Engineered Herpes Simplex Virus Type 1 (HSV-1)-based Vectors as a Platform for Localized Delivery of Therapeutic Antibodies in the Treatment of Skin Disorders

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Introduction

- In recent years, therapeutic antibodies have become one of the most successful classes of biopharmaceutical drugs
- While antibodies have shown success in the treatment of certain cancers, as well as several autoimmune, cardiovascular, and inflammatory disorders, systemic administration of therapeutic antibodies has several functional limitations, including inadequate pharmacokinetics and tissue accessibility
  - In addition, systemic exposure to certain antibodies has been shown to repress the immune system, exposing the patient to significant risk of infections and other complications
- HSV-1 has emerged as a promising gene therapy vector for the treatment of disorders of the skin and other organ systems, and recently, Krystal Biotech, Inc. (Krystal) has leveraged its HSV-1-based STAR-D (Skin-Targeted Delivery) platform to deliver therapeutic transgenes to patients with debilitating diseases
- Krystal is continually evaluating opportunities to expand beyond rare genetic diseases using its STAR-D platform and has embarked upon a discovery program to assess the potential for its vectors to deliver and locally express therapeutic single-chain antibodies (sc-Abs)
- Results from relevant model systems of two such STAR-D-based vectorized antibodies, KB501 (expressing a tumor necrosis factor [TNF]α-targeting antibody) and KB502 (expressing an interleukin [IL]-4Rα–targeting antibody), are presented herein

Materials and Methods

- Molecular efficacy and disease correction were assessed for KB501 and KB502 *in vitro* (2-dimensional cell culture) and/or *in vivo* (two murine models of atopic dermatitis)

Table 1. Antibodies and ELISA kits

Antibody Description	Source	Catalog No.
Human IgG	Abcam	ab109489
β-actin	LI-COR Biosciences	926-42212
Goat anti-rabbit IgG, Alexa Fluor 594	Abcam	ab150080
Human IgG ELISA kit	Abcam	ab195215
Human TNF alpha ELISA kit	Abcam	ab181421
Mouse TNF alpha ELISA kit	RayBiotech, Inc	ELM-TNFa-CL-1

ELISA, enzyme-linked immunosorbent assay; Ig, immunoglobulin.

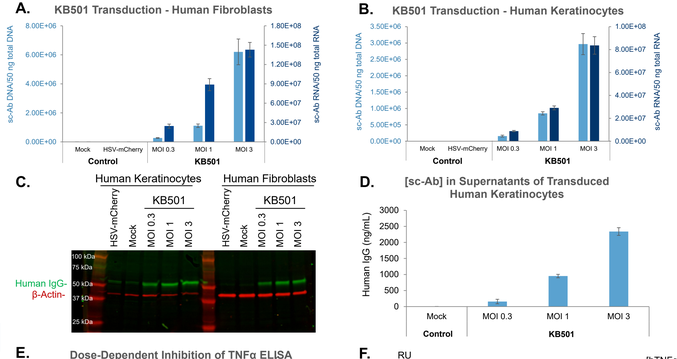
Table 2. Reagents used in murine models

Reagent	Source	Catalog No.	Application Dosage		Application Days
Oxazolone (OXA)	Sigma	E0753-1G	sensitization	OXA/EtOH 2% (w/v)	50 µL/site on Day 0 and Day 1
			challenge	OXA/EtOH 15% (w/v)	10 µL/site on Day 7
Calcipotriol (MC903)	Sigma	C4369-10MG	100 µM in EtOH		25 µL/site on Days 1-5

EtOH, ethanol.

Results: KB501 (an HSV-vectorized TNFα-targeting antibody)

Figure 1. *In vitro* analysis of KB501 in human skin cells[b]



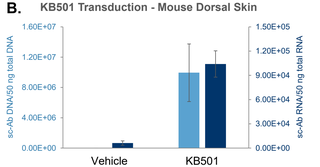
[b]Data presented as average ± SEM. [c]Relative percent of TNFα detected in each condition assuming medium + TNFα control as 100%. MOI, multiplicity of infection.

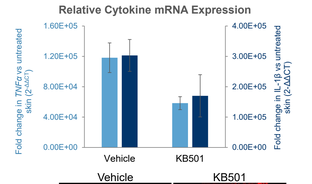
- Dose-ranging detection of vectorized antibody DNA and transcripts in KB501-transduced immortalized human fibroblasts (**Figure 1A**) and keratinocytes (**Figure 1B**), determined via quantitative polymerase chain reaction (qPCR) and real-time quantitative polymerase chain reaction (qRT-PCR) analyses, respectively
- Dose-ranging detection of full-length vectorized antibody protein in KB501-transduced human keratinocytes and fibroblast via western blot (**Figure 1C**) and antibody concentration in cell supernatants harvested from KB501-transduced keratinocytes via ELISA (**Figure 1D**)
- Detection of recombinant human TNFα spiked into cell culture supernatants harvested from KB501-transduced keratinocytes, as assessed by ELISA (**Figure 1E**) and Biacore™ surface plasmon resonance (SPR) analysis quantifying antibody affinity of the KB501-encoded sc-Ab for human TNFα (**Figure 1F**)

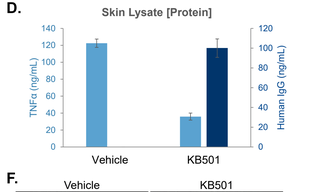
Figure 2. Intradermal injection of KB501 in an oxazolone-induced murine model of TNFα induction[d]

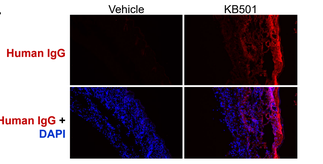
A.

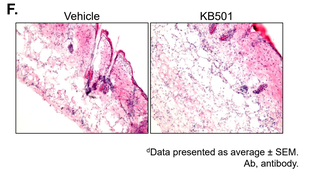
Group No.	Sensitizing Agent	Test Article	Mouse Strain
1	Oxazolone	Vehicle	BALB/c
2	Oxazolone	KB501	BALB/c











[d]Data presented as average ± SEM. Ab, antibody.

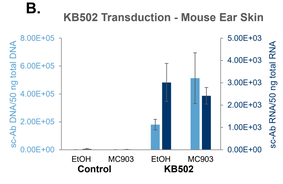
- *In vivo* study outline and design (**Figure 2A**)
- Detection of vectorized antibody DNA and transcripts in KB501-transduced mouse dorsal skin biopsies, determined via qPCR and qRT-PCR analyses, respectively (**Figure 2B**)
- Relative expression of mouse *TNFα* and *IL-1β* transcripts in vehicle and KB501-treated skin vs non-oxazolone–treated murine skin. *GAPDH* was used as the housekeeping gene (**Figure 2C**)
- Mouse TNFα and human IgG protein concentrations in skin biopsies harvested from KB501- or vehicle-treated sites, as assessed by ELISA (**Figure 2D**)
- Representative immunofluorescence images of human IgG in skin biopsies harvested from KB501- or vehicle-treated sites, with DAPI used to stain nuclei (**Figure 2E**)
- Representative hematoxylin and eosin stained dorsal skin biopsies harvested from KB501 or vehicle treated sites (**Figure 2F**)

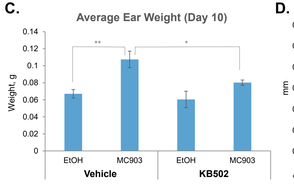
Results: KB502 (an HSV-vectorized IL-4Rα-targeting antibody)

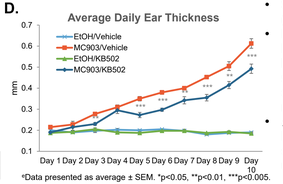
Figure 3. Topical application of KB502 in an acute, MC903-induced atopic dermatitis animal model[e]

A.

Group No.	Sensitizing Agent	Test Article	Mouse Strain
1	Ethanol (EtOH)	Vehicle	C57BL/6J
2	MC903/EtOH	Vehicle	C57BL/6J
3	EtOH	KB502	C57BL/6J
4	MC903/EtOH	KB502	C57BL/6J







*Data presented as average ± SEM. *p<0.05, **p<0.01, ***p<0.005.

- Study outline and design (**Figure 3A**)
- Detection of vectorized antibody DNA copies and transcripts in KB502-transduced mouse ear skin biopsies, determined via qPCR and qRT-PCR analyses, respectively (**Figure 3B**)
- Average whole ear weight, Study Day 10 (**Figure 3C**), and average ear thickness by day, measured using digital calipers (**Figure 3D**)

Results: STAR-D antibody portfolio

Table 3. Concentration of sc-Abs from cells infected with STAR-D-based vectorized antibody constructs

Antibody Name	IgG Type	Target	Concentration in Vero Cell Supernatant (ng/mL)
KB501	Human IgG1	Human TNFα	5844.3724
Ab1Fc2	Human IgG1	Human TNFα	2880.65185
Ab2Fc2	Human IgG1	Human CD20	16132.458
Ab5Fc1	Human IgG1	Human IL-17	>750
Ab5Fc2	Human IgG1	Human IL-17	>750
Ab37Fc1	Human IgG4	Human IL-4Rα	495.7056639
Ab37Fc2	Human IgG4	Human IL-4Rα	>750
KB502	Mouse IgG1	Mouse IL-4Rα	366.1442
Ab66Fc2	Mouse IgG1	Mouse IL-4Rα	357.26944
Ab67Fc2	Human IgG1	Mouse IL-17	>750
Ab68Fc1	Human IgG1	Human CCR4	>750
Ab68Fc2	Human IgG1	Human CCR4	>750

- Krystal has expanded its STAR-D-based antibody portfolio to at least 12 unique products (**Table 3**)

Conclusions

- **Antibody-encoding vectors capably transduce human keratinocytes and fibroblasts, leading to robust expression and secretion of functional sc-Abs**
- **Intradermal administration of KB501 reduced both TNFα transcript and protein expression in an oxazolone-induced murine model of TNFα induction**
  - **KB501 administration also reduced the expression of a downstream marker of TNFα signaling, IL-1β, indicating the molecular efficacy of STAR-D-based vectorized antibodies**
- **Topical application of KB502 to atopic dermatitis-like lesions reduced the atopic dermatitis-like phenotype on a macro-scale, highlighting phenotypic correction induced by STAR-D-based vectorized antibodies**
- **Taken together, these data demonstrate the potential of STAR-D for inducing disease correction following the localized administration of vectors expressing therapeutic antibodies**
- **This novel platform warrants further investigation for the treatment of diseases of the skin and other organ systems amenable to immunomodulatory therapeutics**

Acknowledgments

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Disclosures

All authors were employed by Krystal Biotech Inc during data generation. [a]JCF, BP, ACH, and PA are no longer employees of Krystal Biotech Inc.