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Epidermolysis Bullosa and Associated Therapy: a Literature Review

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Abstract

Background: Epidermolysis Bullosa is associated with poor dermal-epidermal cohesion, leading to the formation of blisters, erosions and scars on the skin and mucous membranes in response to minimal mechanical trauma. It is clinically and genetically heterogeneous, comprising phenotypes with different levels of severity. A definitive cure does not appear to be forthcoming, with symptomatic treatment being the most commonly used approach. However, various studies and clinical trials have been carried out with the aim of testing different theories and finding an effective method for improving the quality of life of people with this condition and mitigating its negative effects. Objectives: The aim of this study is to gather complete and up-to-date information on the main therapeutic strategies used and/or under development for Epidermolysis Bullosa. Material and Methods: A literature review was carried out, searching the Pubmed, Google Scholar and Scielo databases for the keywords "epidermolysis bullosa", "treatment" and "pharmacotherapy", selecting articles using the inclusion and exclusion criteria. Results: Based on the articles included in this review, it was possible to understand the different existing strategies for the treatment of Epidermolysis Bullosa. It was found that there are various types of therapies under development, although the results obtained are promising. Conclusion: As this is a very heterogeneous pathology, it is difficult to find a therapy that is effective and does not cause serious adverse effects. This is an area that should continue to be studied, with the aim of improving the quality of life of those affected.
Keywords: Epidermolysis Bullosa; Dermatosis; Treatment; Pharmacotherapy.

1. Introduction

Rare diseases have a worldwide prevalence of less than 0.05% and can be divided into thousands of different phenotypes, not all of which are known [1]. Around 80% of these pathologies are due to a wide variety of genetic mutations, [1, 2] which makes them difficult to diagnose. Due to the difficulty in researching and understanding these pathologies, the healthcare associated with them is costly and many have no definitive cure [1].

Epidermolysis bullosa (EB), also known as butterfly skin disease, [3] is a rare condition associated with poor dermal-epidermal cohesion, leading to the formation of blisters, erosions and scars on the skin and mucous membranes in response to minimal mechanical trauma. It is often accompanied by damage to nails, teeth and hair. In many cases, the wounds can become chronic, and milia, skin atrophy and depigmentation are also common [4-7]. It is clinically and genetically heterogeneous, comprising phenotypes with different levels of severity. It is divided into 4 main types: Epidermolysis Bullosa Simplex (EBS), Junctional Epidermolysis Bullosa (JEB), Dystrophic Epidermolysis Bullosa (DEB) and Kindler Epidermolysis Bullosa (KEB) [6-9].

This study aims to gather complete and up-to-date information on the main therapeutic strategies used and under development for EB.

2. Types of Epidermolysis Bullosa

2.1. Epidermolysis Bullosa Simplex

EBS is characterised by the formation of blisters in the basal layer of keratinocytes in the epidermis, and is associated with phenotypes with a variable spectrum of severity [7, 10]. It is the most common type and includes 14 clinical subtypes associated with mutations in 7 different genes [6, 7]. Most subtypes are inherited in an autosomal dominant manner, however there are regions of the world where the autosomal recessively inherited form of the disease is more prevalent. The 3 main subtypes are caused by pathogenic variants in the KRT5 or KRT14 genes, which encode keratins 5 and 14, respectively [4, 6, 7, 10]. These proteins form the intermediate filaments that serve as the cytoskeleton of basal keratinocytes. The mutated keratins exert a dominant effect over the functional ones and thus the weakened keratinocytes become susceptible to damage due to mechanical friction, which leads to cytolysis and blistering of the basal epidermis [4, 10].

Localised EBS is the most common subtype, occurring in approximately 60% of individuals [11]. It includes milder phenotypes, which usually appear in early childhood, although some individuals do not manifest the disease until adolescence or early adulthood [7, 11]. The formation of blisters is usually limited to the palms and soles of the feet, although they can appear anywhere as long as there is adequate trauma, and they tend to worsen in hot weather and with sweating [7, 10, 11]. The lesions can be associated with pain and itching, causing individuals' mobility to be reduced during active disease flares [7, 11].

Intermediate EBS occurs in around 15% of individuals, in whom blisters can be present from birth or appear in the first few months of life [7, 11]. Blisters and sores can be severe and fatal in neonates and babies, but generally improve with time [4, 6]. It has a widespread distribution, however it predominantly affects hands and feet, and focal palmoplantar keratoderma can also be observed [7].

Severe EBS occurs in approximately 25% of individuals, usually appearing at birth and with varying severity [11]. It is characterised by large generalised blisters, which can occur in herpetiform clusters and can be haemorrhagic [7]. During childhood, the blisters develop all over the body, mainly on the hands, feet, around the mouth, trunk and neck, and blisters and erosions on the oral and oesophageal mucous membranes are also common [7, 11]. However, the lesions tend to improve with age and generally don't leave a scar [7].

2.2. Junctional Epidermolysis Bullosa

JEB is characterised by cleavage and blistering of the skin's lamina lucida and is a rare type of EB [6, 7]. It is caused by defects in laminin-332, integrin-64 or collagen type XVII (COLXVII), which facilitate the adhesion of basal keratinocytes to collagen in the upper dermis, and the LAMB3, LAMA3 and LAMC2 genes are generally affected [6, 12].

The phenotypic variability of JEB is quite wide, from subtle clinical signs to early lethality, and mutations in 7 different genes can lead to 9 clinical subtypes, inherited in an autosomal recessive manner [6, 7]. The two most frequent subtypes are generalised intermediate JEB or generalised severe JEB (Herlitz JEB) [7, 12].

Intermediate JEB comprises clinically heterogeneous phenotypes and is characterised by a generalised distribution of blisters that heal and lead to subtle atrophy and hypopigmentation, while mucous membranes can also be affected. Alopecia, dystrophy or absence of nails, hypergranulation, chronic wound formation and extracutaneous involvement of the cornea, larynx and urinary tract can also be associated. In this type of JEB, the expression of laminin-332 and COLXVII is reduced, but not completely absent [7, 12].

Severe JEB is characterised by extreme fragility of the skin and mucous membranes, with blisters and mucocutaneous erosions present from birth and on the entire surface of the body, increasing susceptibility to infections [7, 12]. This subtype is associated with loss of expression or complete absence of laminin-332, which contributes to exuberant granulation tissue, especially in the areas of the nose, mouth, buttocks and nail folds [6, 7]. Patients suffer from extreme pain and, in the long term, may experience developmental delays, anaemia and respiratory complications, usually dying within the first few years of life [7, 12].

2.3. Dystrophic Epidermolysis Bullosa

DEB is characterised by cleavage of the skin in the region of the sublamina densa, in the upper portion of the dermis [7, 13]. It is associated with a broad phenotypic spectrum and severity, ranging from mild nail dystrophy to severe extracutaneous involvement and premature death [7].

It develops as a result of mutations in the COL7A1 gene, which codes for type VII collagen (COLVII) [6, 7, 12, 13]. Absent or dysfunctional COLVII leads to fragility of the skin with splitting of the upper layers of the dermis, problems in the healing of mucous membranes and an additional dysfunction of innate immunity [5, 6, 12]. It can be inherited in an autosomal recessive or autosomal dominant manner, making up 2 main groups. It can also be subdivided into 11 subtypes, with manifestations of varying severity [7, 10, 12, 14].

Recessive Dystrophic Epidermolysis Bullosa (RDEB) is generally more severe than the dominant form [7, 10, 14]. It is associated with the formation of blisters all over the body, from birth, and scars that lead to pseudosyndactyly on the hands and feet, resulting in joint contractures. Chronic ulcers, milia, nail dystrophy and even anaemia, developmental delay and squamous cell carcinoma associated with early death are also common [7, 10, 12].

Dominant Dystrophic Epidermolysis Bullosa (DDEB) is characterised by a milder phenotype, with blisters on the skin with a widespread distribution at birth and often with dystrophic nails [7, 12]. It is also associated with albopapuloid lesions, milia, atrophic scars and nail dystrophy[7].

There is also a subtype of DEB, Dystrophic Epidermolysis Bullosa Pruriginosa (DEB-Pr), characterised by intensely pruritic hypertrophic nodules and plaques, mainly on the distal extremities, associated with rashes, erosions and blisters. It is chronic in nature and can be debilitating and reduce patients' quality of life [5, 15].

2.4. Kindler Epidermolysis Bullosa

KEB is a rare type of EB that manifests itself from birth and is difficult to diagnose, since its clinical presentation can contain characteristics usually associated with other types. However, it has peculiar characteristics such as poikiloderma, which combines hyper- or hypopigmentation of the skin, and photosensitivity, which causes erythema and sunburn [6, 7, 10]. The phenotype is generally progressive and also includes blisters and extensive skin atrophy, and cleavage of the skin can occur in multiple layers (intraepidermal, junctional or sublamina densa). In addition to the skin, it can have ocular, gingival, oesophageal, gastrointestinal and genitourinary involvement [6, 7, 10].

It is inherited in an autosomal recessive manner and is caused by mutations in the FERMT1 gene, which encodes kindlin-1[6-8]. This protein is associated with integrins and focal adhesions and, in the skin, it is located next to the basal keratinocytes and link the actin filaments to the membrane proteins [6, 7, 10]. 84 different pathogenic variants in FERMT1 have already been described, most of which are associated with the formation of a premature stop codon and consequent loss of function of the protein, although there is evident clinical variability [6, 7].

2.5. Epidermolysis Bullosa Acquisita

Epidermolysis Bullosa Acquisita (EBA) affects the skin and mucous membranes and is characterised by the presence of autoantibodies targeting COLVII. It results in a loss of dermal-epidermal adhesion and, consequently, skin fragility and the formation of vesicles and blisters [16, 17].

There are two main clinical types of EBA: mechanobullous EBA and inflammatory EBA. In the mechanobullous variant, the fragility of the skin and the formation of blisters, together with pain and itching, are evident in areas prone to trauma, while in the inflammatory variant they can also occur in non-traumatic areas. The recurrent appearance of blisters can gradually lead to the appearance of scars on the skin. In addition to the skin, different mucous membranes such as the oral and nasal cavity, throat and anogenital regions can also be affected, with associated complications [17].

This type of EB can appear at any age but is most common after the age of 50 [16, 17].

3. Diagnosis

To confirm the diagnosis of EB, various laboratory and genetic tests are used to not only exclude other diagnoses (such as infections, autoimmune blistering disorders and other genodermatoses), but also to provide a complete characterisation of both deoxyribonucleic acid (DNA) and proteins. In the case of newborns with a phenotype that may be indicative of EB, they should immediately undergo blood sampling and skin biopsy tests so that treatment can begin as soon as possible. In these cases, the first method of choice will be immunofluorescence mapping (IFM) via skin biopsy using labelled antibodies. Although a direct genetic test provides a definitive diagnosis, the time it takes to obtain the results means that it is not the most recommended method. As in some cases the pathology can appear and evolve with age, a child or adult with characteristic manifestations of EB should be referred for genetic testing, using Next-Generation Sequencing or Sanger Sequencing. For a more complete diagnosis, IFM or transmission electron microscopy tests can also be carried out [18].

4. Therapeutics

Although studies and clinical trials are being carried out to prove different theories for therapy, there is still no definitive cure for EB, and symptomatic treatment is the most commonly used approach [19-21]. This can include pain control, blister prevention, skin protection and generalised prevention of complications and infections [20, 21]. Two-layer skin substitute dressings have been developed with the aim of acting as a permanent covering [22]. Physiotherapy is also a way of keeping the consequences of the disease under control. When the disease is not in its most active phase, physical and weight-bearing activities should be encouraged to facilitate the accumulation of healthy bone minerals [23].

Pharmacological therapy generally focuses mainly on analgesics and antipruritic drugs, as a form of control to maintain a better quality of life. However, it can be associated with limited efficacy and various side effects, which is why many individuals choose to stop treatment or only use it for short periods [21]. Many of the clinical trials currently underway test genetic and cell therapies, and it is necessary to know the existing genetic defect in order to understand which method to use [8]. Methods in which the gene without the mutation is administered and in which there is intradermal injection (ID) of allogeneic or genetically modified fibroblasts are being studied [19]. In dominant forms of the disease, another potential strategy to be used is the elimination of the allele with the mutation through ribonucleic acid (RNA) strategies [8]. The effect of gentamicin in increasing protein production through mutation reading has also been tested [19].

The systemic nature of EB has also led to the investigation of stem cells as alternative sources for cell therapies, due to their regenerative capacities and proven effectiveness in improving various pathologies [20]. The removal of exons with pathogenic RNA variants is also a promising technique for mitigating the effects of the disease, the aim of which would be to produce messenger RNA with alternative splicing, in which the exon with the mutation would be removed [10]. A definitive cure for EB doesn't seem to be in sight any time soon, but various studies and clinical trials have been carried out with the aim of testing different theories and finding an effective method for improving the quality of life of people with this pathology and mitigating its effects.

5. Material and Methods

This study consists of a literature review, with the aim of providing a summary that covers research carried out on the topic in question.

Data was collected from scientific databases such as Pubmed, Google Scholar and Scielo, using the keywords "epidermolysis bullosa", "treatment" and "pharmacotherapy" alone or in combination.

The researcher accessed full access articles, mainly published from 2018 onwards. However, articles before 2018 that proved to have information relevant to the topic in question were also used. Any study that did not meet the inclusion criteria or that proved irrelevant after reading was excluded.

The PRISMA methodology was used to select the articles, and 20 articles were included in the sample, according to the criteria referred above.

6. Ethical implications of the study

During the study's literature review, all bibliographical references were duly cited and identified, showing ethical respect for the principles of copyright and following the Helsinki treaty, so that no plagiarism was committed [24].

7. Results

Table 1 shows the results obtained for different treatments in 9 of the articles used to write this literature review.

Table-1. Articles obtained and corresponding results

Author/year	Treatment	Type of EB	Results
Szymański, *et al.* [17]	Intravenous Immunoglobulin (IVIG)	EBA	In one reported case, treatment was characterised by 4 pulses of 2 g/kg, at 4-6 week intervals, which resulted in a reduction in the severity of the disease for 12 months. Due to a re-exacerbation of the disease, treatment was carried out again, at 4-8 week intervals, which reduced the number and frequency of blisters, however the improvement did not prove to be persistent. In another case, therapy began with 6 pulses of 2 g/kg, at irregular intervals ranging from 4-12 weeks. The severity of the disease gradually reduced over 12 months. Blisters appeared less frequently and healed more easily. There was no complete clinical remission, however the improvement was significant over 46 months, with no adverse effects appearing.
	Rituximab		Treatment with 2 administrations of 1g of rituximab, 2 weeks apart, led to gradual healing of the erosions and no new blisters appeared. Clinical remission was thus achieved for 2 years. In another reported case, 2 administrations of 1 g were carried out together with daily prednisolone treatment. This treatment resulted in complete healing of the erosions. After 12 months, there was less fragility of the skin and the appearance of some rashes as a result of the patient's increased physical activity. However, the erosions healed more quickly.
	Infliximab		A case was reported of a patient with EBA and Chron's Disease (CD) who started treatment with infliximab. The treatment consisted of administering 12 pulses of 350 mg at 8 week intervals. During treatment, both pathologies went into remission. After 2 years, there was no progression in CD or the appearance of new EBA eruptions. Although skin fragility remained, it was significantly reduced, and wound healing was faster.
Niti, *et al.* [20]	Hematopoietic stem cell transplantation	JEB	A clinical trial was carried out in which an initial improvement in the wound healing process was observed, however complications related to graft rejection arose which led to the death of the patients.
		RDEB	In the first clinical trial carried out with children after immunosuppressive chemotherapy, although there was an increase in the expression of COLVII and donor cells in the recipient skin and a reduction in blister formation, there was also toxicity associated with the treatment.

			In another trial, children showed an increase in donor chimerism in whole blood and skin, which improved their clinical situation. However, these results were temporary.
	Cytotherapies with mesenchymal stem cells	RDEB	ID or intravenous (IV) injections of mesenchymal stem cells resulted in a reduction in disease activity, pain and itching and thus an increase in quality of life.
	Genetically corrected autologous epidermal stem cell and fibroblasts therapies	JEB	Epidermal stem cell therapy was applied to the LAMB3 gene, which demonstrated continuous clinical benefits and laminin-332 expression for more than six and a half years, as well as no adverse effects. In other cases, the results of 16 year follow-ups indicate the possible long-term safety and effectiveness of these therapies.
		RDEB	ID injection of genetically modified autologous fibroblasts was evaluated. A clinical trial revealed functional expression of COLVII and formation of new anchoring fibrils. Another trial showed increased COLVII expression at the injection site for 12 months, but no functional anchoring fibrils were formed.
du Rand, *et al.* [25]	Epidermal skin substitutes	EBS	A commercially available epidermal allograft applied twice a week for 3 weeks has been reported to result in a 50% reduction in the surface area of wounds.
		JEB	Epidermal autografts created from areas of skin not affected by blisters were first studied. Two cases were reported in which complete epithelialisation was observed 7 and 10 months after transplantation, while in another case epithelialisation was only partial. Subsequently, reversible mosaicism techniques were used. One case was reported in which this method was used and, 4 months after transplantation, the graft appeared healthy and scar-free. However, a loosening of the skin was noticed. Another strategy is based on the genetic correction of autologous keratinocytes. One year after the transplant using this method, there were no blisters, inflammation or infection, which continued for 16 years. In another case, the graft area remained healed for 2 years with a healthy basal membrane and epidermis. Fibrin-based grafts are more robust and 80% epidermal regeneration has been reported with their use, which has remained stable after 5 years. Cases reported that epidermal allografts resulted in wound epithelialisation after 6 months, which lasted for 8 months. However, graft rejection was also observed in several places, with the formation of blisters and infection.
		RDEB	With the study of epidermal autografts from areas of skin not affected by blisters, complete stabilisation of 2 wounds was achieved in 12 months and more than 50% closure in another. Using the reversible mosaicism technique, cases of 100% epithelialisation of ulcers in 76 weeks have been reported. However, there were also episodes in which it was necessary to carry out another transplant, from which 52,6% epithelialisation was possible at 76 weeks, and cases in which there was mechanical displacement of the keratinocyte sheets, which led to poor wound healing. Tests using the method of genetic correction of autologous keratinocytes reported wound healing within 2 years, but with significant variability. The use of epidermal allografts has also been studied and faster wound healing has been observed within 7 days.
	Dermal skin substitutes	RDEB	The first dermal allografts in culture were applied twice a week for the first 2 weeks, and then weekly, with complete epithelialisation after 3 weeks in one case and partial epithelialisation after 4 weeks in another. In other reported cases, transplantation twice a week for 2-6 weeks increased wound granulation by 1 week, and epithelialisation appeared on the wound edges after 4 weeks. It was also reported, 2 weeks after transplantation, that 28% of the wounds had reduced in size by 70%, with 5% of the wounds having healed completely.

			A dermal substitute is commercially available whose reports have shown epidermal regeneration of 80-100% in 2 weeks, some of which, however, broke down in the following weeks.
	Composite skin substitutes	EBS	A skin substitute composed of fibroblasts and keratinocytes was tested and rapid wound healing was recorded at the graft sites after 3 days. 20 days later, it was found that around 40% of the body surface was covered with the graft and mostly free of blisters.
		RDEB	Through tests with acellular dermal substitute with different types of collagen, complete dermal regeneration was observed. After 10 days, the wound was covered with autografts composed of fibroblasts and keratinocytes. Complete wound healing and improved physical activity were observed 2 years later.
So, *et al.* [26]	EB-101	RDEB	5 years after the application of autologous grafts with genetic correction of keratinocytes, it was observed that 70% of the grafted sites showed more than 50% healing and 63,3% showed more than 75% healing. Pain and itching decreased completely in the grafted sites that showed more than 50% healing. Skin durability improved in 52,8% of the sites and the appearance of blisters after trauma was reduced in 48,6% of the sites. 85,7% of the adverse effects that appeared were classified as mild, and there were 2 cases of localised infections that resolved with oral antibiotics and did not prevent the wound from healing in the long term.
Gurevich, *et al.* [27]	B-VEC	RDEB	In clinical trials to study the effect of topical application, all the wounds closed and remained healed 3 months after application, except for one chronic wound that only partially healed 1 month after treatment. The wounds treated with B-VEC required less time to heal and the time they remained healed was longer, demonstrating its efficacy for dermal-epidermal cohesion. The area of the wounds also decreased significantly with application. Most of the healed samples also showed linear deposition, indicating the expression of complete and functional COLVII in the correct location on the basal membrane.
Kueckelhaus, *et al.* [28]	Autologous transgenic epidermal cultures	JEB	A 5-year follow-up was carried out on a child after the transplantation of autologous transgenic epidermal grafts. After 5 years, the transgenic epidermis was stable and robust and showed no tendency to blister with friction or when healing wounds. The transgenic epidermis was stratified in a similar way to normal epidermis. It was observed that the skin's immune system had been reconstituted, as well as regular expression of COLVII. The transgenic epidermis showed normal levels of water loss and hydration, as well as melanin and haemoglobin levels. However, the transgenic skin showed mild dermal fibrosis, marked by a decrease in elastin microfibrils, as well as mild erythema and neuropathy.
Vermeer, *et al.* [10]	Skipping of exon 70 (COL7A1)	RDEB	Restoration of the protein was observed by immunofluorescence in primary keratinocytes transfected with antisense oligonucleotides and in human skin equivalents, although with low efficiency.
	Skipping of exon 73 (COL7A1)	DEB	The antisense oligonucleotide Q-313 was shown to facilitate COLVII synthesis in mutant fibroblasts and keratinocytes. In the carbomer hydrogel formulation, exon skipping efficiency was increased by around 30-35% after 2 weeks of treatment. It also improved epidermal attachment in DDEB skin models.
	Skipping of exon 80 (COL7A1)	RDEB	Antisense oligonucleotides reached up to 90% of fibroblasts in primary culture and exon skipping was 50% efficient in keratinocytes 48 hours after transfection.
	Skipping of exon 7 (COL17A1)	EBJ	The treatment restored COLXVII expression in primary cultured keratinocytes derived from the patient. It was also possible to observe the expression of collagen in the skin basement membrane in human skin equivalents generated from pre-treated keratinocytes.
		JEB	Topical or injectable administration resulted in improved wound healing and a reduction in the number of blisters at the sites.

			In another trial, IV administration followed by intramuscular (IM), or just IM, was tested and improved the stability of the patients' skin and therefore their quality of life.
		RDEB	Topical administration was tested, as well as localised injection, which induced the formation of anchoring fibrils, improved wound closure and reduced the number of blisters at the sites.
	Betulin	RDEB	The semi-solid gel formulation was well tolerated, however its effects on wound healing were not statistically significant.
	Diacerein	EBS	The 1% diacerein-containing ointment significantly reduced the number of blisters in patients during a 4-week treatment. This effect increased further during 3 months of follow-up.
	Serlopitant		Taking it orally led to a reduction in patients' pruritus, but the results were not statistically significant.
	Epigallocatechin-3-gallate		A 50% reduction in the number of blisters was observed in patients, however these results were not statistically significant.
	Botulinum toxin	EBS	With ID injections, a reduction in pain and the number of blisters was observed in patients. However, these results lasted less than 4 months.
		RDEB	Injection into a patient's internal anal sphincter for 2 years resulted in relief from spasm and pain due to anal blisters. The effect lasted for 4 years after treatment.
Santi, *et al.* [16]	Corticosteroids	EBA	Systemic corticosteroids are the first-line treatment, at dosages of 0.5-1.5 mg/kg/day, with dosages of more than 15 mg/day to control disease activity. They can be used as monotherapy in mild cases of the disease, but most patients need adjuvant treatment for better control of the disease or to avoid undesirable side effects due to prolonged use of corticosteroids. It has been reported that the use of *methylprednisolone* at a dosage of more than 8 mg/day led more quickly to remission of the disease compared to the use of a lower dosage. Some patients also had a good response to pulse therapy with 500 mg of methylprednisolone for 3 days.
	Anti-inflammatory agents		Commonly used dosages of *dapsone* range from 25-100 mg/day or 1-2 mg/kg/day, achieving favourable responses in adults and children. However, it can have adverse effects such as haemolysis and methemoglobinaemia. Efficacy has been reported with *colchicine* at dosages ranging from 0,5-2 mg/day. It is considered a first-line agent for adjuvant treatments, and positive clinical responses have also been reported when used as monotherapy. However, side effects such as diarrhoea may limit its use. A response to the use of 200 mg/day of *minocycline* together with systemic corticosteroids was reported in a patient who had already been treated with various therapeutic options, which suggests the use of this drug as a therapeutic option.
	Immunosuppressants		*Mycophenolate mofetil* is effective in refractory cases of the disease, and successful treatments have been carried out with 2-3 g/day. *Cyclosporine* is usually used in doses of 4-9 mg/kg, leading to improvement in recalcitrant cases.
	IVIG		It is usually administered at a dose of 2 g/kg/cycle for 3-5 days as monotherapy or in combination with other drugs, with positive responses in cases of recalcitrant disease. One study assessed the response after administering 16-31 cycles for 30-52 months, and there was no recurrence of the disease during the average 53.9-month follow-up, suggesting that this method leads to prolonged remission of the disease.
	Extracorporeal photophoresis		This method has proven to be effective in inducing partial to complete remission in cases of recalcitrant disease, and is considered a well-tolerated therapy. European guidelines state that the treatment can

			be used in severe and refractory cases of the disease.
	Rituximab		Cases of recalcitrant disease in which the response was effective have been reported, and the therapeutic regimen is usually 375mg/m^2 weekly for 4 weeks. Another proposed regimen is 1 g administered on day 0 and day 14. With this regimen, patients need a new cycle of treatment after 6 months, but the responses are positive in terms of bringing the disease into remission.

8. Discussion

In EB, it is necessary to know which gene or protein is affected in order to choose the approach to be used. Different approaches have been developed and studied, such as gene therapy, cell therapy and pharmacotherapy, which should be applied to the individual case of each patient [8, 10, 29]. As these methods are still under development, the most widely used approaches continue to be symptom-relieving therapies, in order to improve patients' quality of life [8].

Different gene therapy strategies have been studied with the aim of repairing patient-specific genetic defects or mutations [4, 5]. The emerging tools of gene editing have shown great promise from a therapeutic point of view, as they are precise and there are few off-target effects [4].

Gene editing based on programmable nucleases, such as the CRISPR/Cas9 system, seeks to permanently correct the existing genetic defect in the DNA [13]. To do this, it induces the DNA strand to break and the strand is then restored [8, 13]. This method has already been tested to assess the formation of functional COLVII with its use and has proven to be effective [12]. It has also been successfully used to abolish the expression of the KRT14 mutant allele and pathogenic keratin, thus managing to fully restore a functional intermediate filament network [4]. However, this technique has the limitation that its effect is still unpredictable off-target and can cause unintended genetic modifications if it binds to similar genomic sites off-target. Strategies have been developed to limit the cleavage of this system, so that it is safer and there is no risk of tumourigenesis [13].

In vivo gene therapy approaches have used herpes simplex virus type 1 (HSV-1) vectors to deliver therapeutic genes [27, 30]. These viruses stand out for effectively infecting cells and resisting immune elimination [27]. They are therefore modified to manifest with reduced immunogenicity and act as vectors to deliver the full-length complementary DNA of the COL7A1 gene [8]. By using this strategy, procedures requiring hospitalisation, anaesthesia or invasive surgery are avoided, as it can be applied during routine dressing changes, minimising additional trauma [13, 27]. This vector has been shown to be safe, however its continued long-term application is necessary [8, 13, 30].

Several technologies capable of modulating the biosynthesis of mature messenger RNA have been explored, one of the most promising being exon skipping, mediated by antisense oligonucleotides [13]. Antisense oligonucleotides are short, single-stranded DNA or RNA that are designed to bind to a specific complementary pre-messenger RNA sequence. In this way, they interfere with the binding sites of the splicing enzymes, skipping the mutated exon. This method leads to the expression of shorter but functional proteins and is particularly practical for genes such as COL7A1, since it can act on several exons of this gene [12, 13, 30]. Antisense oligonucleotides generally have low toxicity and limited adverse effects, as well as being easy to manufacture and administer [30]. Their topical administration on wounds using a carbomer hydrogel formulation improves wound healing and stabilises the skin at the site where it is applied, however a systemic treatment would have the advantage of preventing blister formation even on mucous membranes inaccessible through topical treatment [10].

Another intriguing option is "natural gene therapy", which is based on the use of cells or tissues derived from reversible mosaic patches of the patient's skin. This is a phenomenon that occurs in genetic disorders, in which there is a spontaneous reversal of the mutation, which restores the expression of the protein and results in areas of normal skin [8, 29]. This process occurs mainly in keratinocytes and has already been described in various EB subtypes, with successful clinical applications [12, 29].

Another approach to administering COL7A1 to patients involves introducing the transgene into the individual's own cultured cells. Thus, autologous fibroblasts are patched with a vector that integrates the transgene into the genome, and these cells are then injected directly into the edges of the patient's wounds. This approach requires several injections, which can be painful [8]. In another method, the corrected cells are expanded into epidermal layers, which are then grafted onto patients' chronic wounds. This technique has been shown to generate functional correction, with COLVII deposition, the formation of anchoring fibrils and dermo-epidermal stability months after the graft has been placed [12, 13].

When it is not possible to use autologous skin grafts, the use of skin substitute dressings can be an alternative. These products are designed to accelerate wound healing and regenerate the skin, and have good biodegradability, low toxicity and a low probability of immunological rejection. Composite skin substitutes consist of an epidermal layer of keratinocytes and an underlying dermal layer made up of fibroblasts. These products provide dermal-epidermal adhesion and are more similar to normal skin, with the wound-healing properties of the cells that compose them [25].

Therapies based on mesenchymal stem cells have also shown to be promising for aiding wound healing [14]. These cells are characterised by their ability to self-renew and be undifferentiated, as well as having immunomodulatory and anti-inflammatory effects, stimulating tissue regeneration [12, 14, 20]. There are various sources of mesenchymal stem cells for potential therapeutic application, such as bone marrow, adipose tissue, umbilical cord blood and amniotic fluid [12]. Pre-clinical studies with this type of cell have shown encouraging results, and ID and IV administration have also been studied [12, 20]. ID administration of the cells has the advantage of generating a large concentration of cells in a localised region, while IV administration can have a systemic impact [12].

In addition to the therapeutic approaches described, efforts are being made to try to improve the quality of life of people with EB, which may involve the reuse of medicines or the development of new therapies in an attempt to improve symptoms [12].

There are cases of EB that have nonsense pathogenic variants, which lead to premature stop codons. One possible approach in these cases is to promote ribosomal read-through so that the RNA is produced at its full length and thus generates the functional protein [12]. Gentamicin, an aminoglycoside antibiotic, has proven effective in several cases of mutations in the COL7A1, COL17A1 and LAMB3 genes, when administered both topically and IV, and has improved the skin phenotype for months [5, 30].

Fibrosis is also a clinical feature of some EB subtypes, which can severely limit patients [12]. It has been identified that TGF-β signalling is an important driver of fibrosis and its severity [8]. Therefore, therapies that can reduce its signalling have the ability to slow down the progression of the disease [12]. Losartan is a drug approved for the treatment of hypertension, but it has also been shown to neutralise TGF-β signalling [12, 29].

Pregabalin and ropivacaine also have the ability to improve pain by inhibiting calcium currents and the flow of sodium ions, respectively, in nerve cells [9].

Diacerein, a compound isolated from rhubarb root, has been shown to increase skin stability by inhibiting pro-inflammatory IL-β signalling. Betulin, derived from birch bark, also acts on the inflammatory phase of the wound healing process, as it modulates inflammation factors such as COX-2 or IL-6 [9, 29, 30].

Dupilumab is a monoclonal antibody that blocks IL-4 and IL-13 signalling, thereby relieving pruritus and improving the patient's quality of life [12, 15].

Also, infliximab, directed against TNF-α, reduces infiltration, adhesion and chemotaxis of inflammatory cells, as well as reducing IL-1 and IL-6 levels, thus reducing inflammation and leading to long-term clinical remission [17].

9. Conclusion

This literature review shows that there is a wide range of different therapies for Epidermolysis Bullosa. However, they are in the clinical trial phase and there is still no treatment that cures the disease and is universal for all individuals.

Since this is a pathology with different subtypes and phenotypes, it is very difficult to find a therapy that is effective and does not cause serious adverse effects.

The therapy associated with Epidermolysis Bullosa is an area that should continue to be studied, with the aim of enabling those affected to improve their quality of life and reduce the suffering caused by the condition. It is important to monitor the methods that are currently being developed and to wait for new promising results.

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