KB105: An HSV-Based Gene Therapy Vector Engineered to Deliver Functional TGM1 to Autosomal Recessive Congenital Ichthyosis (ARCI) Keratinocytes

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SID 2019
May 9, 2019

INTRODUCTION

Autosomal recessive congenital ichthyosis (ARCI) is a genetically and phenotypically heterogenous group of rare cornification diseases affecting ~1:200,000 persons worldwide. The most common cause of ARCI is an inactivating mutation in the gene encoding transglutaminase 1 (*TGM1*). Functional TGM1 protein crosslinks proteins such as loricrin and involucrin during the formation of the cornified cell envelope. This layer, known as the stratum corneum, acts as a mechanical barrier to protect against water loss and infectious agents.

In ARCI patients lacking functional TGM1, proper cornification does not occur, leading to disruption of the epidermal barrier, dehydration, exposure to microorganisms/viruses, and increased risk of infection. ARCI patients are typically born encased in a collodion membrane that sheds after ~2 weeks. After shedding the collodion membrane, individuals are often left with large, dark, plate-like scales covering the skin, and often suffer from and may experience complications such as fluid and electrolyte imbalances, failure to thrive, and heat intolerance. Patients also suffer from ectropion, eclabium, and transepidermal water loss.

Current therapeutic options are inadequate, providing limited symptomatic relief without restoration of epidermal barrier function or molecular correction of the underlying disease. To this end we have engineered KB105, a novel replication-defective HSV-1 gene therapy vector encoding an optimized *TGM1* transgene, amenable to topical treatment to correct the impaired epidermal barrier in ARCI patients. Here we demonstrate *in vitro* efficacy of KB105 in TGM1-deficient patient keratinocytes. Poster 383 details *in vivo* preclinical safety and pharmacology of KB105. A Phase 1 clinical trial is planned for 2019.

MATERIALS/METHODS

Test Article

KB105: Krystal Biotech, Inc.'s propriety replication-defective HSV-1 vector encoding optimized human *TGM1*

Table 1. Primary and Immortalized Cells

Cell Type	Diagnosis	Mutation
ARCI patient keratinocytes	ARCI	e5 hom c.877-2 A>G (splice site mutant)[2]
Normal Patient Keratinocytes	Normal	Assume normal

Table 2. Antibodies

Antibody Description	Source	Cat no.
Rabbit anti-hTGM1 (polyclonal)	Abcam	ab103814
Goat anti-rabbit IgG, AP-conjugated antibody	Sigma	A3687
Goat anti-rabbit IgG, Alexa-Fluor 488-conjugated Antibody	Thermo Fisher	11034

Table 3. *In situ* transglutaminase assay reagents

Reagent Description	Source	Cat no.
TGM-1 specific substrate – K5	Zedira	B007
Z-DON (TGM-2 inhibitor)	Zedira	Z006

RESULTS

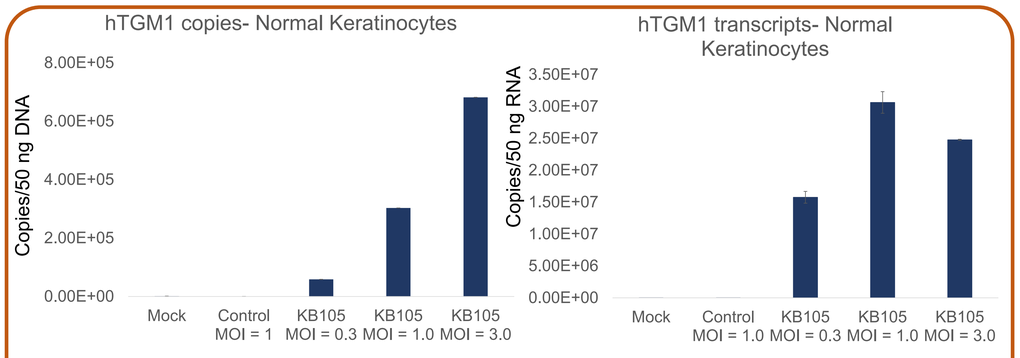

Figure 1. MOI-dependent increase in TGM1 DNA and transcript levels upon KB105 infection in immortalized normal human keratinocytes

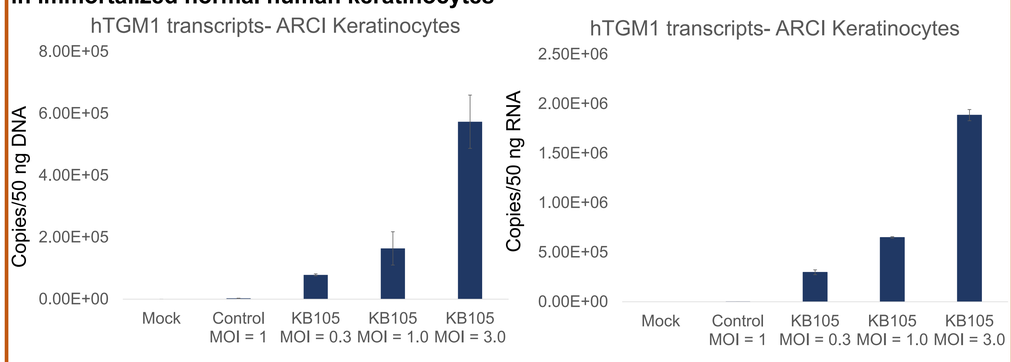

Figure 2. MOI-dependent increase in TGM1 DNA and transcript levels upon KB105 infection in immortalized TGM1-deficient ARCI human keratinocytes

RESULTS (CONTINUTED)

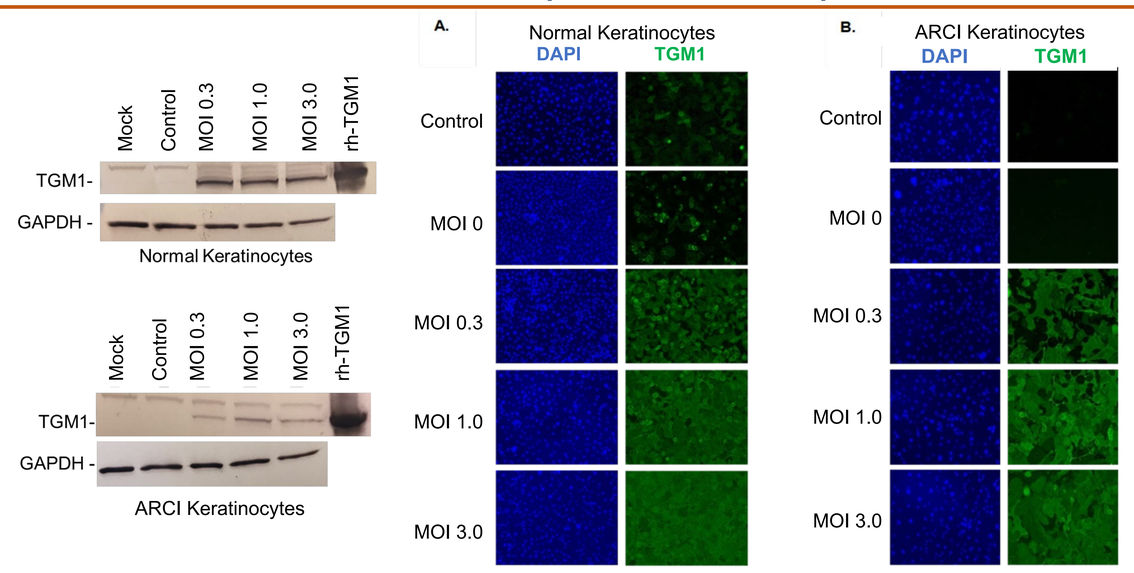

Figure 3. MOI-dependent increase in TGM1 protein levels upon KB105 infection in immortalized normal human and TGM1-deficient ARCI human keratinocytes

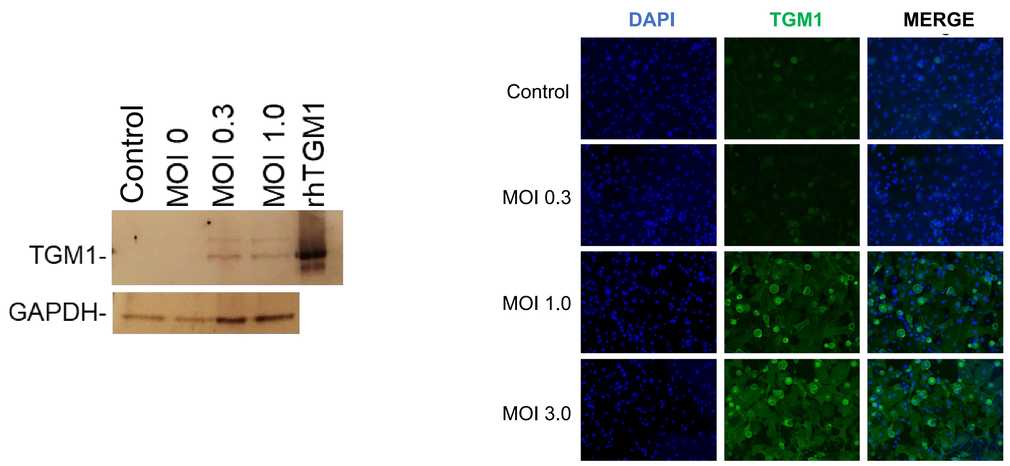

Figure 4. Dose-dependent TGM1 protein expression in primary ARCI patient keratinocytes infected with KB105

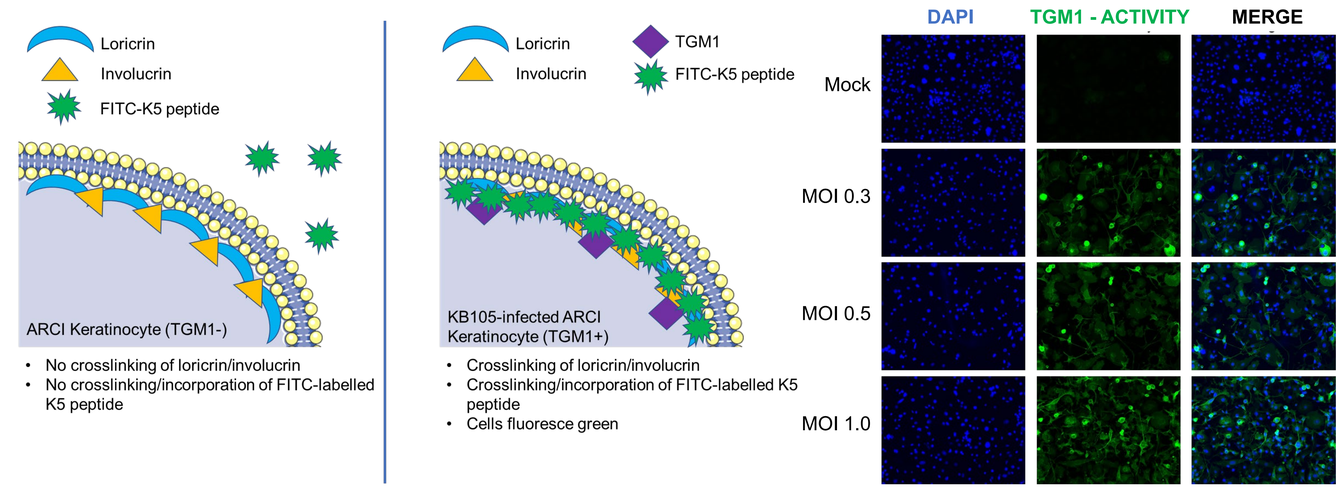

Figure 5. Dose-dependent TGM1 activity as measured by conjugation of a fluorescent peptide in immortalized ARCI keratinocytes infected with KB105

CONCLUSIONS

- KB105 can efficiently transduce TGM1-deficient patient cells, resulting in a dose-dependent increase in TGM1 expression at the DNA, RNA, and protein level.
- KB105-expressed TGM1 produced in ARCI-derived patient cells is functional, and restores transglutaminase enzymatic activity in these cells
- These data, along with *in vivo* data presented at this conference, support application of KB105 for treatment of ARCI

ACKNOWLEDGEMENTS

We kindly thank Dr. Keith Choate (Yale University) for providing us with primary and immortalized normal and ARCI patient-derived keratinocytes.