Eur J Dermatol 2019; 29(5): 459-67

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Article accepted on 13/05/2019

Thymosin β4: potential to treat epidermolysis bullosa and other severe dermal injuries

Thymosin β4 is a naturally-occurring regenerative protein present in almost all cells and body fluids, including wound fluid. In multiple preclinical injury models, it promotes dermal repair and tissue regeneration. Thymosin β4 acts by increasing keratinocyte/epithelial cell migration, angiogenesis, and cell survival, and by decreasing inflammation, apoptosis, and scarring. It also modulates cytokines, including those that cause itching. Thymosin β4 promotes faster repair in various chronic human wounds, including pressure ulcers, stasis ulcers, and epidermolysis bullosa lesions. The faster healing time with increased keratinocyte migration and angiogenesis and reduction in both inflammation and scarring are especially important for epidermolysis bullosa patients who suffer from slow healing and inflammation that leads to itching, infections, pain, fluid loss, scarring, and tissue damage. These multiple mechanisms of action support thymosin β4's role in accelerating dermal repair and suggest the potential to treat various types of severe wounds, including epidermolysis bullosa patients who suffer from frequent blistering wounds that can be life threatening. There is an urgent need at this time to develop a therapeutic, such as thymosin β4, for epidermolysis bullosa. Despite progress in gene/stem cell therapy, there is no cure for this disease and careful wound management is the standard of care.

Key words: thymosin β4, epidermolysis bullosa, dermal healing, migration, clinical trials

Dermal wound healing is a multistep process that is impaired or delayed for many individuals, such as aged, immobilized, and/or diabetic patients. Furthermore, many patients suffer from deep and/or large wounds that do not heal quickly and result in significant scarring and thus loss of tissue function [1, 2]. Patients with epidermolysis bullosa suffer from repeated dermal blisters that result in painful wounds that in some forms of the disease are life-threatening [3, 4]. At this time, there is little that can be done to treat such wounds beyond meticulous wound care to prevent infection, reduce discomfort, and promote natural healing [5, 6]. A naturally-occurring molecule, thymosin β4, has shown promise in many preclinical animal models for repairing injured tissues, including dermal lesions and burns, ocular injury, myocardial infarct, stroke, fibrosis of the kidney and lung, traumatic brain injury, and spinal cord damage [7, 8]. Several Phase 2 clinical studies have also shown accelerated healing and tissue repair with thymosin β4 [9]. Many biological activities/mechanisms of action important in dermal repair have been shown for this molecule. The recent clinical successes with severe and moderate ocular injury further support the use and safety of thymosin β4 in severe dermal injuries [10, 11] (NCT01387347, NCT01393132, NCT02994907, NCT02597803). This review will discuss the data that support the rationale for its use in the clinic to treat problematic wounds, especially in epidermolysis bullosa patients.

Epidermolysis bullosa (EB)

EB is a rare genetic disease affecting 1 in 50,000 worldwide. The United States Epidermolysis Bullosa Registry found that the overall incidence and prevalence of inherited EB were 19.57 and 11.07, respectively [12]. It is a blistering disease in which the skin does not adhere well to the underlying tissue, resulting in lesions induced by mild trauma. EB involves defects in 20 different genes [13]. There are four main EB types: simplex (EBS: mutations in the *KRT5* and *KRT14* genes), dystrophic (DEB: defect in collagen VII), junctional (JEB: defect in laminin-332), and Kindler syndrome (mixed pattern of gene defects) [3]. Laminin-332 and collagen VII are basement membrane components that promote tissue integrity and keratinocyte migration. Mutations in the genes comprising these proteins (three distinct laminin chains and two distinct collagen chains) underlie some of the more severe forms of this disease [14-16]. These EB patients suffer from pain, fluid loss, anaemia, malnutrition, infections, and scarring. One complicating issue is itching which is severe and can lead to additional injury in the skin [4, 5, 13]. There is no specific treatment or cure for EB at this time beyond meticulous wound care requiring rebandaging every one to three days that can take up to two hours per day. Such care is painful for the patients and difficult for the caregivers [5]. Patients with DEB develop

doi:10.1684/ejd.2019.3642

To cite this article: Yang WS, Kang S, Sung J, Kleinman HK. Thymosin β4: potential to treat epidermolysis bullosa and other severe dermal injuries. *Eur J Dermatol* 2019; 29(5): 459-67 doi:10.1684/ejd.2019.3642

significant scarring leading to loss of tissue function, such as use of the hands [17]. RDEB patients more often develop squamous cell carcinoma and a recent report indicated that actually all EB patients could develop squamous cell carcinoma [17]. The quality of life is greatly diminished and life expectancy is reduced for many of these EB patients.

Clinical studies on EB

There is an urgent need to find an effective treatment for EB patients. A variety of current clinical studies are focusing on gene and/or cell therapy as well as on either topical or systemic drug treatments [18-23] (clinicaltrials.gov) (*table 1*). Such studies are progressing and offer hope for the patients. While gene therapy using either viral vectors or genetically modified autologous cells has the potential to cure EB, this treatment approach is at a very early stage and may require invasive procedures [6]. Furthermore, gene therapy will have to be developed for each mutated gene and may require harvesting of the patients own cells for gene delivery. Some gene therapy approaches have used or will use intradermal injections of genetically modified autologous fibroblasts (FCX-007), intravenous infusion of genetically modified stem cells (IMP-allo-APZ2-EB), topical gene therapy (KB103), viral transfer of the collagen VII gene to keratinocyte sheets (LZRSE-Col7A1), autologous genetically modified epidermal cells as grafts, and genetically corrected epidermal autografts (ATMP). Cell-based therapy using autologous stem cells is progressing in the clinic with several sites starting trials for EB patients using either adipose stem cells or mesenchymal stromal cells [18, 20, 21]. The expected development time for gene and/or cell therapy and potential high cost suggest that these approaches may not be available soon or to all patients.
Topical treatments are also being tested on EB patients. Topical gentamicin has recently shown effectiveness in increasing collagen VII synthesis and healing wounds in DEB patients with nonsense mutations in collagen VII [16, 24]. A Phase 1/2 trial (NCT03526159) using systemic and topical gentamicin treatment is recruiting. A topical treatment, Oleogel S-10, consisting of a plant extract that reduces inflammation and promotes keratinocyte migration, has advanced to Phase 3 trials [25]. A topical anti-inflammatory, Diacerein, has completed a small 15-patient Phase 2 trial with efficacy for EB simplex [26], and is recruiting by invitation for its long-term safety trial (NCT03389308). Another topical treatment, Zorblisa, unfortunately failed to show significant efficacy in a recent Phase 3 trial (clinicaltrials.gov, NCT02384460), and oral polyphenon E failed to show efficacy in a Phase 2 trial [27]. Based on preclinical studies, a topical antisense nucleotide (QR313) is being tested (NCT03605069) for patients with an exon 73 mutation in *Col7* [28]. Because of the immediate need to treat EB patients, effective topical treatments for now have the potential to promote healing, relieve the symptoms, and improve the quality of life of EB patients [6]. Thymosin β4 is one topical treatment that is multifunctional, and preclinical and clinical studies have provided the scientific rationale for its use with all EB patients [7, 29]. Thymosin β4, as detailed below, has multiple mechanisms of action that not only reduce inflammation but also protect the tissue and drive the endogenous healing process, resulting in fast wound healing. In an ad hoc analysis, thymosin β4 also showed a trend toward efficacy in JEB and DEB patients in a small 30-patient multi-dose Phase 2 trial (NCT00311766) (*figure 1*).

Thymosin β4 regenerative activities

Thymosin β4 distribution and functions

Originally described for its effects on the immune system, thymosin β4 has, in the last 20+ years, showed consistent strong effects on promoting tissue repair and regeneration in many diverse organs and in various preclinical models of tissue injury [7-9, 30-35]. It is a small 4.9 kDa molecule that is present in almost all cells and body fluids, including wound fluid [36-40]. It has multiple activities (*table 2*) that explain its ability to improve healing in so many organs with different causes of injury. It reduces inflammation by preventing NFkappa B activation, resulting in a reduction in the secretion of various inflammatory mediators [41-44]. Scarring is reduced, in part, by blocking the presence of fibroblasts and by allowing for normal collagen synthesis with faster and appropriate organization of the collagen fibrils [45] (*table 3*). A reduction in scar tissue has been observed by multiple research groups in different animal models of injury, including excisional wounds, myocardial infarct, stroke, and fibrosis of the lung, liver, and kidney [44-51] (*table 2*). Thymosin β4 reduces oxidative stress by targeting anti-oxidative genes [52-57]. It reduced toxicity by inhibiting inducible nitric oxide synthetase (iNOS) and cyclooxygenase-2, resulting in decreased reactive oxygen species (ROS), decreased secretion of NO, and decreased prostaglandins. Thymosin β4 decreased apoptosis by increasing anti-apoptotic proteins, such as caspases and decreasing the Bax/BCL2 ratio [58-60]. This results in cell/tissue survival. Angiogenesis is important for healing wounds as the vasculature provides a way to remove the debris and to supply nutrients for the forming tissue [61]. Thymosin β4 promotes VEGF synthesis and endothelial progenitor cell migration/recruitment and differentiation, leading to new blood vessel formation [61-68]. Thymosin β4 also prevents microbial infection [69]. This activity was unexpectedly discovered when researchers were identifying antimicrobial peptides in activated platelets. Platelets are known to be important in host defense and thymosin β4 was found to be one of seven antimicrobial peptides in platelets. The mechanism of action of thymosin β4 on microbes is not known. An especially important activity is the ability to promote cell migration in various injury models, and particularly the migration of keratinocytes which cover the wound and protect from fluid loss and infection [7, 46, 60, 65, 70, 71]. The migration activity of thymosin β4 is, in part, mediated by its binding to actin. Thymosin β4 binds to actin, and the actin binding domain has been identified as the site responsible for cell migration [57]. Thymosin β4 coordinates actin polymerization with metalloproteinase synthesis to promote cell migration. One mechanism proposes that profilin-dependent dissociation of the G-actin-thymosin β4 complex liberates actin for filament assembly [71]. Thymosin β4 binds to integrin-linked kinase in the lamellipodia to both activate Akt2 and increase metalloproteinase production [72]. In addition,

Table 1. Representative EB clinical trials out of a total of 77 listed in clinicaltrials.gov, as of March 1, 2019.

Number/status	Sponsor	Indication/phase	Treatment
Completed trials			
NCT02384460 Completed/failed	Scioderm/Amicus	EB, Phase 3	SD101/Zorblisa cream, topical, 150 participants.
NCT01538862 Completed	Vanderbilt U	DEB, Phase 2	GCSF, subcutaneous injection, 7 participants.
NCT00951964 Completed/failed	Centr. Hosp. Univ	RDEB, Phase 2	Polyphenon E, topical epigallocatechin 3, 17 participants.
NCT00311766 Completed/failed	ReGeneRx	DEB/JEB, Phase 2	Topical thymosin β4, 30 participants
Active, not recruiting trials			
NCT02323789 Active, not recruiting	King's College	RDEB, Phase 1/2	Mesenchymal stromal cells, 2 infusions, 10 participants, Estimated completion July 2017..
NCT01263379 Active, not recruiting	Stanford U, Stanford U	RDEB, Phase 1/2	Engineered autologous epidermal sheets LZRSE-Col7A1Viral transfer of gene to keratinocyte sheets, grafts, about 10 participants, Estimated completion 2025.
NCT02654483 Active, not recruiting	Menlo Therapeut	EB, Phase 2	Neurokinin-1 receptor antagonist for itch (VPD-737.) oral, 14 participants, Estimated completion February 2019
Recruiting trials			
NCT02579369 Recruiting	Anterogen Co., LTD	DEB, Phase 1/2	ALLO-ASC-DFU, hydrogel with adipose stem cells, topical, about 5 participants, Estimated completion 2017 (no updates)
NCT03068780 Recruiting	Amryt Research LTD	EB, Phase 3	Oleogel-S10, birch bark extract, topical, about 192 participants, Estimated completion 2020.
NCT03536143 Recruiting	Krystal Biotech Inc	RDEB, Phase 1/2	KB103 topical gene therapy (collagen VII) about 6 participants, Estimated completion 2024.
NCT03154333 Terminated	Castle Creek Pharm	EBS, Phase 2	Diacerein, topical anti-inflammatory, about 80 participants, Estimated completion, 2018.
NCT02810951 Recruiting	Fibrocell Technologies	RDEB, Phase 1/2	FCX-007, intradermal injection modified autologous fibroblasts, about 12 participants, Estimated completion 2033.
NCT03490331 Recruiting	Holostem Terapie Avazante S.R.I	JEB, Phase 1/2	Autologous cultured epidermal grafts with genetically modified epidermal stem cells, surgery, about 12 participants, Estimated completion 2020.
NCT03605069 Recruiting	ProQR Therapeutics	RDEB, Phase 1/2	QR-313 antisense nucleotide for patients with Recruiting Exon 73 mutation in Col7A1, topical, about 8 participants, Estimated completion 2019.
NCT01033552 Recruiting	Masonic Cancer Ctr	Severe EB, Phase 2	Mesenchymal stem cell transplants after cyclophosphamide, fludarabine, cyclosporin A, etc. and whole-body irradiation, 75 participants, Estimated completion 2019.
NCT01033552 Recruiting	Masonic Cancer Ctr	Severe EB, Phase 2	Mesenchymal stem cell transplants after cyclophosphamide, fludarabine, cyclosporin A, etc. and whole-body irradiation, 75 participants, Estimated completion 2019.
NCT02984085 Recruiting	Holostem Terapie Avazante	RDEB, Phase 1/2	Genetically corrected epidermal autograft (ATMP), Harvest skin, surgically implant graft, about 12 participants, Estimated completion 2018.
NCT03578029 Recruiting	Lenus Therapeutics	JEB, DEB, Phase 2	Thymosin β4, topical, about 15 patients Estimated completion 2019.
NCT03526159 Recruiting	USC	RDEB, Phase 1/2	Gentamicin sulphate, 3 participants topical, 3 participants systemic, Estimated completion 2020.
Not yet recruiting trials			
NCT03529877 Not yet recruiting	RHEACELL	GmbH & CoRDEB, Phase 1/2a	ABCB5+ stem cells, IV infusions of IMP allo-APZ2-EB, about 18 participants.

Shown are examples of the different types of therapies used for EB. Where one type of therapy has been studied under multiple trials, generally only the most recent one is shown. Estimated completion dates are from clinicaltrals.gov. EB: epidermolysis bullosa; DEB: dystrophic epidermolysis bullosa; JEB: junctional epidermolysis bullosa; EBS: epidermolysis bullosa simplex; USC: University of Southern California; GCSF: granulocyte colony stimulating factor.

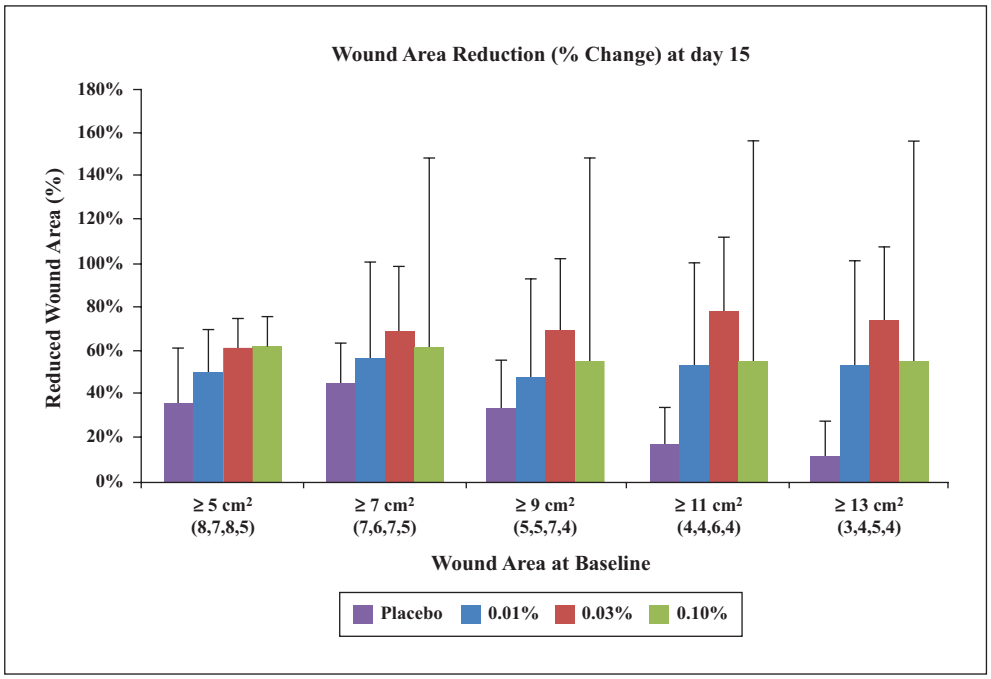

Figure 1. Thymosin β4 decreases the EB wound area (% change) after 14 days of topical treatment more effectively than the placebo, especially for the larger wounds. Patients were treated topically daily, and the wound area was measured at Day 15. These results show the faster healing by thymosin β4 and its ability to be effective for large wounds. Note that all doses of thymosin β4 were more effective than the vehicle control for all wound sizes. Data were obtained based on an ad hoc analysis of NCT-311766.

thymosin β4 increases laminin-332 synthesis which is a known migration factor for various epithelial and endothelial cells, including keratinocytes [70, 73, 74]. Laminin-332 also promotes tissue integrity by increasing keratinocyte adhesion to the underlying extracellular matrix [14, 75]. It should be noted that the underlying defect in JEB involves mutations in laminin-332. This mutation results in the loss of the function of this molecule and the blistering of the skin in JEB [13-16]. Taken together, thymosin β4 has the potential to heal and regenerate dermal injuries of various causes. The multiple mechanisms of action of thymosin β4 are important in all of the major stages of dermal wound healing, including inflammation, proliferation, and remodelling. Such a large range of activities provide the scientific rationale for its potential to heal epidermolysis bullosa wounds caused by different genetic defects.

Thymosin β4 active sites

How can one molecule have so many biological activities? Several active sites within the molecule have been identified [43, 44, 47, 49, 62, 76-78]. The amino terminal SDKP sequence (amino acids 1-4) is naturally found in plasma and is responsible for the anti-inflammatory and anti-fibrotic effects [43, 44, 47, 49, 79]. Thymosin β4 is the only protein known to contain the SDKP sequence. It is interesting to note that decreased serum levels of SDKP result in organ fibrosis, thus demonstrating its protective effect [79]. The amino terminal 15 amino acids (amino acids 1-15) contain the tissue protective and anti-apoptotic activity [78]. The central LKKTETQ sequence (amino acids 17-23) binds to actin and is found naturally in wound fluid [39]. LKKTETQ promotes cell migration, angiogenesis, hair growth, and dermal repair [7].

Table 2. Key activities of thymosin β4 in dermal repair, especially in epidermolysis bullosa.

Activity	Significance
Migration	Re-epithelialization of the wound surface reduces infection, fluid loss, pain
Anti-inflammation	Reduces swelling, pain, itching, tissue damage
Anti-fibrotic	Prevents scarring, loss of tissue function, tissue damage
Angiogenesis	Helps promotes wound healing, tissue survival
Anti-microbial	Prevents bacterial infection, tissue damage
Anti-apoptosis	Prevents cell death from infections, loss of blood supply, cell detachment
Laminin-332	Mediates keratinocyte migration and adhesion, promotes integrity of the skin
Stem cell	Promotes dermal regeneration, hair growth, stem cell maturation to skin and vessels differentiation

Thymosin β4 preclinical dermal repair studies

Almost 30 years ago, the first animal study showing the effectiveness of topical thymosin β4 on rat dermal wounds

Table 3. Examples of anti-fibrotic activity of thymosin β4 in various models.

Tissue/cell: model	Result	Mechanism	References
Skin: incisional wound	Organized collagen	Decreased myofibroblasts	45
Liver: CCL_4 treated	Decreased fibrosis	Decreased Notch signalling Decreased proliferation	98
Lung: bleomycin treated	Decreased fibrosis	Decreased IL-17 Decreased collagen	51, 99
Heart: infarct model	Decreased scar	Decreased cell death Decreased inflammation Increased angiogenesis	46, 100
Kidney: ureteral damage	Decreased fibrosis	Decreased PDGF receptor Decreased HGF	49, 102

CCL4: carbon tetrachloride; IL-17: interleukin 17; PDGF: platelet-derived growth factor; HGF: hepatocyte-derived growth factor.

Table 4. Preclinical dermal healing studies with thymosin β4.

Wound type	Route	Outcome	References
Rat punch	Topical or IP	Improved healing, angiogenesis	30, 62, 76, 84
Mouse punch, aged, diabetic	Topical	Improved healing	83
Rat skin flaps	IP	Increased flap survival	82
Mouse burn	Intradermal	Increased healing, angiogenesis	81
Rat diabetic hind, limb ischemia	Sponge	Increased healing, angiogenesis	66

IP: intraperitoneal.

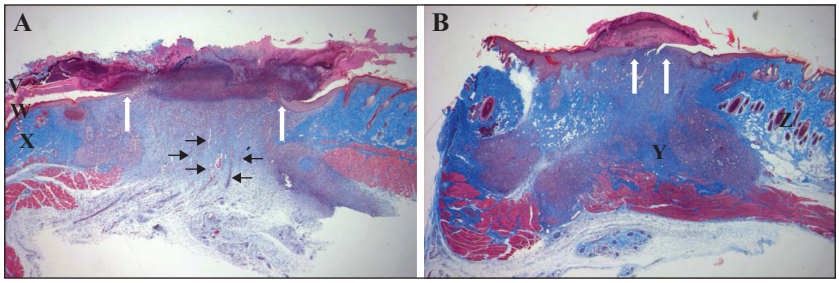

Figure 2. Thymosin β4 increases wound closure and collagen deposition after seven days, in rat skin full-thickness punch wounds: **A**) vehicle; **B**) thymosin beta 4. Sections were stained with Masson's trichrome to visualize collagen I (blue). Animals received six 6-mm full-thickness punch wounds and were treated with either placebo or thymosin β4 on Days 0 and 2. Note in the treated tissue, the increase in granulation tissue (Y) containing collagen I (darker blue staining) in the centre of the wound, the increase in hair follicles (Z) at the wound edge, and increase in keratinocyte migration (white arrows indicate leading edge of the migrating keratinocytes) to cover the wound gap. The figure is taken from [28], representing work done by HKK while a US government employee. V: crust; W: epidermis; X: dermis; black arrows indicate blood vessels.

reported an acceleration of healing (*table 4*). When compared to placebo-treated wounds, the treated rat dermal wounds healed more quickly with increased keratinocyte migration across the wound surface, faster deposition of granulation tissue, increased collagen deposition and maturation, and increased angiogenesis [30] (*figure 2*). Additional studies in mice, aged mice, diabetic mice, mice with dermal burns, and steroid-treated rats confirmed the efficacy of thymosin β4 in accelerating dermal wound closure [7, 9, 45, 58, 66, 76, 80, 81]. It is important to note that thymosin β4 showed good efficacy with both normal and impaired healing models. The survival of skin flaps was also improved by thymosin β4 [82]. Most studies used topical thymosin β4 that was synthetically prepared and commercially available. Recombinant thymosin β4 was equally effective as the synthetic form [83] and a dimeric form was found to be more active [84]. In some studies, thymosin β4 was injected either intraperitoneally or intradermally and showed good efficacy. One unexpected finding was that thymosin β4 accelerated hair growth in several models, including aged mice, diabetic mice, nude mice, rats (*figure 2*), and mice treated with chemotherapeutic agents [7, 85-87]. Furthermore, genetically modified cashmere-producing goats and mice that were both overexpressing thymosin β4 in their skin show increased hair production [88, 89]. For hair growth, thymosin β4 activated the resting follicles by increasing progenitor cell migration from the bulge region to the root and promoted differentiation [90]. In summary, thymosin β4 is active in accelerating dermal repair in various preclinical models of both normal and impaired healing. The healing effects do not appear to depend on the mode of administration.

Thymosin β4 clinical studies

Dermal studies

Thymosin β4 was shown to be safe and well tolerated in all of the clinical trials to date. Phase 1 trials for safety of topically as well as intravenously delivered thymosin β4 have been performed. Its safety was initially confirmed when used in high doses, either topically or systemically, for all of the 15 healthy control patients in the Phase 1 dermal topical safety studies and for all of the 40 patients in the Phase 1 systemic studies [7, 9, 91]. In Phase 2 trials, three doses were tested topically and daily for pressure ulcers (Stages 3 and 4) in 71 patients (NCT00382174) and for venous stasis ulcers in 72 patients (NCT00832091). The studies reported that the mid dose (0.02/0.03%) of thymosin β4 in both trials accelerated healing over that seen with the placebo groups. The median time to complete healing of the pressure wounds that healed in the treated vs placebo pressure ulcer groups was 22 vs 57 days (not significant due to the small sample size of 17-19 patients per arm). The median time to complete healing of the stasis ulcers that healed in the treated vs placebo stasis ulcer groups was 39 vs 71 days (again, not statistically significant due to small sample size of 17-19 patients per arm).

A small study (NCT00311766) was performed with 30 epidermolysis bullosa patients (JEB and DEB) investigating three topical doses (0.01, 0.03, and 0.10 %) of thymosin β4 [7]. At Day 14, ad hoc analysis found that all three doses showed a trend to better efficacy than that in the placebo group (*figure 1*). The primary endpoint of complete healing at 56 days was not met. This is a difficult endpoint for JEB and DEB patients. Recognizing this problem, the FDA has recently revised the guidelines for EB patients and 100% healing is no longer required [92]. The data were analysed based on wound size at baseline at Day 15. All three doses trended toward better efficacy than the placebo for all wound sizes at baseline. The large wounds healed very poorly in the placebo group (not statistically significant due to small sample size). The treated large wounds healed faster than those of the placebo group with similar efficacy to that in all of the wound size groups. These data, in combination with the other dermal trials, suggest the faster healing potential for treating EB patients with thymosin β4. Furthermore, there were no safety or tolerability issues in these fragile patient populations (elderly, diabetic, or JEB/DEB) from which a combined total of 129 patients received thymosin β4.

Rationale for potential of thymosin β4 to treat EB patients

The faster healing observed in the preclinical animal models and in the Phase 2 dermal studies would greatly benefit EB patients [7, 9]. It should be noted that many of the animal models represent impaired healing, such as aged, diabetic, and steroid-treated animals. All three clinical trials with pressure ulcers, stasis ulcers, and EB patients also represent fragile and compromised patient populations. Such studies indicate the safety of thymosin β4 for EB patients. Furthermore, EB patients would likely have faster healing with thymosin β4. The faster healing in the dermal studies further reinforces the efficacy of thymosin β4 and its appropriateness for treating EB patients. Reduction in the wound size by even 50% would greatly benefit the EB patient by reducing the pain, fluid loss, itching, and opportunity for infection.

The mechanisms of action, including anti-inflammation, keratinocyte migration, adherence of the keratinocytes to the wound bed, and upregulation of extracellular matrix proteins (laminin-332), are also important to the EB dermal repair process [7] (*table 2*). The reduction in inflammation would reduce the pain, itching, and scarring. Laminin-332 is upregulated by thymosin β4 and is involved in keratinocyte migration and adhesion and the structural integrity of the epidermal-dermal junction [7, 8, 16]. In JEB, laminin-332 is mutated, indicating the importance of this gene to dermal tissue maintenance.

Summary and future applications

Why do we need a topical agent like thymosin β4 when gene therapy has the potential to cure EB and other severe wounds and is progressing so well toward the clinic? Unfortunately, it is not clear when gene therapy for these indications will be available and if it will be available for all patients and for all forms of the disease given that 20 genes have been described with defects [6, 13]. Patients need immediate effective treatment because the standard of care involving bandaging and symptom relief is not effective in preventing the pain, loss of tissue function, and early death of many EB patients.

The multifunctional regenerative protein, thymosin β4, has shown significant efficacy for healing in different impaired healing animal models with different underlying causes [7, 8]. Thymosin β4 has the potential to be used systemically as well since in preclinical models involving injury to the skin, heart, and brain, it showed good efficacy when given systemically [35, 46, 48, 50, 57, 60, 64, 93]. Furthermore, the efficacy was observed with a single treatment in the skin and heart and with treatments every third day for the brain. In addition, a Phase 1 systemic study showed an excellent safety profile. Safety was further observed with a genetically modified mouse with thymosin β4 overexpression in the skin [89]. This mouse survived and produced offspring with the only observable differences being an increased amount of hair and discoloured teeth. Finally, naturally increased endogenous serum levels have been associated with better survival in scleroderma, septic shock, and liver fibrosis patients [94-96]. Higher serum levels are also associated with corollary capillary development in heart patients [63]. Thus, in the future, systemic thymosin β4 has the potential to be an effective and safe treatment for all EB patients and for other serious dermal injuries.

If systemic therapy is considered, the dosage must be carefully adjusted as thymosin β4 is a biological molecule with at least one known receptor, ATP synthase, that acts by purinergic signalling [97]. Systemic dose response studies in animal models have found that the higher doses may be less effective [50]. A similar 'bell shaped' curve of activity was also seen in the venous stasis and pressure ulcer Phase 2 trials [9].

Finally, other severe dermal injuries might benefit from thymosin β4. Preclinical studies have reported efficacy in healing dermal burns and promoting skin flap attachment/survival, the latter of which may be important for mastectomy patients and for skin grafts [81, 82]. Surgical

wounds and trauma may also benefit from treatment with thymosin β4. Systemic thymosin β4 would be expected to repair and regenerate tissue in patients with internal injuries. For example, elevated systemic thymosin β4 reduces liver fibrosis and bleomycin-induced lung damage [98, 99]. In addition, when implanted in a slow-release complex, it also prevents tissue loss after myocardial infarction [100]. A viral vector overexpressing thymosin β4 has shown efficacy in a pig model of reperfusion injury in the heart [101], and this gene therapy approach may be used in the future for EB patients and for patients with other severe dermal injuries. One advantage is that viral delivery of thymosin β4 would have the potential to benefit all genetic forms of EB. ■

Disclosure. *Financial support: none. Conflicts of interest: WSY is an employee of Lenus Therapeutics which is developing timbetasin for the treatment of epidermolysis bullosa. SK and JS are employees of GTreeBNT which is the parent company of Lenus Therapeutics. HK is a consultant for Lenus Therapeutics.*

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