

Clinical Pharmacology BLA Review

Division of Clinical Evaluation General Medicine (DCEGM)

Office of Clinical Evaluation (OCE), Office of Therapeutic Products (OTP)

Submission Number: BLA 125774/0

Product Name: Beremagene geperpavec (Vyjuvek)

Proposed Indication: Treatment of wounds in patients 6 months of age and older with dystrophic epidermolysis bullosa (DEB)

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Table of Contents

1. Executive Summary	3
2. Recommendations	4
3. Background	5
4. Summary of Clinical Pharmacology Findings	6
5. Clinical Pharmacology Labeling Comments	9
6. Comprehensive Clinical Pharmacology Review	12
6.1. General Pharmacology and Pharmacokinetics	12
6.2. Pharmacodynamic Assessments	14
6.3. Immunogenicity Assessments	16
7. Appendix	19
7.1. Study#1- A Phase 1/2 Study of B-VEC, a Non-Integrating, Replication-Incompetent HSV Vector Expressing the Human Collagen VII Protein, for the Treatment of Dystrophic Epidermolysis Bullosa (DEB) (KB103-001)	19
7.2. Study#2- A Phase 3 Efficacy and Safety Study of Beremagene Geperpavec (B-VEC, Previously KB103) for the Treatment of Dystrophic Epidermolysis Bullosa (DEB) (B-VEC-03)..	

1. Executive Summary

Vyjuvek (Beremagene geperpavec) is an engineered replication-incompetent herpes simplex virus type 1 (HSV-1)-based vector coding human collagen type VII alpha 1 chain (COL7A1).

In this BLA submission, the applicant proposes Vyjuvek for treatment of wounds in patients 6 months of age and older with dystrophic epidermolysis bullosa (DEB). The clinical pharmacology assessment of this BLA is based on two clinical studies (Phase 1/2 and Phase 3) that characterized the pharmacokinetics, pharmacodynamics, and immunogenicity of Vyjuvek.

Pharmacodynamic activity (expression, secretion, and localization of COL7 transgene) was demonstrated in skin biopsies (n=6 subjects) in the Phase 1/2 study. Also, the pharmacodynamic activity is demonstrated in limited number of subjects with baseline anti-HSV-1 and anti-COL7 antibodies (anti-drug antibodies, ADA) suggesting that serum antibodies against HSV-1 and COL7 did not affect local transduction and transgene generation following multiple topical administration of Vyjuvek. In the Phase 3 study, anti-COL7 antibody at baseline was detected in 4.5 % subjects (1 out of 22), and 72% of subjects (13 out of 18) who were seronegative at baseline developed anti-COL7 antibody at Week 26 following treatment with Vyjuvek. Based on the observed clinical benefit following multiple dosing it appears that there is no significant impact of ADA on efficacy; however, data are limited to fully evaluate the impact of ADA on clinical outcome and pharmacodynamic activity.

Pharmacokinetic assessments showed that skin swabs from most subjects (61-100%) were positive for viral vector with limited systemic exposure following topical administration of Vyjuvek. Negative shedding of skin swabs (i.e., confirmed with three measurements below limit of detection) were achieved in 6 out of 9 subjects (67%; Phase 1/2 study) and 16 out of 19 subjects (84.2%; Phase 3 study) within 6 to 8 weeks following

treatment with Vyjuvek. Overall, the clinical pharmacology data support the proposed topical multiple dosing regimen of B-VEC for treatment of wounds in patients with DEB.

2. Recommendations

The clinical pharmacology information in this BLA is acceptable to support approval from a Clinical Pharmacology perspective. Labeling recommendations are provided in section 5.

3. Background

The major collagens in human skin are types I and III which account for approximately 80% and 10% of the total bulk of collagen, respectively. In addition to these major collagens, human skin contains several minor collagens that play a crucial role in providing integral stability to the skin. One of them is type VII collagen, which is the major component of anchoring fibrils (AF). In human skin, anchoring fibrils extend from the lower portion of the dermal-epidermal basement membrane to the underlying upper papillary dermis^{1, 2}.

Dystrophic epidermolysis bullosa (DEB) is a rare genetic blistering disease caused by mutations in collagen type VII alpha 1 chain (*COL7A1*), the gene encoding type VII collagen (COL7). The most severe form of DEB is recessive DEB (RDEB), in which COL7 protein expression is severely diminished or completely absent in the patient's skin due to null mutations in the *COL7A1* gene. Typical morphological findings in DEB patients include dermal-epidermal cleavage below the basement membrane zone (BMZ) and abnormal or structural absence of the anchoring fibrils due to lesions in the *COL7A1* gene. DEB associated blisters and erosions affect skin as well as certain mucosa exposed to disruptive external environments, including the oropharynx, esophagus, rectum, genitourinary system, and eyes.

In this BLA, the applicant proposes Vyjuvek (beremagene geperpavec, previously known as B-VEC or KB103) for treatment of wounds in dystrophic epidermolysis bullosa patients 6 months of age and older. Vyjuvek is an engineered, replication-incompetent herpes simplex virus type 1 (HSV-1)-based vector coding human *COL7A1* that can be applied topically to promote functional COL7 expression in the skin. The proposed mechanism of action depends on the ability of HSV-1 to transduces keratinocytes and fibroblasts.

¹ Sakai LY, Keene DR, Morris NP, et al. Type VII collagen is a major structural component of anchoring fibrils. *J Cell Biol* 1986; 103:1577–86

² Chung HJ, Uitto J. Type VII Collagen: The Anchoring Fibril Protein at Fault in Dystrophic Epidermolysis Bullosa. *Dermatol Clin*. 2010; 28: 93–105.

4. Summary of Clinical Pharmacology Findings

The clinical pharmacology assessment of Vyjuvek were based on two clinical studies:

- **Study#1-** A Phase 1/2 Study of B-VEC, a Non-Integrating, Replication-Incompetent HSV Vector Expressing the Human Collagen VII Protein, for the Treatment of Dystrophic Epidermolysis Bullosa (DEB) (KB103-001)
- **Study#2-** A Phase 3 Efficacy and Safety Study of Beremagene Geperpavec (B-VEC, Previously KB103) for the Treatment of Dystrophic Epidermolysis Bullosa (DEB) (B-VEC-03)

The major clinical pharmacology findings from these two clinical studies are summarized in the following sections.

Pharmacokinetics: Viral vector biodistribution and shedding

- Following topical administration, no viral vector DNA was detected in blood (Phase 2 & Phase 3).
- Viral vector was detected only in one urine sample (3.2%, 1/31; Phase 3).
- Viral vector DNA was detected in skin swab samples in all treated nine subjects with maximum level ranging from 5.1×10^4 to 4.1×10^8 vector genomes. In 6 out of 9 subjects (67%) negative shedding was confirmed with three measurements below limit of detection within 8 weeks (Phase 1/2).
- Skin swabs from 19 out 31 subjects (61.3%) were positive for viral vector following treatment with B-VEC. Skin swabs from 12 out of 31 subjects (38.7%) did not show detectable viral vector at any timepoint during the 26 weeks of treatment (Phase 3).
- The skin swabs maximum viral vector ranges from 5.4×10^2 to 5.3×10^7 vector genomes in 19 subjects with detectable viral vectors. Negative shedding was

achieved in 16 out of 19 subjects (84.2%) within six weeks following treatment with B-VEC (Phase 3).

- Most wound dressings (93.5%, 29/31) contained detectable vector genomes, ranging from 5.2×10^2 to 4.2×10^8 genome copies (Phase 3).

Pharmacodynamics (PD):

The expression, secretion, and localization of COL7 transgene in skin biopsies was evaluated in the Phase 1/2 study. Immunofluorescence (IF) detection of non-collagenous domain 1 (NC1) and domain 2 (NC2) of COL7, and immunoelectron microscopy (IEM) methods were used for pharmacodynamic analysis. The following is a summary of the PD results from the Phase 1/2 study:

- At baseline (pretreatment), skin biopsies (n=12) from the 9 subjects (9 unique and 3 re-enrolled subjects) were negative for NC2 domain of COL7. A lower expression of NC1 domain of COL7 was noted in all 12 skin biopsies.
- Following B-VEC administration, nine skin biopsies at different timepoints were evaluated for expression of NC1 and NC2 domain of COL7 from 6 subjects.
- Following topical administration of B-VEC, fluorescence intensity for NC1 domain was increased in all 9 skin biopsies and the NC2 domain was expressed in 8 out of 9 skin biopsies. In one treated subject no expression of NC2 domain was observed and a small increase in NC1 domain expression from baseline was noted.
- For the 3 subjects whose biopsies were analyzed by immunoelectron microscopy, < 25% of normal skin NC1 and NC2 staining was observed at baseline. Following topical administration of B-VEC, immunoelectron microscopy analysis revealed detectable (>25-100% of normal skin) and appropriately localized AFs at the basement membrane zone (BMZ).

Immunogenicity Assessments

1. Antibodies against HSV:

- Antibodies against HSV-1 were evaluated using a plaque reduction neutralization test (PRNT).
- Antibodies against HSV-1 were detected in 7 out of 12 subjects (58%) at baseline with PRNT50 ranging from 1:80 to 1280. Post-treatment anti-HSV-1 antibodies developed in all 12 subjects (100%) with PRNT50 ranging from 1:40 to 5120. Based on the limited data, no impact of anti-HSV1 antibodies on pharmacodynamic activity of B-VEC (Phase 1/2) was observed.
- At baseline, 63.6% of subjects (14/22) had detectable anti-HSV-1 antibodies. Six of the 8 seronegative subjects seroconverted within 26 weeks following treatment with B-VEC.
- A post-hoc analysis of response rate in primary wound pairs among baseline anti-HSV-1 seropositive vs. seronegative subjects did not reveal any difference in the efficacy of B-VEC (Phase 3).

2. Antibodies against COL7:

- Anti-COL7 antibodies at baseline were detected in four of nine subjects, and two additional subjects developed anti-COL7 antibodies post-treatment within 34-151 days. In these 4 subjects with baseline anti-COL7, pharmacodynamic activity was demonstrated in three skin biopsies but samples from one subject did not express NC2 domain of COL7 protein with moderately increased NC1 domain (Phase 1/2).
- Anti-COL7 antibodies at baseline were detected in one of 22 subjects (4.5%). Thirteen of 18 subjects (72 %) who were seronegative at baseline developed anti-COL7 antibodies at Week 26.
- A post-hoc analysis of response rate in primary wound pairs among subjects with and without anti-COL7 antibodies did not reveal any difference in efficacy of B-VEC (Phase 3).

5. Clinical Pharmacology Labeling Comments

The following is a summary of the clinical pharmacology labeling comments that were communicated to the Applicant. The updated labeling is acceptable from the clinical pharmacology perspective.

- Request to remove detail product description from the mechanism of action as this is already described in section 11
- Request to revise the mechanism of action reflecting only data-supported statements. For example, we could not find data demonstrating “microtubules mediated transport to nucleus and production of precursor protein (procollagen 7)”.
- Request to remove disease pathology that was described elsewhere in the label.
- The following is the recommended language for section 12.2:

12.2. Pharmacodynamics

The pharmacodynamic activity (expression and localization of COL7 transgene) of VYJUVEK was demonstrated in an initial clinical study (n=6 patients). The non-collagenous domain 1 (NC1) and domain 2 (NC2) of COL7 and linear deposition at the dermal-epidermal junction was demonstrated in skin biopsies harvested post-VYJUVEK treatment.

- Recommended to summarize the pharmacokinetic information obtained from the two relevant clinical studies
- Requested to remove speculative language on exposure levels from both nonclinical and clinical studies and updated the parameters per FDA analysis.
- The following is the recommended language for clinical studies in section 12.2:

12.3. Pharmacokinetics

In an initial clinical study, viral vector DNA was detected in skin swab samples in all nine treated patients with maximum level ranging from 5.1×10^4 to 4.1×10^8 vector genomes. In 6 out of 9 patients (67%), negative shedding was confirmed with three measurements below limit of detection within 8 weeks of treatment with VYJUVEK. No viral vector DNA was detected in blood and urine.

In the 31-patient randomized, double-blind, intra-patient placebo-controlled trial, systemic and potential environmental exposure assessments were conducted at weekly clinical site visits via quantification of VYJUVEK genomes in blood, urine, skin swabs, and bandage samples (vector shedding) using a validated qPCR assay, and detection of infectious viral particles in skin swabs (infectivity) using a validated plaque titer assay.

All blood samples and all but one urine sample collected throughout the study were below the limit of detection. Skin swabs from 19 of the 31 patients (61.3%) were positive for viral vector following treatment with VYJUVEK. Negative shedding from skin swabs was achieved in 16 of the 19 patients (84.2%) within six weeks following treatment with VYJUVEK. Most wound dressings (93.5%, 29/31) contained a range of detectable vector genomes. However, no extracellular infectious particles were detected on the skin surface of any patient at any timepoint tested, after topical VYJUVEK application.

- Recommend moving immunogenicity to section 12.6 under the CLINICAL PHARMACOLOGY per updated FDA guidance.
- The following is the recommended language for section 12.6 (Immunogenicity)

There was minimum potential for systemic exposure to VYJUVEK. Antibodies against viral vector (HSV-1) and transgene protein (COL7) were evaluated in clinical studies. A total of 63.6% of evaluated patients (14/22) were anti-HSV-1 antibody positive at baseline. Six of the 8 anti-HSV-1 seronegative patients seroconverted by Week 26 following treatment with VYJUVEK. For patients with available matched baseline and end of study serum samples, anti-drug antibodies

(ADAs) to COL7 were detected in 72.2% (13/18) of patients treated with VYJUVEK for up to 26 weeks.

Data are limited to perform correlative assessment on the impact of ADA on pharmacodynamic activity and clinical response.

6. Comprehensive Clinical Pharmacology Review

6.1. General Pharmacology and Pharmacokinetics

Vyjuvek (beremagene geperpavec) is an engineered, replication-incompetent HSV-1-based gene therapy product coding human *COL7A1*. Non-replicative HSV-1 vector is generated by deleting parts of the viral genome essential for viral replication. The active ingredient is mixed with excipient gel (hydroxypropyl methylcellulose) at the site of care prior to topical application to wounds.

Following topical administration of Vyjuvek the proposed mechanism of action (MOA) involves the following sequential events:

- Entry into cells (e.g., keratinocytes and fibroblasts)
- Transport to nucleus and expression of COL7A1 transgenes
- Secretion of COL7 protein
- Assembly of secreted COL7 protein into anchoring fibrils which hold the epidermis and dermis together.

The data supporting the MOA were generated from proof-of-principle nonclinical studies including in vitro experiments in fibroblast and keratinocytes. In the in vitro study, fibroblasts and keratinocytes obtained from normal individuals and patients with RDEB were treated with Vyjuvek. The COL7 expression was monitored by immunofluorescence or western blot and qRT-PCR analysis. The results demonstrate dose-dependent expression and secretion of COL7 in patient derived fibroblast and keratinocytes following treatment with Vyjuvek. Furthermore, a 3-D organ culture analysis constructed with patient derived or normal fibroblasts and keratinocytes revealed COL7 expression at the basement membrane. The COL7 expression was also evaluated in COL7 hypomorphic (animal model that express about 10% of normal levels of COL7). Vyjuvek was administered by intradermal administration in these mice, and human COL7 DNA and transcript levels, and protein expression in the skin were assessed at different time points post administration. The data generated from the mice model showed that Vyjuvek transduced skin cells resulting in expression and linear BMZ deposition of COL7.

Overall human cell derived in vitro studies, mice model and the clinical study (see pharmacodynamics section below) demonstrate that Vyjuvek can transduce fibroblasts and keratinocytes resulting in secretion of functional COL7.

The FIH study (Phase 1/2) explored different routes of administration (intradermal vs topical) and dose ranges and dosing frequency. In the Phase 1 portion of the study, two adult subjects with two wounds $\leq 10 \text{ cm}^2$ (each wound randomized to Vyjuvek, or placebo administered the excipient gel). The intradermal dose of Vyjuvek was 1×10^8 plaque-forming units (PFU) at Visit Days 0, 2, 28, and 30 (Subject 1) and Visit Days 0, 2, 14, 28, 30 and 42 (Subject 2). In the Phase 2 portion of the study, 18 wounds $\leq 20 \text{ cm}^2$ were administered a unit topical dose between 2.0×10^8 and 6.0×10^8 PFU for 5 consecutive treatments, daily or every other day.

In the Phase 3 study, the unit dose administered weekly to the primary wounds was determined based on the wound area, age at baseline, safety, and efficacy information from the Phase 1/2 study. Subjects were dosed topically for up to 26 weeks. The maximum weekly dose of 3.2×10^9 PFU/subject (6 years of age and older) and the unit dose by wound area of 4×10^8 PFU/ 20 cm^2 were based on preliminary efficacy and safety data obtained from similar dosing regimens in the Phase 2 study.

Traditional clinical pharmacology studies such as absorption, distribution, metabolism, and excretion (ADME) were not warranted considering product characteristics, mechanism of action and route of administration. The pharmacokinetics of Vyjuvek were characterized in Phase 1/2 and Phase 3 studies as part of viral vector biodistribution and shedding assessments. A qPCR method was used to quantify the vector DNA copy number in skin swabs, wound dressings, blood, and urine samples. For Phases 1/2 and 3 studies, the limit of detection (LOD) for vector genomes in blood and urine samples was 100 copies/qPCR reaction, which equates to 2×10^4 copies/mL of sample. The LOD for vector genomes in skin swab samples was 50 and 100 copies/qPCR reaction, which equates to 500 and 1000 copies/skin swab for Phase 1/2 and Phase 3 study, respectively.

Following topical administration, no viral vector DNA was detected in blood (Phase 2 & 3), and one urine sample (3.2%, 1/31) has detectable viral vector (Phase 3). These data suggest limited systemic distribution of the viral vector following topical administration.

Viral vector DNA was detected in skin swab samples in all treated nine subjects (100%) with maximum level ranging from 5.1×10^4 to 4.1×10^8 vector genomes. In 6 out of 9 subjects (67%) negative shedding was confirmed with three measurements below limit of detection within 8 weeks (Phase 1/2). Skin swabs from 19 out 31 subjects (61.3%) were positive for viral vector following treatment with B-VEC. Skin swabs from 12 out of 31 subjects (38.7%) did not show detectable viral vector at any timepoint during the 26 weeks of treatment (Phase 3).

The skin swabs' maximum viral vector ranged from 5.4×10^2 to 5.3×10^7 vector genomes in 19 subjects with detectable viral vectors. Negative shedding was achieved in 16 out of 19 subjects (84.2%) within six weeks following treatment with B-VEC (Phase 3). Skin swabs from 3 subjects (15.8%) did not achieve negative shedding during the 26 week sampling period because of short follow-up period after the most recent dose of B-VEC. Most wound dressings (93.5%, 29/31) contained detectable vector genomes, ranging from 5.2×10^2 to 4.2×10^8 genome copies (Phase 3). Based on the skin infectivity test, no extracellular infectious particles were detected on the skin surface of any subject at evaluated timepoints (Phase 3).

Overall, the viral vector kinetic data from the Phase 1/2 and 3 studies indicate that there was no systemic exposure (blood) following topical administration and most subjects (67-84%) achieved negative shedding from skin swabs within 6-8 weeks after maximum level was achieved.

6.2. Pharmacodynamic Assessments

DEB patients have formation of functionally deficient anchoring fibrils (AFs) between the epidermal basement membrane and the underlying dermal connective tissue. Typical morphological findings in these patients include dermal-epidermal cleavage below the basement membrane zone (BMZ) and abnormal or structural absence of the AFs fibrils due to mutations in the *COL7A1* gene. Immunofluorescent staining of skin of patients

with the most severe recessive DEB (RDEB) demonstrated lack of type VII collagen epitopes and morphological changes or absent AFs ^{3,4}.

Microscopic detection of the non-collagenous domain 1 (NC1) and domain 2 (NC2) of COL7 and linear deposition of both domains at the dermal-epidermal junction, or basement membrane zone (BMZ) are considered pharmacodynamic endpoints that demonstrate functional, full-length protein expression.

The pharmacodynamics of VYJUVEK were evaluated in a subset of subjects who participated in the Phase 1/2 study. Biopsies of intact/healed skin following B-VEC treatment were evaluated for expression and localization of both NC1 and NC2 domains of COL7 at the dermal-epidermal junction (the BMZ) via immunofluorescence (IF) analysis. Further, biopsies were evaluated for the presence of AFs after B-VEC treatment via immunoelectron microscopy (IEM) analysis. However, it should be noted that the IF and IEM methods are non-validated subjective analyses used to generate proof-of-concept pharmacodynamic activity in the Phase 1/2 study. The IF and IEM assessments were not pursued in the Phase 3 study due to technical challenges and ethical concerns with collecting skin biopsies in a disease state that leads to chronic and painful wounds after skin perturbation.

At baseline (pretreatment), skin biopsies from the 12 subjects (9 unique and 3 re-enrolled subjects) were negative for NC2 domain of COL7. A lower expression of NC1 domain of COL7 (< 20% of normal skin) was noted in all 12 subjects (Table 1). Post-treatment skin biopsies were collected from nine (6 unique and 3 reenrolled subjects) and three subjects did not provide post treatment skin biopsies. Following topical administration of B-VEC fluorescence intensity for NC1 domain was increased in 8 out of 9 skin biopsies (fluorescence intensity of 30-100% of normal skin) and the NC2 domain was expressed in 8 out of 9 biopsies (30-100% of normal skin, Table 1). In one treated subject with

³ Bruckner-Tuderman L, Mitsuhashi Y, Schnyder UW, et al. Anchoring fibrils and type VII collagen are absent from skin in severe recessive dystrophic epidermolysis bullosa. *J Invest Dermatol.* 1989;93:3-9.

⁴ McGrath JA, Ishida-Yamamoto A, O'Grady A, et al. Structural variations in anchoring fibrils in dystrophic epidermolysis bullosa: correlation with type VII collagen expression. *J Invest Dermatol.* 1993;100:366-72.

baseline anti-COL7 antibodies, no detection of the NC2 domain and a small increase in expression of the NC1 domain (30% of normal skin) was observed (Table 1).

Three out of nine biopsies were analyzed by immunoelectron microscopy. Skin biopsies from six subjects were not analyzed by immunoelectron microscopy due to dermal-epidermal separation occurring during fixing skin biopsies. For the 3 subjects whose biopsies were analyzed by immunoelectron microscopy < 25% of normal skin NC1 and NC2 staining was observed at baseline. Following topical administration of B-VEC, immunoelectron microscopy analysis revealed detectable (> 25-100% of normal skin) and appropriately localized AFs at the BMZ.

Overall, following B-VEC administration, the COL7 transgene secretion and localization at the BMZ was demonstrated and these data provide supportive pharmacodynamic information informing efficacy of B-VEC.

6.3. Immunogenicity Assessments

Development of antibodies against viral vector (HSV-1) and transgene (COL7) were evaluated in both the Phase 1/2 and Phase 3 studies. A plaque reduction neutralization test (PRNT) was used for detection of antibodies against HSV-1 in serum samples collected pre-and post-treatment with B-VEC. In the Phase 1/2 study, antibodies against HSV-1 were detected in 7 out of 12 subjects (58%) at baseline with PRNT50 ranging from 1:80 to 1280. Post-treatment anti-HSV-1 antibodies were developed in all 12 subjects with PRNT50 ranging from 1:40 to 5120. Based on the limited data, no impact of anti-HSV1 antibodies was observed on pharmacodynamic activity of B-VEC. In the Phase 3 study, at baseline, 63.6% of subjects (14/22) had detectable anti-HSV-1 antibodies and 91% of subjects (20/22) had anti-HSV-1 antibodies following treatment with B-VEC up to 26 weeks. A post-hoc analysis of response rate in primary wound pairs among baseline anti-HSV-1 seropositive vs. seronegative subjects did not reveal any difference in efficacy of B-VEC.

In the Phase 1/2 study, anti-COL7 antibodies at baseline were detected in four of 12 subjects (33%), and two additional subjects (6/12; 50%) developed anti-COL7 antibodies

post-treatment (Table 1). In the Phase 3 study, anti-COL7 antibodies at baseline were detected in one of 22 subjects (4.5%). Thirteen of 18 subjects (72 %) who were seronegative at baseline developed anti-COL7 antibodies at Week 26. A post-hoc analysis of response rate in primary wound pairs among subjects with and without anti-COL7 antibodies did not reveal any difference in efficacy of B-VEC.

Overall, the limited immunogenicity data suggest that pre-existing or post-treatment anti-HSV1 and anti-COL7 antibodies did not appear to impact pharmacodynamic activity. However, data are limited to fully evaluate the impact of ADA on clinical outcome and pharmacodynamic activity.

Table 1: Summary of pharmacodynamic activity of before and after B-VEC Administration

Subject	Visit	NC1 IF	NC2 IF	Baseline Anti-HSV-1 Serostatus ^a	Anti-COL7 Seroconversion ^{b,c}
KB103-001 ^{(b) (6)}	Baseline	10%	0%	Seropositive	No
	Week 4	80%	80%		
KB103-(b) (6)	Baseline	20%	0%	Seropositive	Baseline seropositive
	Week 8	80%	80%		
KB103-001- ^{(b) (6)}	Baseline	10%	0%	Seronegative	Yes (Day 34)
	Week 4	80%	70%		
KB103-001 ^{(b) (6)}	Baseline ^c	10%	0%	Seronegative	No data
KB103-001 ^{(b) (6)}	Baseline	5%	0%	Seronegative	Baseline seropositive
	Week 4	30%	0%		
KB103-001 ^{(b) (6)}	Baseline	10%	0%	Seropositive	Yes (Day 151)
	Week 4	90%	90%		
KB103-001 ^{(b) (6)}	Baseline	10%	0%	Seropositive	Yes (Day 151)
	Week 8	90%	90%		
KB103-(b) (6)	Baseline	5%	0%	Seronegative	Baseline seropositive
	Week 1	30%	30%		
	Week 4	20%	0%		
KB103-001 ^{(b) (6)}	Baseline	5%	0%	Seropositive	No
	Week 2	80%	90%		
	Week 4	80%	90%		
KB103-001 ^{(b) (6)}	Baseline	10%	0%	Seropositive	No
	Week 2	100%	100%		
	Week 13	100%	100%		
KB103-001 ^{(b) (6)}	Baseline ^c	10%	0%	Seronegative	Yes (Day 34)
KB103-001 ^{(b) (6)}	Baseline ^c	10%	0%	Seropositive	No

For NC1 and NC2 IF, numbers are expressed as % fluorescence intensity compared to normal human skin

a: For study subjects that enrolled in a subsequent phase of the study, their anti-HSV-1 serostatus is reported at their earliest baseline visit. b: For study subjects that enrolled in a subsequent phase of the study, the indicated day of seroconversion is relative to their earliest baseline visit.

c: Positivity in anti-COL7 assay is determined as a signal >20 RU/mL, per the manufacturer's protocol.

d: Subject KB103-001^{(b) (6)} re-enrolled as subject KB103-001^{(b) (6)}. e: Subject KB103-001^{(b) (6)} dropped out and was unavailable for post treatment skin biopsy; subjects KB103-001^{(b) (6)} and KB103-001^{(b) (6)} declined post-treatment skin biopsies. f: Subject KB103-001^{(b) (6)} re-enrolled as subject KB103-001^{(b) (6)}.

g: Subject KB103-001^{(b) (6)} re-enrolled as subject KB103-001^{(b) (6)}.

Source: Response to Clin pharm IR#1

7. Appendix

7.1. Study#1- A Phase 1/2 Study of B-VEC, a Non-Integrating, Replication-Incompetent HSV Vector Expressing the Human Collagen VII Protein, for the Treatment of Dystrophic Epidermolysis Bullosa (DEB) (KB103-001)

Objectives: The primary objectives are to evaluate safety and demonstrate molecular correction by establishing the presence of functional COL7 expression.

The clinical pharmacology relevant secondary objectives include assessment of pharmacokinetics and HSV-1/COL7 antibodies.

Study Design: The Phase 1/2 study was an interventional, single center, open-label, randomized, intra-subject placebo-controlled study to assess safety and efficacy of topical B-VEC for the treatment of DEB. The study was divided into 4 phases that corresponded to protocol revisions: Phase 1, Phase 2a, Phase 2b, and Phase 2c. Nine unique patients participated in the study. Three of the 9 subjects enrolled in both Phase 2a and Phase 2b. These three subjects contributed different wounds for treatment and evaluation in Phase 2a and Phase 2b, except in the case of one subject whose chronic dorsal foot wound treated in Phase 2a that achieved partial closure was selected to continue treatment in Phase 2b. The roll over subjects from Phase 2a had different identification number in Phase 2b, hence the study has 12 unique identification numbers.

- **Phase 1:** enrolled 2 adult subjects and 2 wounds $\leq 10 \text{ cm}^2$ were selected in each subject; 1 wound was randomized to B-VEC and the other to placebo. Wounds randomized to placebo were administered the excipient gel and intradermal dose of B-VEC was: $\sim 1 \times 10^8$ plaque-forming units (PFU)) at Visit Days 0, 2, 28, and 30 (Subject 1) and Visit Days 0, 2, 14, 28, 30 and 42.
- **Phase 2a:** enrolled 4 subjects: 2 adults and 2 pediatric subjects. Three wounds $\leq 20 \text{ cm}^2$ were selected per subject; two were randomized to B-VEC and one was randomized to placebo. Each wound randomized to B-VEC was topically

administered 3×10^8 PFU at Day 1, 2,3,4, 5 and additional administration at Day 30,44 and 60. One subject was given a dose of 6×10^8 PFU of topical B-VEC at day 5 and later withdrew from the study due to inability to travel to clinical site.

- **Phase 2b** (protocol v3) specify that age 13 and older will be administer intradermal injection at discretion of investigator. Five subjects were enrolled: 2 pediatrics and 3 adults. For re-enrolled subjects a wash-out period of ~ 3months between treatments in Phase 2a and Phase 2b. Dose: 1×10^8 to 1.2×10^9 PFU per administration day (~ every 2-3 days for 2 consecutive weeks and then Visit Days 30, 60 and 90 if the wounds were open during the visit).
- **Phase 2c** (protocol v4) include large chronic wounds and enrolled one pediatric with wound measure 65.9 cm^2 was treated with topical dose ranging from 8×10^8 to 1.6×10^9 PFU per wound per administration. This subject was followed only for safety due to significant wound size difference between placebo and treatment.

COL7 Analysis by Immunofluorescence (IF) and Immunoelectron Microscopy (IEM): Biopsies of treated skin processed with antibody reagents that bind to the non-collagenous domain 1 (NC1) and domain 2 (NC2) of COL7 were analyzed for the presence and proper localization of functional COL7. For IF analysis, skin punch biopsy samples were fixed, sectioned, and stained using antibodies raised against the NC1 or NC2 domain of human COL7. For IEM analysis, skin punch biopsies were rinsed with serum free medium and then immersed in primary anti-human COL7 antibody (NC1 or NC2 domain). After extensive washing, samples were immersed in a solution containing ultrasmall colloidal gold-conjugated secondary antibody. Biopsies were assessed for AF formation by determining the presence of gold conjugate particles with characteristic features of AFs (density, thickness, curvature, arching, and looping).

Immunogenicity Assessment: Samples (collected at Pre-dose, Days 14 and 84) were evaluated for antibodies against HSV-1 using a plaque reduction neutralization test (PRNT). Serum samples were evaluated for IgG antibodies targeting COL7 using an ELISA assay to qualitatively assess the presence/absence of antibodies against COL7 pre- and post-B-VEC exposure at Days 14 and 84.

Biodistribution and Shedding Assessment: The extent of systemic biodistribution and shedding of viral vector was evaluated using blood and urine samples. Viral shedding at target wounds was evaluated using skin swab samples. Skin swab samples for viral vector shedding was collected at pre-dose and post-dose at Days 1,3, 15, 30, 60 and 90. A qPCR method was used for the detection of viral DNA copy number. For the Phase 1/2 study, the limit of detection (LOD) for vector genomes in skin swab samples was 100 copies/qPCR reaction, which equates to 1×10^3 copies/skin swab. The LOD for vector genomes in blood and urine samples was 100 copies/qPCR reaction, which equates to 2×10^4 copies/mL of sample. If a qPCR reaction for a blood or urine sample produced a signal in the qPCR assay but the signal was below the limit of detection (BL0D), then the sample was reported as BL0D. Alternatively, if a qPCR reaction for a blood or urine sample produced no detectable signal in the qPCR assay, it was reported as negative.

Results

Pharmacodynamics (PD):

Pharmacodynamics were assessed by establishing the presence of functional COL7 expression and the formation of anchoring fibril (AF) post administration of B-VEC.

1. COL7 Analysis by Immunofluorescence

Skin biopsies from all 12 subjects (9 unique and 3-reenrolled subjects) at pretreatment (baseline) showed lower expression of NC1 (%fluorescence intensity compared to normal human skin), which is commonly observed among RDEB subjects (Table 1). All 12 biopsies were NC2 negative at baseline. Following B-VEC administration skin biopsies were collected from 9 out of the 12 subjects (since one subject dropped out of the study and two subjects declined to provide post-treatment biopsies). Following B-VEC-administration fluorescence intensity for NC1 domain was increased in 9 out of 9 subjects (30-100% of normal skin; Table 1) while the NC2 domain is expressed in 8 out of 9 subjects (30-100% of normal skin). In one treated subject with baseline anti-COL7 antibodies no detection of NC2 domain and a small increase in expression of NC1 domain (30% of normal skin) was observed (see for details the immunogenicity section).

2. Anchoring Fibril Analysis by Immunoelectron Microscopy

Detection of mature AFs at the BMZ is provide supportive information on functional COL7 expression. Of the 9 biopsies that were collected, post-treatment biopsies for 6 subjects could not be analyzed due to dermal-epidermal separation occurring during overnight transport of unfixed IEM skin biopsies. At baseline, subjects whose biopsies were analyzed by IEM showed less than 25% of normal skin NC1 and NC2 staining, while IEM analyses of B-VEC-treated skin revealed clearly detectable and correctly localized AFs at the BMZ. The AFs were observed as early as Week 2 and as late as Week 13.

Reviewer comments: The IF detection of COL7 and protein localization are subjective analyses but reasonable choice to generate proof-of-concept supporting efficacy. The observed increased expression of COL7 is further supported by in vitro experiments that demonstrate functional COL7 expression in patient derived fibroblast and keratinocytes.

Blood and Urine Vector Shedding:

Samples were assessed for the presence of B-VEC DNA (viral/vector shedding) using a validated qPCR assay. The limit of detection (LOD) for the assay was 100 copies per the qPCR reaction. No vector DNA was detected above the LOD in any of the blood and urine samples analyzed.

Skin Vector Shedding:

Skin swabs were assessed in the presence of B-VEC DNA using the same qPCR assay used for blood and urine samples (LOD 100 copies per PCR which corresponds to 1×10^3 per skin swab). Viral vector DNA was detected in skin swab in all treated nine subjects with maximum level ranging from 5.1×10^4 to 4.1×10^8 vector genomes. In 6 out of 9 subjects (67%) negative shedding was confirmed with three measurements below limit of detection within 2-8 weeks following treatment. Skin shedding data were not adequate to

confirm negative shedding in 3 out of 9 subjects (33%) due to short follow-up period after the last dose of B-VEC.

Reviewer comments: The viral vector kinetic analysis demonstrates no systemic exposure (blood and urine) following topical administration of B-VEC. Negative shedding was not confirmed in 33% due to inadequate follow-up after the last dosing. The available skin viral kinetic data indicate clearance within 2 to 8 weeks following treatment.

Immunogenicity Assessment:

1. Evaluation of Immune Response to HSV:

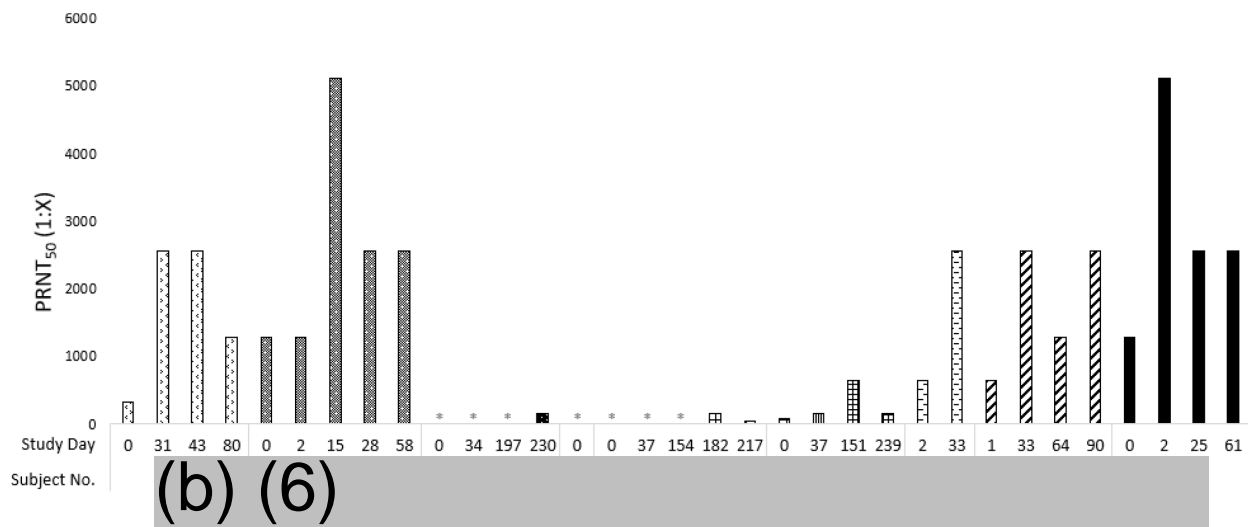
Antibodies against HSV-1 were evaluated using plaque reduction neutralization test (PRNT). The PRNT assay determines the percent reduction in B-VEC-mediated plaque formation in complementing cells in the presence of serially diluted subject sera and is reported as PRNT50 which is the serum dilution at which a ~50% reduction in plaques is observed. An increase in the PRNT50 value over time is suggestive of an increase in anti-HSV-1 antibodies in the sera. Antibodies against HSV-1 were detected in 7 out of 12 subjects at baseline with PRNT50 ranging from 1:80 to 1280 (Figure 1). As expected, post-treatment anti-HSV-1 antibodies were developed in all subjects (Figure 1). Among subjects who demonstrated a clear increase in anti-HSV-1 antibodies from baseline a pharmacodynamic activity is still demonstrated.

2. Evaluation of Antibody Response to COL7

Serum samples collected pre-dose were also evaluated for IgG anti-drug antibodies (ADAs) targeting COL7 by enzyme-linked immunosorbent assay (ELISA). This ELISA qualitatively determines the anti-COL7 serostatus of subjects based on a 20 RU/mL threshold. Anti-COL7 antibodies at baseline were detected for 4 subjects and 2 other subjects seroconverted post-treatment within 34 to 151 days. In these 4 subjects with

baseline anti-COL7 the pharmacodynamic activity was demonstrated in three skin biopsies but samples from one subject did not express NC2 domain of COL7 protein.

Figure 1: Anti-HSV-1 Antibodies in Phase 1/2 Subject Sera



Source: KB103-001 CSR Appendix 16.2.8

Reviewer comments: Although the sample size is limited, pharmacodynamic activity is demonstrated in subjects with baseline anti-HSV-1 antibodies. Also, with the current limited data it appears that pre-existing anti-COL7 antibodies did not impact pharmacodynamic activity. These data suggest that serum antibodies against HSV-1 and COL7 are not affecting local transduction and transgene generation following topical administration of B-VEC. However, these data are limited to fully evaluate the clinical impact of ADA and future study with appropriate sample size is recommended.

7.2. Study#2- A Phase 3 Efficacy and Safety Study of Beremagene Geperpavec (B-VEC, Previously KB103) for the Treatment of Dystrophic Epidermolysis Bullosa (DEB) (B-VEC-03)

Objectives: The primary objective was to determine whether topical administration of B-VEC in addition to standard of care improved wound healing as compared to placebo in children, adolescents, and adults with DEB.

Study Design: Study B-VEC-03 (GEM-3) was a multicenter, intrasubject randomized, placebo-controlled, double-blind, Phase 3 study of B-VEC for the topical treatment of DEB wounds. The investigator selected 2 matched wounds (primary wound pair) in each subject that were similar in size, located in similar anatomical regions, and had similar appearance. The matched wound pair was randomized to treatment with investigational product (IP) such that one wound received weekly treatment with topical B-VEC and the other received placebo.

Dosing and Administration: The unit dose administered weekly to the primary wounds was determined based on the wound area and age at baseline. Subjects were dosed topically for up to 26 weeks or until wound closure. The maximum weekly dose of B-VEC based on the subject's age was:

- ≥ 6 months to <3 years dosed at 1.6×10^9 PFU/week
- (b) (4)
- ≥ 6 years dosed at 3.2×10^9 PFU/week.

The maximum weekly dose of 3.2×10^9 PFU/subject (6 years of age and older) and the unit dose by wound area of 4×10^8 PFU/20 cm² were based on efficacy and safety data obtained from similar dosing regimens in the Phase 2 study.

HSV-1 Antibody Assay: Serum was collected, when possible, per the schedule of events to screen for antibodies against HSV-1 using a validated plaque reduction neutralization test (PRNT).

Type VII Collagen Antibody Assay: Serum was collected, when possible, per the schedule of events to screen for antibodies against COL7 using a commercially available CE-IVD (CE-marked in vitro diagnostic) anti-COL7 enzyme-linked immunosorbent assay (ELISA).

Biodistribution and Viral Shedding: Whole blood, urine, and skin and bandage swabs were collected, when possible, per the schedule of events to evaluate viral shedding by a validated quantitative polymerase chain reaction (qPCR) assay. The limit of detection (LOD) for vector genomes in blood and urine samples was 100 copies/qPCR reaction, which equates to 2×10^4 copies/mL of sample. If a qPCR reaction for a blood or urine sample produced a signal in the qPCR assay but the signal was below the limit of detection (BLOD), then the sample was reported as BLOD. Alternatively, if a qPCR reaction for a blood or urine sample produced no detectable signal in the qPCR assay, it was reported as negative. The LOD for vector genomes in skin swab samples was 50 copies/qPCR reaction, which equates to 500 copies/skin swab. Skin swabs were reported as BLOD if they were below 50 copies/reaction limits of detection.

Infectivity: Skin swabs were collected per the schedule of events to evaluate viral infectivity by validated plaque titer analysis.

Demographic and Baseline Characteristics

The mean age of subjects (N=31) was 17.2 years (range 1 to 44), and 19 (61.3%) subjects were pediatric (range 1-18 years). Most subjects were male (64.5%) and white (64.5%). Ethnicity was about equally distributed between Hispanic or Latino (51.6%) and not Hispanic or Latino (48.4%). All subjects had RDEB except 1 who had DDEB.

The primary wounds were well matched in size between the treatments with medians of 10.6 cm² for B-VEC and 10.4 cm² for placebo. Primary wound sizes varied in size from

approximately 2.3 cm² to over 50 cm². Large secondary wounds exceeding 100 cm² were also treated with B-VEC.

Evaluation of Vector Shedding and Infectivity

All blood samples sample collected throughout the study were below the limit of detection/quantification for all subjects. A urine sample from one subject (3.2%) was positive for viral vector (Table 2). Thus, the blood and urine viral kinetic data indicate no significant systemic exposure following topical administration of B-VEC.

Skin swabs from 19 out 31 subjects (61.3%) were positive for viral vector following treatment with B-VEC. Skin swabs from 12 out of 31 subjects (38.7%) did not show detectable viral vector at any timepoint during the 26 weeks of treatment. The skin swabs maximum viral vector ranged from 5.4×10^2 to 5.3×10^7 vector genomes in 19 subjects with detectable viral vectors. Negative shedding (i.e., confirmed by 3 subsequent measurements below LOD) was achieved in 16 out of 19 subjects (84.2%) within six weeks following treatment with B-VEC. Skin swabs from 3 subjects (15.8%) did not achieve negative shedding during the 26 week sampling period due to short follow-up period after the last treatment. Based on skin infectivity test no extracellular infectious particles were detected on the skin surface of any subject at evaluated timepoints. Most wound dressings (93.5%, 29/31) contained detectable vector genomes, ranging from 5.2×10^2 to 4.2×10^8 genome copies (Table1).

Reviewer comments: The viral vector kinetic results from this Phase 3 study and previous Phase 2 study indicate no systemic exposure (blood) following topical administration. It appears that most subjects (>84%) reached below the limit of quantification for skin swab samples within 6 weeks after maximum level was achieved.

Table 2: Summary of Biodistribution and Vector Shedding Data

Body fluid/site	Number (%) of subjects with detectable DNA during treatment (N=31 subjects)
Skin swab	19 (61.3 %)
Wound dressing	29 (93.5 %)
Blood	0 (0 %)
Urine	1 (3.2 %)

Source: Response to Clin Pharm IR#2; Table 2

Immunogenicity Assessment

1. Evaluation of Immune Response to HSV:

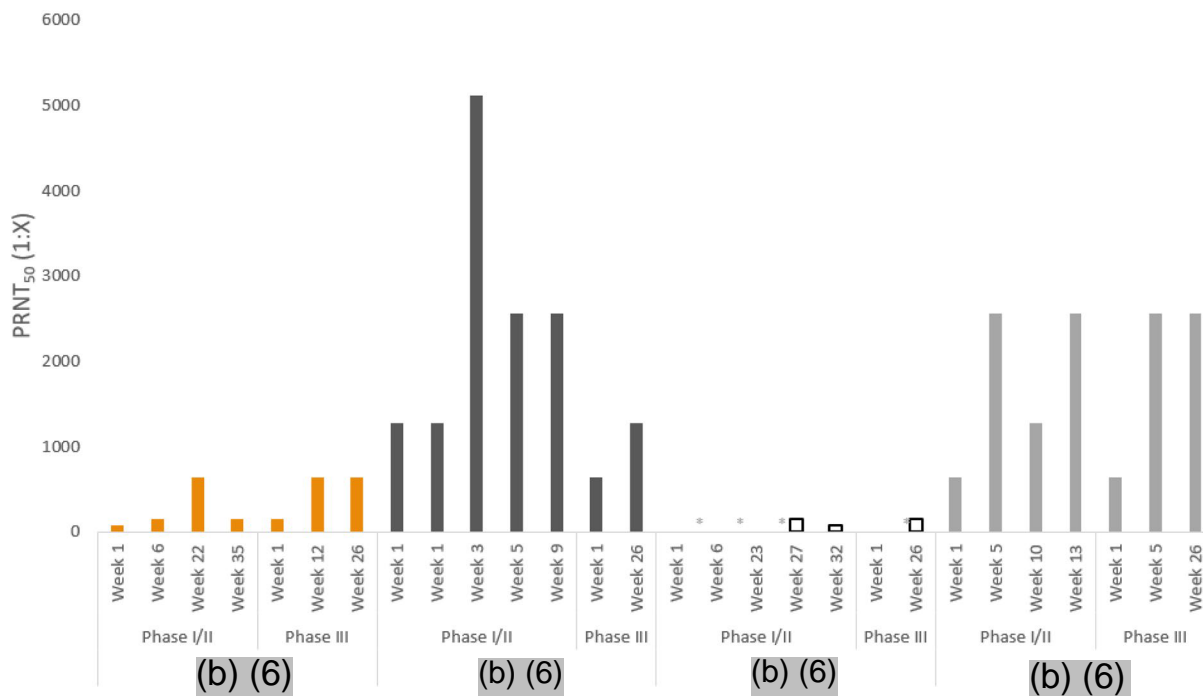
Due to the difficulty of blood draws for DEB patients owing to skin fragility, 22 of the 31 subjects (71.0%) were able to provide a serum sample for anti-HSV at baseline. Matched serum samples antibodies were also obtained at the Week 26 visit for 19 of these subjects. At baseline, 63.6% of subjects (14/22) were anti-HSV-1 antibody positive. Six of the 8 seronegative subjects seroconverted by Week 26. For baseline seropositive subjects, where quantitative differences at study completion could be calculated, antibody responses were variable, and the ratio of the post-treatment increase to baseline anti-HSV-1 antibody titer were below 4-fold (Figure 2). A post hoc analysis of response rate in primary wound pairs among baseline anti-HSV-1 seropositive vs. seronegative subjects was suggestive of similar efficacy of B-VEC.

A retrospective analysis of anti-HSV-1 antibody titers in 4 of the Phase 3 subjects where serum samples were available (Subjects (b) (6)) who also participated in the Phase 1/2 study indicates that anti-HSV-1 antibody levels are transient,

Figure 2: Anti-HSV-1 Antibodies, Subjects with Matched Sera Sample



Figure 3: Anti-HSV-1 Antibodies in Subjects who Participated in Both Phase 1/2 and Phase 3 Studies



Source: Figure 6; Module 2.7.3

2. Evaluation of Anti-COL7 Antibody Response to COL7

The same pre- and post-exposure sera samples collected and tested for anti-HSV-1 antibodies were also evaluated for IgG anti-drug antibodies (ADAs) targeting human COL7 using a commercially available assay. One of 22 (4.5%) subjects was determined to be anti-COL7 antibody positive at baseline. Thirteen of 18 subjects (72 %) who were seronegative at baseline developed ADAs at Week 26. A post hoc analysis of response rate in primary wound pairs among subjects with and without ADAs did not reveal impact on efficacy of B-VEC.