidermis’), which detaches from the deeper dermis layer, forming blisters. EB is caused by mistakes (mutations) in any one of several known genes which code for structural proteins that hold these two skin layers together – these mistakes result in the encoded proteins being faulty or completely absent. EB comprises a genetically, as well as clinically, heterogeneous group of skin disorders. EB varies widely in severity and disease characteristics, depending on the location of the mutation and impact the mutation has on the protein’s ability to function adequately in the skin. Currently, about 30 clinical subtypes are known.

Two decades ago, EB entered the molecular era with the identification of mutations in specific genes expressed within the cutaneous basement membrane zone. So far, mutations in 14 genes have been identified and these form the basis for the development of novel molecular therapies for EB. The aim of the conference was to give an update of current achievements, and:
• to review progress and barriers in fundamental EB research and the development of clinical solutions
• to consider research aspects of EB not addressed to date
• to identify unexplored opportunities and relevant research from complementary areas
• to reach research- and clinical-community consensus on scientific and development priorities

A scientific report of the major topics of the conference has been published recently [1] and here we give a short summary of the main progress in the field of EB research towards improving medical care and raising hope of alleviating and/or curing this serious and rare disease.

Four major sessions of the conference, encompassing up to 6 talks each, addressed various aspects of EB: Modifying Gene Expression (1), Cell and Protein Therapies in EB (2), Cancer (3), and Clinical Trials and Beyond (4).

1. Modifying Gene Expression

This session focused on gene therapy. Proof-of-principle for a grafting approach using an ex-vivo gene therapy for one type of EB has already been demonstrated and published in 2006 [2]. The objective was to correct the consequences of a genetic mutation in a patient with junctional EB (JEB). In brief, this was performed by introducing wild-type complementary DNA into the patient’s own skin-derived stem cells, which were then grown to produce epithelial sheets for transplantation. Keratinocytes from a JEB patient (mutation in the laminin 332 gene = LAMB3) were cultured and transduced in cell culture. Corrected cell populations with stem-cell characteristics were grown to form epithelial sheets that were then transplanted to the patient’s prepared skin. Four years after this procedure, the grafted areas of skin are still expressing sufficient functional laminin. This proof-of-principle for a successful gene therapy is encouraging the development of similar technologies with other genes, to treat other types of EB. In a co-operation between the EB House Austria in Salzburg, and Italy (Universities of Modena and Bolzano), within the EU Project Interreg IV, this technology will be applied to another subtype of JEB (mutation in the COL17A1 gene) as well as adapted for use with the collagen 7 gene (COL 7A1) to treat dystrophic EB (DEB). Other laboratories in France and in the United States are pursuing similar gene therapy technology to attempt to treat RDEB.
2. Cell and Protein Therapies in EB

Local and systemic cell-based therapies
The intradermal injection of fibroblasts (skin cells from the dermis) from donors without EB, close to the edges of an area of blistering, has been tested in a limited number of RDEB patients [3] and resulted in local recovery of the skin. Reduced blistering and improved wound healing were observed for several months, even though the allogeneic fibroblasts only survived in the patients’ skin for a few weeks. Although some patients reported the injections to be painful, particularly near wounds and in areas of scarring, the therapy has not to date caused any side effects. The phase II clinical trial of this fibroblast therapy is currently planned for late 2010.

Cell-based therapies for RDEB have been extended more recently to the use of stem cells. The early-stage trial of one type of stem-cell therapy, a bone-marrow transplant, has been based on successful preclinical research in a type 7 collagen “knock-out” mouse model which was used as a target for bone marrow-derived cell transfer [4, 5].

The first bone marrow cell transfer trial on patients with RDEB was initiated in 2007 and the results were published in August 2010 [6]. Seven children between the ages of 15 months and 14 years have been treated to date. One child died before receiving the transplantation, probably as a result of the essential pre-transplantation medical treatment, and another child died some six months after receiving the transplantation, because of transplant rejection and infection. In the six patients who received transplants, all showed some improvement in wound healing and reduced blister formation, at least for a period of time after transplantation. This trial shows that, while bone-marrow transplant is a severe medical procedure, not to be undertaken without serious consideration of the possible consequences, it can, in principle, provide some apparent benefits to some patients with severe RDEB. However, a great deal of further work is needed to refine the procedure, and to study what the long-term effects – both benefits and unwanted side-effects – are for the people treated to date.

A further clinical trial, at the University of Columbia in New York, with a reduced intensity of the chemotherapy, which must precede transplant of the donor bone marrow, has already been initiated [7]. This new “gentle” approach is expected to have lower morbidity, and mortality, as it may reduce the susceptibility to infection.
Bone marrow transplantation to treat EB is at an early stage of development and the identity of the specific cells leading to repair of the structural integrity of the fragile EB skin is still unknown, although further research on this is underway.

**Protein Replacement Therapy**

In protein therapy, the aim is to introduce directly into the skin, by intradermal injection, the correctly structured protein, which is completely missing or mutated in EB. The therapeutic protein is produced in large quantities in the laboratory as a recombinant human protein, and then purified. Inserting the correct protein occurs by topical application or local injection.

Experiments with intradermal injections of human type 7 collagen into collagen 7-deficient “knock-out” mice showed a reduced level of blistering and extended the survival of these mice [8]. The length of time for which the injected protein persists in the skin depends on the type of the protein and the turnover of this protein in the skin. The half-life of the type 7 collagen molecules in human skin has not yet been determined, but recent studies in mice have suggested that type 7 collagen has a long half-life [9]. This observation, together with the fact that dermatologists are familiar with intracutaneous injections makes the approach very promising at least for some EB subtypes. Unfortunately, attempts to date to apply the same approach to treat JEB with laminin protein failed because it proved too technically difficult to produce the laminin in the laboratory. Compared with gene therapy, the protein replacement is an attractive and apparently safe method because no viruses or living cells are involved in its delivery. Based on these and other observations, clinical trials for phase I and II studies will be initiated.

3. Cancer

Squamous cell carcinoma (SCC) occurs specifically in patients with RDEB, most commonly in their third or fourth decade and primarily on the extremities (hands and feet) [10]. Multiple tumours arise independently, and at the same time. They have a high tendency towards metastatic spread and only surgical removal is an effective therapeutic option. In chronic wounds, a major diagnostic challenge is to identify areas where cancers are initiating or may be already malignant. Three years ago, a DEBRA Task Force was established to develop new diagnostic and therapeutic approaches to skin cancer in EB. Systematic investigation of the mechanisms and molecular events leading to the clinically aggressive behaviour of SCC in EB are currently underway. Among contributing factors,
it seems likely that the continuous tissue repair associated with chronic inflammatory processes in persistent wounds is responsible for the severe outcome of SCC in EB skin.

4. Clinical Trials and Beyond

Pilot studies and clinical trials have been initiated, especially for the more severe subtypes of EB such as JEB or RDEB (Table 1). Various technologies, mostly in the advanced stages of preclinical research or already in early-stage clinical trials, have been reported [11, 12]. Development of potential therapies must assess the risk/benefit ratios for particular patient groups, and consider likely technical and regulatory challenges in establishing their efficacy and safety.

Conclusion

The identification of mutations in known EB genes provides the starting point for the development of novel, particularly molecular therapies, for EB. The aim of the conference EB 2009 was to give an update of the current achievements and it was highly successful in assessing the present state of EB research [1]. A range of technologies is now available that may be appropriate for different types of EB. In addition to gene therapy, which was predominant at the time of the previous DEBRA conference in 2006, cell-, small-molecule- (siRNA), and protein-treatment approaches have developed rapidly in the past five years. Each technology has its benefits and risks and, it is unlikely that any single therapeutic approach is going to be suitable for all patients, or even all patients with a particular type of EB. However, early types of treatments are now emerging which, as they are refined, should help people with EB, either as a single therapy or, in many cases, in the form of a combination of therapies. For a rare disease like EB, it is obvious that only a close collaboration among experts can speed up translational research. At EB 2009, clinicians and researchers established new international networks for future collaborations to facilitate the challenging treatment of EB patients and to raise hope of alleviating and/or curing this serious rare disease.
Table 1*: Examples of molecular therapies under development for different forms of EB

<table>
<thead>
<tr>
<th>Target disease</th>
<th>Nature of therapy</th>
<th>Approach</th>
<th>Research Stage/Clinical Trial</th>
</tr>
</thead>
<tbody>
<tr>
<td>JEB</td>
<td>Gene therapy</td>
<td>Grafting ex vivo genetically modified</td>
<td>Pilot study [2]</td>
</tr>
<tr>
<td>RDEB</td>
<td>Gene therapy</td>
<td>Grafting ex-vivo genetically modified</td>
<td>Planned for clinical trial 2010</td>
</tr>
<tr>
<td>EBS/DDEB</td>
<td>Gene therapy</td>
<td>Silencing of the dominant mutant allele by siRNA</td>
<td>Preclinical development</td>
</tr>
<tr>
<td>RDEB</td>
<td>Cell therapy – localized</td>
<td>Application of chimeric skin equivalents (fibroblasts from healthy donors and patient keratinocytes)</td>
<td>Phase IIb ongoing</td>
</tr>
<tr>
<td>RDEB</td>
<td>Cell therapy – systemic</td>
<td>Allogeneic bone marrow transplantation (standard procedures)</td>
<td>Phase I/II [6]</td>
</tr>
<tr>
<td>RDEB</td>
<td>Cell therapy – systemic</td>
<td>Allogeneic bone marrow transplantation (reduced intensity conditioning)</td>
<td>Phase I/II [7]</td>
</tr>
<tr>
<td>JEB</td>
<td>Cell/Gene Therapy</td>
<td>Grafting of autologous keratinocytes from patients with revertant mosaicism</td>
<td>Pilot study [13]</td>
</tr>
<tr>
<td>RDEB</td>
<td>Protein Therapy – localized</td>
<td>Protein replacement by intradermal injection (type 7 collagen)</td>
<td>Phase I [8]</td>
</tr>
</tbody>
</table>

Table adapted from Uitto et al, 2010 [1]

Abbreviations: DDEB, dominant dystrophic epidermolysis bullosa; EBS, epidermolysis bullosa simplex; JEB, junctional epidermolysis bullosa; RDEB, recessive dystrophic epidermolysis bullosa; siRNA, small interfering RNA

References


Correspondence:
Gabriela Pohla-Gubo, PhD
Department of Dermatology, EB House Austria
Paracelsus Medical University Salzburg
Muellner Hauptstrasse 48
5020 Salzburg
AUSTRIA
mailto:g.pohla-gubo@salk.at