First in Human use of a Novel *In Vivo* Gene Therapy for the Treatment of Autosomal Recessive Congenital Ichthyosis: Results of a Phase I/II Placebo Controlled Trial

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Relevant Conflict of Interest: Dr. Paller is an Investigator for Krystal Biotech, Inc.

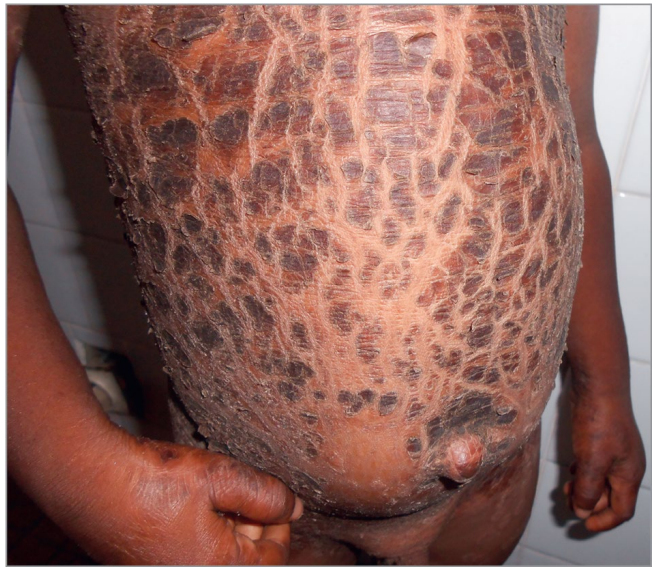
Autosomal Recessive Congenital Ichthyosis (ARCI): *TGM1* variants

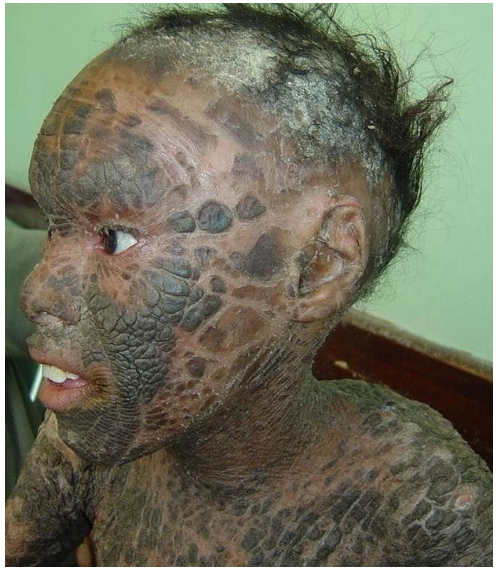
Transglutaminase 1 (TGM1)

- Crosslinks cornified envelope proteins (*e.g.*, loricrin)
- Plays critical role in skin barrier formation

Biallelic loss-of-function variants in *TGM1* lead to lamellar ichthyosis phenotype of ARCI

- Thick, plate-like scaling overlying variable erythroderma, often with ectropion and scarring alopecia
- Increased risk of dehydration, heat shock (hypohidrosis), infections, and conductive hearing loss
- Significantly decreased quality of life (*e.g.,* ostracism, life-long bullying, *etc.*)
- High burden of disease related to risks, time required for care, and psychosocial issues





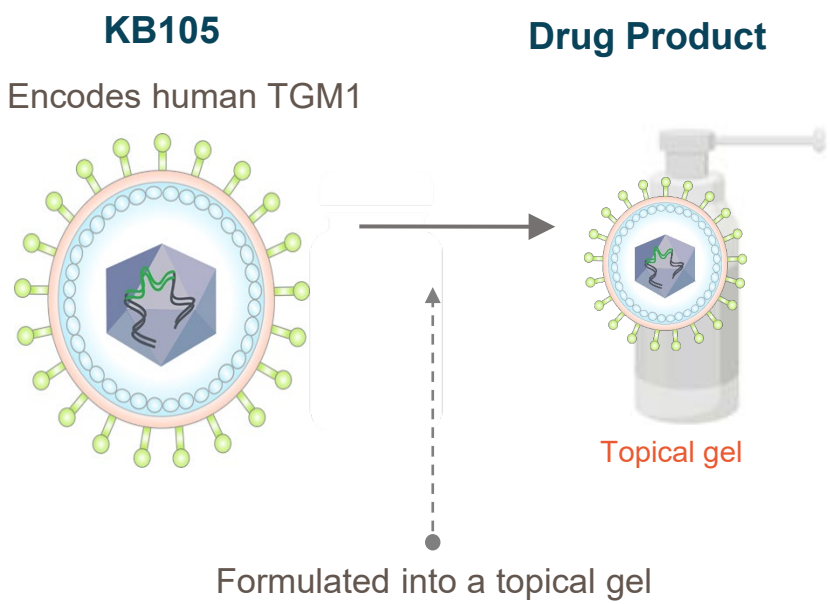
Current Standard of Care

No approved treatments for ARCI- TGM1

Topical and systemic retinoids and time-consuming supportive treatments (up to 4 hours a day of skin care) are most often used

KB105- Treatment approach

Modified Herpes Simplex Virus (HSV-1) vector with inserted multiple copies of optimized functional form of *TGM1*



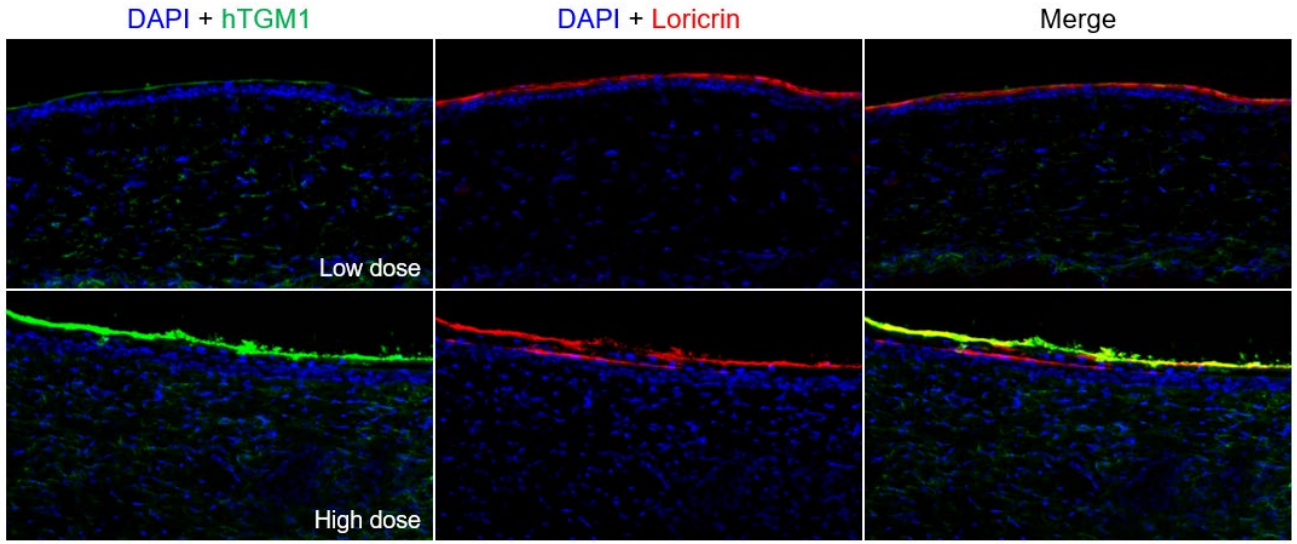
- Formulated into gel for direct application to skin
- Vector is non-integrating, non-replicating, and can accommodate multiple gene copies
- High transduction efficiency and reproducible manufacturing
- Backbone clinically validated in Phase I/II trials (11 patients with COL7A introduction topically for RDEB*); no immune response, vector shedding, or drug-related serious adverse events
- No detected systemic exposure (localized to skin)
- Vector can easily and frequently be reapplied to large areas
- Therapy can be administered by any healthcare worker or caregiver after reconstitution
- Only known disease-correcting therapy in clinical development

*http://ir.krystalbio.com/select-scientific-publications

Summary of Preclinical Data

Safety and preclinical efficacy well established for KB105

- **Efficiently transduces patient keratinocytes *in vivo* and *ex vivo*** to express functional human TGM1
- **Efficiently transduces barrier-impaired mouse skin** (tape stripped X 9 to simulate LI) to express human TGM1 in a dose-dependent manner. Exogenous TGM1 colocalized with loricrin, confirming correct localization.



Dose-dependent human TGM1 detected after topical application in mice

- **Mouse** good laboratory practice/**GLP biodistribution and toxicity study: KB105 can be safely and repeatedly administered to the skin at high doses without systemic vector exposure or adverse effects**
  - Five **weekly topical administrations** of 1.07 x 10^9 pfu/day KB105 to the dorsal mouse skin was **well tolerated** NOAEL dose: 1.07 x 10^9 pfu/day
  - **No KB105-related mortality**, clinical observations, body weight or food consumption changes, macroscopic findings, or effects on organ weight parameters were noted

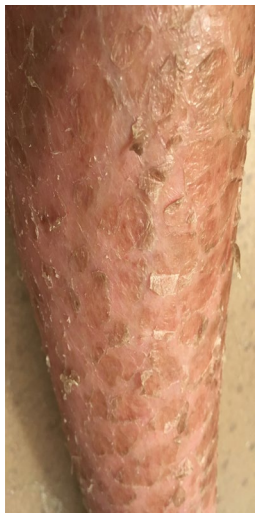
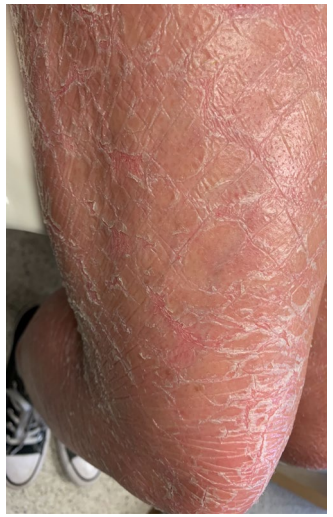
Summary of Phase 1 Trial Design: Exploratory

Study Design	Intra-subject, open label, placebo-controlled study – Exploring technique
Clinical Sites	Paddington Testing Company, Philadelphia – 3 subjects completed dosing Northwestern University, Chicago, Illinois – active, initiated enrollment
Inclusion Criteria	≥18yo with lamellar ichthyosis and genetic diagnosis of TGM1-deficient ARCI IGA (Investigator Global Assessment) score of 3-4 in target areas
Key Objectives	Demonstrate safety (incidence of adverse events, clinical pathology, immunogenicity) Demonstrate efficacy (molecular correction, phenotypic improvement)
Dose and dosing regimen	$2x10^9$pfu KB105 to treat circular skin areas of $20cm^2$ each Baseline and repeat dosing (Is microneedling required? What is optimal schedule?)
Evaluation	• Imaging of target areas – onsite and home images • Biopsies: TGM1 expression (DNA, protein) and functional activity • Skin swabs, blood, urine -Vector shedding: Pre-treatment, Days 3/ 14/ 30/ 60 • Serum - HSV and TGM1 immunogenicity: Pre-treatment, Days 3/ 14/ 30/ 60 • Vital signs, physical examination, routine chemistry and hematology

Patient Demographics and Disease Severity

Subject	Age	Gender	Genotype	Medication
1	39	Male	TGM1 c.430 G>A p.G144R & TGM1 c.456_458delCCT p.L153del	35mg oral acitretin (retinoid) daily
2	24	Female	TGM1 c.2060 G>A p.R687H	None
4*	20	Female	c.2526 A>G and c.2391 T>C	None

*Subject 3 enrolled but could not keep return visits

	1	2	4
			
IGA: Disease Severity	**4**	**3**	**3**

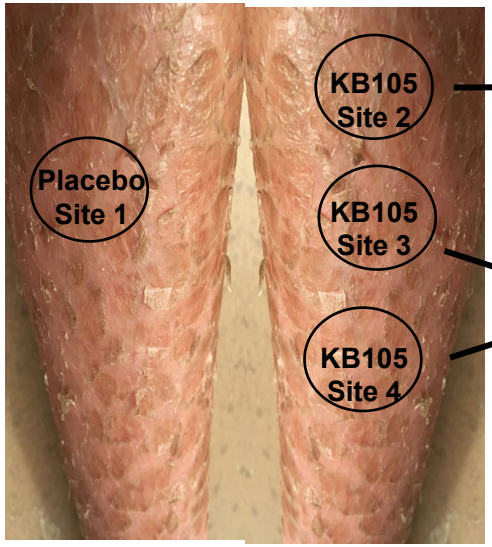
Subject 1: Dosing Regimen – No microneedling

Biopsies for transglutaminase 1 expression and function; and for qPCR for TGM1 DNA (bisected)

Screening -60 to -14	D-14 to D0	D1-D3		D15-D17		D26		D36**		D43		D57 EOS
Genetic testing	Exfoliation (per patient regimen)	Visit 1 Treated D1,D3 Biopsies (D2, D3) VS/ADA		Visit 2 Re-Treatment Biopsies (D15, D16) VS/ADA		Visit 3 Re-Treatment		Visit 4 Re-Treatment** VS/ADA		Visit 5 Re-Treatment Biopsies		Visit 6 (EOS) VS/ADA

20 cm^2 areas treated with $2x10^9$ PFU

R, L shins



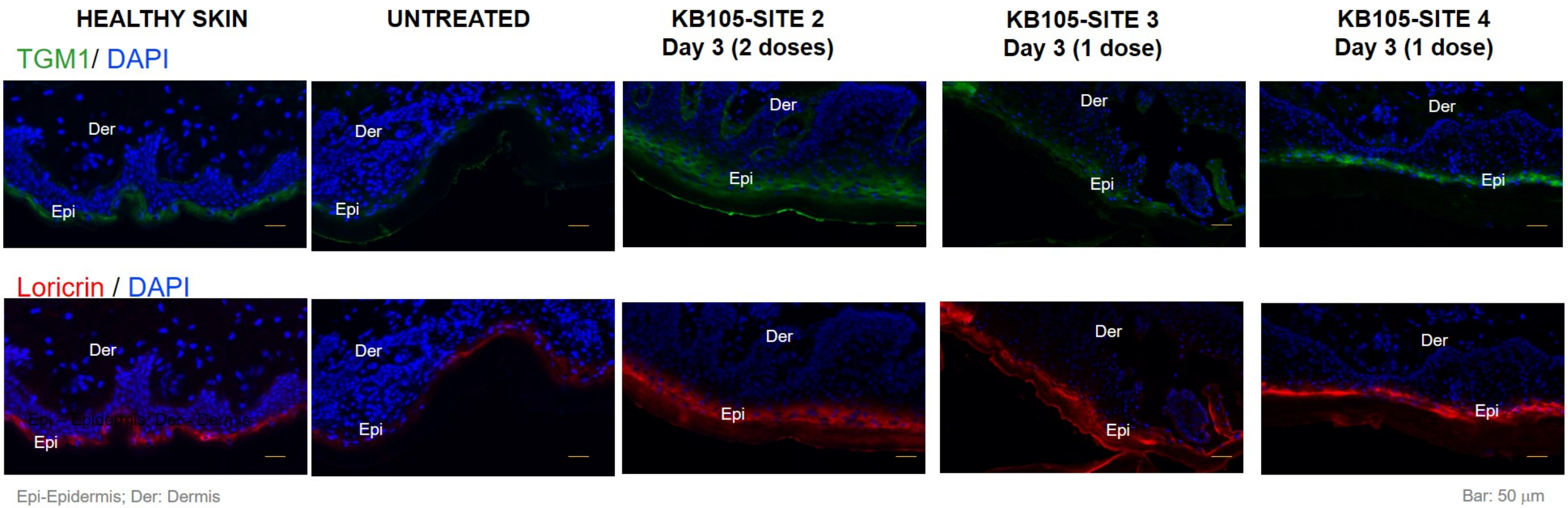
Treated D1, biopsied D2
Treated again D2; biopsied D3

Treated D1 only
Biopsied D3

VS = viral shedding (HSV)

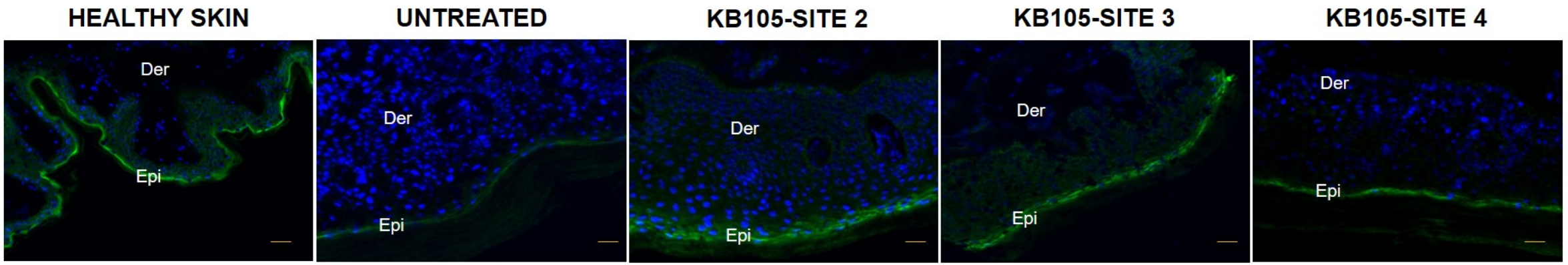
ADA = anti-HSV, anti-TGM1 antibodies

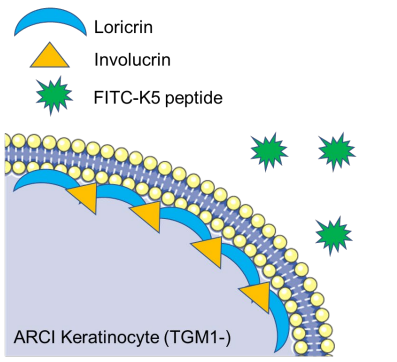
Subject 1: Treatment Restored TGM1 Expression to Normal Levels



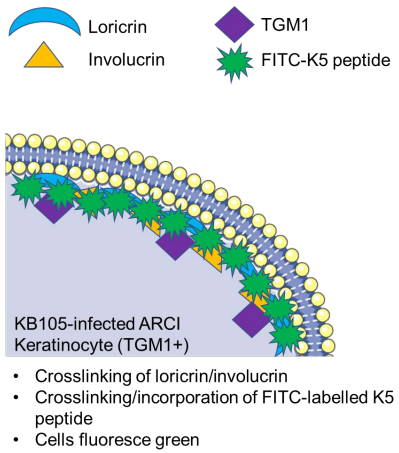
- Subject showed no TGM1 expression in untreated area.
- TGM1 expression was seen in <u>all three treated sites</u> within 48-hours
- TGM1 properly colocalized with its substrate, loricrin, in epidermis

Subject 1: TGM1 Expression Correlated with Increased *In Situ* Activity





- No crosslinking of loricrin/involucrin
- No crosslinking/incorporation of FITC-labelled K5 peptide



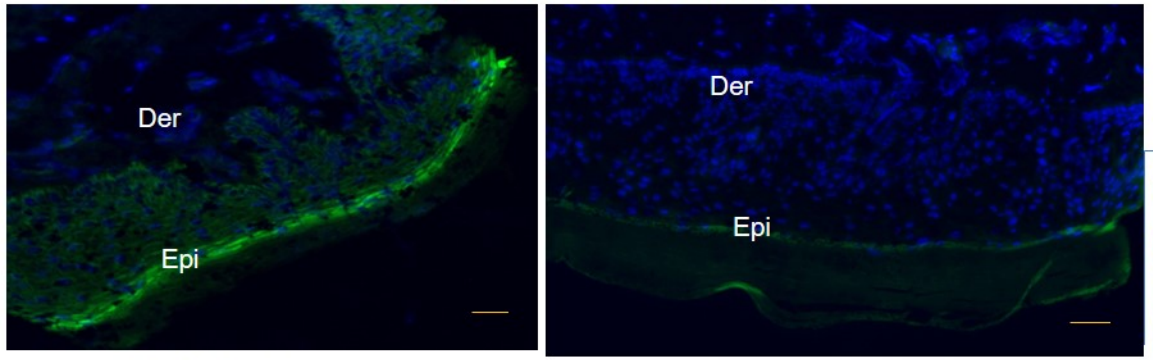
- Crosslinking of loricrin/involucrin
- Crosslinking/incorporation of FITC-labelled K5 peptide
- Cells fluoresce green

TGM1 activity was detected by an in situ fluorescent enzymatic assay that specifically detects TGM1 function*

*TGM2 inhibitor added to inhibit non-specific detection of TGM2 function

Subject 1: Repeated Administration of KB105

Site 2 - Dosing on **D1** and ~~**D2**~~ only

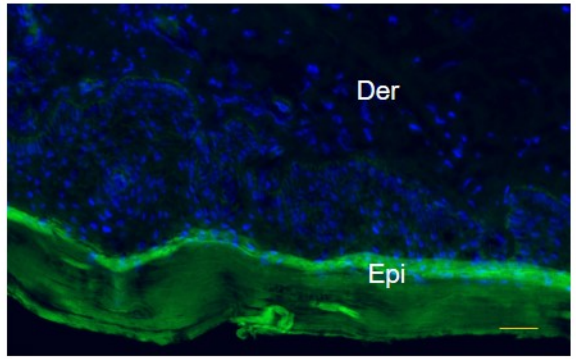


TGM1 Activity restored
Day 3

Activity declines to baseline levels
Day 15

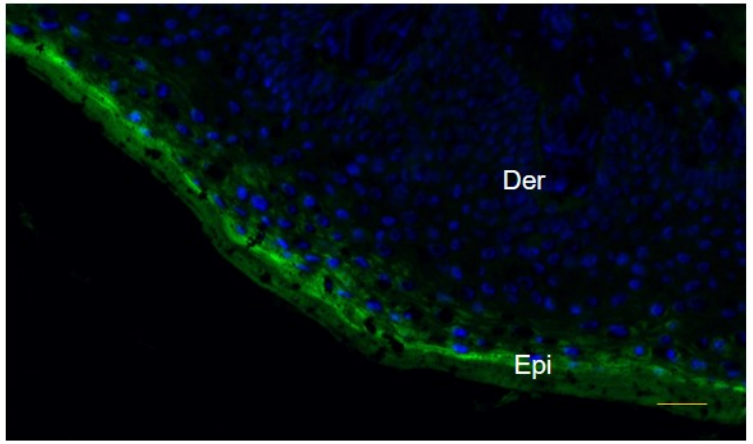
Epi-Epidermis;
Der: Dermis

Retreatment on **Day 15**



Activity increases to normal levels
Day 16

Retreatment on **Day 36**



Bar: 50 μm

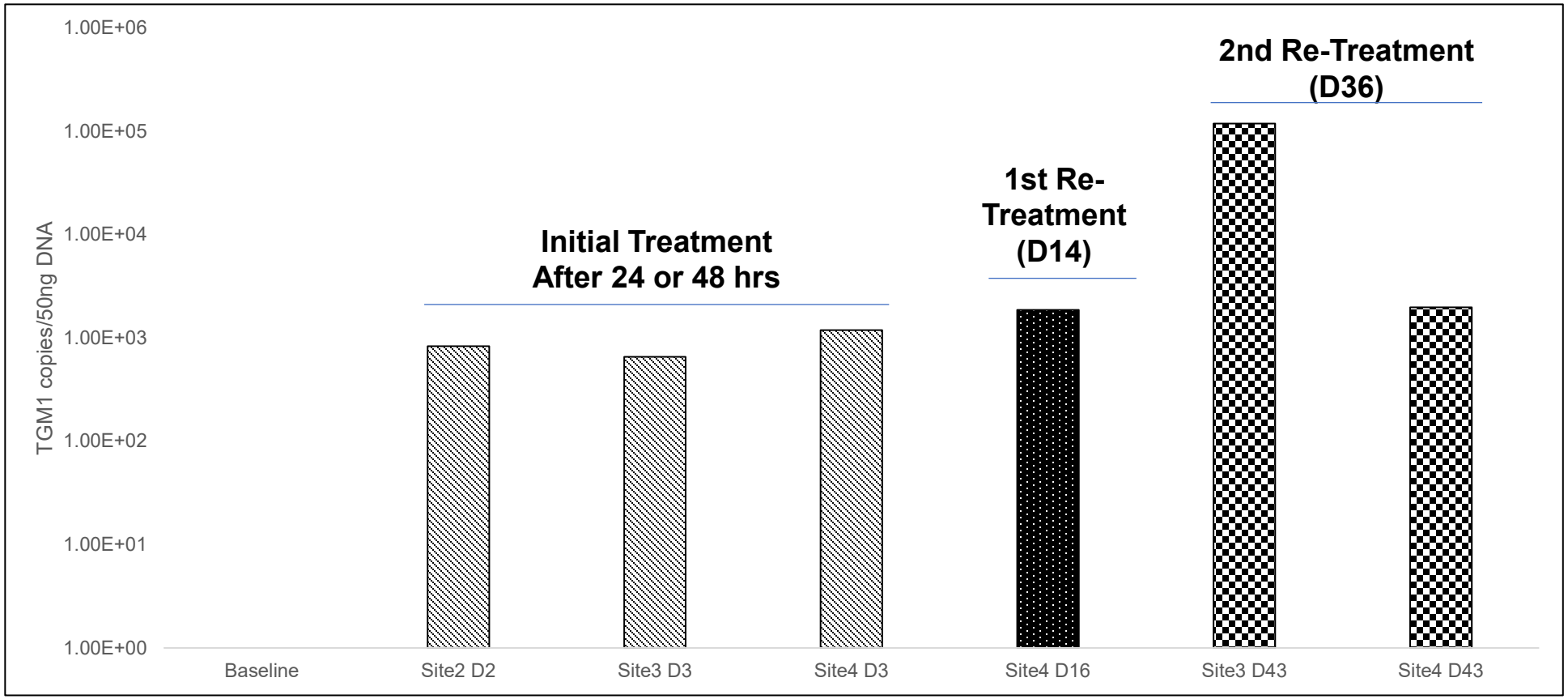
Day 43
Activity persists 7 days later

Re-treatment on D15 and D36 boosted activity to normal levels.

Expression levels correlated with changes in functional activity (not shown here)

Suggests that weekly topical administration would suffice

Subject 1: Good expression of TGM1 DNA in skin and no reduction with re-treatment



TGM1 DNA detected using a transgene-specific Taqman qPCR assay specific to KB105

No adverse events

No neutralizing HSV or TGM1 IgM or IgG antibodies throughout ~2 months

Subject 2: Dosing Regimen – Is microneedling better?

Screening -60 to -14	D-14 to D0	D1-D3		D8		D15-D16		D27-D29		D57 EOS
Genetic testing	Exfoliation (per patient regimen)	Visit 1 Treatment D1,D2, D3 Biopsies (D3) VS/ADA		Visit 2 Re-Treatment Biopsies		Visit 3 Re-Treatment		Visit 4 Re-Treatment Biopsies (D29) VS/ADA		Visit 5 (EOS) VS/ADA

$2X10^9$ PFU per 20 cm^2

Left and Right Arm

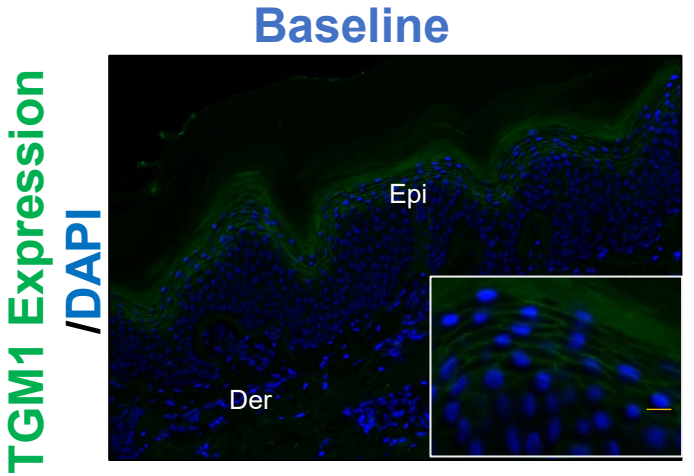
μ-needle

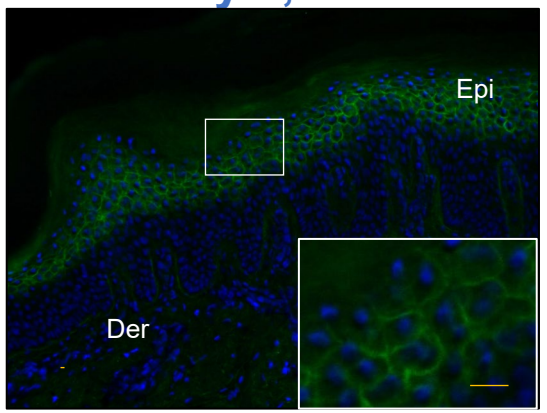
No μ-needle

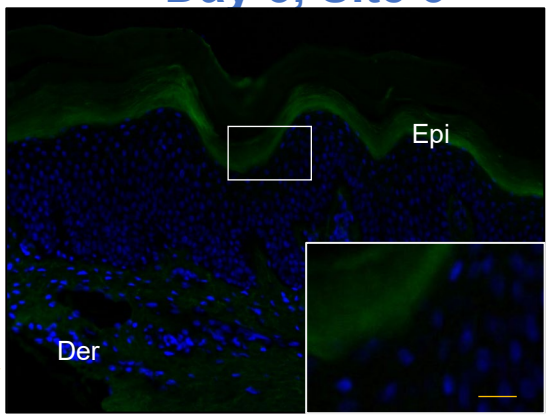
μ-needle

VS = viral shedding (HSV)

ADA = anti-HSV, anti-TGM1 antibodies



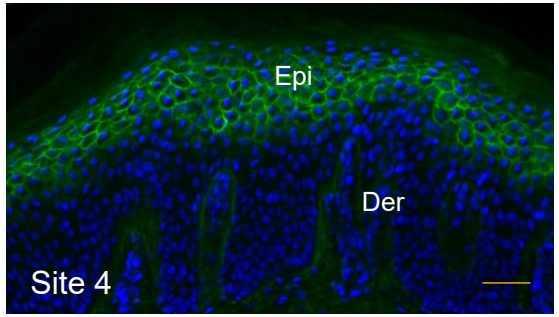




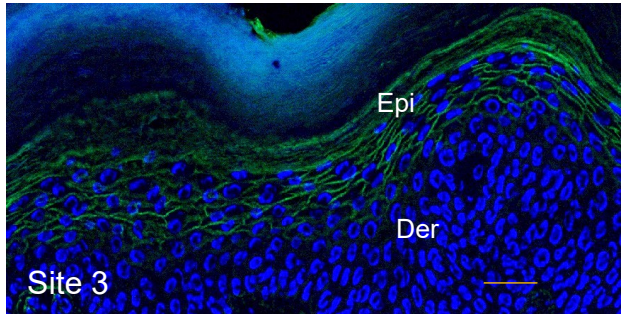
Subject 2: KB105 Was Successfully Repeat Administered

TGM1 levels persist on Day 8 and are boosted by retreatment on Day 27

Dosing on **D1-D3**

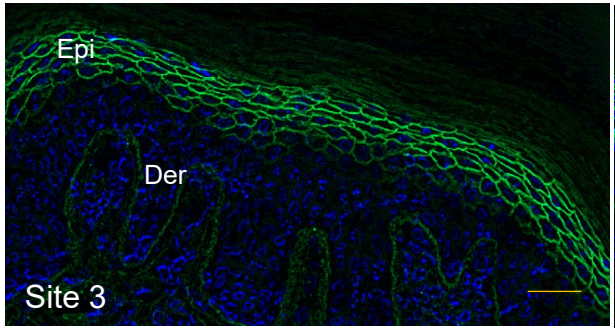


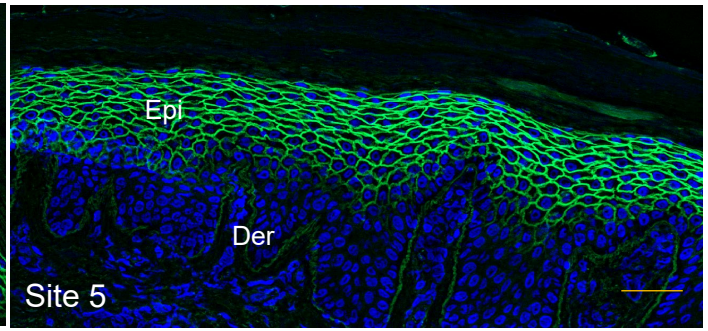
Increased expression relative to baseline
Day 3



TGM1 expression persists
Day 8 (no μ-needle)

Retreatment on **Day 27**



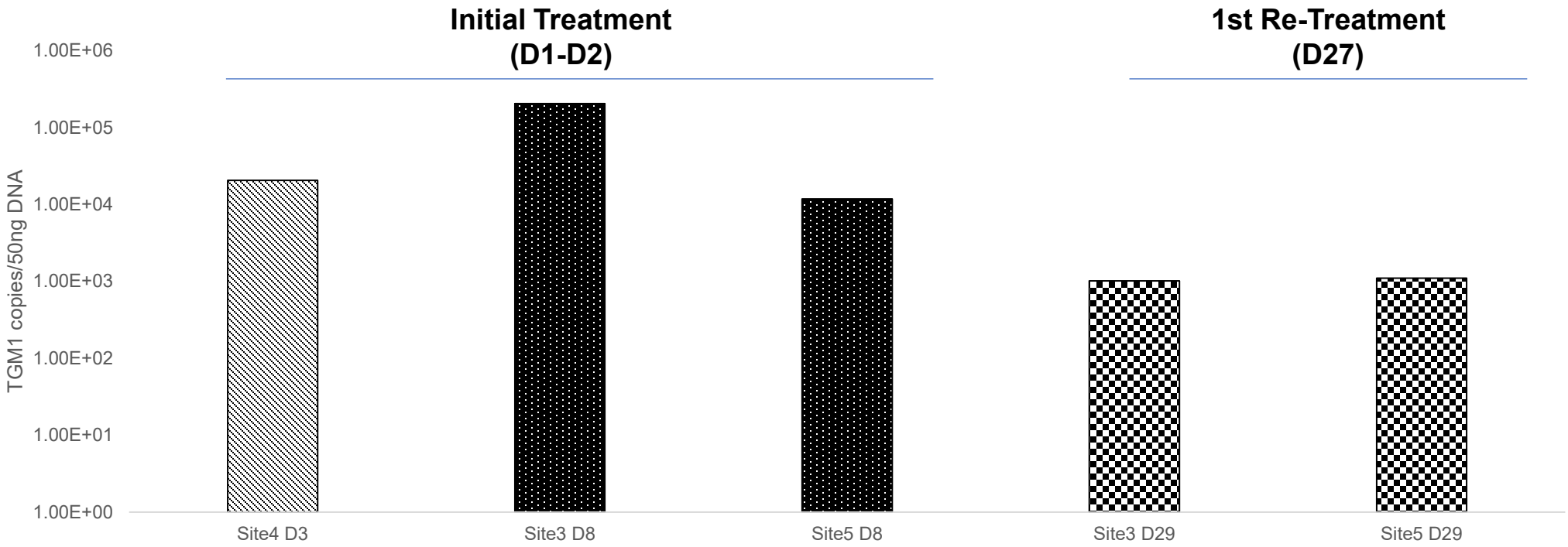


TGM1 expression enhanced upon re-treatment
Day 29 (no μ-needle)

Bar: 50 μm

In situ functional activity levels correlated with changes in TGM1 expression

Subject 2: KB105 DNA Detected Upon Initial and Repeat Administration



DNA extraction from Baseline and Site 3 (D3) biopsies was not feasible.
Drop in DNA levels on Day 29 likely due to biopsy processing issues – TGM1 protein levels were equivalent at all time points

Positive for HSV antibodies at screening (known recent infections)
No AEs; No increase in HSV antibodies; No TGM1 antibodies

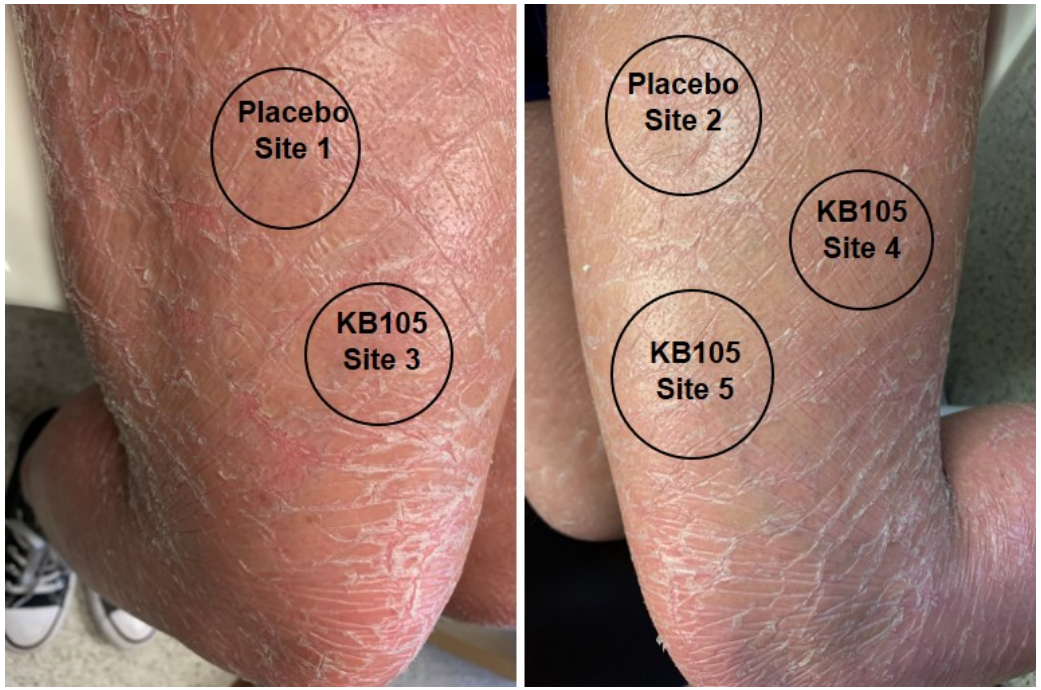
Subject 4: Dosing Regimen

Screening -60 to -14	D-14 to D0	D1-D3		D11		D17		D53-D55		D81 EOS
Genetic testing	Exfoliation (per patient regimen)	Visit 1 Treatment D1, D3 Biopsies (D3) VS/ADA		Visit 2 Re-Treatment		Visit 3 Re-Treatment VS/ADA		Visit 4 Re-Treatment VS/ADA		Visit 5 (EOS) VS/ADA

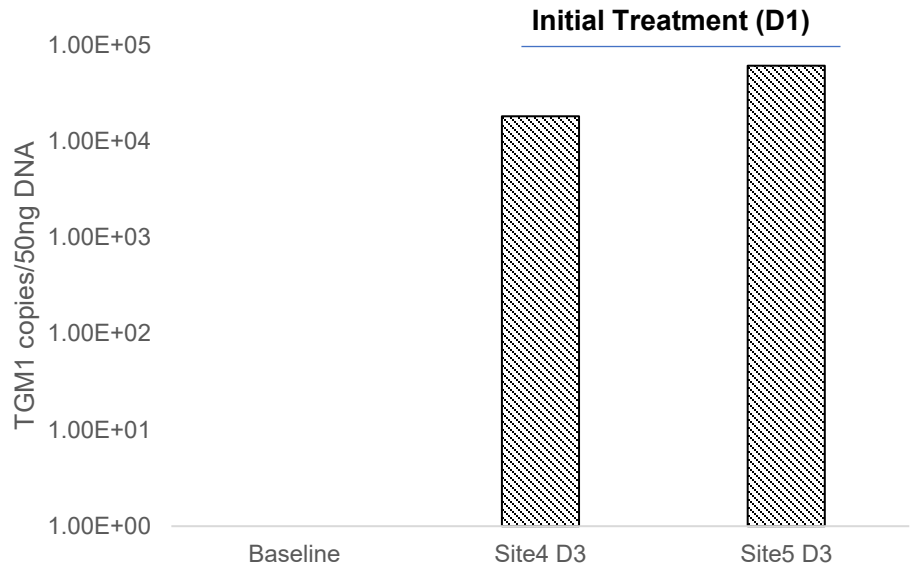
Left and Right Thigh

Same dose

No μ-needle



TGM1 DNA Levels Pre- and Post-Treatment

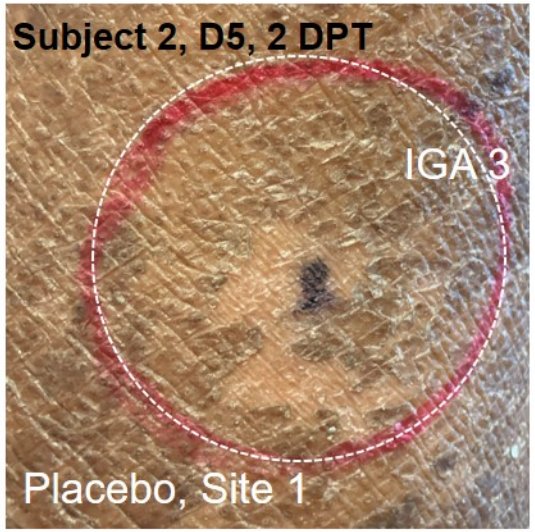


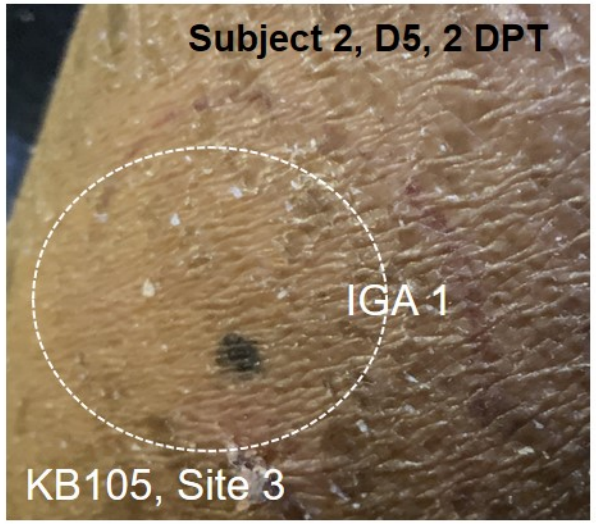
Baseline – Untreated area.
No biopsies collected beyond Day 3 due to Patient's refusal.

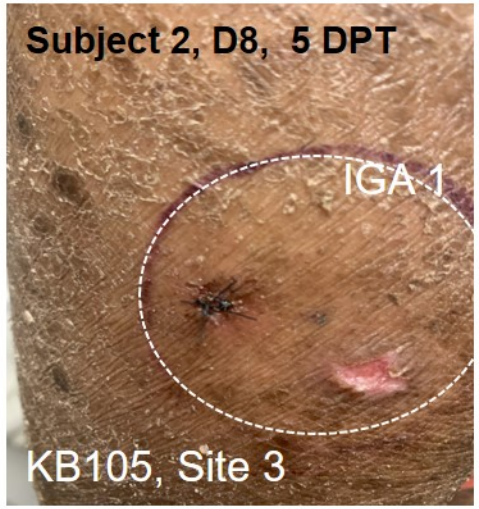
Subject showed increased TGM1 expression and activity in KB105-treated areas
No drug-related AEs noted; No HSV or TGM1 antibodies throughout the study

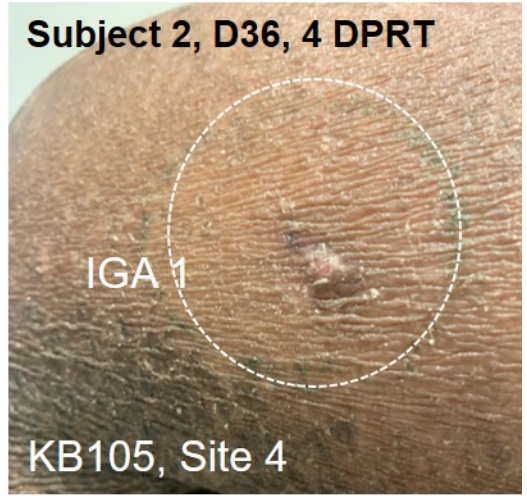
Molecular Correction Correlates with Phenotypic Improvement

Limited phenotypic evaluation feasible due to small target areas, but preliminary results are encouraging

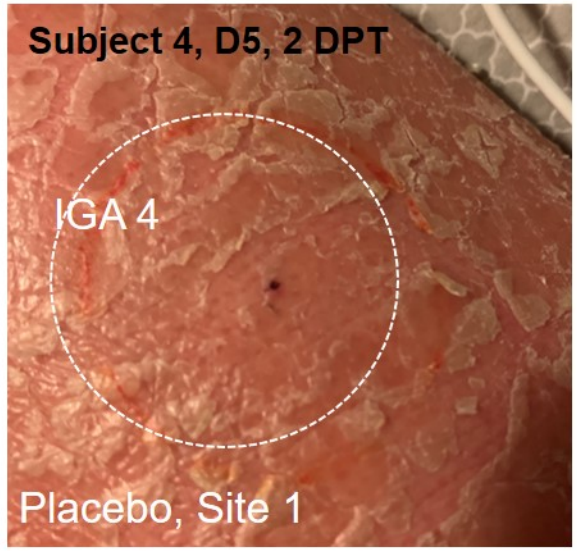


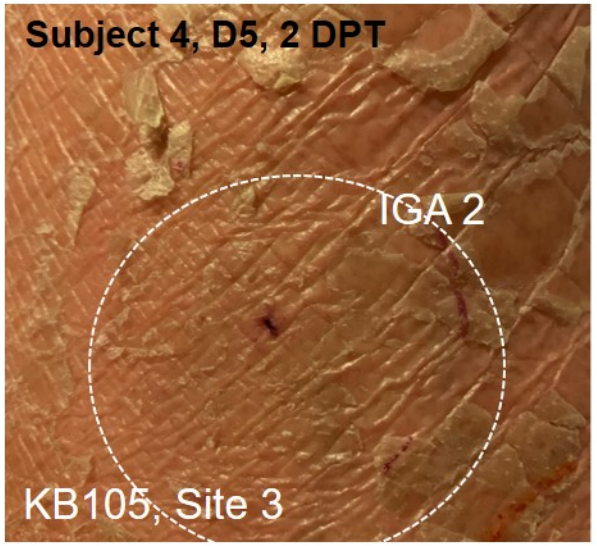






Representative Images





DPT: Days Post Treatment
DPRT: Days Post Re-Treatment

Summary and Lessons Learned

- Repeat dosing with KB105 was well-tolerated with **no drug related AEs and no immune response to HSV or TGM1.**
- **No vector shedding detected in swabs, blood or urine** in all three patients.
- KB105 treatment **restored functional TGM1 protein expression and activity** in all treated sites.
- **KB105-expressed TGM1 was correctly localized in the epidermis**, colocalizing with Loricrin, and was functionally active.
- qPCR, IF, and in situ analyses demonstrated **similar delivery efficacy of TGM1 DNA from single and repeat administration.**
- Similar delivery efficacy with and without microneedling, so no microneedling required in future studies.
- Phenotypic evaluation limited by small treatment areas, but KB105 treated areas showed reduced reversion to ichthyotic scaling phenotype

TGM1 pharmacokinetics suggest optimal dosing frequency may be every week

Future steps

Treat larger areas to optimize dose and dosing regimen.

Optimize dosing regimen based on natural history and skin turnover variability from patient to patient.

Enroll pediatric subjects following Agency review of Phase 1 adult study.

Utilize new Ichthyosis Severity Score when validated

Continue to work with Agency on including home dosing prior to pivotal trial

Thank you for your attention