Workshop Report

CIF in EB: Repurposing treatments for EB

CIF (Chronic inflammation, Fibrosis, Cancer):

repurposing treatments and novel targets in EB

Workshop Spring 2023

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CONTENT

1. Introduction

EB RESEARCH NETWORK (EB RESNET), DEBRA AUSTRIA, AND OUR PARTNERS LIFEARC, HELD AN EXPERT ROUND-TABLE WORKSHOP IN MAY 2023 TO CONSIDER OPPORTUNITIES FOR REPURPOSING THERAPEUTICS FROM OTHER INFLAMMATORY AND FIBROTIC CONDITIONS, TOGETHER WITH IDENTIFYING POTENTIAL UNEXPLOITED INTERVENTION TARGETS TO PREVENT DISEASE PROGRESSION IN CIF (CHRONIC INFLAMMATION, FIBROSIS AND CANCER INITIATION) IN EPIDERMOLYSIS BULLOSA (EB). THIS FORMED PART OF A BROADER RESEARCH-PRIORITIZATION PROCESS FOR INVESTMENT IN THERAPY DEVELOPMENT FOR ALL TYPES OF EB.

The focus of the workshop was on a major and current unmet medical need in EB: how to interrupt the progression from acute to chronic wound, chronic inflammation, and fibrosis (CIF) to cancer initiation, seen primarily in dystrophic EB (DEB), and especially in recessive DEB (RDEB). By considering research gaps in biological understanding and technological challenges in therapy delivery, the goal was to consider known disease mechanisms and hence drug candidates for repurposing, as well as to identify novel unexploited mechanisms and novel targets.

At present, there is no cure for any form of EB, although multiple technologies are being pursued, and clinical trials are in progress for therapies addressing either the underlying defects or symptom relief, for all types of EB. In the past year, a couple of therapies have received regulatory approval. However, efficacy, safety, durability, and the need for systemic 'curative' therapies remains challenging. Addressing defective wound healing and preventing disease progression presents opportunities for novel or repurposed therapeutics, as sole, or complementary, treatments.

2. What is EB

EPIDERMOLYSIS BULLOSA (EB) IS A GROUP OF RARE INHERITED DISEASES CHARACTERISED BY FRAGILE SKIN AND MUCOSA THAT BLISTERS EXTREMELY EASILY, OWING TO A LACK OF, OR DEFECTS IN, THE PROTEINS RESPONSIBLE FOR EPITHELIAL ADHESION.

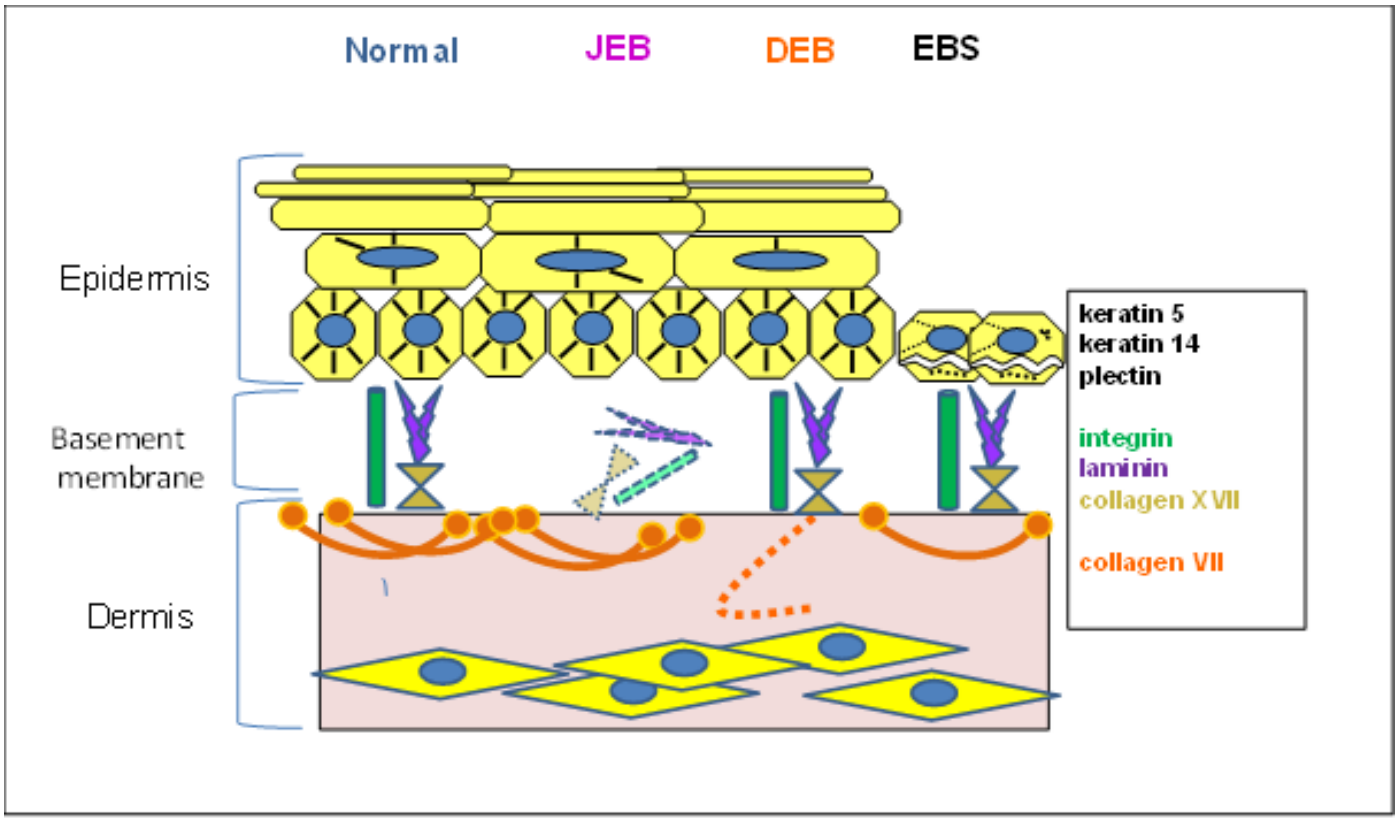
The symptoms, severity and prognosis for patients vary widely among EB subtypes, according to the protein affected and the effects of the specific mutation. Among the ~30 clinical subtypes, which are classified in 4 main types according to the depth within skin at which blistering occurs, recessive dystrophic EB (RDEB), along with severe generalized junctional EB (JEB), is one of the most severe, and characterised by CIF.

To date, RDEB has been the major focus of both academic and industry groups to develop therapeutic approaches to restore collagen VII protein production via various molecular, protein, cell, and gene therapy technologies.

What is EB?

- EB is a group of rare inherited disorders (incidence: ~ 20 per 1 million live births; prevalence: ~11 per 1 million population) resulting in extreme fragility of the skin and mucous membranes.
- EB results from any of a large number of mutations in at least 19 genes.
- 4 main subtypes (EB Simplex, ~70% of cases; Dystrophic EB, ~25%; Junctional EB; ~5%; Kindler syndrome, very rare), vary in severity, with death in infancy for severe subtypes.
- Clinical variability in all subtypes is owing to nature of mutation/ modifier genes; at least 30 clinical subtypes exist. Inheritance may be recessive or dominant; recessive subtypes are usually more severe.
- There is no cure. Treatment includes palliative wound management, pain control, nutritional support, and supportive treatment of complications.

Ref: Consensus reclassification of inherited epidermolysis bullosa and other disorders with skin fragility
C. Has et al. British Journal of Dermatology, Volume 183, Issue 4, 1 October 2020, Pages 614 -627, https://doi.org/10.1111/bjd.18921



Where EB has its effects

Blistering in EB occurs where the epidermis is joined to the dermis – the exact level of separation depends on which skin protein is defective or absent. The outer epidermis consists mainly of keratinocytes – the stem cells in the basal layer of these cells divide continuously, with cells moving towards the surface. In most EBs, either keratin 5 or keratin 14, which together make up the cytoskeleton, is faulty, resulting in the keratinocytes in the basal layer rupturing. Below this epidermis lies the dermis made up of fibroblast cells embedded in the extracellular matrix (ECM): skin and mucosal tissue integrity depends on the linked ECM proteins forming a structure connecting basal epidermal keratinocytes to the underlying dermis. In JEB, protein links in the 'chain' that ties the epidermis and dermis together are faulty or missing. In DEB, collagen 7 'anchors' into the dermis are missing or faulty. Collagen VII, secreted from both epidermal keratinocytes and dermal fibroblasts trimerizes in the upper dermis to form anchoring fibrils. In DEB, the collagen VII, and anchoring fibrils, are defective.

EB type	% total EB cases	Most commonly gene mutations (proteins)	Site of skin cleavage	Most common modes of Inheritance	Clinical presentation
EB Simplex (EBS)	~70%	75% affect *KRT5* and *KRT14*; *PLEC* (Keratin 5; Keratin 14; Plectin),	Within epidermis (outer layer of skin) owing to rupture of fragile epidermal cells lacking internal keratin 'skeleton'	Majority autosomal dominant; some recessive	Broad spectrum of severity, with hands and feet most commonly affected; in severe subtype, blistering in mouth
Dystrophic EB (DEB)	~25% (of which ~ 5% RDEB)	100% *COL7A1* (Collagen VII)	Blisters in dermis beneath basement membrane owing to deficient or absent collagen 'anchors' between skin layers	Autosomal recessive (RDEB) or dominant (DDEB)	RDEB much more severe than DDEB. In RDEB: • Severe blistering • Chronic wounds, scarring • Joint contractures; fusion of fingers and toes 'mitten' deformities • Mouth, GI mucosae compromised. • Multiorgan hyperfibrosis & failure • High risk of SCC
Junctional EB (JEB)	5%	*COL17A1; LAMA3; LAMB3; LAMC2; ITGA6; ITGB4 and ITGA3* (Collagen XVII; Laminin 332; Integrin α6β4)	Blisters within basement membrane, a layer connecting the outer epidermis and deeper dermis	Vast majority autosomal recessive	Depending on subtype: • Death in early infancy • Chronic ulceration, nail dystrophy/ loss. • Scarring. • Mucosal involvement
Kindler EB (KEB)	<1%	FERMT1 (Kindlin)	Blisters across skin layers	Autosomal recessive	Blisters, scarring on backs of hands and feet; photosensitivity and changes in pigmentation. Mucosal involvement in mouth, GI, eyes, and increased risk of SCC in skin, and oral mucosa.

Key features of the four main types of EB. (For more in-depth information: Has, C et al. "Consensus reclassification of inherited epidermolysis bullosa and other disorders with skin fragility.") Brit. J . Dermatol. 4 Feb. 2020

3. Challenges of treating EB

AT PRESENT, CARE INVOLVES AVOIDANCE OF INJURY THROUGH USE OF NON-ABRASIVE EVERYDAY ITEMS, SUCH AS CLOTHING, UTENSILS, AND FOODS, PROTECTIVE BANDAGING TO PREVENT OPEN WOUNDS AND BLISTERS FROM SPREADING, AND WOUND-CARE TO MINIMISE PAIN, ITCH, INFECTION AND TO AID HEALING.

Challenges in developing treatments for EB relate to both features of the disease itself, and to the compromised health and metabolic burden experienced by the patient population. For this reason, drugs that may be considered in other patient groups may not be suitable in EB.

- Anaemia is a major problem in EB patients, so the risks of gastrointestinal bleeding associated with anti-inflammatories such as aspirin outweigh the potential benefits.
- Steroids are also not recommended in EB patients since they suppress the immune system and inhibit healing.
- Inhibition of endogenous TGF β R1 activity may lead to tumour progression in a subset of RDEB patients: some clinical trials of TGF β inhibitors have led to spontaneous development of Squamous Cell Carcinoma (SCC) reflecting the tumour suppressor effects of TGF β .
- Checkpoint inhibitors used in Head and Neck Squamous Cell Carcinoma (HNSCC), and shown to have some efficacy in a small proportion of DEB patients, may not be widely useful owing to cutaneous side effects.
- Narrowing of the oesophagus and difficult venous access in some patients may require modified drug delivery methods.

While the skin is the most obviously affected tissue, EB is in fact a systemic disease. The overall health is compromised due to multi-organ dysfunction caused by inflammation and fibrosis. Organ failure, extending beyond the skin, is a significant contributor to disability and mortality, particularly in severe cases of EB. Disruption of the barrier function of the skin and mucous membranes leads to chronic tissue damage and associated infection and inflammation. Itch is also a substantial unmet need: open and healed wounds, but also intact skin may be itchy. Scratching aggravates the disease.

Severe recessive EB often presents severe complications including fusion of digits (pseudosyndactyly) also known as ‘mitten’ hands and feet, narrowing of the oesophagus and aggressive untreatable SCC. Clinical presentation of a particular subtype can vary widely, even with the same mutation. All disease stages - from acute wound to cancer - may be present simultaneously in a patient. There are significant differences in wound healing according to body site, from patient to patient and according to age. Prevention of disease progression in young children, and reversal of disease/fibrosis in adults, probably require completely different approaches and biological understanding. Each disease stage may be associated with a distinct molecular profile hence the need to profile patients to enable accurate therapeutic targeting.

Clinical needs in treating EB

- Faster wound healing
- Reduced:
  - Pain and itch
  - Wound infection
  - Inflammation
  - Fibrosis
  - Risk of cancer

Clinical considerations for therapy development:

- Risk- benefit profile
- relative to EB subtype
- Age at treatment: before
- health deteriorates and
- tissue damage accrues
- Mode of therapy delivery
- suitable to patient condition
- Treatment protocol compatible
- with patient's other needs

For more info:

Anna L. Bruckner et al. Orphanet Journal of Rare Diseases Vol 15, Art. 1 (2020) https://ojrd.biomedcentral.com/articles/10.1186/s13023-019-1279-y
Bardhan, A., Bruckner-Tuderman, L., Chapple, I.L.C. et al. Epidermolysis bullosa. Nat Rev Dis Primers 6, 78 (2020). https://doi.org/10.1038/s41572-020-0210-0

Clinical challenges in treating EB

Most focus is on visible skin and mucosae, but disease is systemic with life-threatening consequences in severe forms of EB:

- Skin
  - Mix of recurrent vs chronic wounds
  - More chronic and extensive with age
  - Colonisation or infection of wounds always present
  - Impaired wound healing

- Fibrosis:
  - Especially DEB
  - Mitten deformities of hands and feet
  - Flexion deformities of larger joints
  - Mucosal scarring: mouth, airway, oesophagus, anus, urethra, eye
  - Predisposition to cancer

- Secondary effects:
  - Nutritional compromise
  - Impaired growth
  - Pubertal delay
  - Anaemia
  - Reduced bone density
  - Fatigue
  - Pain and itch
  - Renal impairment
  - Cardiomyopathy

4. What are the causes and consequences of chronic inflammation and fibrosis (CIF)?

CHRONIC INFLAMMATION AND NON-RESOLVING WOUNDS ARE A PRIORITY UNMET MEDICAL NEED IN ALL TYPES OF EB.

The fragility of epithelial tissues in EB results in acute wounds from only minimal trauma, such as from walking, the rubbing of clothing, scratching, or eating.

Although it is the lack of specific proteins which causes fragility, the absence of the protein does not cause the damage directly: birth is the first trauma that can lead to extensive damage. It is the recurrent blistering and poor healing which leads to damage that accrues with age, together with consequences including infection, pain, itch, and secondary complications. Aberrant wound healing underpins poor health in patients with a severe type of EB, recessive dystrophic EB (RDEB): chronic wounds and scar formation are the link between continuous blistering and disease complications.

RDEB is characterised by a lack of collagen VII (C7) protein, which results in fragile skin that undergoes an unrelenting cycle of trauma, inflammation, fibrosis and development of SCC. Patients with RDEB vary in the severity of disease, from limited localised blisters to extensive skin and mucosal involvement, according to their specific C7 mutation, as well as their genetic background and environmental factors. Repeated injury leads to infection and persistent inflammation, which compromises wound healing.

Opportunities and needs

Opportunities…

- Newborns and infants have no fibrosis; therefore, this stage of the disease may offer opportunities for early intervention.

Needs….

- Identifying disease and inflammation markers, ideally unique hallmarks of each disease stage, is necessary.
- Characterising the molecular natural history of a single wound
- Correlation of exome/genome data from patients and tissue/clinical data

In turn, this leads to other complications including fibrotic scar formation that restricts joint movement as well as formation of pseudosyndactyly, and oesophageal stenosis (narrowing of the throat impacting the ability to swallow). Fibrotic tissue and chronic inflammation favours development of aggressive cutaneous squamous cell carcinoma (cSSC). Treatment should therefore focus on babies and younger patients before disease progression leads to loss of health and cumulative damage.

The lack of C7 protein (either through absence or mutation), not only determines skin fragility, but also directly affects wound healing by changing cell behaviour and fate, and there is a direct correlation between skin inflammation and disease severity in RDEB individuals.

Restoring C7 levels is the goal of 'curative' gene/cell therapies being developed. As excessive and persistent inflammation is a driver in both chronic wound development and fibrosis, targeting inflammatory mediators is also a therapeutic approach to reduce disease severity.

What drives chronic inflammation and fibrosis?

In DEB, the absence of C7 alone does not initiate severe inflammation and fibrosis – it is the initial trauma that acts as the primary trigger. However, the lack of C7 results in heightened fibrosis together with an altered extracellular matrix (ECM) and tissue environment: collagen in the ECM may also be disorganised via crosslinking and other post-translational modifications. RDEB presents with heightened pro-inflammatory immunity and macrophage infiltration, ultimately resulting in exhaustion of the immune system.

The lack of C7 impacts the secretory profile of fibroblasts. Little is known of the heterogeneity of activated fibroblasts in inflammation (iCAFs): some show enhanced contractility (myofibroblasts), but others do not. Whether contractile fibroblasts revert phenotype after wounds have healed (e.g. after gene therapy) is unclear. A key therapeutic concept is to switch the behaviour of cells by changing the signals they receive.

Some drugs being repurposed, or with the potential to be repurposed, include:

- Ang 1-7 peptide, acting via the Angiotensin Type 1 receptor, downmodulates fibroblast activity and leads to a reduction of IL-6 and contractility. This peptide re-educates fibroblasts and macrophages and is a pro-resolving peptide (it may exert a longer-lasting effect than inhibitors because it activates genes and pathways downstream).
- Porcupine inhibitors and inhibitors of ROCK 1-2 and Wnt signalling, also gastrointestinal-administered.

Role of infection in failed wound healing and inflammation

Chronic wounds in EB patients are heavily colonised, and infection undoubtedly increases inflammation and contributes to failed wound healing. Separating the contribution of infection from other inflammatory triggers is difficult, as is the clinical problem of treating infections.

It is known that the absence of C7 leads to a deficiency of endogenous antimicrobial compounds but, if skin heals, protection against infection is increased and any poor innate immunity becomes less critical.

Infection of skin and mucosal wounds results in persistent inflammation. This inflammation triggers changes in gene expression, which in turn results in decreased cellular proliferation and migration. Additionally, it causes pathological remodelling of the extracellular matrix, which then acts as a seed-bed for epithelial cancers. Re-epithelialisation is essential in preventing and reversing fibrosis and inflammation.

As yet, it is not known whether the aberrant innate immunity that allows bacterial colonisation seen in a mouse model of RDEB (hypomorphic mouse) is also the case in RDEB patients, and whether deficiencies in innate immunity arise from the skin, or are a systemic problem. Identifying the source of such deficiencies would guide therapeutic approaches, and some evidence should result from studies of neonates' skin wounds healed with gene therapy: this will not only show the impact on EB progression but also whether colonisation and innate immune deficiency are present.

Opportunities and needs

Opportunities...

- Microbiome engineering may reduce infection, inflammation and progression to cancer.
- Biofilms likely to hinder therapeutic efficacy of topical, and possibly systemic, 'curative' therapies.
- Does lack of SCC in fibrotic mucosa of fibrotic skin indicate potential druggable targets for intervention?

Needs....

- Characterise microbiome changes and reduction in species diversity as a wound progresses.
- Understand signalling when many microbes are present, and many pathways are activated, which may be confounders in single-wound molecular profiling.

Role of the immune system in disease progression

Pro-inflammatory immunity activity is associated with progression of fibrosis in RDEB, and the fibrosis develops through heightened cellular and structural immune exchange. Progressive fibrosis correlates with an increase in innate immune infiltrate major histocompatibility complex (MHC) II expression and transition from an activated to exhausted phenotype of local adaptive immune cells.

Downregulation of pro-inflammatory immune-system activity with an anti-inflammatory peptide targeting the renin-angiotensin system reduced the fibrosis-enhancing ability of fibroblasts in a mouse model, resulting in reduced progression to multi-organ fibrosis and increased survival.

Opportunities and needs

Opportunities…

- Selective down-modulation of pro-inflammatory immunity can reduce injury-induced fibrosis.
- Other fibrotic conditions may offer indicators of possible targets for design of novel or repurposed drugs.

Needs….

- Molecular diversity exists across fibrotic conditions. – cannot extrapolate directly to EB
- Recognition of, and a detailed mechanistic understanding of, differences in fibrosis in different conditions

Analysis of proteome changes during disease progression in an animal model of RDEB shows that fibrosis is a consequence primarily of altered organization of the extracellular matrix rather than changes in amounts of structural proteins present.

5. Preventing progression to cancer

SQUAMOUS CELL CARCINOMA (SCC) IS CURRENTLY AN INEVITABLE COMPLICATION OF RDEB DISEASE PROGRESSION CHARACTERISED BY ALTERED ECM AND INFLAMMATION. THE SCCS SEEN IN RDEB PATIENTS OCCUR EARLIER IN LIFE (TEENS AND 20S) AND ARE APPRECIABLY MORE AGGRESSIVE AND INVASIVE THAN SPONTANEOUS OR PHOTO-DAMAGE SCCS AND ARE USUALLY FATAL.

Other types of DEB as well as junctional EB (JEB) and Kindler syndrome (KS) are also associated with cancer predisposition, but usually later in life and the cancers are generally less aggressive.

Lack of C7 in EB results in loss of core components of the basement membrane: laminin and integrin levels are reduced and, in the ECM, collagens 1, 3 and 5 are increased. EB subtypes that lack laminin can also develop tumours but not to the same extent as seen in RDEB.

Kindler syndrome is a very rare type of EB, in which keratinocytes show an up-regulation of proinflammatory cytokines interleukin (IL)−1β, IL-6, and tumor necrosis factor-α (TNF-α), and a defect in $\alpha 6 \beta 2$ integrin cytoskeleton. These patients have a lot of fibrosis and a high proportion (although less than RDEB) get cancer at around 40-50 years of age. For these reasons, current focus is on preventing SCC in RDEB, but successful treatments may translate to other EB subtypes.

RDEB SCC tumours are homogeneous, meaning distinct parts of the same tumour share the same driver mutations. Development of SCC in EB is driven by APOBEC mutagenesis, which appears to be a more critical contributor than other causative factors, e.g. reactive oxygen species (ROS). There is no evidence of increased mutational burden that causes the APOBEC mutagenesis, for example, in tumour-adjacent sites, although this has not been explored in non-cancerous EB tissue. APOBEC mutagenesis is driven by infection and the immune response, and may coincide with tumour initiation. The APOBEC signature is not unique to EB SCC, as it is also seen in 90% of HNSCC tumours. No tumour genetic markers have been identified to differentiate EB SCC from HNSCC and other cancers.

Opportunities and challenges

Opportunities

- Similarities to HNSCC may lead to more therapies that can be repurposed, but there are few approved SCC drugs.
- Known mechanisms:

(1) PK1 (Polo kinase 1) is increased in RDEB SCC, and some response has been seen from systemic delivery of a PLK1 inhibitor (Rigosertib) in two clinical trials.

Rigosertib is known to inhibit multiple kinases. The evidence is that 'most' activity is through PLK1, but alternate mechanisms would likely be beneficial rather than detrimental.

(2) APOBEC mutagenesis could be a therapeutic target, e.g., APOBEC3A (A3A) inhibitors.

TNF-alpha drives A3A expression, thus TNF-alpha inhibitors may be able early to block APOBEC A3A mutagenesis

- Checkpoint inhibitors are being explored; 10-20% of patients respond.
- It may be possible to use radiotherapy/chemotherapy in a palliative setting to increase the vulnerability of EB SCC in conjunction with Rigosertib.
- Adopting a mixed approach, with Rigosertib + Cemiplimab, may be advisable to reduce tumour selection pressure.

Challenges

- Stiffness of fibrotic tissue is likely to impede delivery of some therapeutic agents.
- Tumours appear variable in behaviour and progression: some tumours remain dormant for a prolonged time, while some others grow explosively and metastasise.
- It is possible that variation in mechano-transduction is mediating a difference in response to a stiffer ECM in some tumour types.
- Possibility of using an oncolytic virus to deliver a therapeutic payload to the tumour and bypass the restrictive ECM environment.
- Heterogeneous response to PK1 inhibitors (some primary tumours respond well, secondary tumours less well).

6. Intervening in inflammation and fibrosis: how, when, and where to target?

INFLAMMATION FOLLOWING INJURY IS A PROTECTIVE RESPONSE, WHICH IN NORMAL TISSUES HAS A PROINFLAMMATORY PHASE FOLLOWED BY A RESOLUTION PHASE. THIS RESOLUTION PHASE IS CONSIDERED AN 'ACTIVE' PROCESS, NOT SIMPLY 'NOT-INFLAMMATION', AND ACTIVELY RETURNS TISSUES TO A NORMAL NON-INFLAMED STATE. ANY LACK OF RESOLUTION THEREFORE RESULTS IN THE DEVELOPMENT OF CHRONIC INFLAMMATION, AND A FAILURE OF AN INJURY TO HEAL NORMALLY.

Current approaches to combat inflammation and fibrosis rely heavily on inhibiting the pro-inflammatory phase and neglect mechanisms that agonise pro-resolution pathways: strong inhibition of the pro-inflammatory phase leads to immunosuppression, which is not desirable in patients with heavily colonised wounds and prone to SCC development.

Most existing treatments try to prevent inflammation, but there is scope to 'activate' resolution instead. Approaches to create a ‘pro-healing’ tissue environment to tackle disease morbidity require a better understanding of the cellular and molecular processes of normal and aberrant wound healing, to identify opportunities for ‘pro-healing’ therapeutics and potential intervention points for both novel and repurposed drugs.

Activating resolution pathways instead is advantageous because it exploits the patient's own pro-resolving mechanisms, does not require complete inhibition, and thus should lead to fewer side effects common to many anti-inflammatory agents. Pro-resolution is designed for chronic disease.

Current ‘resolution’ drugs in development generally have specific targets, but multiple effects.Systemic delivery may have off-target effects, by changing the behaviour of cells in various tissues, but off-target effects from systemic delivery may be reduced by inherent feedback/control mechanisms in the body.

Approved drugs as candidates for repurposing in EB might include:

- Alpha-MSH agonists, shown to be anti-inflammatory in the skin
- Alpha1 Antitrypsin, which prevents lung fibrosis
- G-Protein Coupled Receptor (GPCR) agonists
- Cannabinoids, have pro-resolution effects, e.g., creams exist to treat pain and itch
- IL31 inhibitors may also work as itch inhibitors for EB

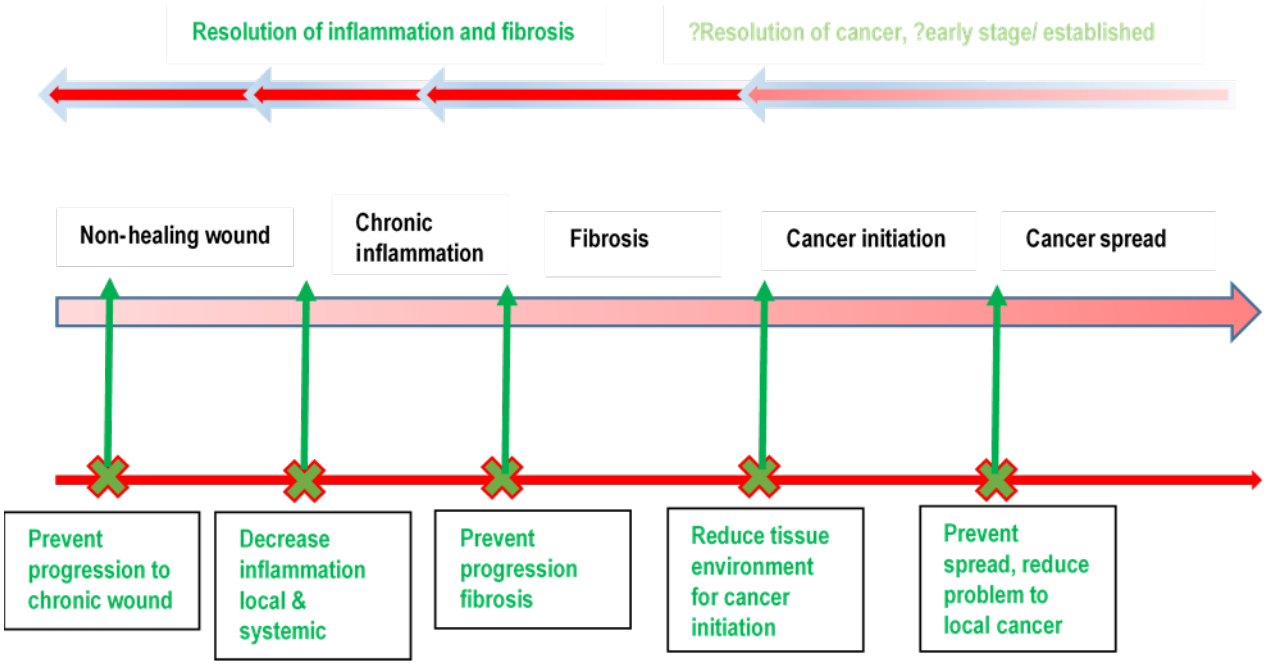


Figure: Approaches to develop novel/ repurposed drugs. *Blocking progression of acute injury to non-healing wounds, fibrosis and cancer-initiation via anti-inflammatories/ anti-fibrotics / anti-cancer drugs or, alternatively, promoting resolution of inflammation and fibrosis via pro-healing pathways. Targeting resolution pathways*

7. Reversing inflammation and fibrosis: resolution pharmacology?

EARLY EVIDENCE THAT INFLAMMATION AND FIBROSIS ARE REVERSIBLE IN EB HAS BEEN DERIVED FROM STUDIES INVOLVING LOCAL INJECTION OF ALLOGENEIC OR AUTOLOGOUS FIBROBLASTS, OR MESENCHYMAL STEM CELLS.

Additional evidence has arisen from multiple studies and clinical trials of gene/cell therapies to replace the missing collagen VII, with reversal of blistering, inflammation and fibrosis.

Within the realm of gene/cell therapy studies, there lies the potential to investigate the factors that contribute to inflammation and fibrosis. This includes not only understanding how changes at the molecular level can affect inflammation and fibrosis, but also exploring whether adjusting the tissue's inflammatory condition before or during gene/cell therapy could enhance the therapy's success rate. In the oral cavity of EB patients, fibrosis occurs but the incidence of cancer is low.

Fibrotic conditions in different disease states are highly variable, and this is reflected in the molecular diversity seen. Fibrotic plaques in EB are heterogeneous and not directly comparable to other fibrotic conditions. Thick, crusty plaques may correspond to an irreversible stage of the disease's course. However, thinner plaques may still revert to earlier stages if treated in a timely manner, hence the need to intervene as early as possible.

The faster the wound re-epithelialises, the faster gene therapy can correct the skin. Keratinocytes produce basement membrane as they migrate. Consideration can therefore be given for the role of antifibrotics and anti-inflammatories in future treatment scenarios; not only as sole treatment but also as a supplement to 'curative' therapies.

Post-surgical prevention of inflammation and fibrosis could significantly enhance outcomes and durability of treatment. This is especially true for surgeries frequently performed in severe EB cases, such as oesophagus dilatations or the separation of fused fingers in mitten hands.

Opportunities and needs

Opportunities…

- Potentiating pro-resolving pathways (wound-healing augmentation) to heal wounds is an alternative approach to attempting to prevent inflammation.
- Development of anti-inflammatory/ anti-fibrotic or pro-resolution drugs or cell-derived factors as standalone treatments, or as adjunct to 'curative' therapies:
    - enhance success and durability of surgeries, preventing re-stenosis or re-fusion of digits
    - enhance efficacy and success of gene-cell therapies
- Pro-resolution approaches may have fewer side-effects than anti-inflammatories.
- Pro-resolving mediators such as small molecule agonists could be used as maintenance therapies as well as to treat acute disease.
- Multiple pro-resolution molecules developed for other conditions: need to identify agonists appropriate for EB
- Clinical trials testing combinations of anti-inflammation and pro-resolution therapies with gene-corrective therapies

Needs….

- Better clinical understanding of DEB progression from birth to adult
    - (i.e. unaffected skin>>erosions>> inflammation>>fibrosis>>cancer)
- Better clinical understanding of regression of DEB after molecular therapy:
    - (i.e. heal erosions>>reverse inflammation>>reverse fibrosis>>? reverse cancer)
- Better understanding of molecular / cellular natural history of:
    - fibrosis and inflammation during DEB disease progression……, and
    - its reversal following C7 molecular correction by gene/cell therapy

8. Complementing curative gene-cell therapies

ANTIFIBROTICS, ANTI-INFLAMMATORIES, AND PRO-RESOLUTION TREATMENTS HAVE A ROLE IN FUTURE TREATMENT SCENARIOS, BOTH AS SOLE TREATMENT OR AS ADJUNCT TO 'CURATIVE' THERAPIES. ENHANCING UNDERLYING WOUND HEALING WILL ALLOW DISEASE-MODIFYING THERAPIES TO WORK BETTER – NOT ONLY UNTIL EFFECTIVE, SYSTEMIC, 'CURES' ARE AVAILABLE, BUT TO COMPLEMENT AND IMPROVE OUTCOMES OF SUCH TREATMENTS.

Therapies aiming to replace, or augment, the defective protein are in development for all types of EB, using various technologies to deliver the proteins, either directly, or mainly via gene- and cell-therapies, topically or systemically. Drugs to modify the patient's own gene expression, and non-specific treatments to aid wound healing or reduce itch or inflammation are also being developed.

The majority of curative treatments at late stages of development – in clinical trials, with a couple approved for clinical use – are topical treatments, aiming to treat local wounds. Systemic delivery of a protein therapy has been explored but a key limitation is getting to the basement membrane through the inflammatory environment; topical delivery might be more successful.

Recent approval of a topical gene therapy ('BVEC') for DEB, restoring C7 production, has achieved long-term clinical reversal of blistering, inflammation and fibrosis: durable for 17 months at least, post-therapy.

However, EB is a systemic disease, and systemic treatments are needed. Stem-cell therapies in development aim to be 'curative' (i.e. to correct the causative genetic defect, through provision of a gene-correct(ed), durable and self-renewing cell population able to synthesize adequate levels of the missing protein); other, mainly drug therapies, that aim to modify symptoms or provide transient increases in missing protein, require repeat dosing. More ablative approaches, such as bone marrow transplantation, have had a mortality higher than the disease itself and the clinicians would not recommend them.

Whether the goal is curative or symptom-relief, therapies need to offer a treatment which balances the risk – benefit profile relevant to the EB subtype, and be acceptable and appropriate in its mode and frequency of delivery to the patient subpopulation to be treated.

Considerations for CIF targeting to complement curative therapies

- Inflammation can prevent effective therapy in several ways, e.g, itch leading to scratching and trauma.
- Aspects of the wound environment interfere with molecular therapies, e.g., presence of matrix metalloproteinases (MMPs). C proteases may solubilise collagen 7 and hence impair the formation of anchoring fibrils.
- Consider where collagen and re-epithelialisation is coming from when applying topical gene therapy: C7 needs to be renewed every 1-3 months and renewal could come from fibroblasts or keratinocytes.
- Basement membrane needs to be restored before reepithelialisation, both of which needed before, e.g. a gene therapy can be effective.
- Autoimmune-related inflammation, which some patients develop due to exposure to gene-therapy products.
- Earlier treatment may also improve tolerance - less likely to develop anti-collagen VII antibodies.
- Potential for combining molecular therapies with cell therapies like ABCB+5 mesenchymal stem cells.

Needs:

- Better understanding of molecular natural history of fibrosis and inflammation during DEB progression.
- Better understanding of disease regression after molecular therapy.
- Combination of molecular curative therapies with anti-inflammatories and wound-healing augmentation therapies.

9. Developing new and repurposed therapies

While EB presents challenges for effective therapeutics development, there are unexploited opportunities for both repurposed and novel treatments. Although EB is a rare group of diseases, it has the advantages of worldwide EB research and clinical communities that are strongly interconnected, and collaborate across diverse research, clinical and supporting infrastructure initiatives.

The patient community is well engaged, participating in clinical studies and trials, in dialogue with clinical-trial sponsors through patient organisations and clinical networks. Patient organisations initiated, and have built on 30+ years of research, to establish genotype-phenotype and mechanistic understanding of cause and consequence of the EB diseases, animal disease models, and both disease and patient registers, de-risking translational development through funded pre-clinical research and early-stage clinical trials.