A Novel *Staphylococcus epidermidis* Compound for the Topical Treatment of Epidermal Growth Factor Receptor (EGFR) Inhibitor-Induced Dermal Toxicity

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Abstract

Systemic therapeutic agents targeting the EGFR-mediated signaling pathway are used for the treatment of advanced cancers, including of the lung, pancreatic, colorectal, and head and neck. Significant dermal toxicities occur in up to 90% of patients treated with EGFR inhibitors (EGFRIs) and other medications inhibiting downstream signaling pathways. These toxicities may be disruptive to a patient's quality of life and adherence to therapy. Inhibition of the EGFR pathway may suppress host defenses and lead to opportunistic pathogenic colonization or infection. Dermal toxicity is associated with elevated levels of *Staphylococcus (S.)aureus* and IL-36γ.

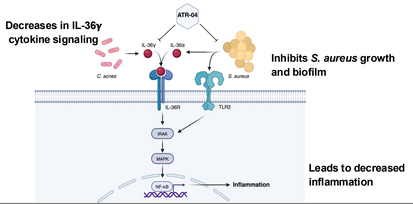
ATR04-484 is a novel *S. epidermidis* strain isolated from a healthy human volunteer, selected for its ability to reduce *S. aureus* colonization and inhibit IL-36γ when applied topically.

ATR04-484 has a promising profile of activities for the treatment of EGFRI-induced dermal toxicity by significantly reducing *S. aureus* growth and completely ameliorating IL-36γ levels. Reconstructed human epidermis (RHE) and *ex vivo* pig skin were utilized to measure the effect of ATR04-484 on *S. aureus* in therapeutic and prophylactic settings with *S. aureus* added prior to or after topical ATR04-484 application, respectively. ATR04-484 inhibited growth of both methicillin-resistant (MRSA) and methicillin-sensitive (MSSA) *S. aureus* in both therapeutic and prophylactic settings. IL-36γ levels were measured on RHE treated with erlotinib alone or in combination with ATR04-484. Application of ATR04-484 reduced erlotinib-induced IL-36γ to a level comparable to non-erlotinib treated RHE ($p<0.001$).

An ongoing multicenter, randomized, double-blind, vehicle-controlled Phase 1/2 clinical study is designed to evaluate the safety and tolerability of topical ATR04-484 (10^9 CFU/g) for the treatment of EGFRI-induced dermal toxicity affecting the face of adult patients. ATR04-484 or its vehicle (3:1 randomization) will be applied in a stable volume to all patients, including the face. The key objectives of the study are to assess the safety and tolerability of topical ATR04-484 and to evaluate efficacy signals including severity of disease, pruritus, and pain. The bioavailability of ATR04-484 and pharmacodynamic parameters (including IL-36γ) are also studied.

ATR04-484: Composition and Expected Mechanism of Action

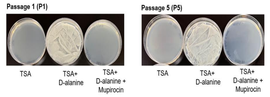
- **ATR04-484 is a live biotherapeutic product,** comprised of an **auxotrophic strain** (**SE484**) of *Staphylococcus epidermidis*
  - Auxotrophic for D-alanine, to control its growth
  - Deleted mupirocin resistance and putative bacteriophage genes



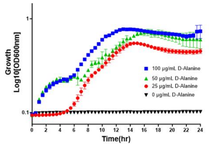
Preclinical Data

D-alanine Auxotrophy and Mupirocin Sensitivity

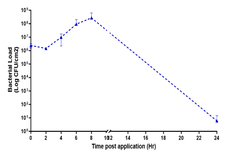
D-alanine auxotrophy and mupirocin sensitivity on tryptic soy agar (TSA)



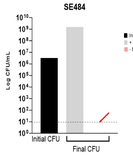
SE484 does not grow without D-alanine *in vitro*



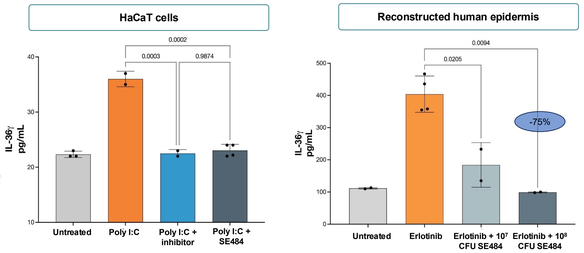
Single dose of SE484 on *ex vivo* pig skin dies within 24 hours



SE484 does not grow in human blood

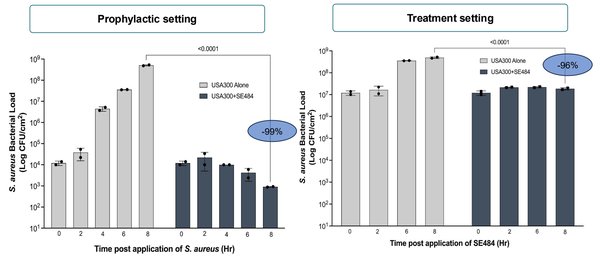


SE484 Significantly Reduces IL-36γ (RHE)



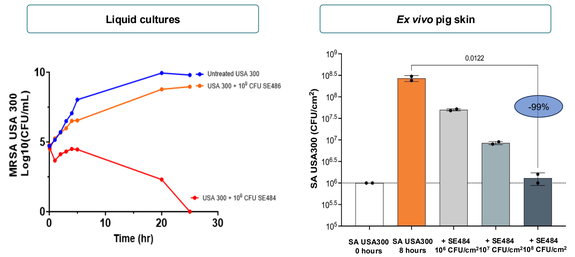
- ✓ **Anti-IL36γ effects** were assessed (1) in HaCaT cells induced to overexpress IL-36γ with Poly I:C and treated with a Poly I:C inhibitor (TLR3/dsRNA); and (2) in reconstructed human epidermis induced to overexpress IL-36γ with erlotinib and treated with SE484
  - ✓ Dose-dependent reduction in IL-36γ observed

SE484 Reduces and Prevents *S. aureus* Growth (RHE)



- ✓ **Anti-*S. aureus* activity** was assessed in reconstructed human epidermis in both a prophylactic setting (pretreated with SE484 then methicillin-resistant *S. aureus* [MRSA] strain USA300 added) and in a treatment setting (pretreated with MRSA then SE484 added)
  - ✓ In RHE models, methicillin-resistant *S. aureus* (MRSA) was reduced by >99% in the prophylactic setting and by 96% in the treatment setting

SE484 Reduces *S. aureus* Growth (*ex vivo* porcine model)

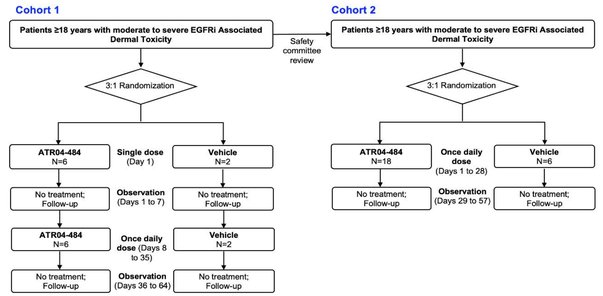


- ✓ **Anti-*S. aureus* activity** was assessed (1) in liquid cultures, and (2) on *ex vivo* pig skin pretreated with MRSA
  - ✓ In liquid cultures, SE484 **reduced** methicillin-resistant *S. aureus* (MRSA) with a **dose-dependent** effect
  - ✓ In *ex vivo* pig skin, *S. aureus* was **reduced by >99%** in the treatment setting with a **dose-dependent** effect

Phase 1/2 Clinical Study Overview

- Multicenter, randomized, blinded, vehicle-controlled Phase 1/2 trial of ATR04-484 in adult patients with moderate to severe EGFRi-related non-infected dermal toxicity affecting the face
  - Additional body sites, including the neck, chest, back, and other areas may also be affected and treated with the study drug
- Dose level: 10^9 CFU / g ATR04-484
- Approximately 32 eligible patients at 4-6 sites in the U.S. will be randomly assigned (3:1 ratio) to treatment with ATR04-484 or its vehicle ointment, in 2 cohorts
  - Cohort 1 (n=8): single dose leading to multiple dose for 28 days
  - Cohort 2 (n=24): multiple dose cohort for 28 days

Clinical Study Schema and Objectives



Objectives	Endpoints
Primary • To evaluate the safety and tolerability of ATR04-484 following topical application to the face, neck, chest, and back	• Frequency and incidence of TEAEs including application site reactions, CS changes in physical and skin examinations, VS, ECG parameters, and clinical labs • Unanticipated EGFRi dose adjustments or rescue therapy for the dermal toxicity will be described.
Secondary • To assess the effect of ATR04-484 on modified CTCAE descriptors for skin toxicity • To assess the effect of ATR04-484 on local pruritus and pain • To evaluate the bioavailability of SE484 in the plasma	• Change from baseline (BL) in Investigator assessment of disease severity based on modified CTCAE. • Change from baseline in numeric rating scales (24-hour reflective assessments) for pruritus and pain. • Bioavailability of SE484 in plasma by qPCR
Exploratory • To assess changes in selected skin and plasma cytokines • To assess changes in the skin microbiome and describe the relative abundance of SE484 • To evaluate health-related QOL (Functional Assessment of Cancer Therapy-EGFRI 18; FACT-EGFRI-18)	• Change from BL in selected skin and plasma cytokines as biomarkers, including IL-36γ. • Change from BL in skin and nasal microbiome assessed by shotgun sequencing. • Change from baseline in health-related quality-of-life assessed by FACT-EGFRI-18 questionnaire.

Summary

- EGFRi-associated dermal toxicity is associated with a **significant unmet need for treatment,** as it **leads to EGFRi discontinuations and poor quality of life** in cancer patients
- ATR04-484, a drug product containing SE484, an auxotrophic strain of *S. epidermidis*, has demonstrated key proof of concept for safety and effect of the drug to treat EGFRi-induced dermal toxicity:
  - **auxotroph** controlled by D-alanine
  - **significantly reduces IL-36γ** induced by erlotinib
  - **significantly reduces *S. aureus*** in multiple models, including *ex vivo* pig skin
- Multi-dose clinical study (NCT06830863) to enroll patients with moderate to severe EGFRi associated dermal toxicity, to assess safety and to evaluate effect following 4 weeks of topical therapy

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