azitra

NYSE: AZTR

The development of a *Staphylococcus epidermidis* strain expressing LEKTI-D6 (ATR12-351) for Netherton syndrome

Mary Spellman MD
American Society of Gene and Cell Therapy

Precision dermatology powered by synthetic biology.

FORWARD-LOOKING STATEMENTS

This document contains forward-looking statements concerning Azitra, Inc. (“Azitra”, the “Company,” “we,” “us,” and “our”). The words “believe,” “may,” “will,” “potentially,” “estimate,” “continue,” “anticipate,” “intend,” “could,” “would,” “project,” “plan,” “expect” and similar expressions that convey uncertainty of future events or outcomes are intended to identify forward- looking statements. These forward-looking statements include, but are not limited to, statements concerning the following:

- our future financial and operating results;
- our intentions, expectations and beliefs regarding anticipated growth, market penetration and trends in our business;
- the timing and success of our plan of commercialization;
- our ability to successfully develop and clinically test our product candidates.

These forward-looking statements are subject to a number of risks, uncertainties and assumptions, including (i) we are an early-stage clinical biopharmaceutical company with limited operating history, (ii) there are no drug products to date that incorporate our microbial library and genetic engineering platform and the clinical and commercial utility of our microbial library and genetic engineering platform is uncertain and may never be realized; (iii) we have only recently commenced Phase 1 clinical studies of our initial product candidates and our product candidates will require extensive additional preclinical and clinical testing; (iv) we expect we will need additional financing to execute our business plan and fund operations, which additional financing may not be available on reasonable terms or at all; and (v) those other risk described in “Risk Factors” section of the prospectus (“Prospectus”) dated June 15, 2023 filed by Azitra with the Securities and Exchange Commission on June 21, 2023.

In light of these risks, uncertainties and assumptions, the forward-looking events and circumstances discussed in this document may not occur and actual results could differ materially and adversely from those anticipated or implied in our forward-looking statements. You should not rely upon forward-looking statements as predictions of future events. Although we believe that the expectations reflected in our forward-looking statements are reasonable, we cannot guarantee that the future results, levels of activity, performance or events and circumstances described in the forward-looking statements will be achieved or occur. Azitra does not undertake and specifically disclaims any obligation to update or revise our forward-looking statements to reflect new circumstances or unanticipated events as they occur, except as required by law.

Personal Disclosures

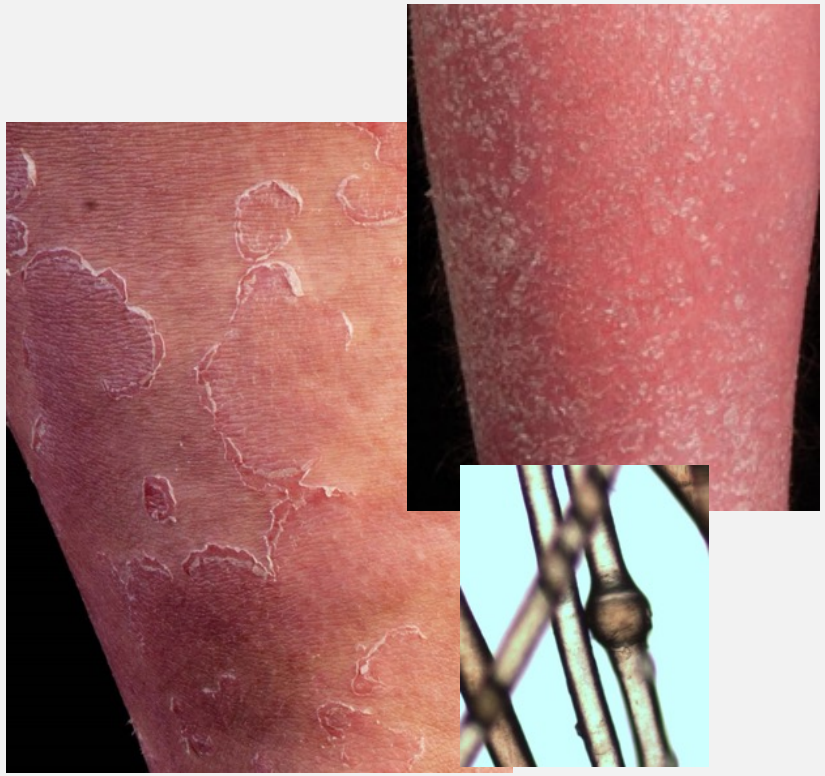
I am an independent contractor and receive consulting fees from Azitra Inc.

I have no other relevant disclosures.

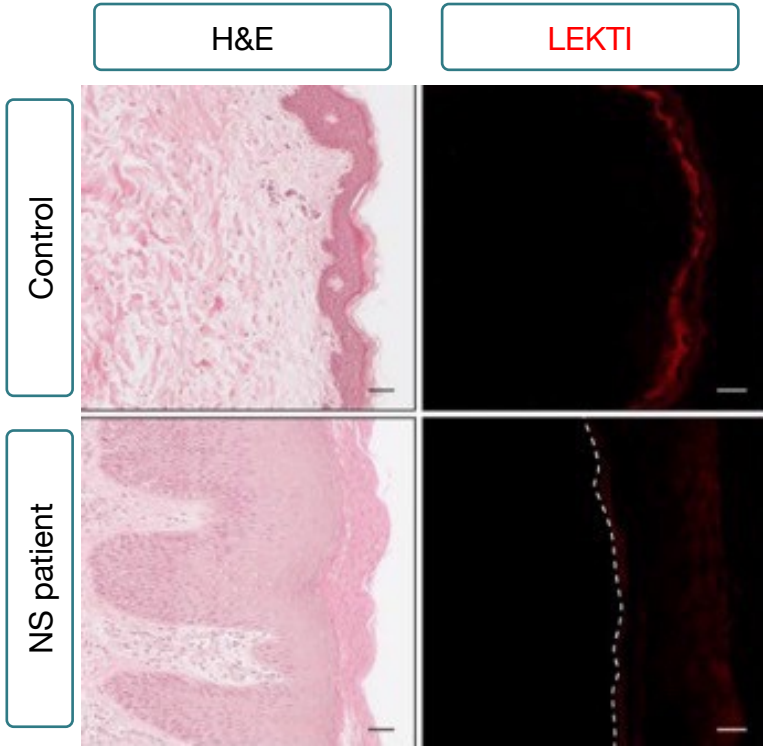
Netherton Syndrome

- **Netherton syndrome (NS)** is a rare, autosomal recessive disease
  - Estimated to occur in 1:100,000- 1:200,000 live births
  - Global prevalence of ~20,000
- Classic clinical triad of congenital ichthyosiform erythroderma, trichorrhexis invaginate (‘bamboo hair”), and an atopic diathesis
  - Caused by mutations in the serine protease inhibitor of Kazal type 5 (*SPINK5)* gene, which encodes the serine protease inhibitor, LEKTI (lympho-epithelial Kazal-type related inhibitor)
  - Loss-of-function mutations in *SPINK5* lead to unregulated kallikrein (KLK) proteolysis and activation of the KLK cascade
  - Overactive proteases cause desquamation and scaling, skin barrier defects, increased transepidermal water loss, pruritus
  - Microbial and allergen penetration trigger release of antimicrobial peptides and activation of inflammatory pathways, including IL-36γ
  - Multisystem complications, including life-threatening dehydration, failure to thrive, and sepsis in infancy with ~10% mortality rate
  - No approved therapies for NS

Clinical Presentation

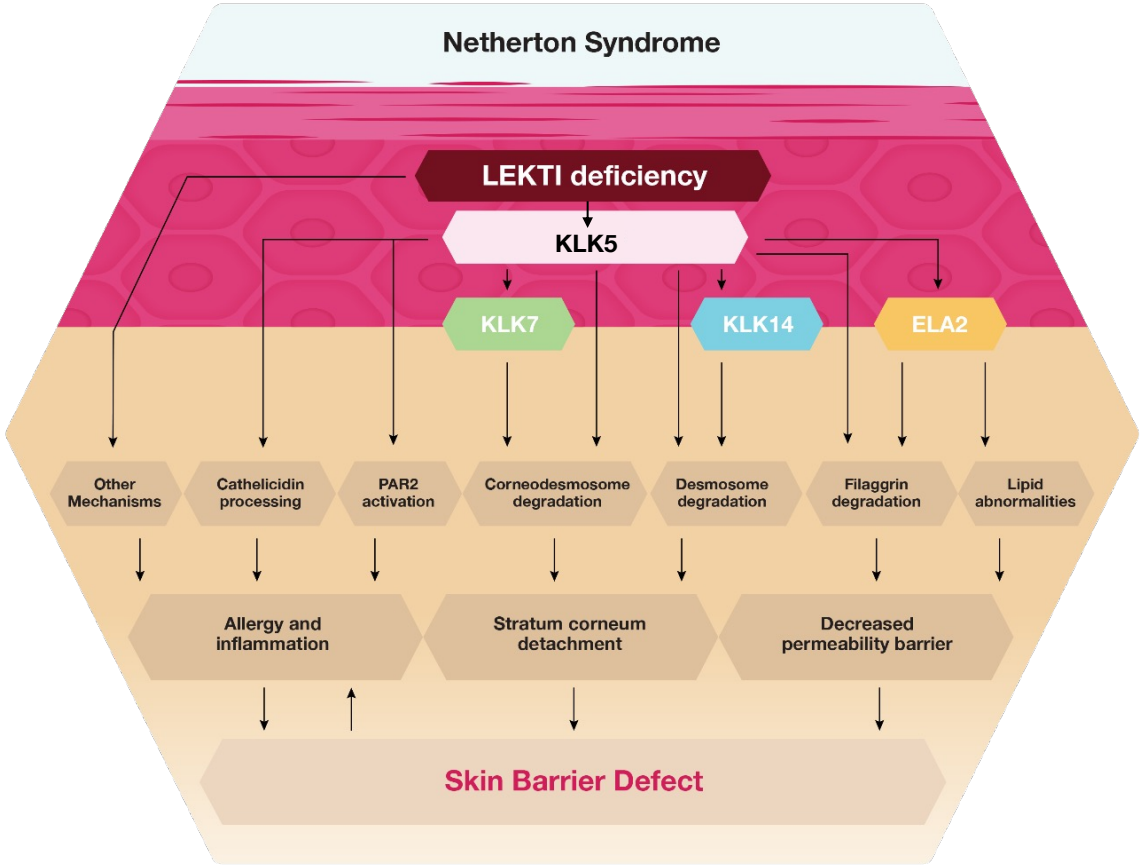


LEKTI is Deficient in Skin Affected by Netherton Syndrome



Mintoff, Fischer, *Mol Genet Genomic Med.*, **2021**, *9*, e1611

Targeting KLK5 in Netherton Syndrome via Expression of LEKTI



- LEKTI fragments inhibit KLK5, KLK7 and KLK14, which are involved in desquamation, PAR-2 activation, degradation of lipid hydrolases
- Uncontrolled serine protease activity leads to defects in skin barrier, and release of pro-inflammatory and pro-allergic mediators, causing the disease manifestations of Netherton syndrome
  - Activation of inflammatory pathways is associated with elevation of IL-36γ

ATR-12: LEKTI-Secreting *Staphylococcus epidermidis*

- **ATR-12 is a live biotherapeutic product,** comprised of an **auxotrophic strain** of *Staphylococcus epidermidis* (SE351) engineered to express domain 6 (D6) of the human LEKTI protein
  - ATR-12 is formulated as a topical ointment (ATR12-351)
- **Mechanism of action:** Delivery of recombinant human LEKTI-D6 (rhLEKTI) in the lower layers of the stratum corneum restores KLK activity, while the *S. epidermidis* strain reduces IL-36γ. Auxotrophic ATR-12 inhibits the overactive proteases through LEKTI fragment secretion.
  - Inhibit skin serine protease activity, improve skin barrier function, reduce underlying cutaneous inflammation, and improve the cutaneous signs and symptoms (appearance, pain, and pruritus) of Netherton syndrome

Key Facts

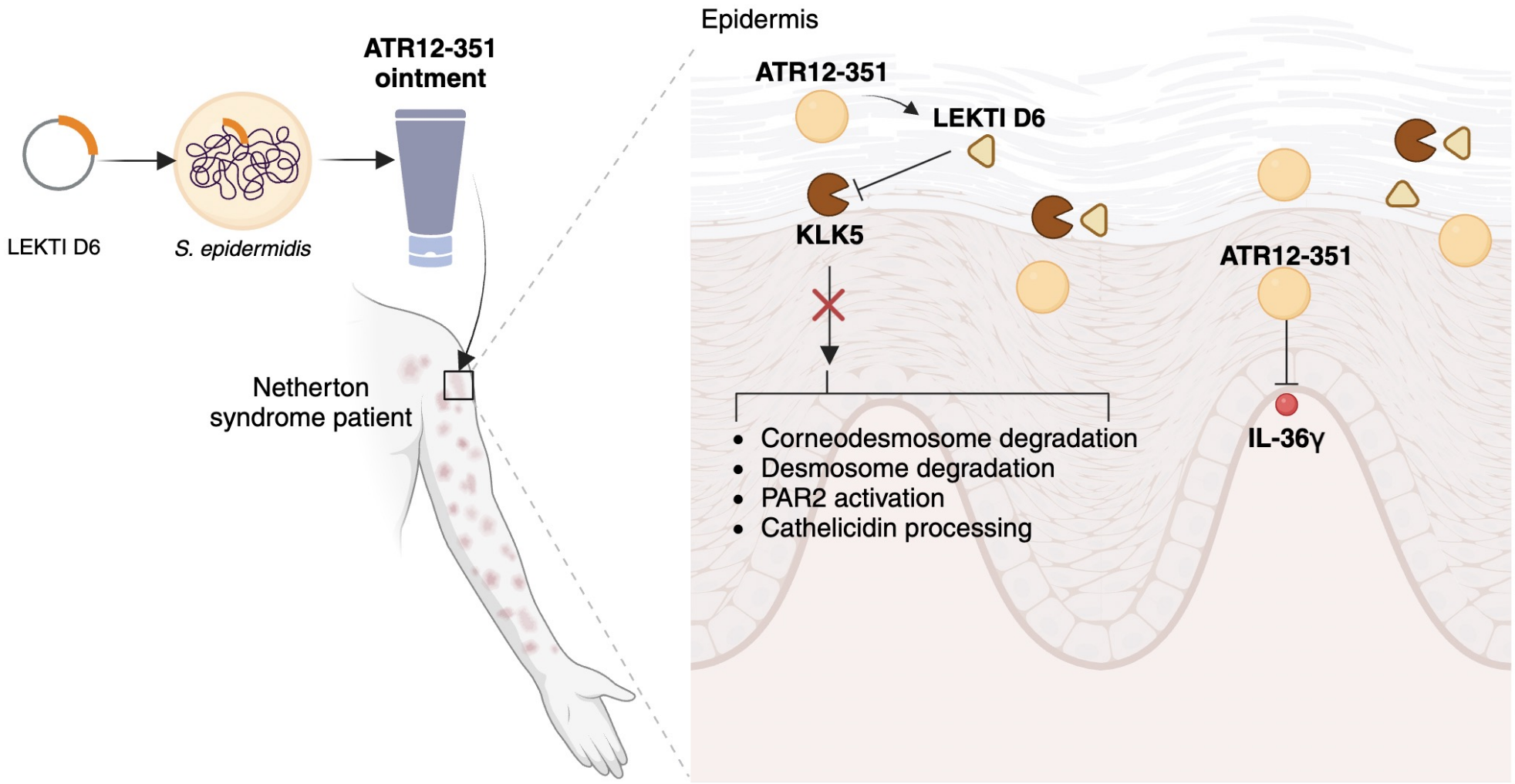
Primary Mechanism:
Restore kallikrein levels

Pharmacologic Effects (on HSE):
Reduction of trypsin-like activity (protease activity) by 7-fold

Topical Formulation:
Effectively and substantially delivers LEKTI throughout skin

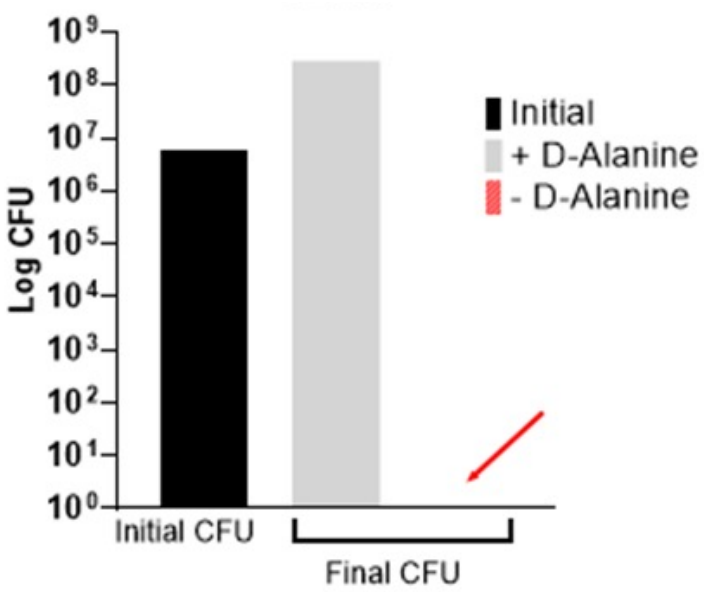
Clinical Development Status:
Phase 1b

Mechanism of Action of ATR12-351 to Treat Netherton Syndrome

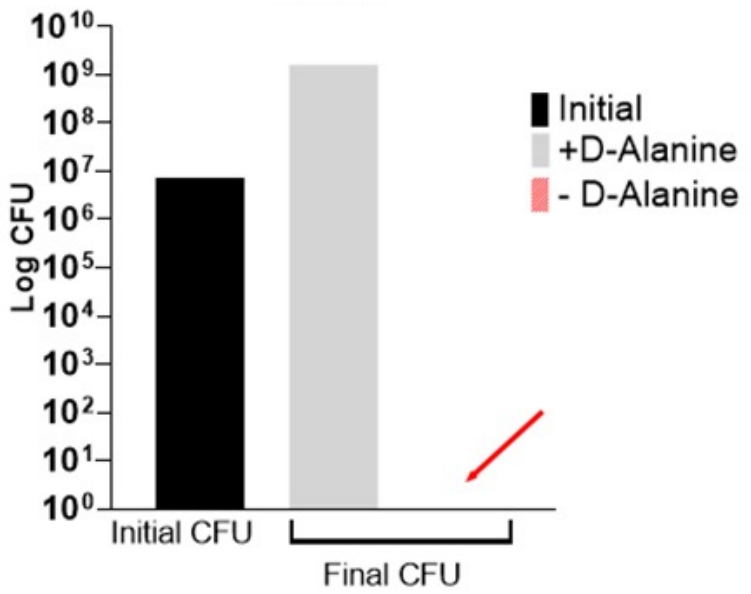


SE351 Does Not Grow in Human or Porcine Blood Without D-alanine

Human blood



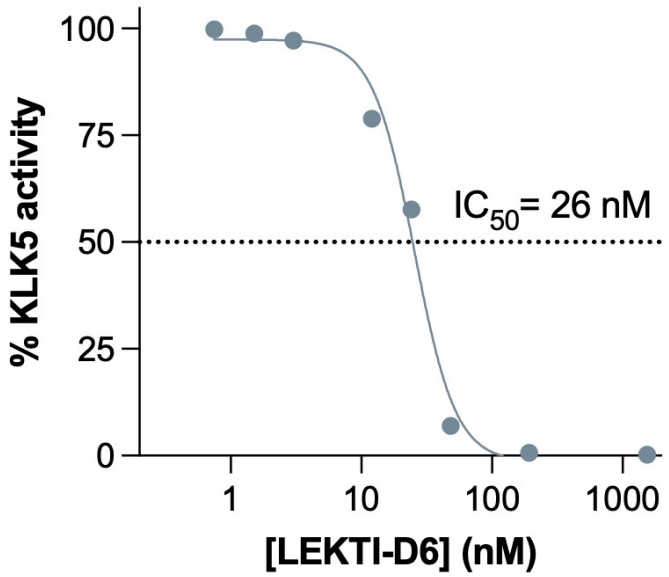
Pig blood



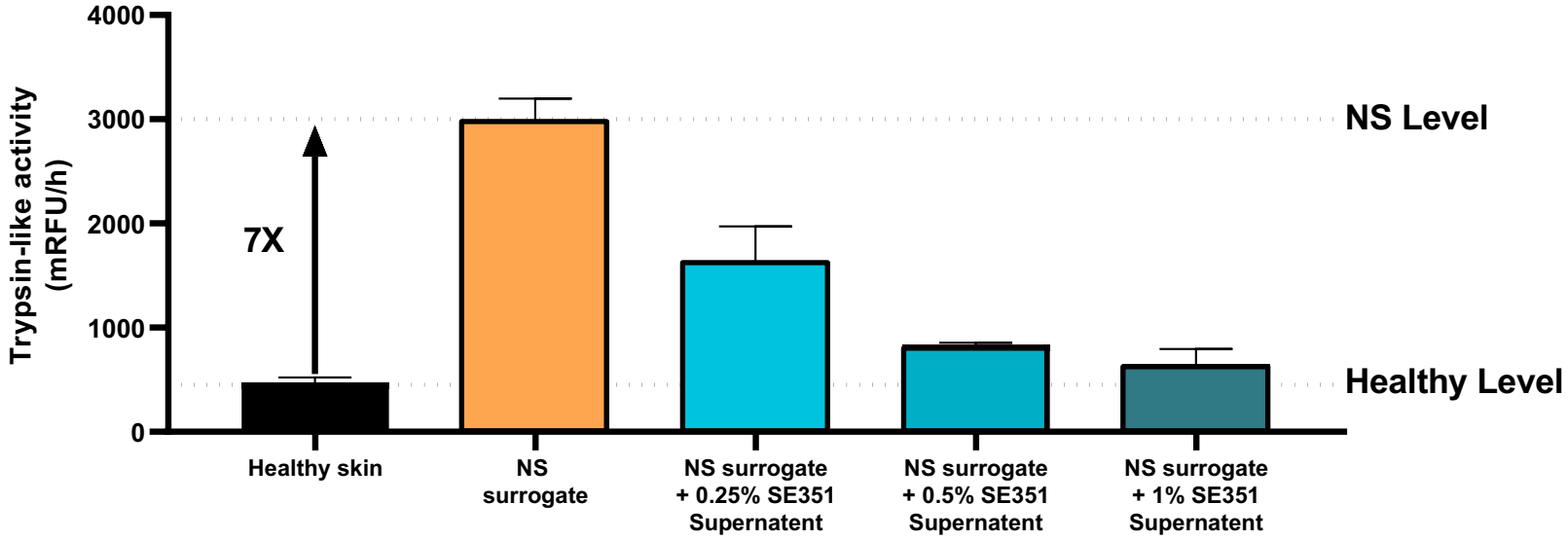
- SE351, an auxotroph, does not survive in the absence of D-alanine (not naturally present in humans)

Confirmation of SE351 Activity

Nanomolar IC50 of KLK5 from SE351 *in vitro*



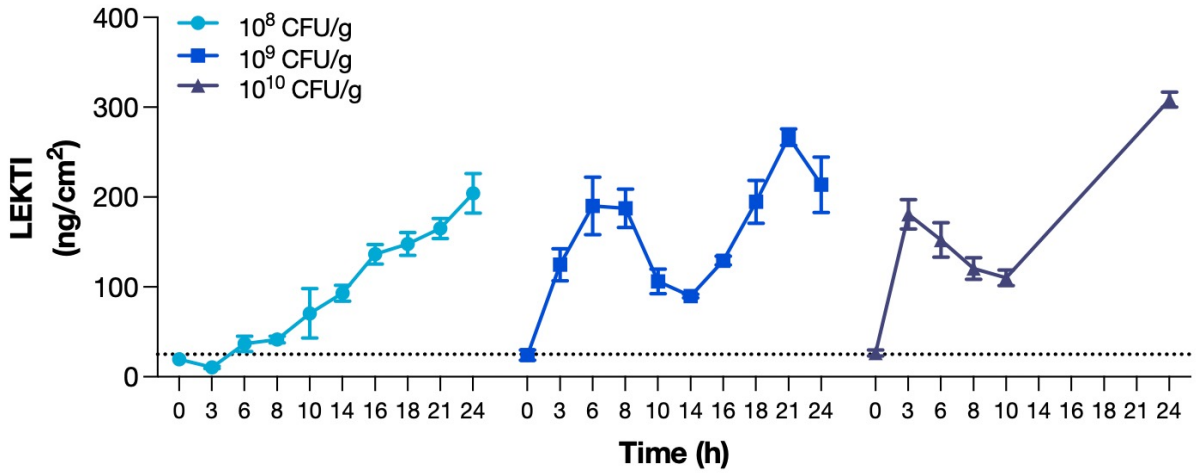
***S. epidermidis* strain SE351 (LEKTI-secreting) spent broth inhibition of human skin tape stripped extracts**



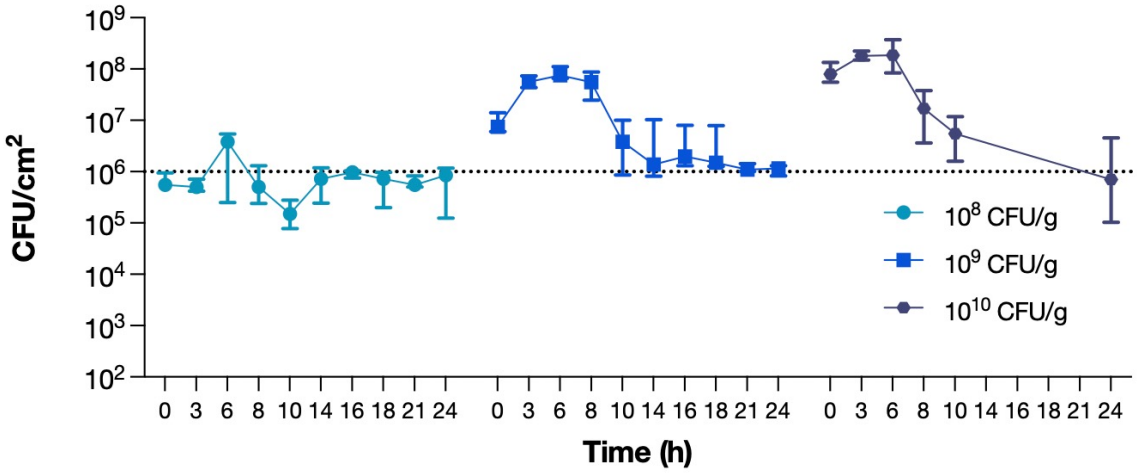
- Trypsin-like activity (key measure of protease activity) decreased after addition of spent broth from LEKTI-secreting strain SE351
- Dose-dependent response across concentrations of supernatant

LEKTI Delivery to *ex vivo* Pig Skin from Single Dose of SE351

LEKTI delivery from SE351 in *ex vivo* pig skin



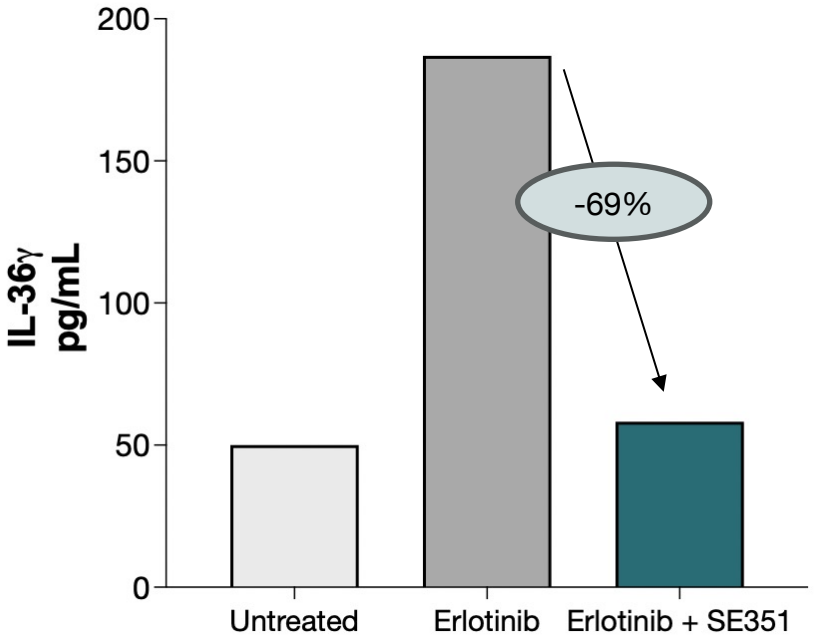
CFU/cm² of SE351 in *ex vivo* pig skin



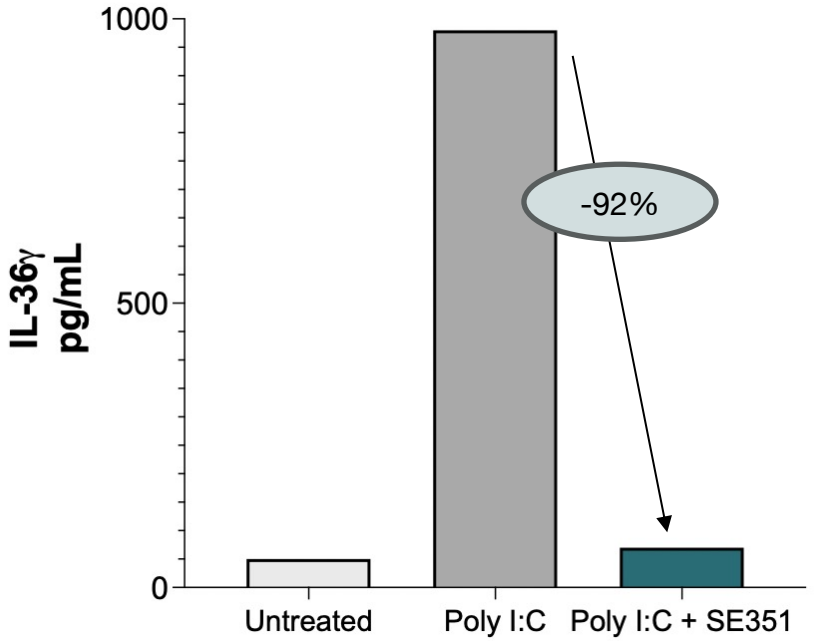
- Dose-dependent LEKTI delivery from SE351
  - Up to 300 ng/cm² LEKTI delivered from a single dose of SE351

SE351 Potently Reduces IL-36γ

Reconstructed epidermis induced with erlotinib and treated with SE351 cells

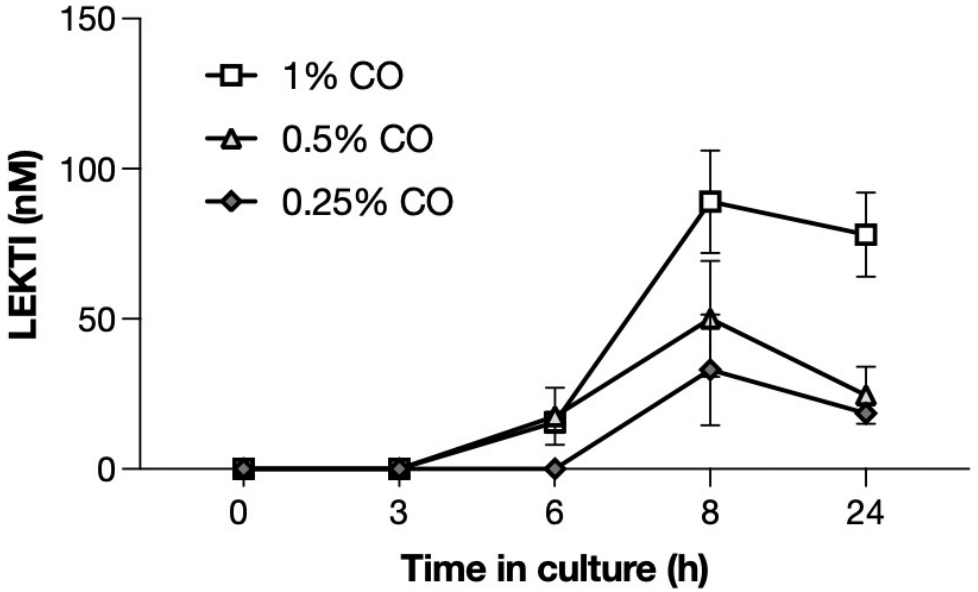


HaCaT cells induced with Poly I:C and treated with SE351 cell-free supernatant

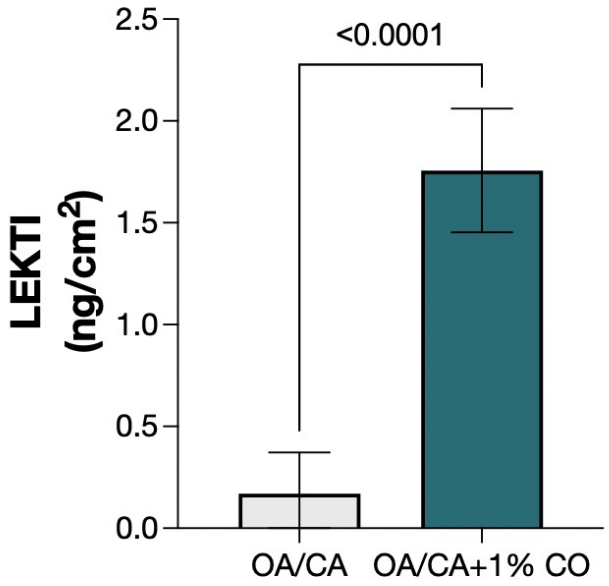


Optimization of Topical Formulation using Colloidal Oatmeal to Increase Delivery of LEKTI

LEKTI expression in culture



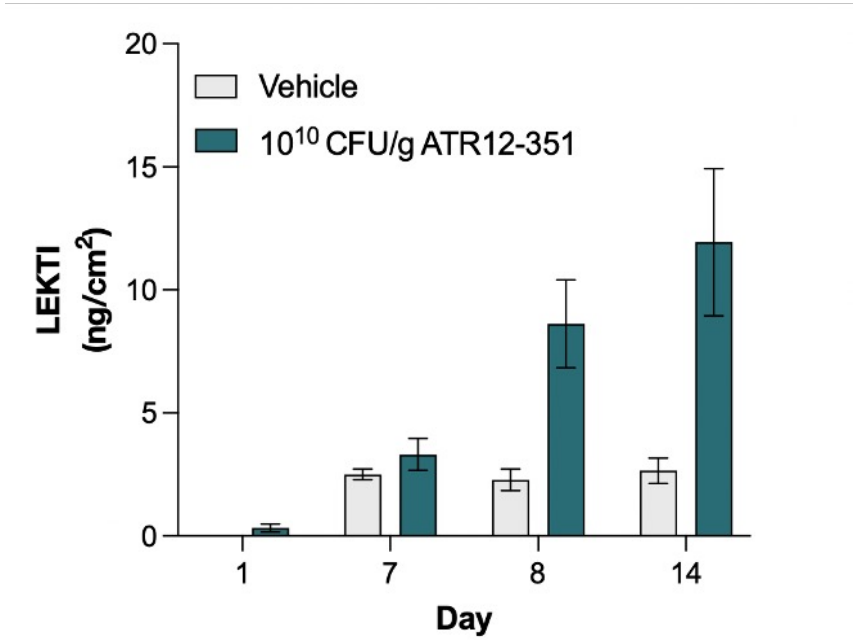
LEKTI delivery on growth plates



OA=oleyl alcohol
CA=cetyl alcohol
CO=colloidal oatmeal

ATR12-351 Delivers High Amounts of LEKTI in Minipigs with Abraded Skin and is Safe and Well Tolerated

LEKTI delivery by ATR12-351 over time



Safety findings

	Control (N=8)	ATR12-351 10^{10} CFU/g (N=8)
Plasma Bioanalysis for rhLETKI-D6, Day13	ND	ND
Presence of SE351 in blood	ND	ND
Died or Sacrificed Moribund	0	0
Body Weight	NC	NC
Food Consumption	NC	NC
Clinical Observations	NC	NC
Dermal (Draize) Scoring	Mild	Moderate
Electrocardiography	NC	NC
Ophthalmoscopy	NC	NC
Hematology	NC	NC
Coagulation	NC	NC
Serum Chemistry	NC	NC
Urinalysis	NC	NC
Bone marrow smears	NC	NC
Organ Weights	NC	NC
Gross Pathology	NC	NC

ND=not detected; NC=no ATR12-351-related change

- ATR12-351 delivers high amount LEKTI to skin of minipigs through 14 days
- ATR12-351 was safe and well-tolerated in minipigs with abraded skin and SE351 was not detected in the blood
  - Transient, inconsequential elevations in Draize scoring due to erythema

A Randomized, Double-Blind, Vehicle-Controlled, First-in-Human Safety, Tolerability, and Proof-of-Concept Study of Topical ATR12-351 in Adults with Netherton Syndrome

NCT06137157

Phase 1b Clinical Trial Design

Study overview

Multicenter, randomized, double-blind, vehicle-controlled study in adults (n=12) with Netherton syndrome

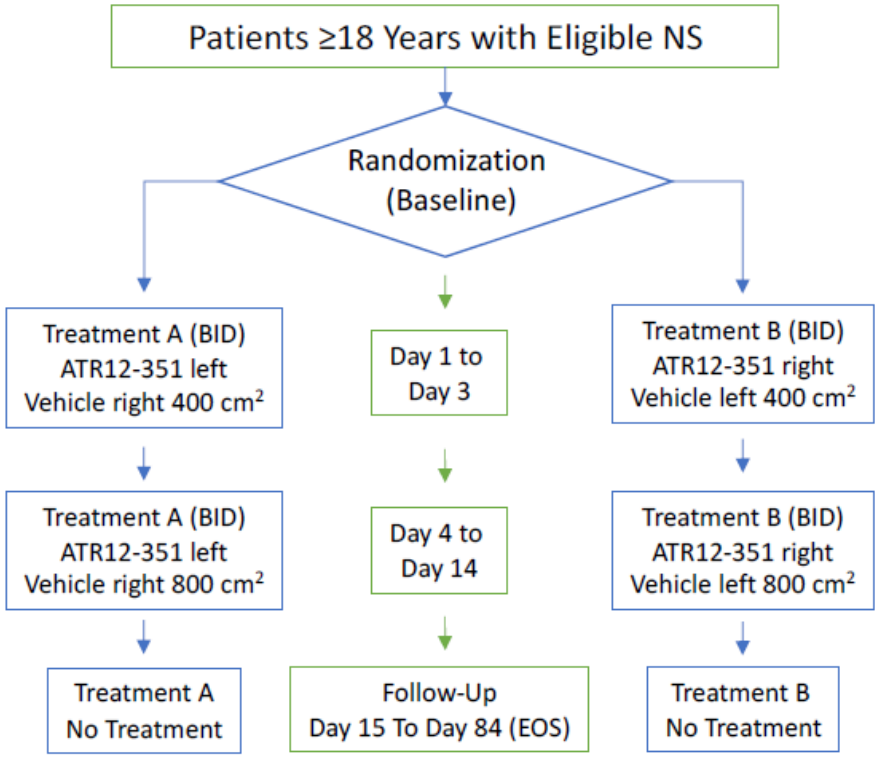
- Dose level: 10^9 CFU / g ATR12-351
- N=12 patients dosed twice daily for 14 days

- Primary objective: to assess the safety and tolerability of topical application of ATR12-351

- Secondary objectives:
  - Evaluate efficacy signals (investigator and patient global assessments, NS-modified SCORAD)
  - Evaluate the skin pharmacokinetics of rhLEKTI-D6

- Exploratory objectives:
  - Evaluate pharmacodynamic parameters, including anti-rhLEKTI response, cytokine responses, biomarkers such as KLK5, KLK7, IL-36γ, TARC/CCL17, trypsin-like activity, and chymotrypsin-like activity

Design



Summary

- ATR12-351 is a topical ointment containing a lyophilized version of a live biotherapeutic product, Staphylococcus epidermidis strain designated SE351.
  - This strain has been modified to be auxotrophic and to express recombinant human LEKTI protein.
- ATR12-351 is intended to address the underlying cause of Netherton syndrome by replacing deficient/dysfunctional LEKTI at affected areas, countering the dysregulated skin serine protease activity.
- ATR12-351, a LEKTI-expressing strain of *S. epidermidis* in development for Netherton syndrome has demonstrated key proof of concept in preclinical studies:
  - ATR12-351 has nanomolar IC50 values to inhibit KLK5, a key driver of Netherton syndrome
  - ATR12-351 delivers functional LEKTI and reduces protease activity to normal levels
  - ATR12-351 delivers LEKTI significantly more effectively than LEKTI delivery alone
- The Phase 1b, first-in-human clinical study (NCT06137157) aims to establish safety and tolerability as well as initial efficacy of ATR12-351 application in patients with Netherton syndrome.


azitra
THANK YOU
Precision dermatology powered by synthetic biology.