Successful *In Vivo* COL7A1 Gene Delivery And Correction of Recessive Dystrophic Epidermolysis Bullosa (RDEB) Skin Using An Off The Shelf HSV-1 Vector (KB103)

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INTRODUCTION

Recessive Dystrophic Epidermolysis Bullosa (RDEB) is an autosomal recessive, inherited skin disease caused by null mutations within the type VII collagen gene (COL7A1). The mutations cause an absence or reduction of functional type VII collagen protein (COL7), which make up anchoring fibrils that maintain binding of the epidermis to the dermis. The disease is characterized by a mechanical fragility and repeated blister formation in the sub-lamina densa, at the level of the structurally defective anchoring fibrils.

There is no effective therapy for RDEB, and death is usually the result of aggressive squamous cell carcinoma, sepsis, or malnutrition. Currently studied *ex vivo* gene replacement or bone marrow replacement therapies for RDEB, require highly specialized facilities and considerable expense.

Krystal Biotech, Inc has developed an off-the-shelf therapy consisting of a proprietary modified replication deficient HSV-1 vector (KB103) encoding COL7A1 for direct *in vivo* administration to RDEB skin either by intradermal injection or by topical administration.

METHODS

Primary Cells

Cells	Diagnosis	*COL7A1* Mutation
RDEB77, fibroblasts	Sg-RDEB	NM_000094: c.2782_2783insGACAC / p.Thr928Argfs*7 Homozygous
RDEB81, fibroblasts and keratinocytes	Sg-RDEB	NM_000094: c.2923_2924insA / p.A975fs Heterozygous, second allele unknown
RDEB84, fibroblasts and keratinocytes	Sg-RDEB	NM_000094: c.8709del7/ G2899del7_fs. Heterozygous, second allele unknown
Br42-45, fibroblasts and keratinocytes	Normal	Analysis not done: assume wild type

Human COL7 Antibodies

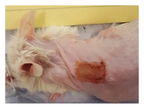
Ab name	epitope	type	ref
NP185	portion of NC1 domain	monoclonal	4
FNC1	portion of NC1 domain	polyclonal	5
LH24	portion of NC2 domain	monoclonal	3
HPA042420	portion of NC1 domain	polyclonal	SIGMA

RDEB mice

Homozygous Col7a1^{flNeo} mice lack both functional copies of Col7a1 and express a~10% of normal levels. Their phenotype closely resembles characteristics of severe human DEB, including mucocutaneous blistering, nail dystrophy, and mitten deformities of the extremities (1).

Xenograft model

NSG mice (NOD *scid* gamma) grafted with xenografts composed of devitalized porcine dermis and RDEB keratinocytes (2).

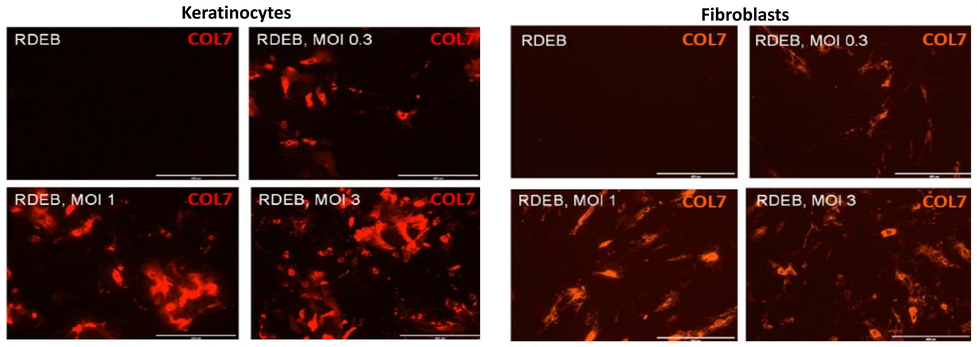


Test Article

KB103 : Krystal Biotech, Inc's proprietary replication deficient HSV-1 vector encoding COL7A1

RESULTS (*in Vitro*)

KB103-mediated COL7 expression in normal and RDEB keratinocytes and fibroblasts

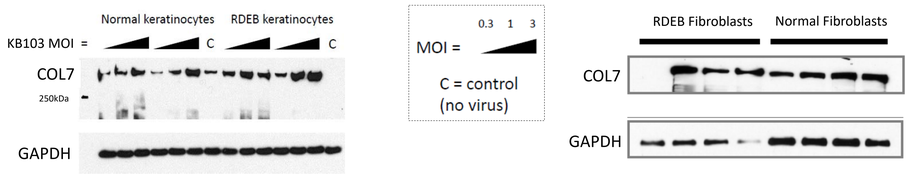


Immunofluorescent staining for COL7 (red) in primary human RDEB keratinocytes or fibroblasts. Cells were infected with KB103 at escalating MOI (Multiplicity of infection = plaque forming units per cell). After 48h cells were fixed and stained with HPA042420 antibody to evaluate COL7 expression (red).

Sample	Col7A1 Fold-Change Over WT-HDK
WT-HDK	1.00
RDEB-HDK, MOI 0	0.79
RDEB-HDK, MOI 0.3	7.00
RDEB-HDK, MOI 1	13.73
RDEB-HDK, MOI 3	26.25

Sample	Col7A1 Fold-Change Over WT-HDF
WT-HDF	1.00
RDEB-HDF, MOI 0	0.34
RDEB-HDF, MOI 0.3	5.13
RDEB-HDF, MOI 1	30.79
RDEB-HDF, MOI 3	60.57

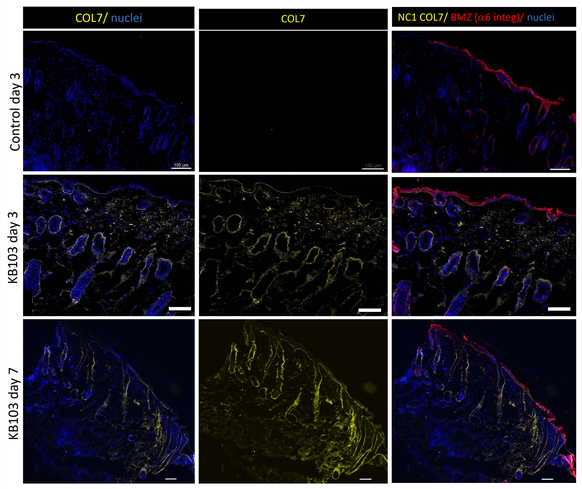
qPCR analysis for COL7A1 in RDEB fibroblasts (HDF) and keratinocytes (HDK). Cells were infected with KB103 at escalating MOI. After 48h, COL7A1 expression was assessed using a SYBR green based COL7A1 assay. Data is presented as fold-change in expression relative to Normal (WT) cells.



Western blot analysis of COL7 expression in KB103-infected RDEB keratinocytes and fibroblasts. Primary normal and RDEB patient keratinocytes and fibroblasts were infected with KB103. 48 hours later, the cell lysates and conditioned media were collected, and COL7 protein was detected by Western blotting (HPA042420 antibody)

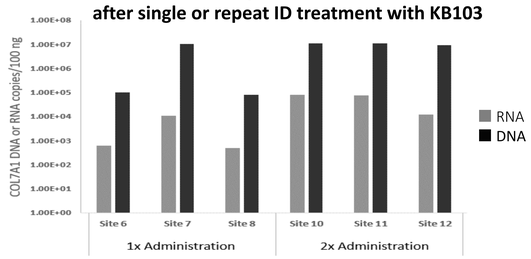
RESULTS (*in Vivo*)

Robust and widespread COL7 expression in KB103-treated RDEB mouse skin



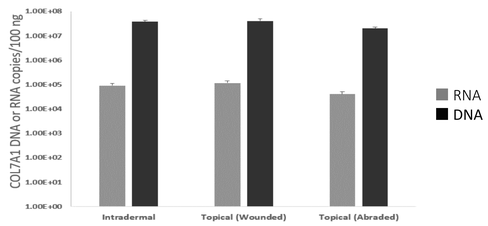
Immunofluorescent staining for human COL7 in RDEB skin. KB103 or control (HSV reporter) treated RDEB mouse skin was harvested 48 hours (Day 3) or 6 days (Day 7) after intradermal treatment on Day 1, and stained with an anti-human COL7 antibody (yellow, HPA042420). An integrin α6 antibody (red) was used to stain for Basement Membrane Zone (BMZ). Nuclear staining done with Hoechst compound . Scale bar is 100 micron.

Human COL7A transcripts detected in mouse RDEB skin after single or repeat ID treatment with KB103



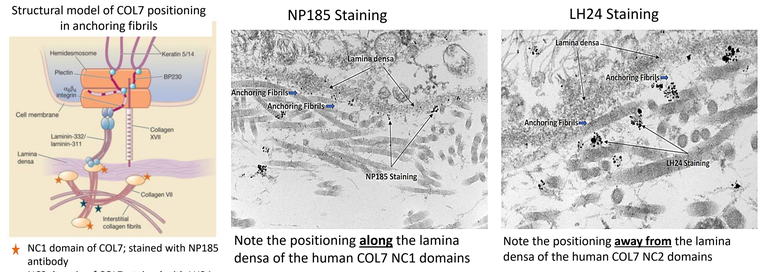
Quantitative RT-PCR analysis of COL7A1. RDEB mouse skin homogenates were assayed on Day 7 following 1X (Day 1) or 2X (Day 1 and 3) intradermal administration of KB103

KB103 can be administered by ID or topical application



Quantitative PCR and RT-PCR detection of COL7A1 in BALB/c mice. BALB/c mice were treated with KB103 by ID injection into intact skin or topical application on wounded or abraded skin. Skin homogenates, harvested 48-hours after treatment were assayed for human COL7A1 DNA and RNA copies.

Initiation of nascent anchoring fibrils formation in KB103-treated RDEB mice



NC1 domain of COL7; stained with NP185 antibody

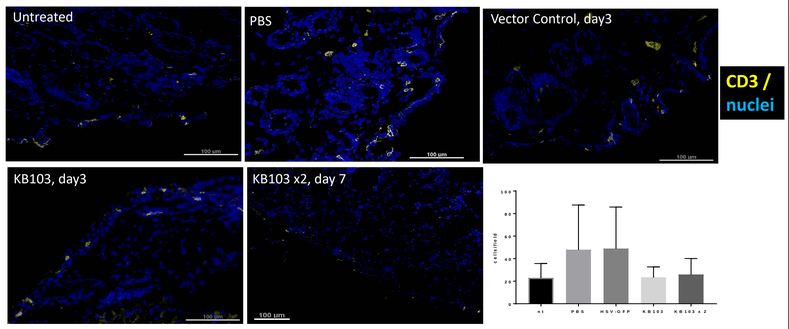
NC2 domain of COL7; stained with LH24 antibody

Note the positioning **along** the lamina densa of the human COL7 NC1 domains

Note the positioning **away from** the lamina densa of the human COL7 NC2 domains

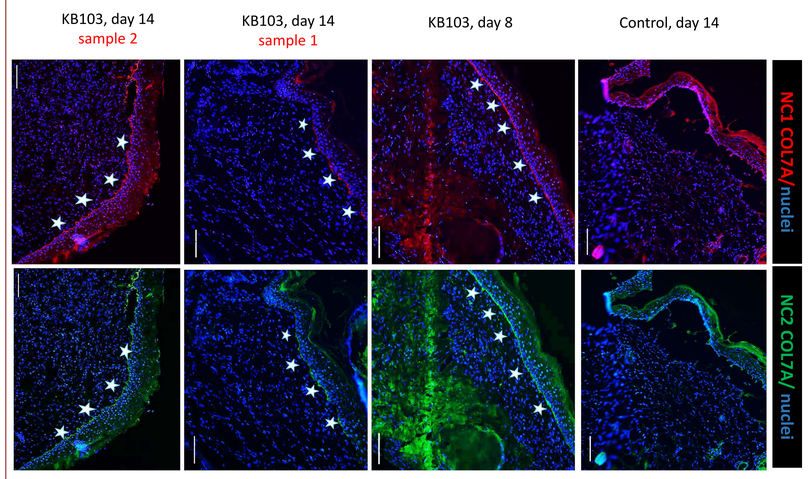
Electron micrographs (EM) of RDEB mouse skin. KB103 was administered by ID injection on Day 1 and processed for EM on Day 3.

No inflammatory infiltration after single or repeat intradermal administration of KB103



Immunofluorescent staining for CD3+ T cells in RDEB skin. RDEB skin treated with KB103, PBS, or Vector Control (HSV reporter) on Day 1 was harvested on Day 3 or 7 and stained with an anti-CD3 antibody. Untreated skin served as a control.

Linear deposition of COL7 at the BMZ and enhanced structural integrity in KB103-topically treated RDEB xenografts



Immunofluorescent detection of human COL7 in RDEB skin xenografted NSG mice. KB103 or a control HSV vector was topically applied on the RDEB grafts. The tissues were harvested and analyzed 8 and 14 days after treatment. Human COL7 was stained with 2 antibodies: NP185 (red), LH24 (green). Hoechst compound stains the nuclei. Scale bar = 100 micron.

CONCLUSIONS

We demonstrate that KB103 efficiently transduces RDEB fibroblasts and keratinocytes *in vitro,* resulting in KB103 dose-dependent supraphysiological human COL7 expression, without any obvious toxicity even at high doses. When administered by intradermal injection to RDEB mouse skin, KB103 showed no toxicity, robust production and distribution of COL7 around hair follicles and surrounding dermis, linear deposition along the BMZ, and most importantly, the human COL7 incorporated into anchoring fibrils with proper structural orientation. Studies in BALB/c mice demonstrated that KB103 can be equally efficiently delivered by topical or ID application. Finally, in primary regenerated human RDEB skin xenografts, *in vivo* topical application of KB103 yielded robust linear deposition of COL7 at the BMZ. Enhanced structural integrity of KB103-treated xenografts was also observed . Together these studies strongly support clinical translation. **An IND application to evaluate KB103 in RDEB patients has been cleared by the FDA, and the clinical trial was initiated in May 2018.**

REFERENCES

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