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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*Concurrent Session: Genetic Disease, Gene Regulation, Gene Therapy*

*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.

- 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

Dose-dependent human TGM1 detected after topical application in mice
**Summary of Phase 1 Trial Design: Exploratory**

<table>
<thead>
<tr>
<th>Study Design</th>
<th>Intra-subject, open label, placebo-controlled study – Exploring technique</th>
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</thead>
<tbody>
<tr>
<td>Clinical Sites</td>
<td>Paddington Testing Company, Philadelphia – 3 subjects completed dosing</td>
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<tr>
<td></td>
<td>Northwestern University, Chicago, Illinois – active, initiated enrollment</td>
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<tr>
<td>Inclusion Criteria</td>
<td>≥18yo with lamellar ichthyosis and genetic diagnosis of TGM1-deficient ARCI</td>
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<td>IGA (Investigator Global Assessment) score of 3-4 in target areas</td>
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<tr>
<td>Key Objectives</td>
<td>Demonstrate safety (incidence of adverse events, clinical pathology, immunogenicity)</td>
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<td>Demonstrate efficacy (molecular correction, phenotypic improvement)</td>
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<tr>
<td>Dose and dosing regimen</td>
<td>2x10⁹ pfu KB105 to treat circular skin areas of 20cm² each</td>
</tr>
<tr>
<td></td>
<td>Baseline and repeat dosing (Is microneedling required? What is optimal schedule?)</td>
</tr>
<tr>
<td>Evaluation</td>
<td>• Imaging of target areas – onsite and home images</td>
</tr>
<tr>
<td></td>
<td>• Biopsies: TGM1 expression (DNA, protein) and functional activity</td>
</tr>
<tr>
<td></td>
<td>• Skin swabs, blood, urine -Vector shedding: Pre-treatment, Days 3/ 14/ 30/ 60</td>
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<tr>
<td></td>
<td>• Serum - HSV and TGM1 immunogenicity: Pre-treatment, Days 3/ 14/ 30/ 60</td>
</tr>
<tr>
<td></td>
<td>• Vital signs, physical examination, routine chemistry and hematology</td>
</tr>
</tbody>
</table>
### Patient Demographics and Disease Severity

<table>
<thead>
<tr>
<th>Subject</th>
<th>Age</th>
<th>Gender</th>
<th>Genotype</th>
<th>Medication</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>24</td>
<td>Female</td>
<td>TGM1 c.2060 G&gt;A p.R687H</td>
<td>None</td>
</tr>
<tr>
<td>4*</td>
<td>20</td>
<td>Female</td>
<td>c.2526 A&gt;G and c.2391 T&gt;C</td>
<td>None</td>
</tr>
</tbody>
</table>

*Subject 3 enrolled but could not keep return visits

**IGA: Disease Severity**

- Subject 1: 4
- Subject 2: 3
- Subject 4: 3
### Subject 1: Dosing Regimen – No microneedling

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

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

20 cm² areas treated with 2x10⁹ PFU
R, L shins

VS = viral shedding (HSV)
ADA = anti-HSV, anti-TGM1 antibodies

VS/ADA

Treated D1, biopsied D2
Treated again D2; biopsied D3

Treated D1 only
Biopsied D3
Subject 1: Treatment Restored TGM1 Expression to Normal Levels

- Subject showed no TGM1 expression in untreated area.
- TGM1 expression was seen in all three treated sites within 48-hours.
- TGM1 properly colocalized with its substrate, loricrin, in epidermis.
Subject 1: TGM1 Expression Correlated with Increased *In Situ* Activity

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

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

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

TGM1 DNA detected using a transgene-specific Taqman qPCR assay specific to KB105
Subject 2: Dosing Regimen – Is microneedling better?

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

2X10⁹ PFU per 20 cm²

Left and Right Arm

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

In situ functional activity levels correlated with changes in TGM1 expression

Retreatment on Day 27

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

Increased expression relative to baseline
Day 3

Dosing on D1-D3

TGM1 expression persists
Day 8 (no µ-needle)
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 AE; No increase in HSV antibodies; No TGM1 antibodies
Subject 4: Dosing Regimen

<table>
<thead>
<tr>
<th>Screening -60 to -14</th>
<th>D-14 to D0</th>
<th>D1-D3</th>
<th>D11</th>
<th>D17</th>
<th>D53-D55</th>
<th>D81 EOS</th>
</tr>
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<tbody>
<tr>
<td>Genetic testing</td>
<td>Exfoliation (per patient regimen)</td>
<td>Visit 1 Treatment D1, D3 Biopsies (D3) VS/ADA</td>
<td>Visit 2 Re-Treatment</td>
<td>Visit 3 Re-Treatment VS/ADA</td>
<td>Visit 4 Re-Treatment VS/ADA</td>
<td>Visit 5 (EOS) VS/ADA</td>
</tr>
</tbody>
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**Left and Right Thigh**
- Same dose
- No µ-needle

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

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- 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.
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