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 makes 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.

METHODS

Primary Cells

<table>
<thead>
<tr>
<th>Cells</th>
<th>Diagnosis</th>
<th>Human COL7 Antibodies</th>
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<tbody>
<tr>
<td>Keratinocytes</td>
<td></td>
<td>HPA042420</td>
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<tr>
<td>Fibroblasts and keratinocytes</td>
<td></td>
<td>NC1 domain</td>
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<tr>
<td>Normal</td>
<td></td>
<td>NC2 domain</td>
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<tr>
<td>RDEB</td>
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RDEB mice

Homozygous Col7a1−/−mice lack both functional copies of Col7a1 and express <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 (NOG and gamma) grafts with xenografts composed of desiccated porcine dermis and RDEB keratinocytes (2).

Test Article

KB103 : Krystal Biotech, Inc's proprietary replication deficient HSV-1 vector encoding COL7A1 (KB103) encoding COL7A1 for direct in vivo administration to RDEB skin either by intradermal injection or by topical administration.

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).

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, COL7A1 expression was assayed using a SYBR green based COL7A1 assay. Data is presented as fold-change in expression relative to normal (WT) cells.

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, COL7A1 expression was assayed using a SYBR green based COL7A1 assay. Data is presented as fold-change in expression relative to normal (WT) cells.

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 bullies and surrounding dermis, linear deposition along the BMZ, and most importantly, the human COL7 incorporated into anchoring fibrils with proper structural orientation. Studies in BA/Bc 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 COL7 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

(2) Ortiz-Urda A, Lin Q, Green D, Keene DR, Marinkovich MP, Khavari PA “Injection of genetically engineered fibroblasts corrects regenerated human epidermolysis bullosa skin tissue”. JCI 2008.