Microbiome Products to Address Skin Health and Appearance

May 22nd, 2019
Skin health and appearance are closely linked to the microbiome

Azittra employs a three-part strategy to address skin disease and skin conditions using *Staphylococcus epidermidis* (SE)

- **SE**, a commensal organism
- **Microbiome**
- **Active drug ingredients from SE**
- **SE expressing therapeutic proteins**
Azitra’s product portfolio addresses the cause of skin disease and the consequences of dysbiosis.

**AZT-01**
- Eczema
- >$7B (US)

**AZT-02**
- Netherton syndrome (Orphan)
- >$800M (US)

**AZT-03**
- Inflammatory disease (e.g. Psoriasis)
- >$6.2B

**AZT-04**
- EGFR inhibitor associated rash
- > 1B (US)

**AZT-14**
- Base strain for consumer health markets

Consumer products based on non-engineered SE

Pharma products based on engineered SE
Current Pipeline

**DISCOVERY**
- **AZT-04**: SE auxotroph for EGFR inhibitor associated rash
- **AZT-02**: LEKTI-secreting SE for Netherton syndrome
- **AZT-01**: Filaggrin-secreting SE for atopic dermatitis
- **AZT-03**: IL-10-secreting SE for psoriasis
- **AZT-05**: Filaggrin protein for ichthyosis vulgaris
- **AZT-14**: Native SE for consumer health

**PRECLINICAL**

**PHASE 1**

**PHASE 2**

**PHASE 3**

Will consolidate phase 1 and 2
Staphylococcus epidermidis (SE) is an excellent colonizer of human skin, naturally abundant and able to grow on all human skin types. And upon skin colonization, SE can:

- Improve hydration
- Improve lipid content
- Decrease inflammation
- Promote tissue repair
- Protect against pathogens

Nodake et al, 2015
Nodake et al, 2015
Lai et al, 2009
Naik et al, 2015
Linehan et al, 2018
Cogen et al, 2010
Lai et al, 2010
Iwase et al, 2010
Naik et al, 2015
SE can be engineered to deliver therapeutic proteins

Live Biotherapeutic Products (LBPs) target the cause of skin disease and address dysbiosis
SE is also a source of bioactive drug products

DoD-supported discovery program targeting MRSA
Azitra’s *S. epidermidis* (SE), AZT-04

Rationale

Selection

Development plan for EGFR inhibitor-associated rash
Robust published body of high-impact evidence around SE

Non-classical Immunity Controls Microbiota Impact on Skin Immunity and Tissue Repair

Microbial guardians of skin health
Skin microbes can promote skin immunity, repair, and antimicrobial defense

Compartmentalized Control of Skin Immunity by Resident Commensals

S. epidermidis Influence on Host Immunity: More Than Skin Deep

Staphylococcus epidermidis Esp inhibits Staphylococcus aureus biofilm formation and nasal colonization

A commensal strain of Staphylococcus epidermidis protects against skin neoplasia

Commensal–dendritic–cell interaction specifies a unique protective skin immune signature
Strains of **SE** have potent anti-inflammatory effects

SE contributes to skin immunity and tissue repair

Liehan JL ... Belkaid Y. Cell. 2018.
SE (AZT-04) grows well on most skin types
Azitra selected its SE from the Azitra/Jackson Lab strain library

**Figure**. Within and between-individual strain variation of *S. epidermidis* in healthy skin over time. (A) Dendrogram of *S. epidermidis* strain similarity based on core SNVs. Similar strains are grouped into clades. (B) Full strain tracking for 2 representative individuals. Sample headings are colored by skin site characteristic. T1, T2, and T3 are time points, with T1-T2 >1 year and T2-T3 <1 month. Each color in a bar represents the relative abundance of a strain from the phylogenetic tree on the left that represents reference genomes. Hp: hand, Vt: forearm, Ac: inner elbow, lc: buttock crease, Id: finger web, Pc: leg crease, Ph: heel, Tw: toe web, Tn: toenail, Al: side of nose, Ea: ear canal, Ba: back, Ch: cheek, Gb: forehead, Mb: mouth, Oc: nape, Ra: behind ear.

NIH grant 1R42AI142971-01 (Azitra/Jackson Lab)
**SE colonizes mouse skin**

SE readily colonizes wildtype and compromise mouse skin and continues to grow rapidly after colonization.

SE (blue) is able to integrate into mouse microbiome (where SE is not native) and take up 20-30% of the total microbiome.

<table>
<thead>
<tr>
<th>Actinobacteria</th>
<th>Bacteroidetes</th>
<th>Chlamydia</th>
<th>Cyanobacteria</th>
<th>Firmicutes</th>
<th>Eukaryota (Fungi)</th>
<th>Proteobacteria</th>
<th>Unclassified</th>
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<tr>
<td>Protectobacterium</td>
<td>Bacteroides</td>
<td>Chlamydia</td>
<td>Microcystis</td>
<td>Clostridium</td>
<td>Alternaria</td>
<td>Alcaligenes</td>
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<td>Rhodococcus</td>
<td>Bacteroides</td>
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<td>Dorea</td>
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<td>Elusimicrobiaceae</td>
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<td>Lachnospiraceae</td>
<td>Parasagittaria</td>
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<td>Parasabacteroides</td>
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<td>Lactobacillus</td>
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Corporate discussion document

**CONFIDENTIAL**
Auxotrophy provides control and avoids antibiotic selection

Azitra’s core strains are auxotrophic strains of SE

✓ Selected Wild Type strain converted to a proprietary auxotroph
✓ Concept:
  • SE requires D-alanine for growth and survival
  • Deletion of D-alanine racemase(s) creates auxotroph
  • Growth and survival is dependent on D-alanine provided by the formulation
✓ Auxotrophy provides for control of growth, proliferation and distribution
✓ Can be used as a cosmetic or a therapeutic
✓ Can be engineered to deliver therapeutic proteins
✓ Is readily manufactured and formulated
AZT-04 can outcompete other microbes on RHE

- **P. acnes** relevant in acne
- **S. capitis** relevant in armpit odor

### High Propionibacterium
- Recipient communities + *epidermidis* challenge

### High Staphylococcus
- Recipient communities + *epidermidis* challenge

Julia Oh’s lab (data unpublished)

Species:
- C. pseudogenitalium
- M. luteus
- P. acnes
- S. capitis
- S. epidermidis

Relative Abundance:
- 1.00
- 0.75
- 0.50
- 0.25
- 0.00

Replicates (n=3)

CFUs/Ct:
- $10^6$
- $10^5$
- $10^4$
- $10^3$
- $10^2$
- $10^1$
AZT-04 suppresses *S. aureus* population *in vitro* (RHE)

*S. aureus*-associated dysbiosis is associated with eczema, psoriasis, and EGFR inhibitor-associated rash.
AZT-04 increases antimicrobial peptide levels in the skin

**SE application on RHE (reconstructed human epidermis) increases human beta-defensin**
EGFR inhibitors cause a rash in up to 70-90% of patients

This rash is strongly linked to *S. aureus* colonization

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**EGFR-induced skin reactions in patients (%)**

- Rash: 80%
- Alopecia: 45%
- Paronychia: 30%
- Nail alteration: 20%
- Xerosis: 20%
- Trichomegaly: 1%

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Fabbrocini G *et al.* Skin Appendage Disord. 2015

Kobayashi T *et al.* *Immunity*. 2015
EGFR Inhibitor associated rash: near term opportunity supported by biology

- Large unmet need >$1B (US)
- Current treatment: oral antibiotics and topical steroids
- Well-defined problem
  - Papulopustular acneiform eruption: 70-90% of patients
  - Rapid onset, dose-dependent, long lasting, and consistent symptoms
- Use of EGFR inhibitors leads to severe impairment of skin homeostasis, inflammation, poor barrier function and impaired host defense
- Strong link to dysbiosis and presence of staphylococcus aureus bacteremia (Li J et al., 2009 JOP, Grenader et al., 2008 Clin Lung Cancer)
EGFR inhibition impairs the cutaneous immune defense of keratinocytes allowing *S. aureus* colonization

Lichtenberger et al., 2013 Sci Translational Med
###Clinical strategy: AZT-04 EGFR inhibitor associated rash

####Clinical Advisor (formal engagement in process)
Mario E. Lacouture, MD
MSKCC, Oncodermatology
World Expert on cancer therapy related skin toxicities

<table>
<thead>
<tr>
<th></th>
<th>Objective</th>
<th>Study Population</th>
<th>Design</th>
<th>Study Arms and Size</th>
<th>Primary Endpoint</th>
<th>Secondary Endpoints</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Initial human study</strong></td>
<td>Safety</td>
<td>Normal human volunteers</td>
<td>Subjects randomized to different doses, double blind, placebo controlled, each subject is their own control</td>
<td>3 arms, 6 subjects per arm 3 doses with single administration</td>
<td>Safety</td>
<td>Microbiome population on the skin over time</td>
</tr>
<tr>
<td><strong>Phase 1b (under IND)</strong></td>
<td>Safety</td>
<td>EGFR inhibitor patients</td>
<td>Randomized, double blind, placebo controlled</td>
<td>16-24 patients per dose</td>
<td>Safety</td>
<td>Visual assessment of skin tone and texture</td>
</tr>
</tbody>
</table>
AZT-04 is readily manufactured and stable in storage & in use

Manufacturing and formulation supports first human use

✓ CRO manufactures AI
  • GMP cell bank
  • GMP production

✓ Production: highly efficient, low cost

✓ AI: excellent storage stability

✓ Formulated product: excellent room temp stability
Engineered protein-secreting SE strains

AZT-01, AZT-02, and AZT-03
Novel microbe-based protein delivery platform

Modular plasmid design

1. Promoters
2. RBS
3. Secretion signal peptide
4. Gene of interest
5. Cell penetrating peptide

+ Kill Switches, Biosensors
AZT-01: eczema (atopic dermatitis)

Three challenges:

1. Atopic dermatitis (AD) most often due to deficiency in filaggrin, a critical skin barrier protein
2. *S. aureus* predominates the skin, leading to dysbiosis*
3. Skin in inflamed with a TH2-driven immune response

✓ >15 million patients in the US
✓ Market value >$7.2 B
✓ Most often affects children & young adults
✓ Recurrent, chronic disease, flares
✓ Limited satisfaction with current therapies
✓ High demand for effective topical therapy

*a shift in the natural microbiome leading to an increase in bacteria not normally present on the skin*
Filaggrin protein is key to skin structure and moisture

* Natural Moisturizing Factors

Azitra filaggrin reduces water loss in *ex vivo* human skin explant

Effect of tape stripping on TEWL

- 5X Increased water loss

- Reduced water loss

<table>
<thead>
<tr>
<th>Time (days)</th>
<th>Normal</th>
<th>Stripped Ctl</th>
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<tbody>
<tr>
<td>1</td>
<td></td>
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<td>2</td>
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<td>5</td>
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</table>

Effect of hFLG on the change in TEWL (relative to baseline stripped)

- TEWL is increased by 5x following damage to the skin barrier
- Rx with exogenous hFLG dose-dependently reduced the TEWL
Azitra filaggrin provides Natural Moisturizing Factors (NMFs)

Significant and consistent levels of NMFs found after application of Azitra filaggrin to human skin
A chronic, severe, orphan disease caused by deficiency in LEKTI protein

- Autosomal recessive disease affecting 260,000 patients WW
- Skin exhibits excessive peeling, dry scales, severe inflammation, pruritus*, and redness
- Infants at risk for failure to thrive: 10% die in their first year
- Delivery of LEKTI to the skin can inhibit skin loss

* itching


AZT-02: Netherton syndrome (NS)
AZT-02 for Netherton syndrome expresses LEKTI

LEKTI delivery by a commensal skin microbe

1. Recombinant LEKTI production
2. Optimized LEKTI secretion
3. Skin colonized by engineered microbe

Therapeutic strategy:

1. Promoter
2. Secretion signal
3. SPINK5

LEKTId6
Overactive KLKs

Staphylococcus epidermidis
AZT-02 for Netherton syndrome expresses functional LEKTI

*In vitro* assay for KLK inhibition

Kallikrein (KLK)

Colorimetric/fluorogenic peptide

Candidate inhibitor

Quantify protease activity by evolution of A405 nm signal

**Enzyme inhibition (% of Ctrl)**

<table>
<thead>
<tr>
<th></th>
<th>LEKTI Domain</th>
<th>KLK5 IC₅₀ (nM)</th>
<th>KLK7 IC₅₀ (nM)</th>
</tr>
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<tbody>
<tr>
<td>D6</td>
<td>160</td>
<td>40</td>
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<tr>
<td>D8</td>
<td>210</td>
<td>80</td>
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<tr>
<td>D10-15</td>
<td>&gt;250</td>
<td>46</td>
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</table>

LEKTI domain 6 is a promising fragment for expression given its high activity on KLK5/7 and its ready expression in prokaryotes
1. Treatment of skin disease with recombinant commensal skin microorganisms (2034)
2. Treating skin disease with recombinant organisms (2038)
3. Auxotrophic strains of Staphylococcus bacteria (2038)
4. Treatment of Netherton syndrome with LEKTI expressing microbes (2038)
5. Cosmetic compositions with engineered bacteria (2038)
6. Treating Atopic Dermatitis with recombinant organisms (2038)
7. Treating inflammatory skin disease with recombinant organisms (2038)
8. Filaggrin subunits for treatment of eczema and ichthyosis vulgaris (2038)
9. Commensal bacteria to treat cancer associated rash (2038)
Highly experienced team

RICK ANDREWS MS SM
CEO

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Bios Partners
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MICROBIOLOGY AND STAPH

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Corporate Strategy

STELLA ROBERTSON, PHD
DRUG DEVELOPMENT

Corporate discussion document
Harnessing the microbiome to treat skin disease.