In Vitro and *In Vivo* Pharmacology of KB301, an HSV-1-Based Gene Therapy, for the Treatment of Superficial Skin Depressions

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INTRODUCTION

Due to the essential role collagen plays in the process of skin biorejuvenation, and the diminution of dermal collagen being a significant contributor to the aged phenotype, direct and indirect collagen stimulation/supplementation/replacement has been a focus of cosmetic product development[1-3]. However, directed supplementation of functional full-length human type III collagen (COL3), produced by and secreted from the subject's own dermal cells, has not been explored clinically. To this end, we engineered KB301, a replication-defective HSV-1 gene therapy vector, for the targeted delivery of human COL3.

OBJECTIVES

Our preclinical program explored KB301's ability to transduce clinically relevant skin cells and express and secrete mature human COL3 *in vitro*, as well as to confirm proper tissue localization of the transgene without toxicity or systemic vector distribution *in vivo*. This preclinical program used, in part, primary human dermal fibroblasts (HDFs) harvested from aged patients (65- to 75-years-old) *in vitro* and 12- to 13-month-old mice (equivalent to 38- to 49-year-old humans[4]) *in vivo* as representative models for studying COL3 supplementation.

MATERIALS & METHODS

Test Article

KB301: Krystal Biotech, Inc.'s propriety replication-incompetent, non-integrating HSV-1 vector expressing full-length human COL3.

Table 1. Critical Reagents

Reagent	Application	Source
Primary human dermal fibroblasts	*In vitro* dose-ranging	Lonza (cat. no. CC-2511)
Anti-human COL3	Western blot/IF (*in vitro*)	Abcam(cat. no. ab7778)
Anti-rabbit IgG (AP conjugated)	Western blot	Sigma (cat. no. A3687)
Anti-human GAPDH	Western blot	Abcam (cat. no. ab9485)
Recombinant human COL3	Western blot	Abcam (cat. no. ab73160)
MTS assay kit	Cell viability	Abcam (cat. no. ab197010)
Anti-rabbit IgG (AlexaFluor® 488 conjugated)	IF (*in vitro*)	ThermoFisher (cat. no. A11034)
Anti-human COL3	IF (*in vivo*)	Origene (cat. no. AF5810)
Anti-mouse IgG (AlexaFluor® 594 conjugated)	IF (*in vivo*)	Abcam (cat. no. 150120)

RESULTS

In Vitro KB301 Dose-Ranging Analysis in Primary Human Dermal Fibroblasts

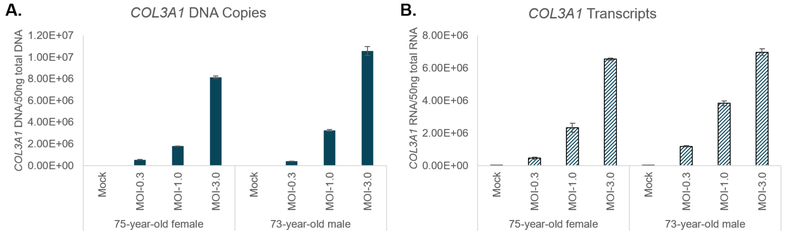

Figure 1. Dose-dependent increases in *COL3A1* DNA (A) and transcript levels (B) upon KB301 transduction of primary aged HDFs.

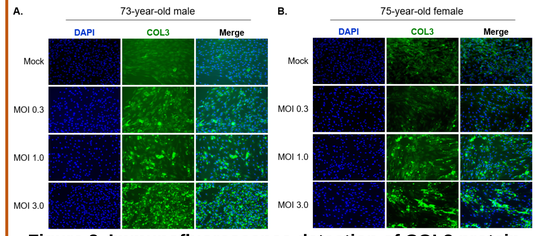

Figure 2. Immunofluorescence detection of COL3 protein expression in male (A) and female (B) primary aged HDFs.

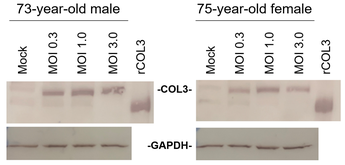

Figure 3. Intracellular COL3 protein expression in KB301-transduced male and female primary aged HDFs.

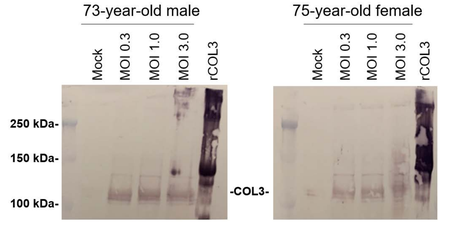

Figure 4. COL3 protein secreted into cell supernatants of KB301-transduced male and female primary aged HDFs.

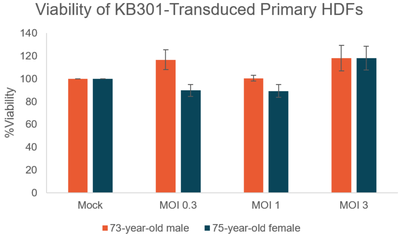

Figure 5. Viability of Male and Female Primary HDFs after KB301 transduction.

In Vivo Pharmacodynamics in Young and Aged Immunocompetent Mice

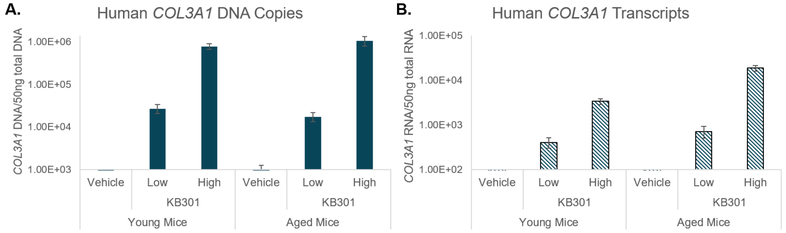

Figure 6. Human *COL3A1* DNA (A) and transcript (B) levels in treated skin 48-hours after intradermal administration of KB301 to young (6-8 weeks old) and aged (13 months old) mice.

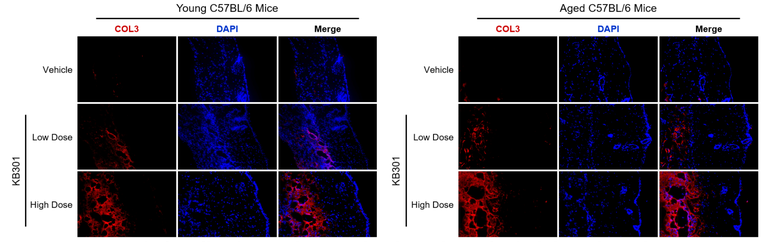

Figure 7. COL3 protein localization 48-hours after intradermal administration of KB301 to young (6-8 weeks old) and aged (13 months old) mice.

Table 2. Human *COL3A1* DNA and RNA biodistribution and pharmacokinetics

Test Article	Termination (hours)	Dose Site (genome copies/50ng DNA)	Dose Site (transcripts/50ng RNA)
Vehicle	4	<LOD	<LOD
	24	<LOD	<LOD
	168	<LOD	<LOD
KB301	4	$2.39\times10^{6}\pm6.97\times10^{5}$	$3.64\times10^{5}\pm1.17\times10^{5}$
	24	$1.95\times10^{5}\pm5.40\times10^{4}$	$2.35\times10^{4}\pm6.10\times10^{3}$
	168	$2.21\times10^{2}\pm9.55\times10^{1}$	$5.63\times10^{1}\pm4.08\times10^{0}$
All other samples (blood, bone marrow, lymph nodes, testis, brain, liver, lungs, heart, spleen, and kidney) were below the limit of detection (LOD).			

CONCLUSIONS

Results from these proof-of-concept studies and safety assessments support the application of KB301 for the treatment of shallow-to-moderately deep wrinkles and other superficial skin depressions. A Phase I clinical trial of repeat-dose KB301 is underway (NCT04540900).

REFERENCES

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2: Liu *et al.* 2005. *Semin Plast Surg* 19 (3): 241-50.
3: Yutskovskaya *et al.* 2014. J Drugs Dermatol 13 (9): 1047-52
4: Flurkey *et al.* 2009. In The Jackson Laboratory Handbook on Genetically Standardized Mice, 329-331.

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