Strain-Selective Bacteriophage Editing of the Skin Microbiome to Deplete Pathogenic *Cutibacterium acnes* in Acne Vulgaris

Funding mechanism: NIH/NIAMS R21 (Exploratory/Developmental Research Grant) — 2 years; up to $275,000 direct costs total, ≤$200,000 in any single year; preliminary data not required.

Project Summary / Abstract

Acne vulgaris is among the most prevalent skin diseases, and decades of broad-spectrum oral and topical antibiotics have driven widespread *Cutibacterium acnes* resistance and collateral disruption of the wider skin microbiome — a recognized antimicrobial-stewardship problem. Acne is increasingly understood not as simple bacterial overgrowth but as dysbiosis, in which specific pathogenic *C. acnes* phylotypes (notably type IA1) expand in lipid-rich pilosebaceous follicles, form biofilms, and provoke inflammation, while other strains behave as commensals (Castillo 2019; Mohammadi 2024). Bacteriophages are well matched to this problem: *C. acnes* phages are highly host-specific yet, as a group, lyse most clinical strains, so a small defined cocktail could deplete pathogenic *C. acnes* while sparing *Staphylococcus*, *Corynebacterium*, and other commensals — the mechanistic basis for "microbiome editing." The most mature human evidence (BiomX BX001: a topical three-phage hydroxyethylcellulose gel) showed in a double-blind, vehicle-controlled Phase 1 *cosmetic* trial that the product was safe and significantly reduced facial *Cutibacterium* versus vehicle at Day 35 (−0.22 log; p=0.036; ~24%), with no increase in phage-resistant bacteria over repeated exposure (Golembo 2022). These data establish topical feasibility and safety but stop short of the rigorous, quantitative preclinical characterization of strain selectivity, biofilm activity, and resistance dynamics that a therapeutic program requires. This exploratory R21 will (1) genomically characterize a defined lytic phage panel and map its host range across phylotype-typed clinical isolates; and (2) quantify cocktail-mediated planktonic and biofilm killing and resistance emergence in vitro, with a purified endolysin (PAC1-type) as a replication-independent comparator. A contingency-funded ex vivo human follicle assay tests strain-resolved depletion-with-sparing. Deliverables are a genomically defined candidate cocktail, quantitative selectivity/biofilm/resistance benchmarks, and go/no-go criteria to justify a future IND-enabling program — replacing a blunt, resistance-driving antibiotic course with a programmable, strain-selective edit, in direct alignment with NIAMS dermatology and antimicrobial-stewardship priorities.

Specific Aims

Acne vulgaris is driven in part by dysbiosis — proliferation of pathogenic *C. acnes* phylotypes (notably IA1) in pilosebaceous follicles — rather than simple overgrowth (Castillo 2019; Mohammadi 2024). Antibiotic therapy is increasingly compromised by resistance and off-target microbiome damage, motivating precision alternatives. *C. acnes* bacteriophages are exquisitely host-specific yet collectively broad against clinical isolates, making a defined cocktail an attractive microbiome-editing agent. Published human data (BX001 topical three-phage gel) establish topical safety and a significant reduction in facial *Cutibacterium* without an increase in phage-resistant bacteria (Golembo 2022) — but that work was a cosmetic study, and rigorous quantitative preclinical characterization of strain selectivity, biofilm activity, and resistance is still lacking. This R21 is deliberately scoped as exploratory feasibility work to generate that missing characterization and the decision criteria for a future therapeutic program. We will:

Aim 1. Assemble and genomically characterize a defined *C. acnes* phage cocktail and map its host range. We will curate lytic *C. acnes* phages, perform whole-genome sequencing and annotation (capsid, tail, endolysin/holin; confirm absence of integrase/toxin/known resistance genes to verify lytic lifestyle), and confirm the expected low genetic diversity and broad intraspecies host range (Castillo 2019; Mohammadi 2024). Using a panel of phylotype-typed clinical isolates spanning IA1 and commensal-associated types, we will define per-phage and cocktail coverage by efficiency-of-plating and liquid-killing assays, confirm specificity against *Staphylococcus* and *Corynebacterium*, and rationally compose a minimal cocktail maximizing IA1 coverage. *Go/no-go:* a ≥3-phage cocktail covering ≥80% of IA1 isolates with no detectable lysis of tested commensal genera.

Aim 2. Quantify killing, biofilm activity, and resistance dynamics in vitro. We will measure planktonic time-kill across multiplicities of infection and biofilm-associated killing by the cocktail, with a purified PAC1-type endolysin as a replication-independent comparator and a phage+antibiotic combination arm to test reported synergy. We will characterize resistance emergence under serial cocktail passage, distinguishing CRISPR-Cas from CRISPR-Cas-independent mechanisms by whole-genome sequencing of resistant isolates, benchmarked against the BX001 clinical finding of *no increase* over baseline insensitivity on repeated exposure (Golembo 2022). *Go/no-go:* cocktail achieves ≥3-log planktonic kill and measurable biofilm-burden reduction, with resistance frequency not exceeding pre-existing baseline insensitivity by a pre-specified margin.

Contingency Aim 3 (milestone-gated). Strain-resolved depletion with commensal sparing ex vivo. Conditional on meeting Aim 1–2 go/no-go criteria, an ex vivo human skin/follicle explant model co-colonized with defined pathogenic and commensal strains will test whether the topical cocktail (in a hydroxyethylcellulose-type gel) reduces pathogenic *C. acnes* burden while preserving *Staphylococcus*/*Corynebacterium* and overall community structure (16S/shotgun metagenomics),

paralleling the human reduction of *Cutibacterium* without broad disruption (Golembo 2022).

Impact: These studies will deliver a genomically defined candidate cocktail and the quantitative selectivity, biofilm, and resistance data — with explicit go/no-go criteria — needed to justify a future IND-enabling clinical program, advancing a programmable microbiome edit as a stewardship-aligned alternative to antibiotics for acne.

Significance

Acne vulgaris is one of the most common skin diseases worldwide and a core component of the NIAMS dermatology mission. The dominant treatment paradigm — broad-spectrum oral and topical antibiotics — has fueled widespread *C. acnes* antibiotic resistance and collateral disruption of the wider microbiome, a recognized antimicrobial-stewardship problem (Castillo 2019; Mohammadi 2024). Critically, the disease biology has shifted: acne is increasingly understood as dysbiosis, in which specific pathogenic *C. acnes* phylotypes (notably IA1) expand in lipid-rich follicles, form biofilms, and provoke inflammation, while other *C. acnes* strains remain commensal (Castillo 2019; Mohammadi 2024). A therapy that retires the blunt instrument of antibiotics and instead selectively thins pathogenic strains would be a meaningful advance.

Bacteriophages are uniquely matched to this need. *C. acnes* phages are tailed dsDNA Siphoviruses that are highly host-specific yet, as a group, kill most clinical strains, so a small cocktail can in principle deplete pathogenic *C. acnes* while sparing *Staphylococcus*, *Corynebacterium*, and other commensals — the mechanistic basis for "microbiome editing." Because acne is a surface disease, phages are topically accessible; they self-amplify on target, and lytic phages and their endolysins can act on follicular biofilms where antibiotics penetrate poorly (Castillo 2019; Mohammadi 2024). The most mature human evidence — BiomX's BX001, a topical three-phage cocktail in hydroxyethylcellulose gel — showed in a double-blind, vehicle-controlled Phase 1 *cosmetic* trial (75 participants, mild-to-moderate acne) that the product was safe and well tolerated and that high-dose BX001 significantly reduced facial *Cutibacterium* versus vehicle at Day 35 (−0.22 log; p=0.036; ~24% reduction, versus a 26% increase under vehicle), with phage-resistant isolates remaining at their ~9% (4/45) baseline prevalence rather than increasing over repeated exposure (Golembo 2022). These data establish feasibility and topical safety but, by design, stop short of rigorous preclinical efficacy and selectivity characterization. This R21 fills that gap, generating the quantitative selectivity, biofilm, and resistance benchmarks — and the decision criteria — needed to move from cosmetic tolerability toward a therapeutic program.

Innovation

This proposal advances several conceptual and technical innovations. First, it operationalizes "microbiome editing" as a measurable endpoint — quantifying not just pathogen depletion but commensal sparing and whole-community preservation — rather than treating phage as a simple antibacterial. Second, it deliberately exploits the unusual biology of *C. acnes* phages: their characteristically low genetic diversity paired with broad intraspecies host range, which makes a minimal cocktail covering most clinical isolates while remaining specific to *C. acnes* tractable to design (Castillo 2019; Mohammadi 2024). Third, it pairs whole phages with a replication-independent endolysin/artilysin comparator (PAC1-type); engineered *C. acnes* endolysins can be tuned for altered or improved lytic activity, and as direct-acting enzymes are mechanistically expected to face low resistance pressure — providing a built-in fallback arm to interrogate alongside the phage cocktail (Rimon 2023). Fourth, it treats the key biological caveats — pseudolysogeny and both CRISPR-Cas and the more recently described CRISPR-Cas-independent resistance in phylotype IA1 strains — as primary experimental variables with explicit measurement, rather than afterthoughts. Together these elements move beyond the existing cosmetic safety framing toward a mechanistically grounded, stewardship-aligned therapeutic candidate.

Approach

Rigor and reproducibility (applies to all aims). Key biological resources will be authenticated (phage identity by whole-genome sequencing; *C. acnes* isolates by phylotyping and 16S where appropriate). In vitro assays use ≥3 independent biological replicates with pre-specified analysis plans; resistance passaging is run in replicate lineages. For the ex vivo contingency aim, donor explants are treated as biological replicates with blinded, randomized assignment of cocktail versus vehicle and blinded enumeration. Sex of explant donors will be recorded and balanced where feasible. All go/no-go criteria are pre-specified below.

Aim 1 — Cocktail assembly, genomics, and host-range mapping

Rationale. A defensible microbiome-editing cocktail must demonstrably cover pathogenic phylotypes (especially IA1) while remaining restricted to *C. acnes*. The reported low phage diversity but broad host range makes a rational, minimal composition feasible (Castillo 2019).

Experimental design. We will curate lytic *C. acnes* phages from collaborating phage-genomics resources and propagate them on reference hosts. Each phage will undergo whole-genome sequencing, assembly, and annotation, confirming lytic lifestyle (endolysin/holin present; integrase/toxin/known resistance determinants absent). A panel of phylotype-typed clinical isolates spanning IA1 and commensal-associated types will be assembled. Host range will be quantified by

spot-titer and efficiency-of-plating, with liquid-culture killing kinetics as a confirmatory readout; specificity will be tested against *Staphylococcus* and *Corynebacterium* spp. A minimal cocktail (target ~3 phages, mirroring the BX001 composition) will be selected to maximize IA1 coverage.

Expected outcomes. A genomically defined, lytic phage panel; a quantitative host-range matrix; and a rationally composed cocktail covering ≥80% of IA1 isolates with no activity against tested commensal genera.

Potential pitfalls & alternatives. Very low phage diversity could limit coverage breadth; if so, we will prioritize the endolysin/artilysin arm (Aim 2) and/or add phages with complementary receptor usage. If pseudolysogeny confounds plaque scoring, liquid-culture killing kinetics become the primary coverage readout.

Aim 2 — Killing, biofilm activity, and resistance dynamics

Rationale. Follicular biofilms limit antibiotic efficacy, and resistance (including CRISPR-based) is the central risk to durable phage activity; quantifying both directly is essential before translation (Castillo 2019; Mohammadi 2024).

Experimental design. Planktonic killing is measured by time-kill across multiplicities of infection. Biofilm activity is assessed in established *C. acnes* biofilm assays by viable counts and matrix imaging, comparing cocktail, purified PAC1-type endolysin, and phage+antibiotic combinations to test reported synergy. Resistance is probed by replicate serial passage under cocktail pressure; resistant isolates are whole-genome sequenced to distinguish CRISPR-Cas from CRISPR-Cas-independent mechanisms. Because the BX001 trial reported pre-existing insensitivity in ~9% of isolates at baseline with *no increase* on repeated exposure (Golembo 2022), our benchmark is whether in vitro emergence remains within a pre-specified margin of baseline rather than rising. Endolysin activity will additionally be tested against any cocktail-resistant isolates recovered, to probe replication-independent killing as a fallback (Rimon 2023).

Expected outcomes. Quantitative killing and biofilm parameters; evidence for or against phage+antibiotic synergy; characterized resistance frequencies and mechanisms; and an assessment of whether the endolysin arm retains activity where phage resistance arises.

Potential pitfalls & alternatives. If cocktail resistance exceeds the pre-specified margin under intense passage, we will broaden the cocktail, increase reliance on the endolysin/artilysin arm, and/or test sequential dosing. If in vitro biofilms poorly mimic follicles, results are interpreted alongside the contingency ex vivo model.

Contingency Aim 3 (milestone-gated) — Strain-resolved depletion with commensal sparing ex vivo

Rationale. The defining claim — depleting pathogenic *C. acnes* while sparing commensals — should be demonstrated in human tissue, paralleling the clinical reduction of *Cutibacterium* without broad disruption (Golembo 2022). This aim is initiated only if Aim 1–2 go/no-go criteria are met, keeping the project within R21 scope.

Experimental design. A de-identified ex vivo human skin/follicle explant model co-colonized with defined pathogenic and commensal strains receives topical cocktail (in a hydroxyethylcellulose-type gel) versus vehicle under blinded, randomized assignment; outcomes include strain-resolved *C. acnes* burden, *Staphylococcus*/*Corynebacterium* survival, and community structure by 16S/shotgun metagenomics. Formulation tolerability on tissue is monitored.

Expected outcomes. Demonstration that topical cocktail reduces pathogenic *C. acnes* while preserving commensal genera and overall community composition, establishing tissue-level proof-of-concept for microbiome editing.

Potential pitfalls & alternatives. Ex vivo follicle penetration may be limited; we will optimize formulation/contact time and use the endolysin arm as needed. A murine *C. acnes* challenge model (including a multidrug-resistant strain) is held as a *future* in vivo extension beyond this R21 rather than committed here, to preserve feasibility.

Timeline

[ILLUSTRATIVE] Two-year R21. **Months 1–10 [ILLUSTRATIVE]:** Aim 1 — phage curation, sequencing, host-range matrix, cocktail selection; Aim 1 go/no-go review. **Months 8–20 [ILLUSTRATIVE]:** Aim 2 — killing/biofilm assays, synergy, resistance passaging and sequencing, endolysin comparisons; Aim 2 go/no-go review. **Months 16–24 [ILLUSTRATIVE]:** Contingency Aim 3 (if milestones met) — ex vivo studies and microbiome sequencing; integration and assembly of the preclinical package and decision memo for a future IND-enabling pathway.

Budget Justification

[ILLUSTRATIVE] Requested within the NIH R21 cap of **$275,000 direct costs total over two years [ILLUSTRATIVE]**, with no single year exceeding $200,000 (R21 is a non-modular small-grant mechanism). To stay within this ceiling, scope is concentrated in Aims 1–2, with Aim 3 milestone-gated.

- **Personnel [ILLUSTRATIVE]:** PI/PD (~1.8 calendar months/yr [ILLUSTRATIVE]); Co-I phage microbiologist (~1.0 month/yr [ILLUSTRATIVE]); one full-time postdoctoral scientist or, alternatively, ~0.6 FTE technician [ILLUSTRATIVE] (not both, to respect the cap).
- **Sequencing & supplies [ILLUSTRATIVE]:** whole-genome sequencing of phages and resistant isolates, plus 16S/shotgun metagenomics only if Aim 3 is triggered; phage propagation/purification reagents; biofilm and tissue-culture consumables.
- **Endolysin & ex vivo [ILLUSTRATIVE]:** PAC1-type endolysin production; de-identified human skin explant procurement for the contingency aim.
- **Other [ILLUSTRATIVE]:** publication and core-facility fees.

No clinical-trial costs are requested in this R21. **SBIR alternative:** given the consumer-product/topical path, this work is also suitable for an NIH SBIR (R43 Phase I feasibility / R44 Phase II development) submission by a small-business partner, using the same Aims 1–2 framework.

Vertebrate Animals

No vertebrate animal work is proposed within the funded scope of this R21. A murine *C. acnes* challenge model is identified only as a *future* in vivo extension beyond this award. Should any animal work be added by amendment, a full Vertebrate Animals Section would accompany it, including justification of species/numbers (set by power analysis [ILLUSTRATIVE]), minimization of pain/distress with appropriate analgesia and humane endpoints, attention to sex as a biological variable, and IACUC approval under AAALAC-accredited oversight; topical, non-systemic administration would limit anticipated distress.

Human Subjects / Clinical Trial

No human-subjects clinical trial is proposed in this R21. The contingency ex vivo aim uses **de-identified human skin explants and banked/clinical *C. acnes* isolates**; human-derived material will be obtained and used under IRB review or exemption determination as applicable, and this work is preclinical and non-clinical. For the future clinical program these data are intended to enable, investigational phage administration in the U.S. would proceed under FDA oversight — including, where appropriate, the emergency/expanded-access IND (eIND) route for investigational phage therapeutics — with prospective IRB approval and informed consent. The prior human evidence cited here (Golembo 2022) was conducted as a cosmetic study, not a pivotal therapeutic trial.

Investigators & Environment

Investigators (review criterion). The assembled team [ILLUSTRATIVE] is designed to cover every methodology in the aims, an explicit NIH review consideration for an exploratory project:

- **Principal Investigator (PI/PD) — [Name, Title, Institution]:** dermatology/skin-microbiome scientist; overall direction, Aims 1–3.
- **Co-Investigator — Phage Biology/Genomics — [Name, Institution]:** phage curation, sequencing, host-range (Aim 1), resistance genomics (Aim 2).
- **Co-Investigator — Endolysin/Protein Engineering — [Name, Institution]:** PAC1-type endolysin/artilysin production and testing (Aim 2).
- **Co-Investigator — Microbiome Bioinformatics — [Name, Institution]:** 16S/metagenomic analysis (contingency Aim 3).
- **Industry partner (optional, SBIR route) — [Small business, e.g., a topical-phage developer]:** formulation and translational/regulatory strategy.

Environment. [Institution] provides BSL-2 microbiology, phage propagation and purification capacity, genomics/sequencing cores, bioinformatics computing, and dermatology clinical-isolate access — sufficient to execute Aims 1–2 and the contingency ex vivo aim.

References

1. Golembo M, Puttagunta S, Rappo U, et al. Development of a topical bacteriophage gel targeting *Cutibacterium acnes* for acne prone skin and results of a phase 1 cosmetic randomized clinical trial. *Skin Health and Disease*. 2022;2(2):e93. doi:10.1002/ski2.93. PMID: 35677920.
2. Castillo DE, Nanda S, Keri JE. *Propionibacterium* (*Cutibacterium*) *acnes* Bacteriophage Therapy in Acne: Current Evidence and Future Perspectives. *Dermatology and Therapy*. 2019;9(1):19-31. doi:10.1007/s13555-018-0275-9. PMID: 30539425.
3. Mohammadi M. *Cutibacterium acnes* bacteriophage therapy: exploring a new frontier in acne vulgaris treatment. *Archives of Dermatological Research*. 2024;317(1):84. doi:10.1007/s00403-024-03585-x. PMID: 39644414.
4. Rimon A, Gelman D, Castro J, et al. Characterization and Engineering Studies of a New Endolysin from the *Propionibacterium acnes* Bacteriophage PAC1 for the Development of a Broad-Spectrum Artilysin with Altered Specificity. 2023. PMC10218239.