azitra

NYSE: AZTR

Engineered *S. epidermidis* as a protein delivery system for treating skin diseases

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American Society of Gene and Cell Therapy

Precision dermatology powered by synthetic biology.

SPECIAL NOTE REGARDING 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

Employment/compensation

Current

- Azitra Inc. (NYSE: AZTR) (Cofounder, COO)*
- Actuate Therapeutics Inc. (Cofounder)*
- Inspired Spaces LLC (Cofounder)*
- LetsImproveHealth LLC (Founder)*
- Umbrex LLC (Healthcare management consultant)*
- Yale University (Assistant Professor Adjunct)

Former

- Bios Partners LP (Former Partner)*
- Bios Research (Former Senior Analyst)
- Cue Biopharma (NASDAQ: CUE) (Former consultant)
- Encore Vision (Former consultant)
- Novartis (Former consultant)
- TFF Pharma (NASDSQ: TFFP) (Former consultant)

Shareholder

- Azitra Inc. (NYSE: AZTR)*
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- i-Lumen Scientific*
- Immusoft Corporation*
- IN8bio (NASDAQ: INAB)*
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- Stream Biomedical*
- Trefoil Therapeutics*

Current Board of Directors

For-profit

- Azitra Inc. (NYSE: AZTR)*
- IN8bio (NASDAQ: INAB)*

Non-profit (No financial interest)

- International Network for Simulation-based Pediatric Innovation, Research, and Education (INSPIRE) (Treasurer)
- International Pediatric Simulation Society (IPSS) (Treasurer)

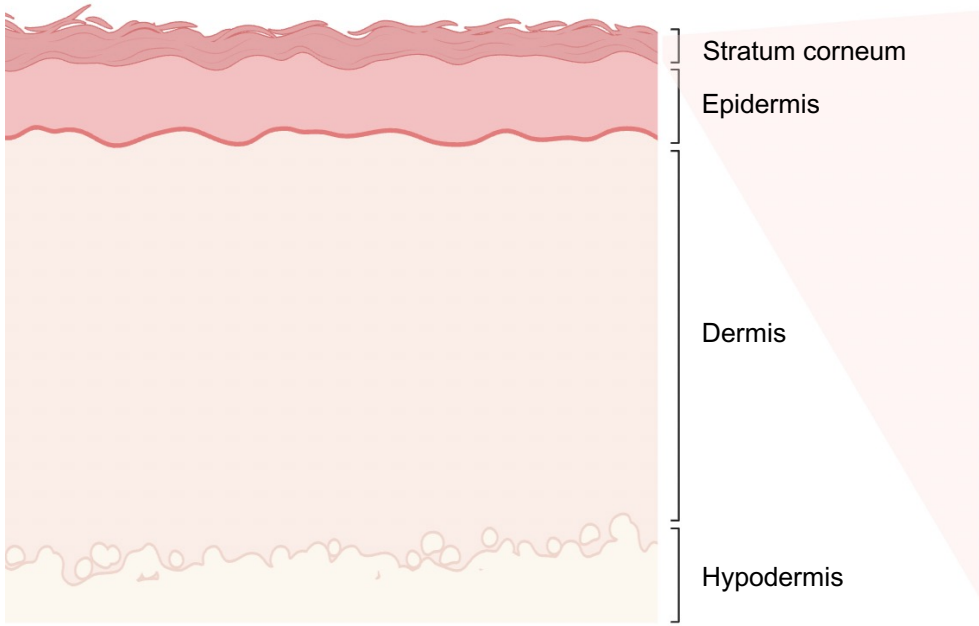
Grant/research support (PI)

- Connecticut Innovations
- Connecticut Biosciences
- Defense Advanced Research Projects Agency (DARPA)
- Department of Defense (DoD)
- Indiana University
- Macy Foundation
- National Institutes of Health – NIAMS
- National Institutes of Health – NIAID
- National Science Foundation (NSF)
- Thiel Foundation
- Yale University

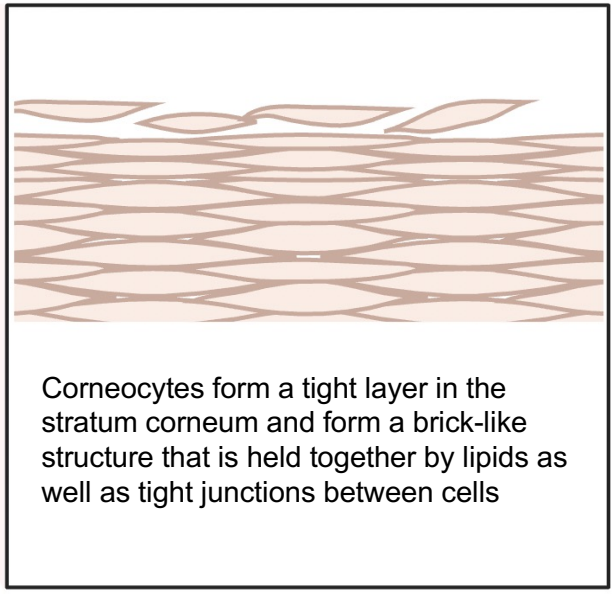
***Current financial interest**

The challenge in topical protein delivery: the skin barrier prevents large molecules

Epidermis



Stratum corneum



Corneocytes form a tight layer in the stratum corneum and form a brick-like structure that is held together by lipids as well as tight junctions between cells

Skin delivery challenges

- The skin barrier **prevents large molecules and proteins** (>500 Daltons) from penetrating to the epidermis and dermis
- Other transepidermal delivery methods are **challenging, ineffective, or uncomfortable** to patients (e.g., microneedles) and require purified protein

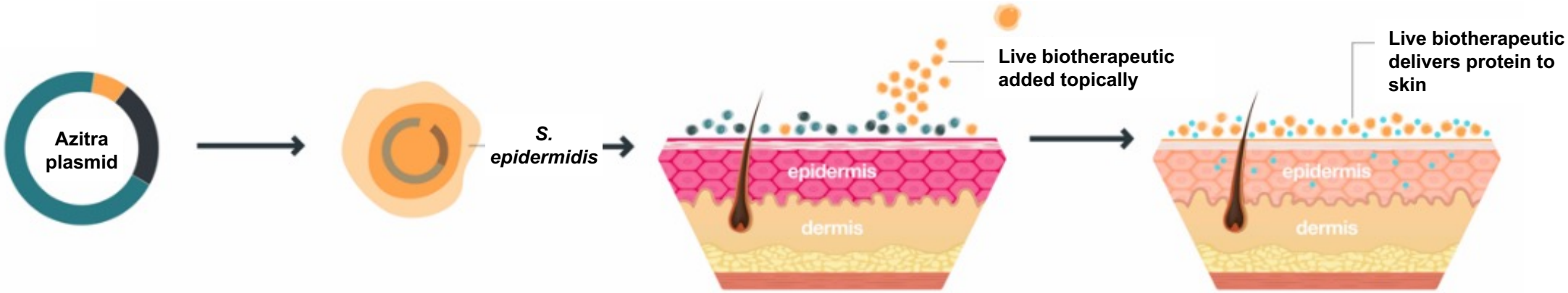
Engineering *S. epidermidis* as a drug delivery system to treat skin diseases

1 **Insert gene fragment that encodes gene of interest**

2 **Insert gene into chromosome of *S. epidermidis***

3 **Colonize skin with live biotherapeutic product**

4 **Secrete protein of interest for therapeutic treatment**



- ✓ *S. epidermidis* as a delivery chassis has numerous **well-documented biological benefits**
- ✓ **Effective transdermal protein delivery** can be achieved by using a living protein factor that continuously secretes protein *in situ*
- ✓ **Modular design** allows for insertion of different genes of interest depending on disease target

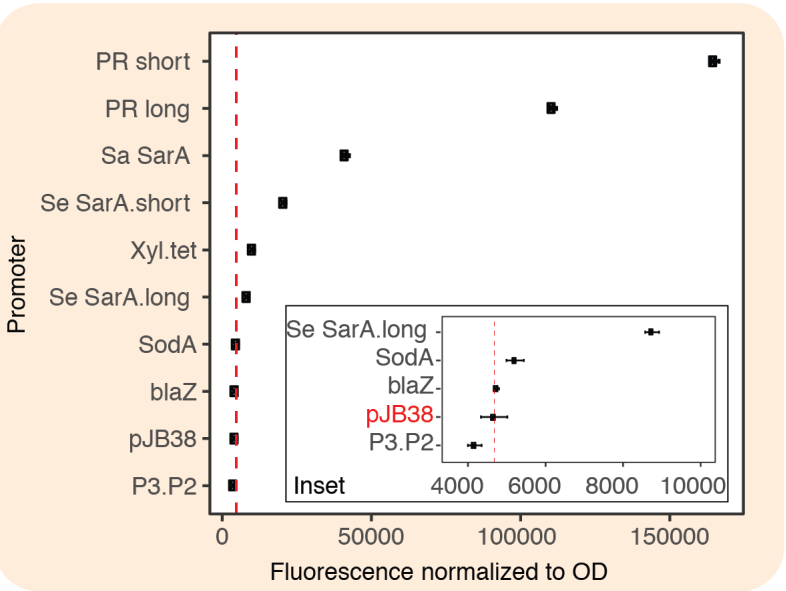
Enhancing protein expression, secretion, and delivery with synthetic biology and libraries

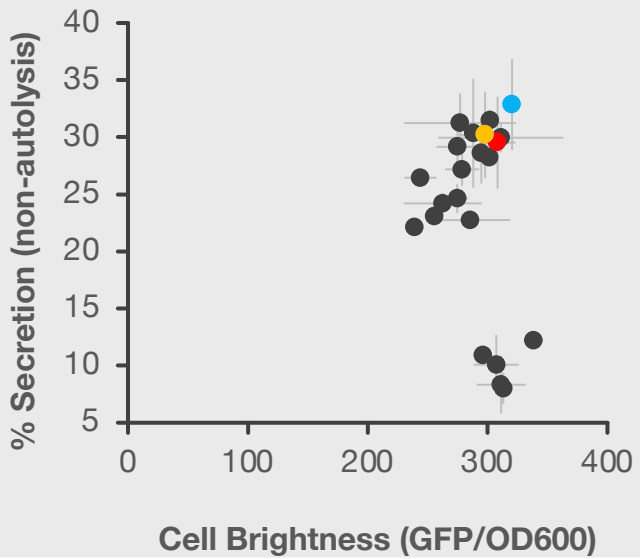
Modular plasmid design

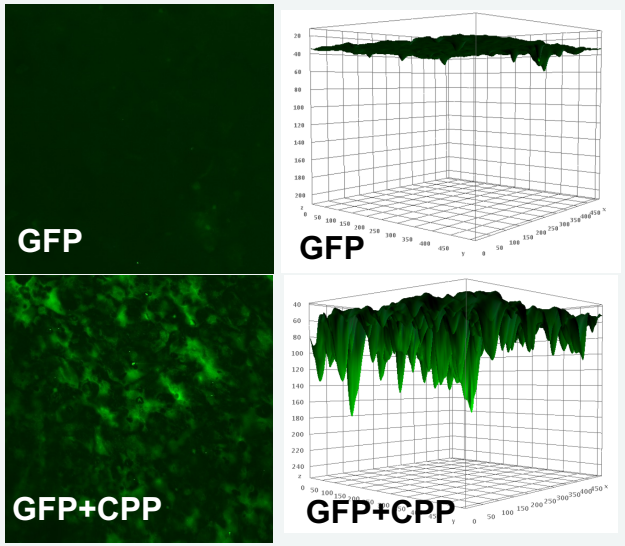
01 Promoters

02 Secretion signal peptide

03 Cell penetrating peptide

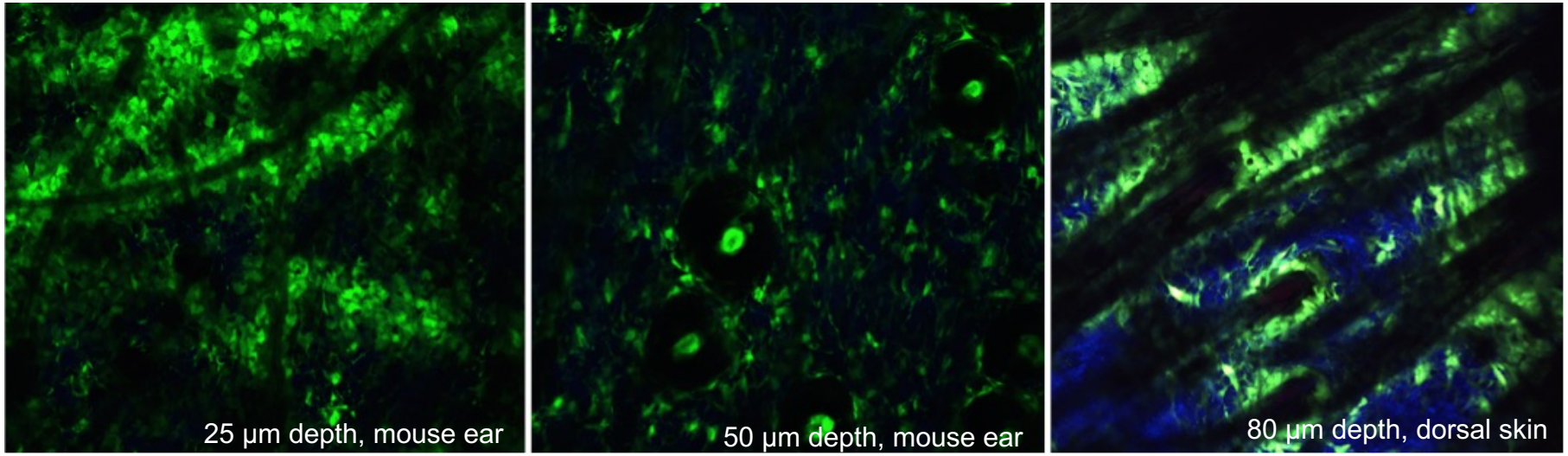




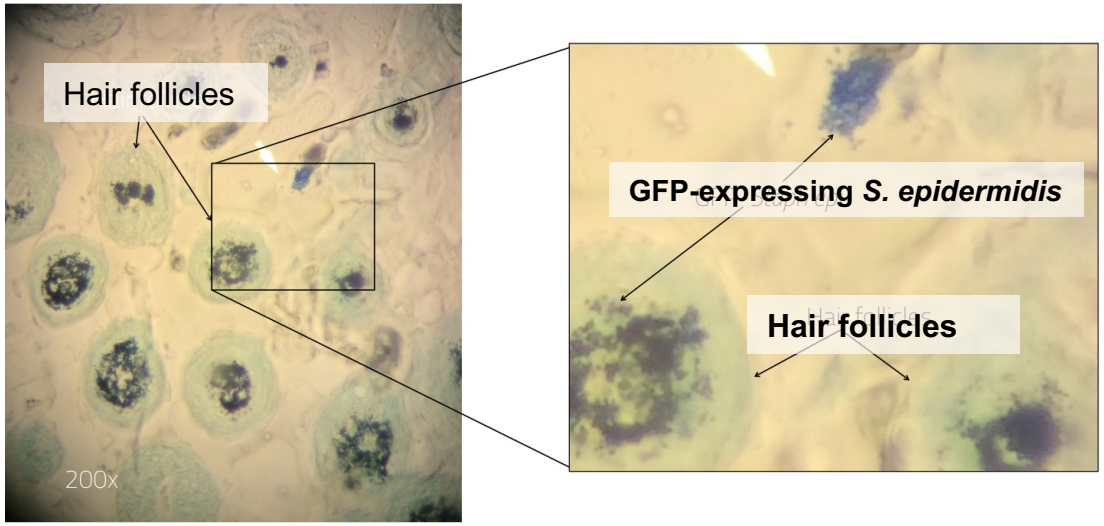


Three days after single application of GFP-expressing *S. epidermidis* shows deep penetration and localization to hair follicles

2-photon microscopy



Light microscopy



ATR-12: LEKTI-Secreting *Staphylococcus epidermidis* for Netherton syndrome

ATR-12 and Netherton syndrome summary

- **Netherton syndrome** is a rare, orphan autosomal recessive disease with no current FDA-approved treatment option
- Characterized by severe inflammation, pruritus, scaling, red, and dehydrated skin
  - Caused by mutations in the *SPINK5* gene, which encodes the serine protease inhibitor, **LEKTI** (lympho-epithelial Kazal-type related inhibitor)
  - Results in overactive proteases causing desquamation, skin barrier defects, and activation of inflammation
  - ~10% mortality rate in infants
- **Mechanism of action:** auxotrophic ATR-12 inhibits the overactive proteases through LEKTI fragment secretion
- **NS as a monogenic disease allows for proof-of-concept for *S. epidermidis* as a novel protein delivery system**

ATR-12 Key Facts

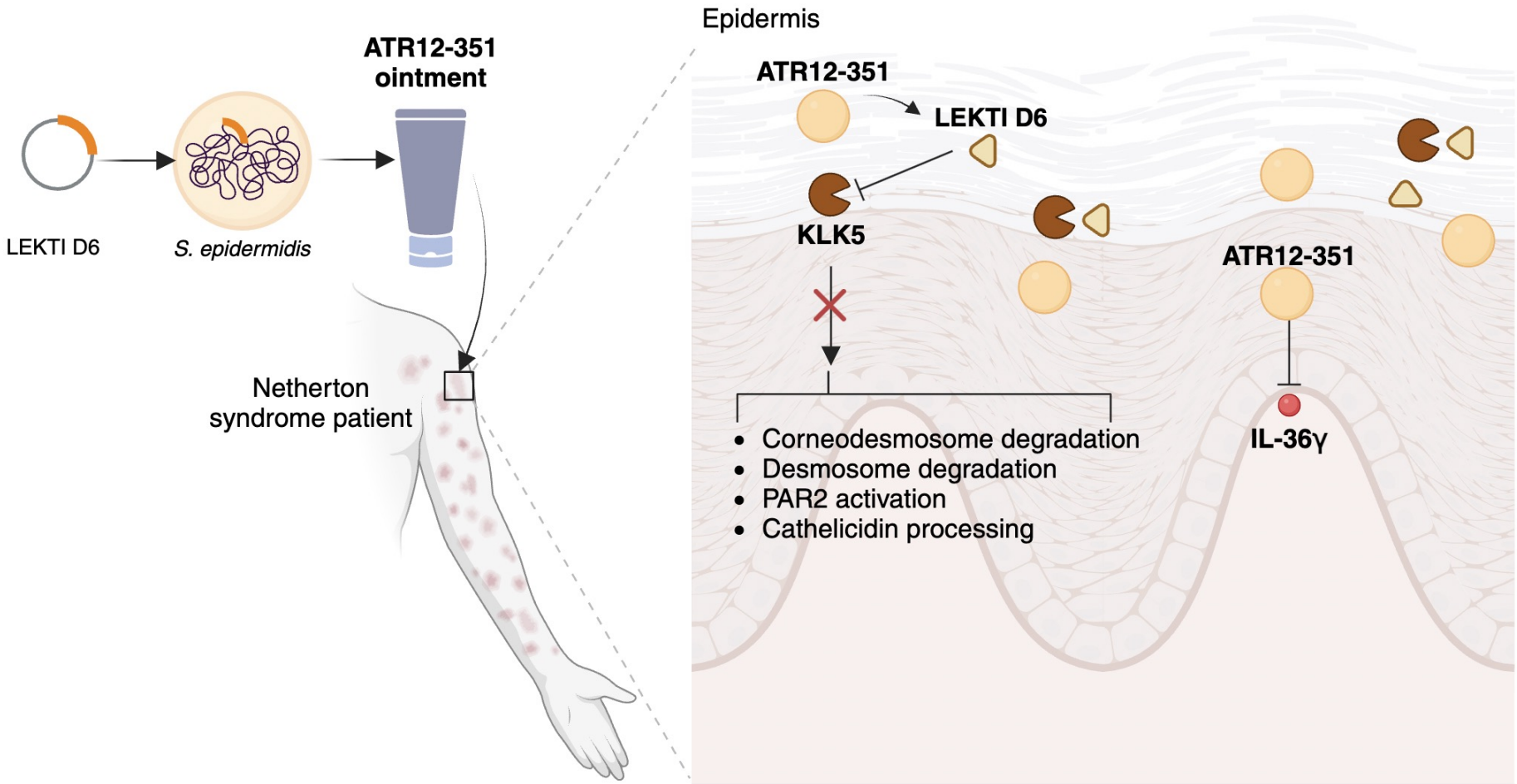
Primary Mechanism:
Protein replacement and kallikrein inhibition

Clinical Status:
Phase 1b

Global Prevalence:
~20K+ Patients

Peak Sales Opportunity:
~$250M

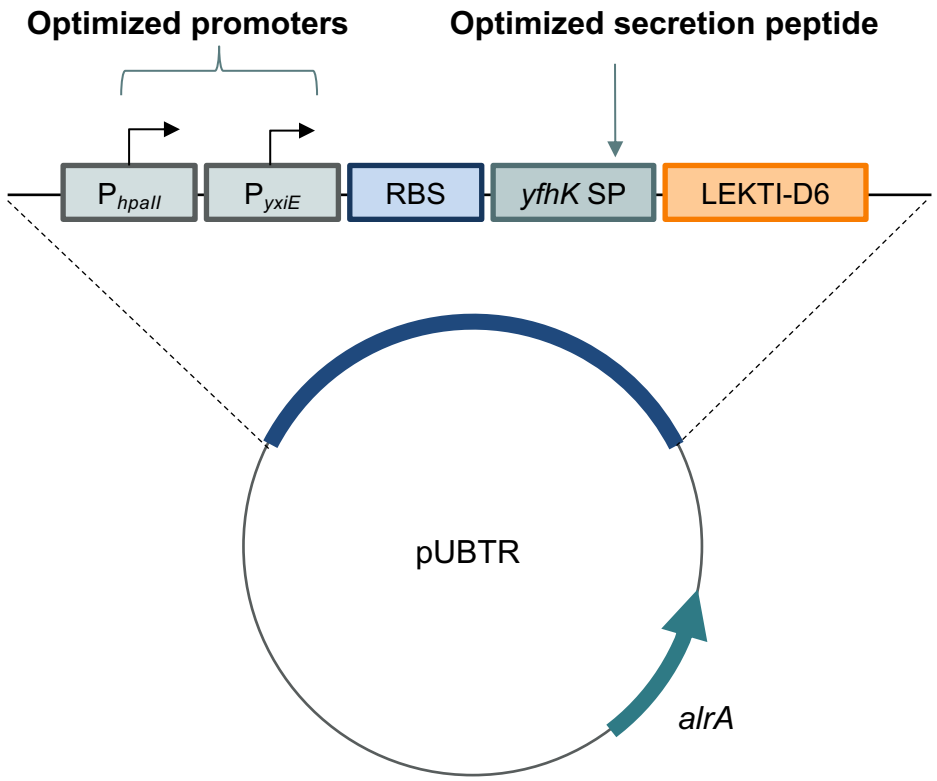
Mechanism of action of ATR12-351



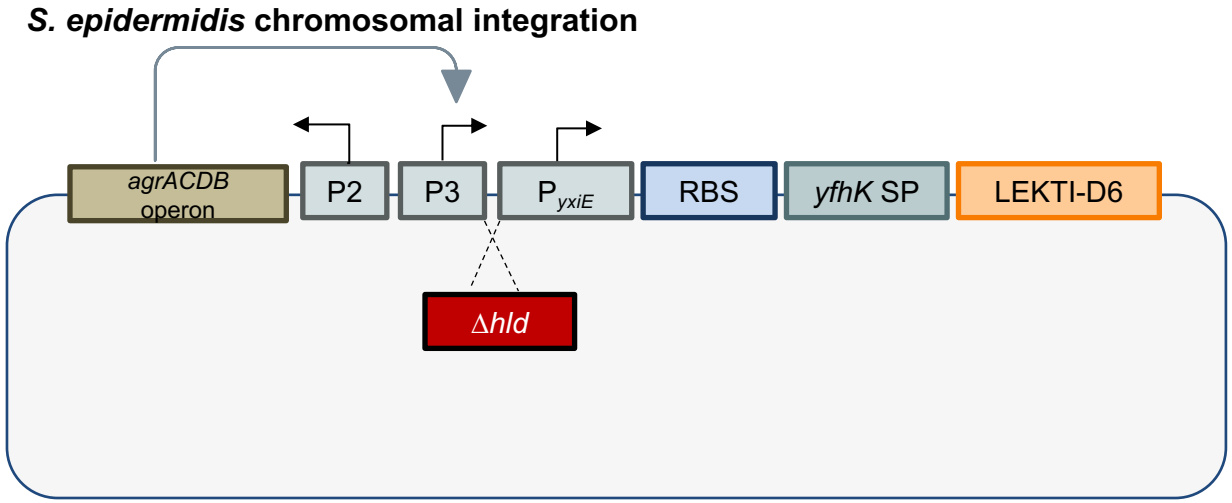
Collectively, ATR-12 addresses Netherton syndrome by LEKTI replacement and IL-36γ inhibition

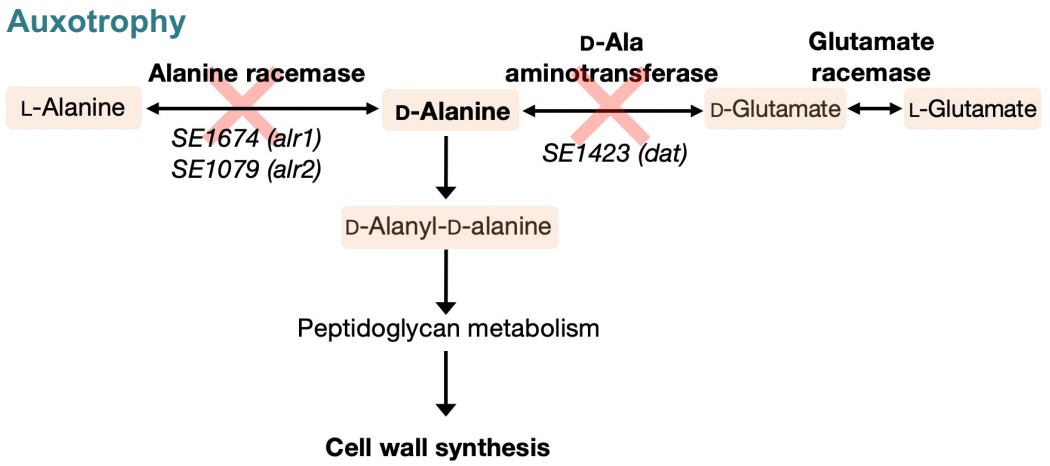
Design of ATR12-351: an auxotrophic, LEKTI-D6 secreting strain of *S. epidermidis*

SE27a: Plasmid expression of LEKTI-D6



SE351: Chromosomal integration of LEKTI-D6 and D-alanine auxotrophy

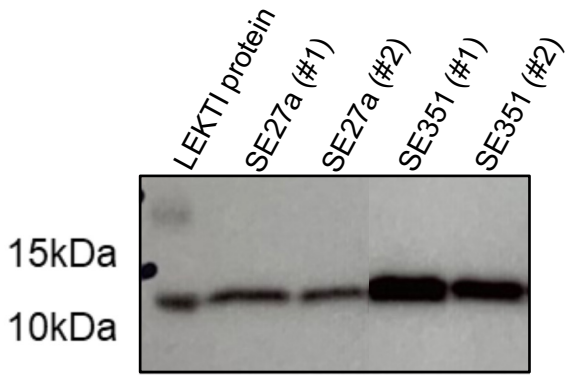




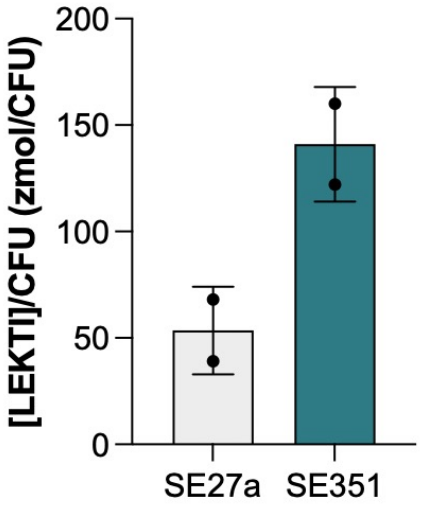
Confirmation of SE351 construction

Increased expression of LEKTI-D6 with chromosomal integration

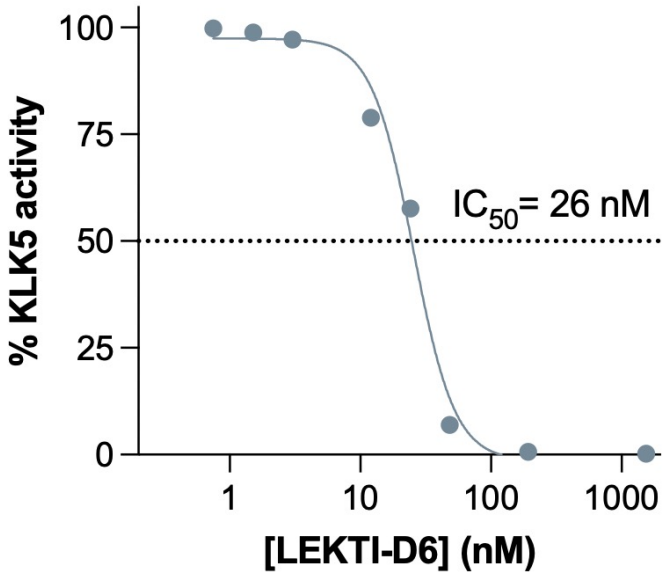
LEKTI protein expression



Active LEKTI/CFU



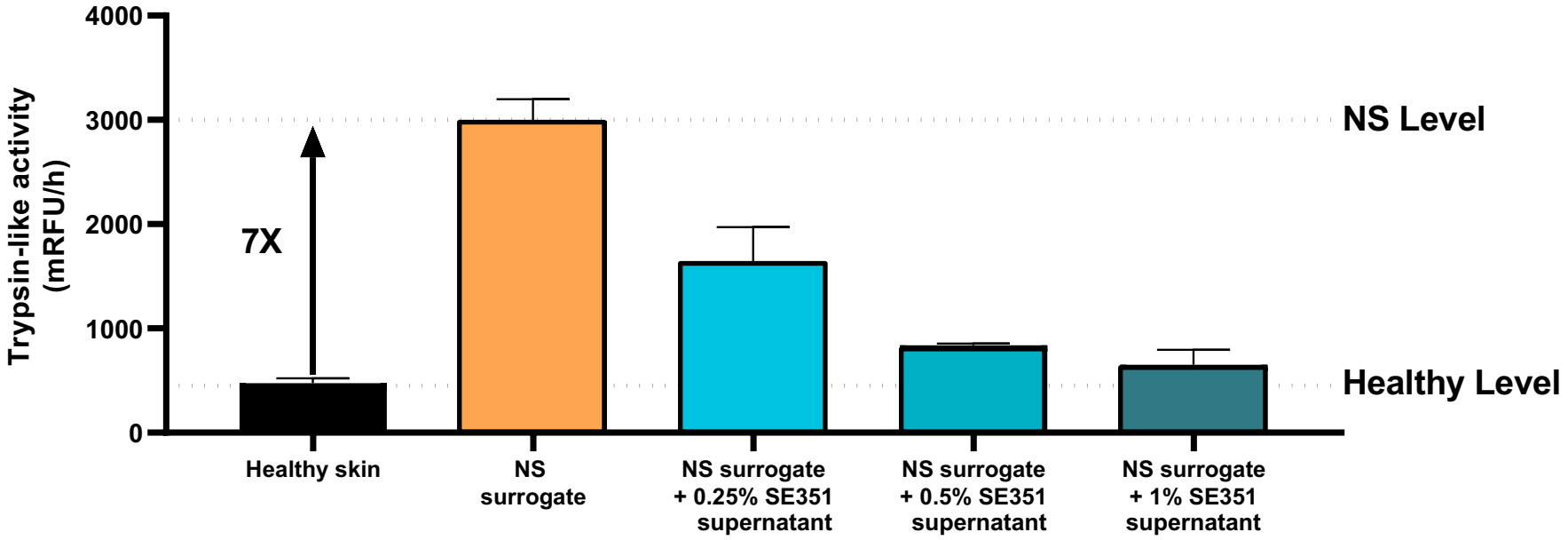
Nanomolar IC50 of KLK5 from SE351



- ✓ Successful chromosomal integration of LEKTI
- ✓ Chromosomal integration results in higher LEKTI expression than episomal LEKTI
- ✓ Nanomolar IC_{50} of KLK from LEKTI produced by SE351

Ex vivo activity of SE351 shows decreased protease activity

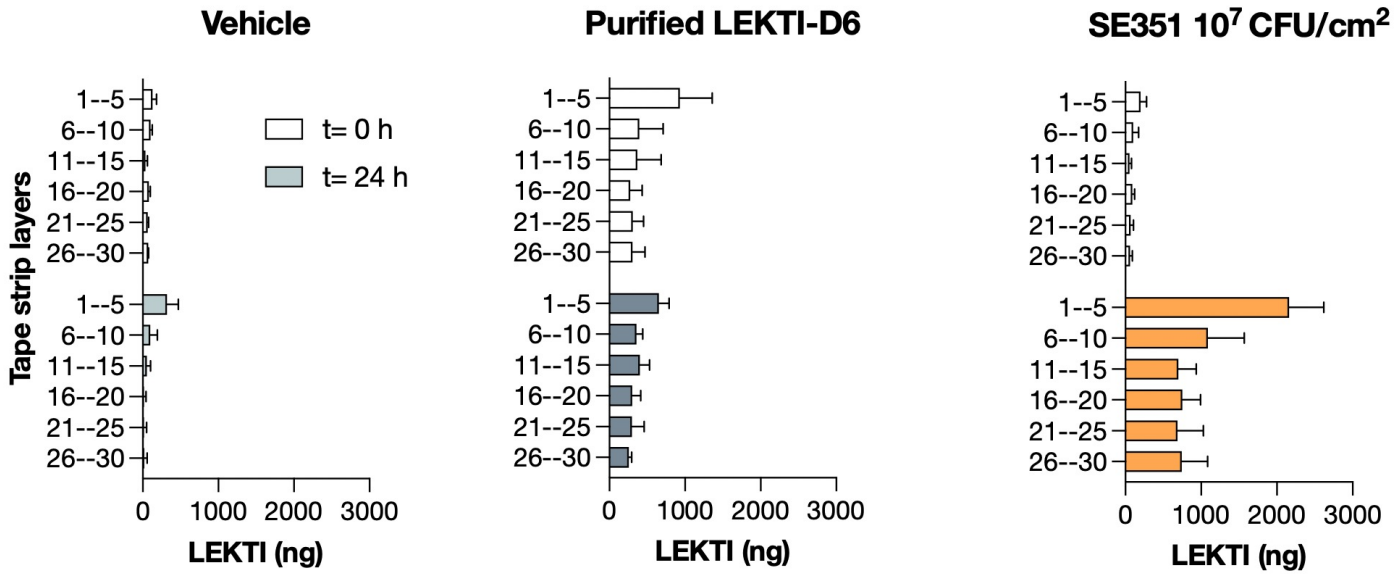
***S. epidermidis* strain SE351 (LEKTI-secreting) spent broth inhibition of human skin tape stripped extracts**



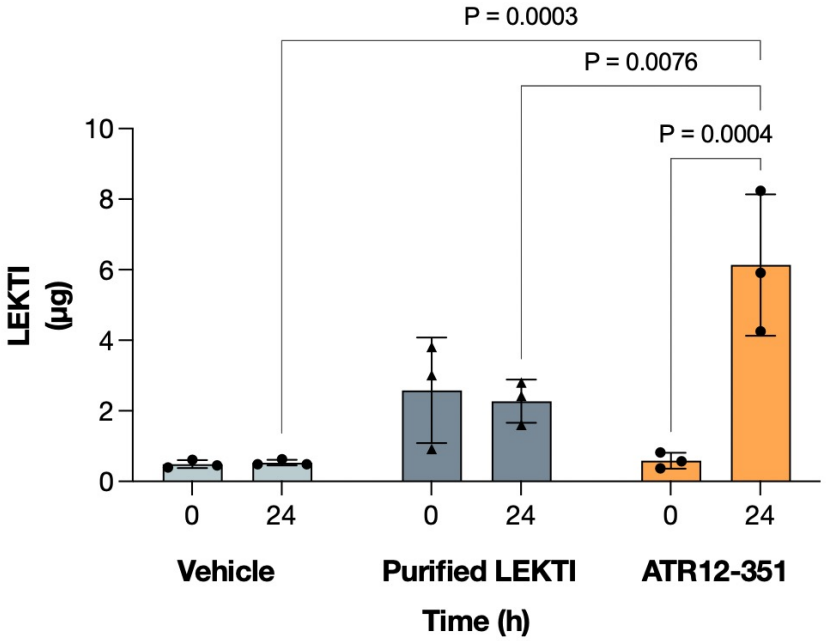
- ✓ Trypsin-like activity (key measure of protease activity in NS patients) decreased after addition of spent broth from LEKTI-secreting strain SE351 in ATR-12
- ✓ Dose-dependent response seen across concentrations of supernatant

ATR-12 provides superior LEKTI delivery compared to topical LEKTI delivery in *ex vivo* full thickness human skin

LEKTI by skin layer



Total LEKTI amounts



- ✓ LEKTI activity is significantly higher after 24 hours compared to vehicle and topical protein alone in all layers following ATR-12 application
- ✓ The LEKTI activity penetrates to at least 30 layers deep in substantial amounts

Summary

- ✓ *S. epidermidis* can serve as a **novel drug delivery system**
- ✓ Synthetic biology tools using chromosomal integration, promoter optimization, and secretion peptides can **enhance expression and delivery of protein**
- ✓ ATR12-351, a LEKTI-expressing strain of *S. epidermidis* in development for Netherton syndrome (NS) has demonstrated key proof of concept in preclinical studies:
  - ✓ ATR12-351 has **nanomolar IC_{50}** values to inhibit KLK5, a key driver of NS
  - ✓ ATR12-351 delivers functional LEKTI and **reduces protease activity to normal levels**
  - ✓ ATR12-351 delivers LEKTI significantly **more effectively than LEKTI delivery alone**
- ✓ Azitra has an open Phase 1b clinical trial in NS (NCT06137157)

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THANK YOU

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