Management of severe epidermolysis bullosa by haematopoietic transplant: principles, perspectives and pitfalls

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Abstract: People with severe forms of epidermolysis bullosa (EB) develop widespread blistering and progressively debilitating multisystem complications that may result in a shortened lifespan. As some wounds in EB individuals are difficult or impossible to access with topical therapy, we examined the potential of systemic therapy with normal haematopoietic stem cells. In both animal models and children with EB, healthy donor cells from the haematopoietic graft migrated to the injured skin; simultaneously, there was an increase in the production of skin-specific structural proteins deficient in EB, increased skin integrity and reduced tendency to blister formation. Even though the majority of evaluable individuals have had a positive response in skin healing, frequently changing their quality of life, the improvement in lifestyle has been varied and the overall clinical response incomplete. To change the current amelioration of disease into a full cure, we propose to (i) increase safety as well as efficacy of haematopoietic cell transplant (HCT) using co-infusion of mesenchymal stromal/stem cells with haematopoietic stem cells and non-myeloablative conditioning for transplant; (ii) optimize homing of donor cells into the skin erosions in animal models of EB; and (iii) discover and test new drugs for EB therapy using patient-specific induced pluripotent stem cells. We conclude that although HCT has always been a risky treatment restricted to those with serious life-threatening or debilitating diseases, by most benchmarks, the results of HCT in EB have shown that HCT has the potential of being a durable, systemic therapy for people with severe forms of EB.

Abbreviations: EB, epidermolysis bullosa; JEB-H, Herlitz variant of junctional EB; C7, type VII collagen; COL7A1, type VII collagen gene; AF, anchoring fibrils; LM-332, laminin-332; LAMA3, laminin α3 gene; LAMB3, laminin β3 gene; LAMC2, laminin γ2 gene; DEJ, dermal–epidermal junction; BMT, bone marrow transplantation; HCT, haematopoietic cell transplantation; GFP, green fluorescent protein; MSC, mesenchymal stem/stromal cells; iPSC, induced pluripotent stem cells; Lin, lineage; PDGFRA, platelet-derived growth factor receptor alpha; HB-EGF, heparin-binding epidermal growth factor-like growth factor; HMGB1, high-mobility group protein B1.

Key words: bone marrow – cord blood – epidermolysis bullosa – genodermatosis – haematopoietic cell transplantation – regenerative medicine

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“How wonderful that we have met with a paradox. Now we have some hope of making progress.” – Niels Bohr.

Why you care

The skin is a complex, constantly changing organ that makes a boundary between us and the rest of the world. Its appearance and function allow the external affirmation of an individual and the internal freedom for self-determination. The unremitting progression of epidermolysis bullosa (EB) destroys all of this. People with severe forms of EB develop widespread blistering and erosions shortly after birth. They can heal rapidly, but because of the underlying defect, they keep blistering and ultimately become infected, which then prevents proper healing. Eventually, they develop progressively debilitating multisystem complications, resulting in a shortened lifespan. By any measure, the effects of this painful and mutilating disease are tragic.

In search of the anchor

Severe forms of EB, such as generalized recessive dystrophic EB (severe generalized RDEB) and the Herlitz variant of junctional EB (JEB-H), are rare, autosomal recessive genodermatoses. Individuals with severe generalized RDEB and JEB-H exhibit severe mucocutaneous blistering, and a variable and heterogeneous spectrum of clinical features caused by malfunction of internal organs such as the lungs, heart, kidneys, bone marrow and lymphohematopoietic system (1–5). The molecular pathology of severe generalized RDEB involves loss-of-function mutations in the COL7A1 gene. This precludes physiological expression of type VII collagen (C7), one of the skin-specific extracellular matrix molecules critical for skin integrity. C7 molecules form homotrimers and then polymerize into large (approximately 780 nm long) anchoring fibrils (AFs). C7 interacts with epidermal and dermal proteins, and AFs connect the skin basement membrane to the papillary dermis in a fashion reminiscent of a two-piece fastener with a mat of loops on one side and nylon hooks on the other (Velcro®). Specifically, C7 interacts with laminin-332 (LM-332), a heterotrimeric protein completely dysfunctional in JEB-H, which can be caused by biallelic disruption of any one of three genes (LAMA3, LAMB3 and LAMC2) that encode LM-332. LM-332 interacts with integrin receptors α3β1 and α6β4 to form focal adhesions and stable anchoring contacts in the dermal–epidermal junction (DEJ)(6–15).

Think global, act global

The progress in understanding the molecular and cellular biology of EB has been extraordinary. After distinct skin fracture lines associated with the different forms of EB were established, mutations in at least 15 genes were found to be associated with various forms of EB, and animal models were created (16). The momentum of this basic research caught the attention of clinician-scientists, and the curiosity-driven investigations rapidly changed into...
research aimed at treatment of the skin wounds. A number of interventions using cell-, protein- or gene-based approaches have been shown to be effective in animal models and are now being introduced to patients with EB in safety-defining, phase I trials (16–34).

All of these are important approaches, but they also illustrate the clinical focus on healing the individual wounds, rather than treating the whole person with severe EB, a person who invariably has wounds difficult or impossible to access with topical therapy (35). In addition, local injections are not expected, with the exception of patient-derived, gene-corrected stem cells (20), to provide durable correction of C7 or LM-332 deficiency. For example, injected cells and transgenes are cleared from skin, and repeated wound treatment can elicit an immune response that limits its benefits (36, 37).

In contrast, systemic therapy with gene-corrected, patient-derived cells (autologous) or stem cells from healthy donors (allogeneic) could in principle provide enduring correction of EB, possibly preventing disease progression, such as development of pseudosyndactyly and squamous cell carcinoma (38–42).

**Stem cells as medicine**

Bone marrow transplantation (BMT) is the prototypical stem cell therapy and has been in existence for more than 40 years (43). Even today, it is the only stem cell therapy with proven therapeutic effect. Interestingly, a skin disease was one of the trigger points leading to the BMT field. For more than half a century, biologists searched for a reliable method of skin grafting for burn victims. During experimentation in mice, they found that the skin grafts only ‘took’ when bone marrow from the same donor was exchanged at the same time (44). Knowing that bone marrow could be exchanged suggested a therapy for leukaemia, which at that time was virtually always lethal. However, the first wave of BMT in adults with leukaemia produced few, if any, cures. It took an additional decade of laboratory investigations and an improved understanding of human leucocyte antigen biology to enable the first successful BMT from a matched sibling donor in 1968 (45). Additional applications of BMT followed in quick succession: BMT from unrelated donor, BMT for lymphoma, BMT for aplastic anaemia, BMT for haemoglobinopathy and the successful use of cord blood as an alternative source of haematopoietic stem cells in transplantation. The number of patients who have benefited from haematopoietic cell transplantation (HCT) has since risen exponentially, and approximately 50 000 people have received HCT to date, most with life-saving results (43).

In one of the most striking examples of ‘adjacent possible’, whereby one discovery at the edge of scientific complexity makes another discovery achievable (46), in the same month the first successful BMT was performed (November 1968), a game-changing (in retrospect) experiment demonstrated the ability of healthy donor cells to replace a missing enzyme or protein and correct the underlying disease. Specifically, fibroblasts from an individual with an enzyme deficiency (mucopolysaccharidosis type I) were cultured with fibroblasts from an individual with another genetic enzyme deficiency (mucopolysaccharidosis type II), and they biochemically corrected each other (47). This immediately suggested that cells from healthy individuals could ‘functionally correct’ the genetic disease. Since the first HCT for mucopolysaccharidosis type I in the early 1980s, HCT has become a new standard-of-care for individuals with several congenital enzymopathies.

Equally established, however, was the notion that only a soluble protein (such as an enzyme, which is widely distributed by blood) can cross-correct the inherent protein deficiency. In contrast, we hypothesized that allogeneic HCT might also correct structural protein-deficient diseases. Because a cure for severe EB was not immediately on the horizon, we examined the potential of normal haematopoietic stem cells to provide C7-producing cells that would migrate to the injured skin. **Right cell at the right time**

To investigate this, mice with targeted inactivation of COL7A1 (C7null/null) were injected with different populations of haematopoietic and non-haematopoietic cells. C7null/null mice mimic human severe generalized RDEB; blisters develop in newborn pups, and most mice die within the first week of life. On the one hand, this early lethality limited the time within which the corrective intervention had to be effective. On the other hand, it provided a robust readout for our stem cell transfer experiments. We found that intravenous infusion of haematopoietic stem cell–expressing measles virus receptor CD150 (48) rescued 15% of animals, and their blisters healed. Importantly, some of the donor cells (labelled with green fluorescent protein, GFP) homed to the vicinity of the DEJ of skin and oral mucosa, approximately 25% of them co-expressed GFP and C7, and ultrastructural examination of skin in transplanted animals revealed developing AFs (49).

Taken together, these results provided the proof of concept that donor-derived, C7-producing cells homed to the right place (i.e. the DEJ) at the right time (i.e. quickly) and provided partial correction of the disease phenotype (50).

**First in human**

With this proof of concept, we opened a clinical trial of myeloablative chemotherapy conditioning and HCT for individuals with severe generalized RDEB (51). In 5 of 6 individuals, light microscopy demonstrated a significant increase in C7 at the DEJ (Fig. 1a,b). Electron microscopy similarly indicated new development of AFs in at least one individual. Most importantly, we observed
resolution of the majority of skin and mucosal lesions (51), and this modest-to-dramatic clinical benefit has been maintained years after HCT (38) (Fig. 1c,d).

At the time of discovery, however, a new approach is fragile, disproportionately influenced by previous experience, and – in the absence of mechanistic proof – without clear separation from potentially confounding variables. For example, it has not been clear how the transient immunosuppression, the relative inactivity of the patient and high level of general medical care surrounding the HCT (52) or the dynamics of aberrant wound healing in severe generalized RDEB [‘regression towards the mean (53)’] may have impacted our observations in these transplant patients.

Thus, to begin to unravel the biological mechanisms whereby infusion of donor C7-producing cells can improve the condition of people with EB, we analysed donor cells in the skin of HCT recipients who had successful HCT (i.e. achieved full donor lymphohaematopoietic engraftment). Quantitatively, we detected unexpectedly high levels of skin chimerism, typically in the range of 10–30%, which confirmed that donor cells home to the injured skin in humans with severe generalized RDEB (38, 51, 54). This is relevant to the clinically meaningful impact of HCT on skin healing in EB, as animal and human data suggest that approximately 30% of wild-type C7 levels in skin may be sufficient to protect from blistering (17, 52, 55, 56). Based on these data, we are able to propose a tentative mechanism whereby donor cells home to recipient skin, engraft long term, and secrete C7. C7 then travels to the basement membrane zone of the skin, increasing the skin stability in the process (Fig. 2). Moreover, donor cells of both haematopoietic and non-haematopoietic phenotype were found in both epidermis and dermis of the recipients after HCT (38, 51).

As this suggests that stem cells from one organ (bone marrow or cord blood) can heal another (skin and mucosa), we are currently testing the hypothesis that non-haematopoietic bone marrow cells, termed mesenchymal stem/stromal cells (MSCs) (57), can further enhance the benefits of HCT. MSCs are multitalented cells that secrete extracellular matrix proteins, facilitate tissue repair and immunomodulate T cells (58). Thus, in principle, they could correct the biochemical deficiency in EB; ameliorate the physical injury of chemotherapy used before HCT; and help control the major immune complication of HCT, which is graft-versus-host disease (59–63).

**Slings and arrows**

Prior to this clinical trial in severe generalized RDEB and JEB-H, it was not known whether the combined risks of HCT (which to enable engraftment causes immune deficiency and sometimes organ dysfunction) and of severe EB (which because of loss of mucocutaneous barrier leads to infections, frequently with resistant microorganisms) would make the procedure unsurvivable. Individuals and families impacted by these severe forms of EB, however, are willing to accept risks that would be unthinkable in healthy individuals. These are diseases with severe effects on longevity and quality of life. In the case of JEB-H, almost all patients die within the first 3 years of life from complications of the disease itself. The risks of stem cell therapy are in part balanced by the potential benefit that it may be the only available treatment to help them survive into later childhood. For patients with severe generalized RDEB, the average life expectancy is longer, but stem cell therapy is still the only systemic treatment currently available that might enable them to live a remotely normal, less pain-filled life.

Nevertheless, we have been willing to undertake the significant risks of HCT only for patients with the most severe EB disease. To help us evaluate the balance of risk versus benefit of HCT for individuals with EB, we have employed the novel strategy of having an external expert review panel assess the severity of disease and appropriateness of risk for each case. This panel review strategy is also used to evaluate transplant outcome. Importantly, we have excluded patients based on the recommendation of the panel when patients were assessed as not having severe enough disease to warrant the risks of HCT today.

Unfortunately, several individuals have died as a result of transplant-related toxicity or of rapid progression of EB, while others have recovered from serious events such as infections and respiratory or renal failure. Although complications were not unexpected, they are powerful incentives to continue to improve the safety of HCT. It is reassuring that HCT itself has – in just 50 years – evolved rapidly from a highly experimental procedure into a life-saving, standard-of-care intervention for thousands of people.

**Setting realistic expectations**

There are few homeruns in experimental medicine. Even though the majority of evaluable individuals have had a positive response in skin healing, frequently changing their quality of life, the improvement in lifestyle has been varied and the overall clinical response incomplete. In agreement with these observations, most individuals showed biochemical correction of C7 or LM-332 deficiency, donor cells in skin and mucosa and significantly diminished mucocutaneous surface area involved. Clearly, a longer follow-up and better objective quantifiable metrics of change are needed, but in those with improved skin healing, we observed a trend towards a reduced need for dressings and pain control, lowered frequency of oesophageal dilatations and a diminished
progression of pseudosyndactyly. Yet, all individuals have one or more remaining lesions. We conclude that we were able to change the severe EB phenotype to that of a milder form of EB (38, 50).

To change the current amelioration of disease (much like HCT has been for congenital enzymopathies) into a full cure, we plan to use the same adaptive experimental approach that has informed our studies to date: we keep what works and adapt what does not. Three main action items underlie this platform.

First, change one variable at a time to increase safety as well as efficacy, such as those aimed at assessment of co-infusion of MSCs with HCT, or non-myeloablative conditioning for transplant. Even though development of new therapies is typically a succession of crisis points, where some failures can be a consequence of previous successes, we think that the clinical questions could be formulated in such a way that meaningful results are available within several years so that decisions whether or not to continue the specific strategy can be made quickly. This outcome-driven approach should lead to the uncovering of biological relationships (reinforced by the parallel studies in animal models and personalized induced pluripotent stem cells, iPSCs, below), the mechanisms of which can then be usefully adapted in subsequent clinical trials.

Second, evaluate interventions in animal models of EB. This should help in identifying the clinically meaningful optimization of the cellular entity from the donor (i.e. cells with the dual ability to home to injured skin and secrete C7 or LM-332) and the growth hormone and cytokine environment in the recipient. Recent examples of these strategies include lineage negative (Lin-), platelet-derived growth factor receptor alpha positive (PDGFRα+) cells that were shown to home into injured EB skin; and gradients of the heparin-binding epidermal growth factor-like growth factor (HB-EGF) and the high-mobility group protein B1 (HMGB1) that enhance homing into injured skin (64, 65).

Third, perform in vitro mechanistic studies, for example, using the patient-specific iPSCs (66), which are adult stem cells resembling embryonic stem cells. iPSCs have been a popular cell choice to study tissue formation (as they can recapitulate some of the inaccessible early stages of human development) and to discover and test new drugs. Furthermore, EB iPSCs can provide an inexhaustible supply of multipotential personalized cells, in which – for example – requirements for the biochemical correction of C7 and LM-332 defects can be determined on the cellular and organoid levels (67–71).

Known unknowns

Major open questions relate to the mechanism of C7 and LM-332 cross-correction, to our ability to deliver the HCT safely and to the future of EB clinical trials in the complex regulatory environment.

First, we know that C7 and LM-332 are expressed at the DEJ at significantly higher levels after HCT than before HCT in people with severe generalized RDEB and JEB-H, respectively. In addition, donor cell chimerism in skin is a potential structural correlate to the clinical responses seen in the majority of HCT recipients, and both haematopoietic and non-haematopoietic donor cells are present in the epidermis and dermis of the HCT recipient. However, we do not yet know their exact phenotypes. Because of the heterogeneous nature of the donor bone marrow or umbilical cord blood graft, it is conceivable that different cells migrate to epidermis than to dermis, and that both cell populations are expressing C7 or individual chains of LM-332. It is equally possible that only a fraction of these donor cells secrete these extracellular matrix proteins. The donor cells presumably respond to injury signals from the EB skin and can also be functional in the skin tissue repair indirectly, creating a permissive environment for productive skin repair by engaging the recipient’s cells in the use of donor C7 and LM-332.

Second, we know that we can use the physiological mechanism that allows cells from blood to integrate to injured skin. However, we do not know yet if the correction in skin is attributed to the haematopoietic stem cells or another cell type (‘collective cell migration’), or if the clinical improvement is at least in part related to changes in expression of other skin proteins, or if it might be related to circulating donor white blood cells (72, 73). Thus, in addition to some exciting examples, such as Lin-/-PDGFRα+ cells and the HB-EGF and HMGB1 gradients described earlier, there are likely additional subpopulations capable of biochemical correction of EB defects and additional mechanisms of homing that can be explored using stem cell graft engineering or pharmacological enhancement of skin homing.

Third, the ultimate goal is bringing useful therapies to clinic. As the regulatory agencies require a significant amount of data about new therapies, the recommendations at different steps of the clinical trial approval process can be different, even contradictory, and funding (especially for rare diseases) may be challenging to obtain and sustain (74).

And then, there are of course the ‘unknown unknowns’.

Focus on the horizon

HCT has always been a risky treatment restricted to those with serious life-threatening or debilitating diseases. By most benchmarks, the results of HCT in EB have shown that HCT has the potential of being a durable, systemic therapy for severe generalized RDEB and JEB-H. Patients and their families have faced, and will continue to face, some incredibly hard choices regarding the benefits and risks involved in either going ahead with HCT or not. There is a narrow but deep contrast in the decision to undergo the acute complications of HCT for severe EB or to endure the progressive complications of untreated severe generalized RDEB and JEB-H. Furthermore, only a few other options are in principle available (3, 75).

The history of EB research – with its impact on molecular dermatology, physiology of skin and use of animal models of skin disease – illustrates that the general medical importance of EB is disproportionate to its actual incidence. Therefore, it is reasonable to expect that the most recent observations from clinical trials in genodermatoses will inform therapy in acquired conditions of skin (such as thermal and chemical burns) and in other extracellular matrix disorders (such as those affecting connective tissue of cardiovascular and musculoskeletal systems).

Understandably, not every physician appreciates the attractions of HCT, but we agree with Stephen Katz that ‘investigative dermatology... is not skin-related research performed by dermatologists’ but rather it is ‘skin biology and skin disease research performed by any scientist in any discipline.‘(76) This is especially relevant today when tremendous logarithmic growth in technology and new discoveries, as well as the existing regulatory requirements, necessitate working together. Our approach has been to share the data as early as they are available so that others can contribute to
References

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