

METHOD FOR TREATING EPIDERMOLYSIS BULLOSA

BACKGROUND

1. Technical Field

5 The present disclosure relates to treatment of epidermolysis bullosa, particularly to methods for treating epidermolysis bullosa by administering to a subject in need thereof a pharmaceutical composition comprising adenine or a salt thereof. Also related is a pharmaceutical composition for use in treating epidermolysis bullosa in a subject in need thereof.

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2. Description of Related Art

Epidermolysis bullosa (EB) is an uncommon form of genodermatosis that frequently leads to medical complications affecting different areas of the body. These complications manifest as the formation of blisters on the skin and mucous membranes.

15 EB is characterized by an extraordinary fragility of the skin, making it susceptible to tears, sores, and blisters even with minor friction or impact. In more severe instances, blisters can also occur internally within the body, posing a life-threatening risk when present in areas such as the mouth, esophagus, stomach, intestines, upper airway, bladder, and genitals.

20 EB arises from genetic mutations that follow either an autosomal dominant or autosomal recessive inheritance pattern. These mutations affect at least one of the 16 genes responsible for the production of up to 14 proteins known for the cohesion and strength of the skin. EB encompasses a collection of disorders characterized by the presence of painful blisters triggered by mechanical trauma. These blisters emerge due

25 to the vulnerability, disruption, and fragility of the dermoepidermal junctions within the skin and other tissues that line or cover epithelial surfaces.

More than 30 subtypes of EB have been identified by researchers, and these

subtypes are grouped under four major types based on the location of skin changes and the specific gene mutation involved. These major types include: (1) epidermolysis bullosa simplex (EBS), which is a common form of EB that affects the outermost layer of the skin (i.e., epidermis), leading to fragility, blistering, and erosion of the skin; (2) 5 junctional epidermolysis bullosa (JEB), which impacts the proteins within the junctional area of the skin, resulting in blistering and erosions primarily in the upper portion of the basement membrane; (3) dystrophic epidermolysis bullosa (DEB), which is characterized by blister formation within the upper layers of the dermis that is caused by issues in the attachment between the basement membrane and the upper dermis; and 10 (4) Kindler epidermolysis bullosa (also known as Kindler syndrome), which tends to produce blisters across multiple layers of the skin, including the basement membrane.

In terms of symptoms, all types of EB phenotypes exhibit fragile skin that is prone to blisters and chronic inflammation. The severity of EB varies depending on the specific mutation present in the patient and the precise location of the affected protein 15 at the ultrastructural level. Additionally, unlike common wounds, EB exhibits a cyclical pattern, where affected individuals with such condition undergo recurring symptoms such as skin blistering and wounds that may reappear after a certain period, rather than persisting as a continuous event. This recurring nature requires long-term and continuous medical management to address the episodic symptoms. That is to say, even 20 with proactive treatment and care, individuals with EB may still encounter cyclical symptoms. Therefore, EB is a progressive condition that may result in scarring and contractures, leading to decreased mobility. It can cause the fusion of fingers and toes, resulting in mitten-like deformities, as well as microstomia. EB can also cause significant disability and even increases the risk of developing skin cancer.

25 In the clinical context, the usefulness of different wound care products designed for normal-healing skin is highly restricted when it comes to EB. For instance, a Phase 3 trial conducted on individuals with EB demonstrated that the topical cream SD-101,

which contains 6% allantoin, did not exhibit any improvement in wound healing (Paller A.S. et al., Orphanet Journal of Rare Diseases. 2020, 15(1):158). Prior to recent developments, there were no approved therapies specifically designed for EB treatment.

Hence, there remains an existing need for enhanced treatment options in EB that
5 can effectively reduce the risk of complications or mortality, effectively manage symptoms, and enhance the overall quality of life.

SUMMARY

In view of the foregoing, the present disclosure provides a pharmaceutical
10 composition for treating epidermolysis bullosa, comprising adenine or a salt thereof. The pharmaceutical composition of the present disclosure can efficiently reduce the size of wounds associated with epidermolysis bullosa, shorten the time required for closure of the wound, and delay wound recurrence, and thus is useful for relieving pain and pruritus, reducing the risk of complications of EB, as well as improving the quality of
15 life of patients with EB.

In at least one embodiment of the present disclosure, a pharmaceutical composition for treating epidermolysis bullosa is provided. The pharmaceutical composition comprises adenine or a salt thereof and a pharmaceutically acceptable excipient thereof.

In at least one embodiment of the present disclosure, the epidermolysis bullosa
20 may be epidermolysis bullosa simplex, junctional epidermolysis bullosa, dominant dystrophic epidermolysis bullosa, recessive dystrophic epidermolysis bullosa, Kindler syndrome, or epidermolysis bullosa acquisita.

In at least one embodiment of the present disclosure, the adenine or the salt thereof is in an amount of from 0.001% to 80% by weight in the pharmaceutical composition,
25 such as 0.001%, 0.002%, 0.005%, 0.01%, 0.02%, 0.05%, 0.08%, 0.1%, 0.2%, 0.3%, 0.4%, 0.5%, 0.75%, 1%, 2%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 70%, and 80%. In some embodiments, an amount of the adenine or the salt

thereof in the pharmaceutical composition has a lower limit chosen from 0.001%, 0.005%, 0.01%, 0.05%, 0.1%, 0.5%, 1%, 5%, 10%, 15%, and 20% by weight, and an upper limit chosen from 80%, 70%, 60%, 50%, 40%, 30%, and 25% by weight. In some embodiments, the adenine or the salt thereof is in an amount of from 0.01% to 50% by weight in the pharmaceutical composition.

In at least one embodiment of the present disclosure, the adenine or the salt thereof serves as a sole active ingredient for treating epidermolysis bullosa in the pharmaceutical composition. In some embodiments, the adenine or the salt thereof may be in combination with an additional active ingredient, e.g., other than the adenine or the salt derivative thereof, for treating the epidermolysis bullosa.

In at least one embodiment of the present disclosure, the pharmaceutically acceptable excipient may be selected from the group consisting of a filler, a binder, a preservative, a disintegrating agent, a lubricant, a suspending agent, a wetting agent, a solvent, a surfactant, an acid, a flavoring agent, polyethylene glycol (PEG), alkylene glycol, propylene glycol, sebacic acid, dimethyl sulfoxide, an alcohol, stearic acid, calcium stearate, sorbitan stearate, glyceryl monostearate, microcrystalline cellulose, silicon dioxide, gelatin, fat, glycerin, dietary fiber, alginate, pectin, carrageenan, amidated pectin, xanthan, gellan gum, karaya gum, rhamsan, welan, gum ghatti, gum arabic, cera alba, sorbitan palmitate, cetyl palmitate, cetyl 2-ethyl hexanoate, sorbitan oleate, phenoxyethanol, petrolatum, sodium chloride, methylparaben, propylparaben, cetearyl alcohol, and any combination thereof.

In at least one embodiment of the present disclosure, the pharmaceutical composition may be in a form selected from the group consisting of a solution, a liniment, a lotion, a spray, an ointment, a foam or foamable formulation, an emulsion, a salve, a cream, a gel, a paste, a patch, a glue, a film, a powder, a granule, and a wound dressing.

In at least one embodiment of the present disclosure, a method for treating

epidermolysis bullosa in a subject in need thereof is provided. In some embodiments, the method comprises administering an effective amount of the above-mentioned pharmaceutical composition to the subject. In some embodiments, the method comprises administering an effective amount of adenine or a salt thereof to the subject.

5 In at least one embodiment of the present disclosure, the pharmaceutical composition is administered to the subject topically. In some embodiments, the pharmaceutical composition is administered to a wound associated with epidermolysis bullosa in the subject.

In at least one embodiment of the present disclosure, the subject suffers from
10 epidermolysis bullosa simplex, junctional epidermolysis bullosa, dominant dystrophic epidermolysis bullosa, recessive dystrophic epidermolysis bullosa, Kindler syndrome, or epidermolysis bullosa acquisita.

In at least one embodiment of the present disclosure, the administration of the pharmaceutical composition reduces a size of the wound associated with epidermolysis
15 bullosa. In some embodiments, the administration of the pharmaceutical composition shortens the time required for closure of the wound.

In at least one embodiment of the present disclosure, the wound associated with epidermolysis bullosa may comprise at least one of a chronic wound and an acute wound. In some embodiments, the wound associated with epidermolysis bullosa may
20 be selected from skin and mucosa blisters and tearing.

In at least one embodiment of the present disclosure, the pharmaceutical composition may be administered to the wound associated with epidermolysis bullosa in an effective amount of the adenine or the salt thereof between 0.001 mg/cm² and 100 mg/cm². In some embodiments, the effective amount of the adenine or the salt thereof
25 administered to the wound has a lower limit chosen from 0.001 mg/cm², 0.005 mg/cm², 0.01 mg/cm², 0.02 mg/cm², 0.05 mg/cm², 0.1 mg/cm², 0.3 mg/cm², 0.5 mg/cm², 1 mg/cm², 3 mg/cm², 5 mg/cm², 10 mg/cm², 15 mg/cm², 20 mg/cm², 25 mg/cm², and 30

mg/cm², and an upper limit chosen from 100 mg/cm², 90 mg/cm², 80 mg/cm², 70 mg/cm², 60 mg/cm², 50 mg/cm², and 40 mg/cm². In some embodiments, the adenine or the salt is administered to the wound in an effective amount of from 0.01 mg/cm² to 50 mg/cm².

5 In at least one embodiment of the present disclosure, the pharmaceutical composition is administered to the subject for a treatment duration. In some embodiments, the treatment duration is at least one week, at least two weeks, at least three weeks, at least four weeks, at least five weeks, at least six weeks, at least seven weeks, at least eight weeks, at least 3 months, at least 4 months, at least 5 months, at
10 least 6 months, at least 7 months, at least 8 months, at least 9 months, at least 10 months, at least 11 months, or at least 12 months. In some embodiments, the treatment duration is lifelong.

In at least one embodiment of the present disclosure, the administration of the pharmaceutical composition reduces the size of the wound by at least about 50% for
15 the treatment duration, such as within one week. In some embodiments, the administration of the pharmaceutical composition reduces the size of the wound by at least about 60%, at least about 70%, at least about 80%, at least about 90%, or at least about 95% for the treatment duration. In some embodiments, the administration of the pharmaceutical composition reduces the size of the wound by at least about 90% within
20 three weeks.

In at least one embodiment, the method of the present disclosure may further comprise applying a dressing to the skin area affected by the wound associated with epidermolysis bullosa that covers the wound. In some embodiments, the examples of the dressings include, but are not limited to, a bandage, a gauze, a mesh, a tubular
25 bandage, a cohesive bandage, a soft silicone tape, a hydrogel impregnated gauze, a biosynthetic cellulose, and a bordered dressing.

In at least one embodiment of the present disclosure, a pharmaceutical composition

for use in treating epidermolysis bullosa in a subject in need thereof is provided. In some embodiments, the pharmaceutical composition comprises adenine or a salt thereof and at least one of the pharmaceutically acceptable excipients thereof.

In at least one embodiment of the present disclosure, a use of a pharmaceutical composition in the manufacture of a medicament for treating epidermolysis bullosa in a subject in need thereof is provided. In some embodiments, the pharmaceutical composition comprises adenine or a salt thereof and at least one of the pharmaceutically acceptable excipients thereof. In some embodiments, a use of adenine or a salt thereof in the manufacture of a medicament for treating epidermolysis bullosa is also provided.

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BRIEF DESCRIPTION OF THE DRAWINGS

The present disclosure can be more fully understood by reading the following detailed descriptions of the embodiments, with reference made to the accompanying drawings.

FIGs. 1A to 1E are a series of photographic images showing the effect of adenine on wound closure as compared to vehicle-treated wounds in five subjects with EB within the treatment duration.

FIG. 2 shows the effect of adenine on reducing the wound body surface area (BSA) in subjects with EB within the treatment duration.

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DETAILED DESCRIPTION OF THE EMBODIMENTS

The description discloses some embodiments in such a detail that a person skilled in the art is able to utilize the embodiments based on the disclosure. Not all steps or features of the embodiments are discussed in detail, as many of the steps or features will be obvious to a person skilled in the art based on this disclosure.

It is further noted that, as used in this disclosure, the singular forms “a,” “an,” and “the” include plural referents unless expressly and unequivocally limited to one referent.

The term “or” is used interchangeably with the term “and/or” unless the context clearly indicates otherwise.

As used herein, the term “about” generally means within 10%, 5%, 1%, or 0.5% of a given value or range. Alternatively, the term “about” means within an acceptable
5 standard error of the mean when considered by one of ordinary skill in the art. Unless otherwise expressly specified, all of the numerical ranges, amounts, values, and percentages, such as those for quantities of materials, durations of time periods, temperatures, operating conditions, ratios of amounts, and the likes disclosed herein, should be understood as modified in all instances by the term “about.”

10 The present disclosure provides a method of treating epidermolysis bullosa in a subject in need thereof. In some embodiments of the present disclosure, the method comprises administering an effective amount of a pharmaceutical composition to the subject, wherein the pharmaceutical composition comprises adenine or a salt thereof and a pharmaceutically acceptable excipient thereof.

15 As used herein, the term “comprising” or “comprises” is used in reference to compositions, methods, and respective component(s) thereof, which are included in the present disclosure, yet open to the inclusion of unspecified elements.

As used herein, the term “treat,” “treating,” or “treatment” encompasses partially or completely preventing, ameliorating, mitigating, and/or managing a symptom, a
20 disorder, or a condition associated with a disease (e.g., epidermolysis bullosa). The term “treat,” “treating,” or “treatment” as used herein refers to application or administration of one or more therapeutic agent or surgery to a subject, who has a symptom, a disorder, or a condition associated with a disease, with the purpose to partially or completely alleviate, ameliorate, relieve, delay onset of, inhibit progression of, reduce severity of,
25 and/or reduce incidence of one or more symptoms, disorders, or conditions associated with the disease. Treatment may be administered to a subject who exhibits only an early sign of such symptoms, disorders, and/or conditions for the purpose of decreasing the

risk of developing the symptoms, disorders, and/or conditions associated with a disease.

As used herein, the terms “patient,” “individual,” and “subject” are used interchangeably. The term “subject” means a human or an animal. Examples of the subject include, but are not limited to, a rodent, a murine, a monkey, a guinea pig, a dog, a cat, a cow, a sheep, a pig, a horse, a rabbit, and a human. In some embodiments of the present disclosure, the subject is a mammal, e.g., a primate such as a human.

As used herein, the phrase “an effective amount” refers to the amount of an active ingredient (e.g., adenine) that is required to confer a desired effect (e.g., reducing the size of wounds) on the treated subject. Effective doses will vary, as recognized by one of ordinary skill in the art, depending on routes of administration, excipient usage, the possibility of co-usage with other treatment, and the condition to be treated.

In at least one embodiment of the present disclosure, the pharmaceutical composition may be topically administered to a wound in the subject. In some embodiments, the pharmaceutical composition is topically administered in an effective amount of the adenine or the salt thereof between about 0.001 mg/cm² and about 100 mg/cm² of the wound.

In at least one embodiment of the present disclosure, the pharmaceutical composition is topically administered in an effective amount of about 0.001 mg/kg to about 100 mg/kg of the adenine or the salt thereof per subject body weight (mg/kg). In some instances, the effective amount of the adenine or the salt thereof to be administered may be about 0.001 mg/kg to about 80 mg/kg, about 0.002 mg/kg to about 70 mg/kg, about 0.005 mg/kg to about 60 mg/kg, about 0.01 mg/kg to about 60 mg/kg, about 0.05 mg/kg to about 80 mg/kg, about 0.05 mg/kg to about 50 mg/kg, about 0.1 mg/kg to about 70 mg/kg, about 0.1 mg/kg to about 30 mg/kg, about 0.5 mg/kg to about 60 mg/kg, about 0.5 mg/kg to about 20 mg/kg, about 1 mg/kg to about 10 mg/kg, or about 1 mg/kg to about 5 mg/kg.

As used herein, the term “administering” or “administration” refers to the

placement of an active ingredient (e.g., adenine) into a subject by a method or route which results in at least partial localization of the active ingredient at a desired site to produce a desired effect.

In at least one embodiment of the present disclosure, the pharmaceutical composition may be administered to the subject 1 to 4 times per day or 1 to 14 times per week for the treatment duration. For example, the pharmaceutical composition may be administered to the subject with a frequency of once a day, twice a day, 3 times per day, 4 times per day, once every two days, 2 times every two days, once every three days, 2 times every three days, 3 times every three days, once per week, 2 times per week, 3 times per week, 4 times per week, 5 times per week, 6 times per week, 7 times per week, 8 times per week, 9 times per week, 10 times per week, 11 times per week, 12 times per week, 13 times per week, or 14 times per week.

The pharmaceutical composition of the present disclosure may comprise the adenine or the salt thereof as a sole active ingredient for treating epidermolysis bullosa. In other words, the adenine or the salt thereof serves as the only active ingredient for the treatment of epidermolysis bullosa in the pharmaceutical composition. In this embodiment, the present disclosure provides a safe and effective therapy for the treatment of epidermolysis bullosa by administering the adenine or the salt thereof alone as the active ingredient.

As used herein, the term “pharmaceutically acceptable excipient” refers to a pharmaceutically acceptable material, composition, or vehicle, such as a disintegrating agent, a binder, a lubricant, and a surfactant, which does not abrogate the biological activity or properties of the active ingredient, and is relatively non-toxic; that is, the material may be administered to an individual without causing an undesirable biological effect or interacting in a deleterious manner with any of the components of the composition in which it is contained.

Many examples have been used to illustrate the present disclosure. The examples

below should not be taken as a limit to the scope of the disclosure.

EXAMPLES

Example 1: Effect of adenine in EB patients

5 In this example, a clinical study, in which 5 subjects with DEB were enrolled, was conducted in Taiwan. Subjects experienced wound sizes ranging from 2 cm² to 20 cm² which were treated daily with the adenine cream or vehicle once a day for 1 month in a single-blind fashion. The adenine cream used herein contained 0.02% adenine and the excipients including cera alba, glyceryl monostearate, sorbitan palmitate, sorbitan
10 stearate, cetyl 2-ethyl hexanoate, stearic acid, sorbitan oleate, phenoxyethanol, propylene glycol, petrolatum, sodium chloride, methylparaben, propylparaben, cetearyl alcohol, and cetyl palmitate. The primary endpoint was the percentage of unclosed wound area on Day 14 (compared to Day 1), and the secondary outcome measures included:

- 15 (1) Time to first achieving 100% closure of the EB target wound;
 (2) Time to first achieving 75% closure of the EB target wound; and
 (3) The proportion of the EB target wounds with first complete closure in 3 weeks of treatment.

 In this preclinical trial, a total of 23 wounds (14 wounds in the adenine treatment
20 group and 9 wounds in the vehicle treatment group) were assessed once per week across 5 individuals.

 Additionally, after the initial study, 3 of the 5 subjects (i.e., P1, P2, and P3) continually received the adenine cream following the same protocol, except that a total of 22 wounds (13 wounds in the adenine treatment group and 9 wounds in the
25 vehicle treatment group) were assessed daily for 1 month. The benefit of recording wounds daily allowed for a clear demonstration of the efficacy of adenine in

accelerating wound healing in EB patients. Data from these 3 subjects, following daily assessments, were presented in Table 1 and Table 2.

Table 1. Effect of adenine on unclosed wound area

Arm		Adenine Cream										P value	Vehicle				
Wound size	(cm ²)	12.31	13.19	10.98	4.66	2.08	11.07	8.64	2.63	3.06	7	0.547	10.37	11.64	13.99	4.22	4.94
The percentage (%) of unclosed wound area	1	100	100	100	100	100	100	100	100	100	100	-	100	100	100	100	100
	2	117	90	136	97	77	74	88	137	139	100	0.329	96	100	97	82	108
	3	62	66	79	61	36	69	67	106	143	97	0.175	80	92.4	95.7	86	148.5
	4	52	86	79	41	24	59	61	104	189	97	0.344	75.2	94.1	66.4	116.7	148.2
	5	56	70	59	42	7	57	35	60	187	101	0.239	78.2	53.8	74.1	143.4	142.5
	6	42	79	43	34	0	48	28	65	163	92	0.172	68.9	60	67.5	125.2	164.1
	7	32	50	48	29	0	45	32	27	150	64	0.095	64	67	50	108	123
	8	32	57	32	14	0	27	24	36	134	61	0.132	62	40	47	141	157
	9	22	49	26	17	0	19	11	15	94	47	0.049	62	45	51	126	141
	10	14	55	5	10	0	17	7	3	64	44	0.028	62	50	41	130	114
	11	7	42	7	3	0	8	6	0	64	33	0.061	51	36	31	147	104
	12	6	51	5	1	0	8	3	0	35	31	0.025	43	39	35	107	68
	13	3	40	3	0	0	3	2	0	24	22	0.056	39	24	18	92	48
	14	1	30	0	0	0	2	2	0	0	15	0.011	37	22	16	51	54
	15	1	29	0	0	0	1	0	0	0	9	0.016	17	20	14	40	46
	16	1	29	0	0	0	0	0	0	0	6	0.022	12	17	12	32	41
	17	2	26	0	0	0	0	0	0	0	5	0.019	10	13	8	22	28
	18	2	18	0	0	0	0	0	0	0	3	0.011	12	15	5	24	18
	19	0	16	0	0	0	0	0	0	0	1	0.004	11	12	4	15	12
	20	0	8	0	0	0	0	0	0	0	0	0.016	11	6	2	12	7

	21	0	8	0	0	0	0	0	0	0	0	0.011	12	7	2	6	6
	22	0	15	0	0	0	0	0	0	0	0	0.41	13	4	0	2	0
	23	0	8	0	0	0	0	0	0	0	0	0.269	10	4	0	2	0
	24	0	9	0	0	0	0	0	0	0	0	0.549	8	2	0	0	0

As shown in Table 1, the percentage of unclosed wound area was zero (complete closure) in 5/10 wounds on Day 14 for the adenine-treated wounds. For the remaining 5 wounds, the percentages of unclosed wound areas were 1%, 2%, 2%, 15%, and 30%, respectively. In contrast, for the 5 vehicle-treated wounds, the percentages of unclosed wound areas on Day 14 were 16%, 22%, 37%, 51%, and 54%, respectively.

With regard to the secondary endpoints, several wounds were selected for treatment on each patient, and an additional patient was included in the analyses. The time to achieve 100% and 75% closure of the EB target wound was summarized in Table 2 below. 100% closure was achieved in 16.7 ± 6.18 days for the adenine-treated wounds as compared to 24.0 ± 3.32 days for the vehicle-treated wounds ($P = 0.0037$). Similarly, the number of days for 75% closure averaged 10.4 ± 3.50 days for the adenine-treated wounds as compared to 15.6 ± 3.74 days for the vehicle-treated wounds ($P = 0.0117$).

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Table 2. Time to achieve 100% and 75% closure of the EB target wound

		Time to achieve (day)	Time to achieve (day)	Time to achieve (day)
	Wound area (cm ²)	>50% wound closure	>75% wound closure	100% complete wound closure
Adenine Cream	12.31	6	9	19
	13.19	9	18	30
	10.98	6	10	14
	4.66	4	8	13
	2.08	3	4	6
	11.07	6	9	17

	8.64	5	8	16
	2.63	7	9	11
	3.06	12	13	15
	7.00	9	13	20
	2.16	10	12	15
	13.3	6	12	24
AVG ± Std	7.6±4.52	6.9±2.61	10.4±3.50	16.7±6.18
Vehicle	Wound area (cm ²)	>50% wound closure	>75% wound closure	100% complete wound closure
	10.37	12	15	26
	11.64	8	13	25
	13.99	8	13	22
	4.22	15	17	23
	4.94	13	18	22
	5.64	10	11	20
	3.70	20	22	30
AVG ± Std	7.8±4.13	12.3±4.27	15.6±3.74	24.0±3.32
P value	0.9248	0.0152	0.0117	0.0037

These results showed that as compared with vehicle, the treatment with adenine resulted in a numerically higher rate of complete 100% and partial > 75% wound closure. It thus can be seen that wounds treated with adenine can reach complete healing earlier than the vehicle-treated wounds. Accordingly, as in the absence of any effective treatment till now, the faster wound healing of adenine has the potential to reduce pain and improve the quality of life for EB patients.

In addition, based on the provided patient feedback and the results of questionnaire surveys, the wounds were less likely to recur after the adenine treatment, implying that adenine may also have the effect of delaying wound recurrence.

Additionally, the results on the further secondary endpoints, i.e., the proportion of the EB target wounds with the first complete closure in 3 weeks of treatment, were presented in Table 3. Complete wound closure was observed in 22/27 wounds (81%) for the adenine-treated wounds as compared to 5/18 wounds (28%) for the vehicle-

treated wounds ($P = 0.0003$). These results demonstrated that the proportion of the EB wounds with the first complete closure in adenine-treated groups was significantly higher than that in the vehicle-treated groups, suggesting the potential for the superior efficacy of adenine in promoting EB wound healing.

Table 3. Proportion of the EB target wounds with first complete closure in 3 weeks
treatment

Arm	Patient code	Wound number	Wound area (cm ²)	Position	Week 1 Closure rate (%)	Week 2 Closure rate (%)	Week 3 Closure rate (%)
Adenine Cream	P1	1	12.31	Back central	67.3	98.2	100.00
	P1	2	13.19	R upper arm	49.6	69.1	91.4
	P1	3	10.98	R waist	51.8	100.00	-
	P2	4	4.66	R upper arm	70.4	100.00	-
	P2	5	2.08	R lateral back upper	100.00	-	-
	P2	6	11.07	R back medial	54.8	97.6	100.00
	P2	7	8.64	R shoulder	67.7	97.5	100.00
	P2	8	2.63	R back medial	72.7	100.00	-
	P2	9	3.06	R shoulder	-50.3	99.2	100.00
	P2	10	1.72	R shoulder	-43.0	95.5	100.00
	P2	11	7.00	R back medial	36.0	84.3	100.00
	P3	12	2.16	R upper arm	43.2	100.00	-
	P3	13	13.30	R waist	61.6	81.8	93.3
	P1	14	37.89	L back medial	73.87	94.49	100.00
	P2	15	33.46	L lateral back upper	60.73	90.81	99.35
	P3	16	25.06	L posterior waist	37.95	80.58	87.55
	P3	17	17.65	L posterior waist	86.54	92.06	100.00
	P1	18	11.62	L shoulder	31.90	56.10	86.42
	P5	19	11.19	R back	84.25	100.00	-
	P2	20	8.11	L lateral back upper	71.63	100.00	-
	P2	21	6.89	L lateral back upper	67.45	95.42	100.00
	P1	22	6.09	R hand	55.65	93.08	100.00
	P4	23	5.45	L back	85.52	100.00	-
	P1	24	5.23	Posterior neck	87.52	100.00	-
	P4	25	4.29	L posterior neck	57.56	100.00	-
	P4	26	3.96	L back	68.87	100.00	-
	P1	27	3.65	R waist	37.09	75.76	100.00
Vehicle	P1	1	39.42	Back medial	20.63	52.60	71.30
	P1	2	10.37	Chest	35.17	62.40	87.80
	P1	3	11.64	L upper arm	32.97	77.70	93.00
	P2	4	13.99	L back medial	49.84	83.40	97.30
	P2	5	4.22	L shoulder	-8.66	48.28	93.25
	P2	6	1.61	L back medial	-345.86	-122.85	9.51
	P2	7	4.94	L back medial	-23.89	45.59	93.83
	P3	8	5.64	L elbow	43.60	92.80	100.00
	P3	9	3.70	Back lower	12.40	47.90	64.60
	P5	10	31.80	L shoulder	52.95	83.56	90.33
	P3	11	24.33	R waist	63.73	82.76	92.00
	P2	12	20.38	R lateral waist	64.56	100.00	-
	P4	13	14.05	R waist	46.32	63.33	77.49
	P2	14	9.46	R lateral waist	88.82	100.00	-
	P1	15	4.97	R lateral neck	0.95	66.29	83.00
	P2	16	4.88	R back	76.80	100.00	-
	P1	17	4.26	L shoulder	82.79	95.01	100.00
	P1	18	1.60	L hand	70.03	81.27	87.13
P Value	-	-	0.6052	-	0.1563	0.0328	0.0265

R: right; L: left

5 In addition to the above, photographic images with quantitative data of the effect of adenine on wound closure, as compared to vehicle-treated wounds, were shown in a series of images in FIGs. 1A to 1E of subjects suffering from EB. Furthermore, the

average percentage of the wound body surface area (BSA) in the 3 subjects (i.e., P1, P2, and P3) was calculated and shown in FIG. 2, in which a total of 22 wounds (13 wounds in the adenine treatment group and 9 wounds in the vehicle treatment group) were assessed.

5 Taken collectively, both the *in vitro* data and the effects of adenine cream on wound closure in subjects with EB provided evidence of the potential effectiveness of adenine for the treatment of EB.

Example 2: Comparison of EB treatment by adenine, Filsuvez, LEAES and B-VEC

10 In June 2022, the European Commission granted approval to the topical gel Filsuvez (Amryt Pharma) for the management of partial thickness wounds linked to dystrophic and junctional EB in patients aged 6 months and above.

 Further, as the first gene therapy trial for RDEB, autologous gene-corrected keratinocytes expressing full-length type VII collagen (C7) using a retroviral vector
15 were developed to treat chronic open wounds in patients with severe RDEB, in which a skin graft, LZRSE-COL7A1 engineered autologous epidermal sheets (LEAES), was derived from the patient's own skin cells that had been genetically engineered to express the missing protein (i.e., C7), and the corrected cells were transplanted back to the patient.

20 In addition, beremagene geperpavec (B-VEC) is a topical investigational herpes simplex virus type 1 (HSV-1)-based gene therapy designed to restore C7 protein by delivering COL7A1 and has been approved by the United States Food and Drug Administration (FDA) for the treatment of DEB.

 In this example, the clinical efficacy of the adenine cream of the present disclosure
25 was compared with the conventional therapies, i.e., Filsuvez, LEAES, and B-VEC, and the results were illustrated in Table 4. Furthermore, the trial design comparison, the closure proportion, and the time to first complete closure of Filsuvez and the adenine

cream of the present disclosure were shown in Table 5 to Table 7, respectively.

Table 4. Comparison of conventional therapies and the method of the present disclosure in terms of EB treatment

	Filsuvez	LEAES	B-VEC	Adenine Cream
Description	Birch bark extract (triterpene extract)	Gene-corrected cell therapy that restores functional collagen VII, <i>ex vivo</i> , through AAV vector and skin re-graft	Topically applied, <i>in vivo</i> , modified HSV-1 vector containing two functional COL7A1 genes	Small molecule (adenine)
Mechanism of action (MOA)	1. Transient up-regulation of inflammatory mediators; 2. Promotion of keratinocyte activities; 3. Enhancement of keratinocyte differentiation	To transfer in functional COL7 gene, expressing collagen VII and anchoring fibril (AF)	To transfer in functional COL7 gene, expressing collagen VII and anchoring fibril (AF)	To increase intracellular ATP, mobilizing cell for migration and closing wound (Hypothesis to be confirmed)
Route of administration	Topical cream	Skin graft	Topical gel	Topical cream
Subject No.	223 (Phase III)	7 (Phase I/IIa)	12 (2 in Phase I; 10 in Phase II)	5 (Preclinical)
Wound No.	NA	42 (Phase I/IIa)	33 (Phase I + Phase II)	45
Clinical Outcome-1	Phase III primary endpoint met: Proportion of patients with first complete closure within 45 days	At 3 and 6 months, 95% of treated sites had $\geq$ 50% healing; 12 months, 68%; 24 months, 71%	9 out of 10 wounds 100% closure following initial administrations	In 14 days among 15 wounds, 8 out of 10 treated wounds reached $\geq$ 98% closure ($p = 0.011$)
Clinical Outcome-2	Phase III secondary endpoint missed: Time to first complete closure (until Day 90)	The continued expression of the NC2 domain of C7 was detected as late as 2 years after treatment in participants Nos. 4 and 6	The average time to 100% closure was 17.4 days (median 14 days)	From a group of 12 treated wounds, 100% closure took average 16.7 ± 6.18 days ($p = 0.0037$); in 3 weeks among total 45 wounds, 81% of the 27 treated wounds reached 100% closure (<i>vs.</i> 28% of placebo)
Summary	1. Only chronic wounds were included ($\geq$ 21 days) 2. The treatment of 45 days was longer than the present disclosure	1. The expression of NC2 or AF was not sustained 2. No data of 100% closure was shown	Safety issues (viral shedding, α -HSV, and α -C7 antibody) should be addressed and kept monitoring	The efficacy was comparable to the gene therapy

NA: not applicable

Table 5. The trial design comparison Filsuvez and adenine cream

	Filsuvez, Phase III	Filsuvez, Phase II	Adenine cream, pilot study
Patient type	JEB, DEB, and Kindler syndrome	JEB, DEB, and Kindler syndrome	DEB
Wound size	10 to 50 cm ² , aged ≥ 21 days and < 9 months	5 to 200 cm ²	2 to 20 cm ²
Treatment duration	90 days	28 days	30 days
Arms	Two arms (Filsuvez and vehicle)	One arm (Filsuvez and non-adhesive wound dressing)	Two arms (adenine and vehicle)
Primary endpoint	Proportion of patients with first complete closure of the EB target wound within 45 days of treatment [Time Frame: 45 ± 7 days]	Intra-individual difference in re-epithelialization of wound (halves) at Day 14 in Recent Wounds or Day 28 in Chronic Wounds [Time Frame: 14 days for recent wounds, 28 days for chronic wounds]	The percentage of wound area closure of each targeted wound at Day 14 compared with Day 1
Secondary endpoint	1. Time to first complete closure of the EB target wound [Time Frame: 90 ± 7 days] 2. Proportion of patients with first complete closure of the EB target wound at [Time Frame: 90 ± 7 days] 3. The incidence of wound infection between baseline and D90 ± 7 as evidenced by adverse events and/or use of topical and/or systemic antibiotics 4. The maximum severity of wound infection between baseline and D90 ± 7 as evidenced by adverse events and/or use of topical and/or systemic antibiotics 5. Change from baseline in total body wound burden as evidenced by clinical assessment using Section I of the EB disease activity and scarring index (EBDASI) at D90 ± 7 6. Change from baseline in itching using the Itch Man Scale in patients ≥ 4 years and up to 13 years of age, and the Leuven Itch Scale in patients ≥ 14 years of age before wound dressing changes at D90 ± 7	1. Percentage of wound epithelialization at Day 7 ± 1 [Time Frame: Day 7 ± 1] 2. Percentage of wound epithelialization at Day 14 ± 1 [Time Frame: Day 14 ± 1]	1. Time to first achieving 75% closure of the EB target wound 2. Time to first achieving 100% closure of the EB target wound 3. Proportion of the EB target wounds with first complete closure in 3 weeks (21 days) treatment

5 Table 6. Comparison of adenine cream with Filsuvez in terms of proportion of

patients with first complete closure

Filsuvez, Phase III		
	Filsuvez Group (N = 104)	Control Group (N = 114)
Primary Endpoint	41.3%	28.9%
Relative risk (95% CI)	1.44 (1.01, 2.05)	
P-value *	0.013	
Filsuvez, Phase II		
	Faster healing of wound treated with Filsuvez (N = 8)	Faster healing of wound treated with dressing (N = 8)
Secondary Endpoint	100%	0%
P-value	0.008	
Adenine cream, pilot study		
	Adenine Group (N = 27)	Control Group (N = 18)
Secondary Endpoint	81%	28%
P-value	0.0003	

*Pre-specified adjustment to account for independent data monitoring committee (IDMC) interim sample size re-estimation. CI: confidence interval.

5 Table 7. Comparison of adenine cream with Filsuvez in terms of time to first complete closure

	Filsuvez, Phase III		Adenine cream, pilot study	
Secondary Endpoint	Time to first complete closure of the EB target wound [Time Frame: 90 ± 7 days]		Time to first achieving 100% closure of the EB target wound [Time Frame: 30 days]	
	Filsuvez Group (N = 104)	Control Group (N = 114)	Adenine Group (N = 12)	Control Group (N = 7)
	37.7 days	44.5 days	16.7 days	24 days
P-value	0.302		0.0037	
	Filsuvez, Phase II		Adenine cream, pilot study	
Secondary Endpoint	Percentage of wound Epithelialization at Day 7 ± 1		Percentage of the target wound healing on Day 7	
	Filsuvez Group (N = 12)	Control Group (N = 12)	Adenine Group (N = 10)	Control Group (N = 5)
	69.7%	57.4%	52%	20.2%
P-value	0.21		0.09	
Secondary Endpoint	Percentage of wound Epithelialization at Day 14 ± 1		Percentage of the target wound healing on Day 14	

	Filsuvez Group (N = 12)	Control Group (N = 12)	Adenine Group (N = 10)	Control Group (N = 5)
	87.7%	79.2%	94%	64.4%
P-value	0.33		0.01	

These results showed that after 12 months from the treatment of LEAES, more than 50% of the subjects lost molecular correction, and more than 30% of the subjects got healing less than 50%, implying that the skin graft was just not efficacious sufficiently. The efficacy of the adenine cream of the present disclosure was comparable to the gene therapy of B-VEC; however, the safety concerns of gene therapy remained to be seen.

Additionally, in comparison with Filsuvez, the adenine cream of the present disclosure kicked in earlier than 45 days, indicating that the adenine-treated wounds closed even faster. Also, both chronic and acute wounds could be effectively by the adenine cream, while Filsuvez targeted wounds only aged ≥ 21 days and < 9 months.

Moreover, the difference in the time to wound healing between Filsuvez group and control group over the 90 days was not statistically significantly different (P-value = 0.302), while by the treatment of adenine, the time to first achieving complete closure was only 16.7 days compared to 24 days in the control group with a significant difference (P-value = 0.0037). Similarly, the intra-individual difference in median percentage of wound epithelialization between the Filsuvez group and the control group was not statistically significantly different (P-value = 0.21 and 0.33, respectively). For the treatment of adenine, the percentage of the target wound healing could achieve to 94.0% on Day 14, as compared to 64.4% in the control group with a significant difference (P-value = 0.01).

From the above, it can be clearly seen that the pharmaceutical composition comprising adenine or a salt thereof provided in the present disclosure can effectively

relieve symptoms of EB, including shortening wound healing time and delaying wound recurrence. Also, the pharmaceutical composition of the present disclosure exhibits superior therapeutic effects compared to the conventional therapies. Therefore, the method of the present disclosure is useful for improving the treatment of EB, which has
5 safety and good efficacy in reducing the risk of complications, such as infections and squamous cell carcinoma, and improving the quality of life of patients with EB.

It is obvious to a person skilled in the art that with the advancement of technology, the basic idea may be implemented in various ways. The embodiments are thus not limited to the examples described above; instead, they may vary within the scope of the
10 claims.

The embodiments described hereinbefore may be used in any combination with each other. Several of the embodiments may be combined together to form a further embodiment. A compound, a composition, or a method disclosed herein may comprise at least one of the embodiments described hereinbefore. It will be understood that the
15 benefits and advantages described above may relate to one embodiment or may relate to several embodiments. The embodiments are not limited to those that solve any or all of the stated problems or those that have any or all of the stated benefits and advantages.

CLAIMS

What is claimed is:

1. A use of a pharmaceutical composition for manufacture of a medicament for treating epidermolysis bullosa in a subject in need thereof, wherein the pharmaceutical
5 composition comprises adenine or a salt thereof and a pharmaceutically acceptable excipient thereof for reducing a size of a wound associated with epidermolysis bullosa in the subject.
2. The use according to claim 1, wherein the epidermolysis bullosa is epidermolysis
10 bullosa simplex, junctional epidermolysis bullosa, dominant dystrophic epidermolysis bullosa, recessive dystrophic epidermolysis bullosa, Kindler syndrome, or epidermolysis bullosa acquisita.
3. The use according to claim 1, wherein the wound associated with epidermolysis
15 bullosa comprises at least one of a chronic wound and an acute wound.
4. The use according to claim 1, wherein the wound associated with epidermolysis bullosa is selected from skin and mucosa blisters and tearing.
- 20 5. The use according to claim 1, wherein the pharmaceutical composition is for topically administering to the subject.
6. The use according to claim 5, wherein the pharmaceutical composition is for administering to the wound associated with epidermolysis bullosa in an effective
25 amount of the adenine or the salt thereof between 0.001 mg/cm² and 100 mg/cm².
7. The use according to claim 6, wherein the pharmaceutical composition is for

administering to the wound associated with epidermolysis bullosa in an effective amount of the adenine or the salt thereof between 0.01 mg/cm² and 50 mg/cm².

8. The use according to claim 1, wherein the adenine or the salt thereof is in an amount
5 of from about 0.001% to about 80% by weight in the pharmaceutical composition.

9. The use according to claim 8, wherein the adenine or the salt thereof is in an amount of from about 0.01% to about 50% by weight in the pharmaceutical composition.

10 10. The use according to claim 1, wherein the adenine or the salt thereof serves as a sole active ingredient for treating epidermolysis bullosa in the pharmaceutical composition.

11. The use according to claim 1, wherein the pharmaceutical composition is for administering to the subject for a treatment duration of at least one week.

15

12. The use according to claim 11, wherein the pharmaceutical composition reduces the size of the wound by at least about 50% for the treatment duration.

13. The use according to claim 11, wherein the treatment duration is at least three weeks.

20

14. The use according to claim 13, wherein the pharmaceutical composition reduces the size of the wound by at least about 90% for the treatment duration.

15. The use according to claim 11, wherein the pharmaceutical composition is for
25 administering to the subject 1 to 4 times per day or 1 to 14 times per week for the treatment duration.

16. The use according to claim 1, wherein the pharmaceutical composition further reduces the time required for closure of the wound.

17. The use according to claim 1, wherein the pharmaceutical composition is in a form
5 selected from the group consisting of a solution, a liniment, a lotion, a spray, an ointment, a foam or foamable formulation, an emulsion, a salve, a cream, a gel, a paste, a patch, a glue, a film, a powder, a granule, and a wound dressing.

18. The use according to claim 1, wherein the pharmaceutically acceptable excipient is
10 selected from the group consisting of a filler, a binder, a preservative, a disintegrating agent, a lubricant, a suspending agent, a wetting agent, a solvent, a surfactant, an acid, a flavoring agent, polyethylene glycol (PEG), alkylene glycol, sebacic acid, dimethyl sulfoxide, an alcohol, calcium stearate, microcrystalline cellulose, silicon dioxide, gelatin, fat, glycerin, dietary fiber, alginate, pectin, carrageenan, amidated pectin,
15 xanthan, gellan gum, karaya gum, rhamosan, welan, gum ghatti, gum arabic, and any combination thereof.