Topical Bacteriophage Cocktail (AB-SA01) for Recalcitrant *Staphylococcus aureus* Chronic Rhinosinusitis: A Randomized, Placebo-Controlled Efficacy Trial of Intranasal Sinus Irrigation

Project Summary / Abstract

Chronic rhinosinusitis (CRS) is a common inflammatory disease of the sinonasal mucosa, and a recalcitrant subset persists despite endoscopic sinus surgery (ESS), prolonged antibiotics, and topical therapy. *Staphylococcus aureus* is a frequent pathogen in this group, and its mucosal biofilm formation is associated with treatment failure and disease severity; biofilm-embedded *S. aureus* is markedly more antibiotic-tolerant than planktonic cells, so antibiotics often fail. Lytic bacteriophages suit this niche: they self-amplify at the infection site, can disrupt biofilm, are deliverable topically by sinus irrigation to diseased mucosa, and can kill methicillin-resistant *S. aureus* (MRSA). Anti-staphylococcal phage reduced biofilm of CRS *S. aureus* ex vivo isolates (Drilling 2014, *AJRA*); topical phage plus EDTA was safe and reduced infection in a sheep sinusitis model (Drilling 2014, *IFAR*); a related cocktail was safely delivered in an in vivo CRS sinusitis model (Fong 2019); and a Phase 1 first-in-human trial of intranasal AB-SA01 met its primary safety/tolerability endpoint with reduced *S. aureus* burden and improved endoscopic appearance (Ooi 2019). No randomized controlled efficacy trial has reported. This R01 will (1) define AB-SA01 breadth, biofilm-reducing activity, and resistance suppression against contemporary U.S. CRS *S. aureus*/MRSA isolates; (2) optimize phage–EDTA and phage–antibiotic regimens in biofilm and sheep sinusitis models; and (3) conduct a U.S. randomized, double-blind, placebo-controlled trial of twice-daily AB-SA01 sinus irrigation under an FDA IND, testing microbiologic and endoscopic efficacy. Success would move topical phage toward routine use for one of otolaryngology's most refractory infections.

Specific Aims

Recalcitrant *S. aureus* CRS is a high-burden, biofilm-associated disease that repeatedly defeats

antibiotics and surgery. A completed Phase 1 trial of intranasal AB-SA01 demonstrated safety, reduced bacterial burden, and endoscopic improvement (Ooi 2019), but the field lacks the controlled efficacy evidence needed for adoption. We will close that gap.

Aim 1 — Define breadth, biofilm-reducing activity, and resistance suppression of AB-SA01 against contemporary U.S. CRS *S. aureus* isolates. We will biobank *S. aureus*/MRSA isolates from U.S. recalcitrant CRS patients and quantify cocktail host range, biofilm-biomass reduction, and emergence versus suppression of phage resistance under single-phage versus full-cocktail exposure. *Hypothesis:* the multi-phage cocktail lyses most isolates, reduces biofilm biomass, and suppresses resistant-mutant outgrowth that single phages permit.

Aim 2 — Optimize adjunctive regimens that enhance biofilm killing. In validated biofilm and sheep sinusitis models, we will compare phage + EDTA and phage + antibiotic regimens against phage alone for biofilm eradication, mucosal safety, and durability. *Hypothesis:* biofilm disruptors and synergistic antibiotics improve eradication without compromising mucosal safety.

Aim 3 — Test clinical efficacy in a U.S. randomized, double-blind, placebo-controlled trial. Adults with recalcitrant *S. aureus* CRS will be randomized to twice-daily intranasal AB-SA01 sinus irrigation (up to 3×10^9 PFU) versus placebo irrigation for 14 days, under FDA IND and IRB approval. *Co-primary endpoints:* change in *S. aureus* burden/eradication and change in endoscopic Lund-Kennedy score; safety and patient-reported outcomes are secondary.

Impact: Moving AB-SA01 from a single-arm Phase 1 safety signal to controlled efficacy data could make culture-matched topical phage rinses an alternative to repeated antibiotics and revision surgery for refractory *S. aureus* CRS.

Significance

CRS carries a large clinical and quality-of-life burden, and the recalcitrant subset that fails ESS, antibiotics, and topical therapy is the central unmet need. *S. aureus* is a frequent pathogen here, and its mucosal biofilm formation is associated with treatment failure and severity. Because biofilm-embedded *S. aureus* is substantially more antibiotic-tolerant than planktonic cells, antibiotics frequently fail and patients cycle through repeated courses and revision surgery. Lytic phages directly address the biofilm problem and can kill MRSA. Anti-staphylococcal phage reduced biofilm of CRS isolates ex vivo (Drilling 2014, *AJRA*), and topical phage with EDTA was safe and reduced infection in a sheep sinusitis model (Drilling 2014, *IFAR*). A controlled efficacy trial is the necessary next step, and a positive result would change management of refractory *S. aureus* sinus disease.

Innovation

This project is innovative in indication, formulation, and delivery. (1) It targets the biofilm biology that defines recalcitrant *S. aureus* CRS rather than planktonic bacteria. (2) It advances a *defined multi-phage lytic cocktail* (AB-SA01) selected for breadth across clinical *S. aureus*/MRSA and tested here for resistance suppression, since multi-phage exposure is expected to limit resistant mutants that single phages select. (3) It exploits *topical sinus-irrigation delivery* of self-amplifying phage onto diseased mucosa, paired with a biofilm disruptor (EDTA) and phage–antibiotic combinations to render tolerant MRSA biofilms treatable. (4) It is, to our knowledge, among the first U.S. randomized controlled efficacy trials of topical phage for CRS, building on first-in-human AB-SA01 data (Ooi 2019). Engineered or antibiotic-resensitizing phages are a future extension, preclinical for CRS and out of scope here.

Approach

Aim 1 — Cocktail breadth, biofilm reduction, and resistance suppression

Rationale. Phages are strain-specific, so value depends on breadth against the local clinical population and on suppressing resistance; anti-staphylococcal phage reduced biofilm of CRS *S. aureus* ex vivo isolates (Drilling 2014, *AJRA*), and these properties must be confirmed against contemporary U.S. isolates.

Design. We will biobank *S. aureus*/MRSA isolates from U.S. recalcitrant CRS patients [N ILLUSTRATIVE: ~80–100]. Host range of AB-SA01 and each component phage will be measured by spot and efficiency-of-plating assays; biofilm activity by crystal-violet biomass and viable-count assays with and without phage; resistance suppression by serial passage under single-phage versus full-cocktail pressure, scoring resistant-mutant frequency. Isolates will be molecularly typed and phage titers verified.

Expected outcomes. The cocktail lyses most isolates, reduces biofilm biomass, and suppresses resistant outgrowth that single phages permit, defining a U.S. coverage profile and a resistance-monitoring assay for Aim 3.

Pitfalls & alternatives. If coverage is lower than expected, we will document non-susceptible lineages and use culture-matching at trial screening rather than altering the fixed product. If biofilm reduction is modest, Aim 2 adjuncts mitigate.

Aim 2 — Adjunctive regimens (phage + EDTA; phage + antibiotic)

Rationale. EDTA and synergistic antibiotics can enhance killing; topical phage + EDTA was already safe and reduced infection in a sheep sinusitis model (Drilling 2014, *IFAR*), and a related cocktail was safely delivered in an in vivo CRS sinusitis model (Fong 2019).

Design. Using Aim 1 isolate biofilms and the sheep sinusitis model (Drilling 2014, *IFAR*; delivery context Fong 2019), we will compare phage alone, phage + EDTA, and phage + antibiotic for biofilm-biomass and viable-count reduction, mucosal histology/safety, and durability. Dosing will bracket the clinically tested range (up to 3×10^9 PFU; Ooi 2019). Animal studies will be randomized, use blinded histologic scoring, and include both sexes where feasible.

Expected outcomes. EDTA and/or antibiotics improve eradication over phage alone without added mucosal toxicity, yielding a lead regimen and dosing rationale for Aim 3.

Pitfalls & alternatives. If an adjunct adds mucosal irritation, we de-prioritize it and advance the better-tolerated arm; ex vivo and in vivo readouts are cross-checked before translation.

Aim 3 — Randomized controlled efficacy trial of intranasal AB-SA01

Rationale. The Phase 1 first-in-human trial showed AB-SA01 sinus irrigation was safe and tolerable, reduced *S. aureus* burden, and improved endoscopic Lund-Kennedy scores, but was open-label and uncontrolled (Ooi 2019). A randomized, placebo-controlled trial is required to establish efficacy.

Design. Adults with recalcitrant *S. aureus* CRS (culture-positive, prior ESS, failed medical therapy) will be randomized 1:1, double-blind, to twice-daily intranasal AB-SA01 sinus irrigation (up to 3×10^9 PFU) versus matched placebo for 14 days, mirroring the Phase 1 regimen [N ILLUSTRATIVE: ~60–80; multi-site U.S.]. **Co-primary endpoints:** change in *S. aureus* burden/eradication and change in endoscopic Lund-Kennedy score. **Secondary:** treatment-emergent adverse events, patient-reported sinonasal outcomes, and on-treatment resistance monitoring (Aim 1 assay). Conducted under **FDA IND** and **IRB** approval with DSMB oversight and ClinicalTrials.gov registration; sample size [ILLUSTRATIVE] powered to the co-primary endpoints with a pre-specified analysis plan.

Expected outcomes. AB-SA01 reduces *S. aureus* burden and improves endoscopic scores versus placebo, with tolerability consistent with Phase 1.

Pitfalls & alternatives. If recruitment of culture-positive recalcitrant patients is slow, we will add U.S. otolaryngology sites and extend enrollment [ILLUSTRATIVE]. Aim 1 screening will culture-match or exclude non-susceptible strains. If the effect size is smaller than the Phase 1 signal suggests, secondary endpoints and the Aim 2 adjunct regimen will inform a definitive trial.

Timeline

[ALL TIMELINE VALUES ILLUSTRATIVE] **Years 1–2:** Aim 1 biobanking, host-range, biofilm, and resistance assays. **Years 2–3:** Aim 2 adjunct optimization; finalize IND package. **Years 3–5:** Aim 3 trial enrollment, treatment, follow-up, analysis, dissemination. Aims overlap to de-risk the clinical phase.

Budget Justification (Modular R01 Sketch)

[ALL AMOUNTS ILLUSTRATIVE] Requested at **$250,000 direct costs/year for 5 years**. **Personnel:** PI (rhinology/ID), co-investigator microbiologist (phage/biofilm), trial coordinator, research nurse, and a graduate/postdoctoral scientist. **Microbiology/phage:** biobanking, host-range and biofilm assays, resistance monitoring, GMP-grade AB-SA01 study product. **Animal model:** sheep sinusitis studies (Aim 2). **Clinical trial:** site costs, endoscopy, microbiologic sampling, data management, DSMB, IND/regulatory support. A categorical budget with institutional rates will accompany submission.

Vertebrate Animals

Aim 2 uses a sheep model of *S. aureus* sinusitis, the established large-animal system for topical sinus phage delivery in which phage + EDTA was shown safe and infection-reducing (Drilling 2014, *IFAR*; delivery context Fong 2019). Procedures (inoculation, topical irrigation, endoscopic/microbiologic sampling, humane endpoints, histology) follow published methodology. Numbers will be minimized consistent with power [N ILLUSTRATIVE]; both sexes included where feasible; all work IACUC-approved with veterinary oversight, anesthesia/analgesia, and humane euthanasia.

Human Subjects / Clinical Trial

Aim 3 is an NIH-defined clinical trial: a U.S. randomized, double-blind, placebo-controlled study of intranasal AB-SA01 sinus irrigation in adults with recalcitrant *S. aureus* CRS [enrollment ILLUSTRATIVE: ~60–80], under **FDA IND** and **IRB** approval at each site, with written informed consent, DSMB monitoring, and ClinicalTrials.gov registration. The regimen mirrors the Phase 1 protocol that met its safety endpoint (Ooi 2019). Inclusion requires culture-confirmed *S. aureus* and prior failure of surgery/medical therapy; protections address the modest known risks (mild, transient effects in Phase 1). The population will reflect the sex, racial, and ethnic diversity of U.S. CRS patients, with enrollment monitored for inclusion.

Team & Environment

The work integrates rhinology, infectious-disease/phage microbiology, and clinical-trials expertise, modeled on the translational program that generated the foundational evidence (Ooi 2019; Drilling 2014; Fong 2019). The U.S. team pairs an otolaryngology PI experienced in ESS and refractory CRS with a phage/biofilm microbiology core, a vertebrate-animal sinusitis facility, and a multi-site clinical trials unit with IND/IRB and GMP product-supply support to deliver all three aims.

References

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