

HSV-1 mediated COL7A1 (KB103) delivery to keratinocytes and fibroblasts for recessive dystrophic epidermolysis bullosa (RDEB) therapy: preparations for Phase-I clinical trials

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Abstract

Current gene therapy for Recessive dystrophic epidermolysis bullosa (RDEB) requires processing of primary cells ex-vivo resulting in significant costs in manufacturing and lead time in production. We have produced a novel engineered replication defective HSV-1 vector encoding COL7A1 transgene (KB103) for off-the-shelf application in RDEB patients. KB103 can be injected or topically formulated for direct application to open wounds. RDEB primary fibroblasts and keratinocytes are efficiently transduced by KB103 *in vitro*, leading to detection of recombinant collagen 7 (C7) by immunostaining and Western blotting. COL7A1 corrected RDEB cells showed functional restoration in adhesion assays and KB103 efficiently deposited C7 at the dermal-epidermal junction in organotypic cultures and in mouse skin after intradermal injection. Clinical grade KB103 is currently being manufactured under GMP and will undergo release testing and characterization for optimal formulation, feasibility and safety in animal studies ahead of phase-I clinical trial.

Goal of the study

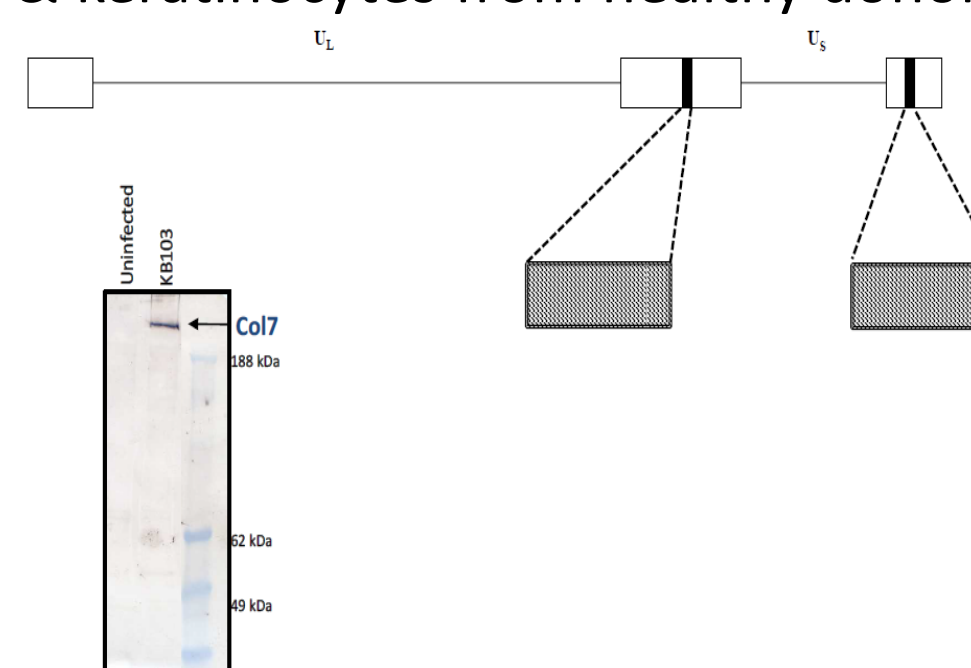
Preclinical assessment of HSV-1 mediated COL7A1 delivery *in vitro* and *in vivo*

Why to use HSV-1 for gene therapy?

Broad host cell range (including epithelial cells)
Highly infectious
Dividing and non-dividing cells targeted
Large payload (150Kb)
Easily purified to high titer
Does not integrate into the host genome
Already proven to be safe for human in clinical trials

Materials and Methods

Figure 1: Replication-deficient HSV-1 engineered to express 2 copies of human type VII collagen under the CMV promoter (KB103). The vector was used to transduced primary fibroblasts & keratinocytes from healthy donors and RDEB patients.



- Indirect-immunofluorescence
- Immuno-blotting
- Q-RT-PCR
- Adhesion to matrix coated tissue culture
- Organ cultures
- Intradermal injection to SKH1-elite hairless mice

Results

Figure 1: HSV-1 delivers COL7A1 to primary fibroblasts and keratinocytes *in vitro* with high efficiency. Observed by Immunofluorescence (A) and Immuno-blotting (B).

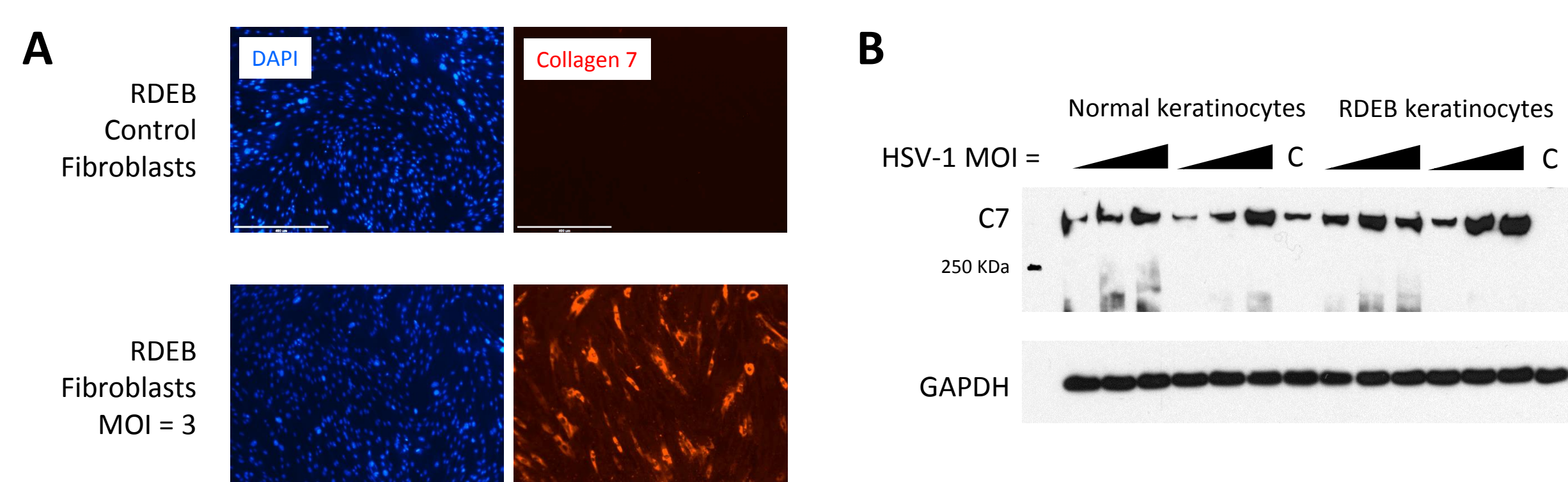


Figure 3: HSV-1 COL7A1 localizes to the epidermal-dermal junction in organ cultures.

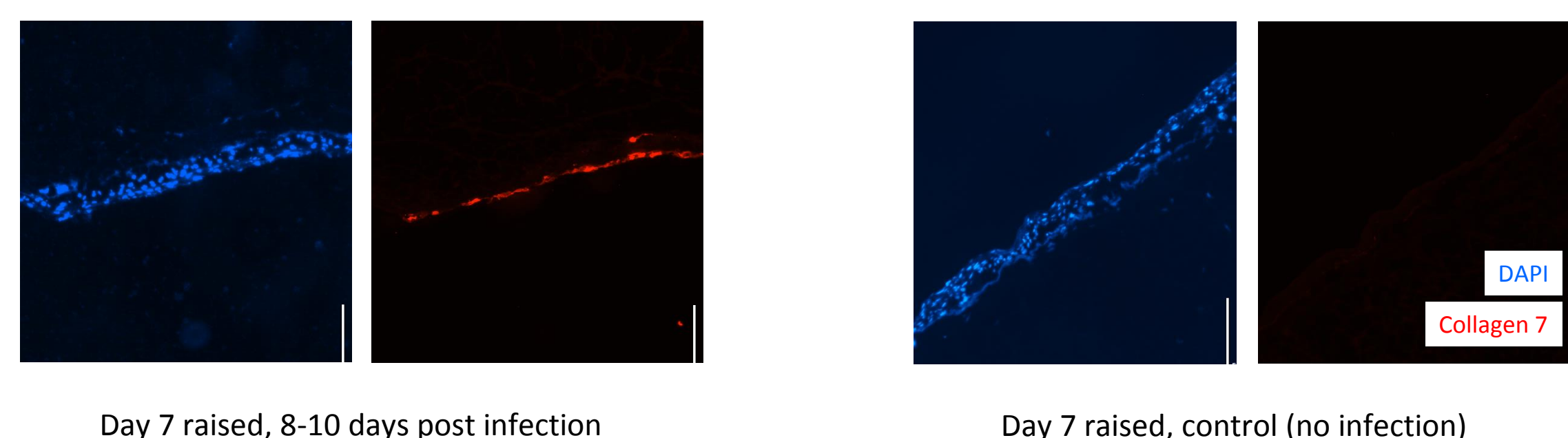


Figure 2: HSV-1 COL7A1 mediates increased adhesion of primary RDEB keratinocytes to fibronectin (A) and type I collagen (B).

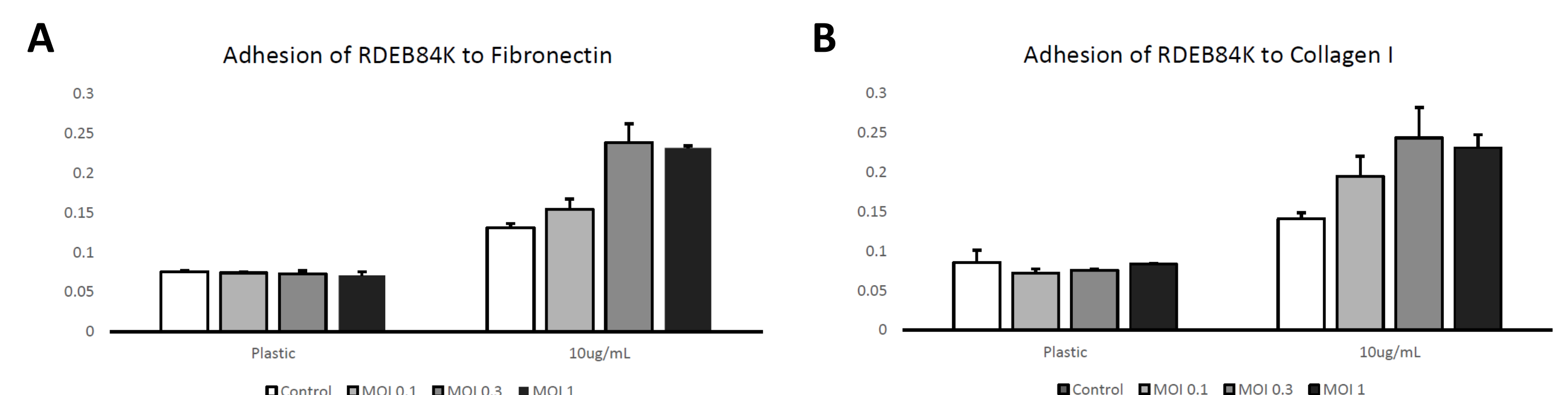
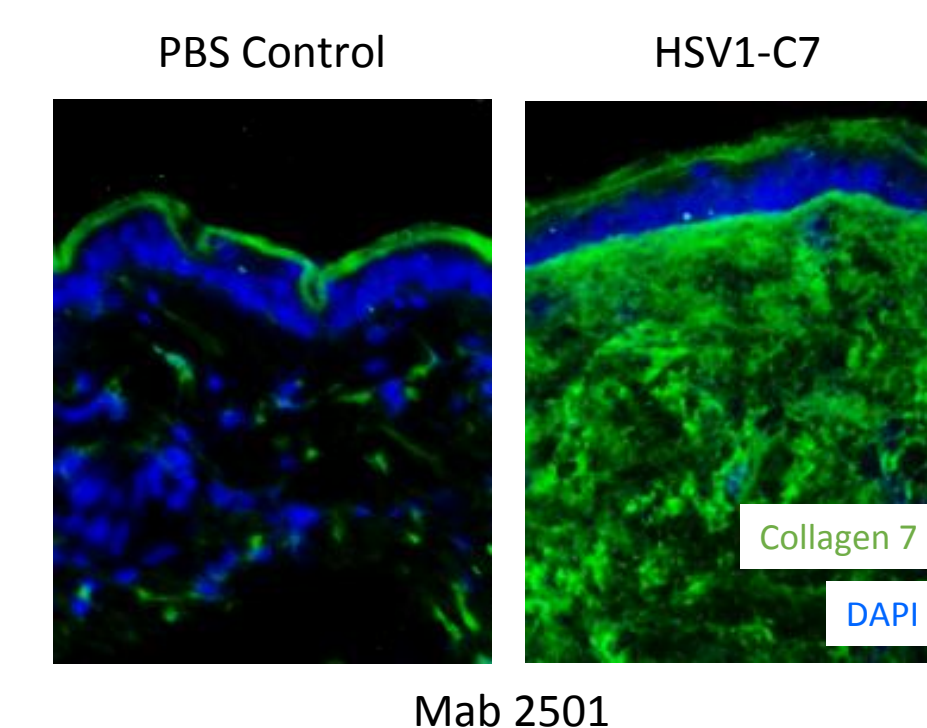


Figure 4: HSV-1 COL7A1 localizes to the epidermal-dermal junction *in vivo*.



Clinical Trial Design: Projected to begin Phase I Spring 2018

Phase I: 3 adult RDEB subjects and Phase II: 6 subjects age 5 and older

Vector will be applied via two approaches:
1) Topical to wounds
2) Intradermal injection to intact skin

End points: Safety, C7 expression, anchoring fibril formation (primary) and duration of wound closure of treated wound compared to untreated wound (secondary)