Engineering and De-Risking a Biofilm-Targeted Lytic Phage Cocktail for Sight-Threatening *Pseudomonas aeruginosa* Keratitis

Project Summary / Abstract

Pseudomonas aeruginosa is the leading cause of contact-lens–associated bacterial keratitis, an ophthalmic emergency that can destroy vision within 24–48 hours through rapid corneal melting. Multidrug resistance, potent secreted toxins, and biofilm on the cornea and contact lenses increasingly blunt fluoroquinolone and fortified-antibiotic therapy, and eyes are still lost to corneal perforation, transplantation, or enucleation. Lytic bacteriophages are mechanistically suited to this niche: they adsorb to bacterial surface receptors and kill through resistance-independent mechanisms, self-amplify precisely at the infection site, penetrate biofilm, and can be delivered as eye-drops with minimal host toxicity. The preclinical foundation, while early, is converging across independent groups. Topical phage KPP12 enhanced bacterial clearance, preserved corneal transparency, and suppressed neutrophil-mediated damage in a mouse *P. aeruginosa* keratitis model (Fukuda 2012); a two-phage cocktail (ΦR18 + ΦR26) suppressed *P. aeruginosa* in a mouse keratitis model (Furusawa 2016); and the biofilm-tropic phage Clew-1, which uses the exopolysaccharide Psl as its receptor and replicates preferentially within biofilm, reduced corneal bacterial burden in a mouse keratitis model (Walton 2025). What is missing is a *defined, manufacturable cocktail* that unites planktonic-active and biofilm-tropic phages and is rigorously benchmarked against standard of care. This proposal assembles a complementary-receptor anti-pseudomonal cocktail, defines the host-range and biofilm-penetration breadth needed for clinical relevance, establishes topical efficacy and phage–antibiotic synergy in an established mouse keratitis model against fortified-antibiotic comparators, characterizes resistance and antibiotic re-sensitization, and generates the ocular-grade purity, stability, and immunologic-safety package required to support a future emergency/expanded-access IND (eIND), consistent with current compassionate-use anti-pseudomonal phage experience (Elfadadny 2025). As a higher-risk exploratory extension, we will pilot the intravitreal route for endophthalmitis to inform feasibility. The work directly serves the National Eye Institute's mission to protect vision by delivering a de-risked, sight-sparing therapeutic for antibiotic-refractory corneal infection.

Specific Aims

Sight-threatening *P. aeruginosa* keratitis remains partly refractory to fluoroquinolone and fortified-antibiotic therapy because of multidrug resistance and biofilm on the cornea and contact lenses. Lytic phages kill through receptor-mediated, resistance-independent mechanisms, self-amplify at the infection site, and can penetrate biofilm — and biofilm-tropic phages such as Clew-1 home to the Psl exopolysaccharide matrix that shields *Pseudomonas* on the cornea (Walton 2025). Independent groups have shown topical phage efficacy in mouse *P. aeruginosa* keratitis (Fukuda 2012; Furusawa 2016). No registered trial yet targets phage therapy for keratitis. **Our central hypothesis** is that a defined cocktail pairing planktonic-active phages with a Psl-targeting, biofilm-tropic phage will achieve broader, more durable killing of clinical keratitis isolates than any single phage and will act synergistically with standard-of-care antibiotics. We will test this and build the translational package across three aims.

Aim 1 — Assemble and characterize a complementary-receptor anti-pseudomonal cocktail with biofilm tropism. We will combine a well-characterized planktonic-active lytic phage (KPP12-type Myoviridae) and a second planktonic phage with a distinct receptor (e.g., a ΦR18-type Podoviridae) with a Psl-targeting, biofilm-tropic Clew-1–type phage. Across a panel of contemporary keratitis clinical isolates we will quantify host range (spot and efficiency-of-plating assays), map receptor usage (LPS, type IV pili, Psl) with defined mutants, confirm genome safety by whole-genome sequencing (no toxin, antibiotic-resistance, or lysogeny genes), and measure biofilm penetration on abiotic and contact-lens surfaces. *Success metric:* a locked 2–3 phage cocktail covering ≥80% of a ≥40-isolate panel with ≥2 distinct receptors and confirmed lytic, temperate-gene-free genomes. *Go/no-go:* coverage ≥80% and demonstrable in vitro biofilm reduction before advancing to Aim 2.

Aim 2 — Establish topical efficacy and phage–antibiotic synergy in an established mouse keratitis model. Using the scarified-cornea mouse *P. aeruginosa* keratitis model, we will test the cocktail alone and combined with sub-inhibitory fortified/fluoroquinolone antibiotic, benchmarked against antibiotic monotherapy and vehicle, in randomized, masked, adequately powered studies using both sexes. *Endpoints:* corneal CFU, masked clinical/transparency score, and neutrophil infiltration (myeloperoxidase). *Success metric:* ≥1-log CFU reduction versus vehicle and non-inferiority (or additivity in combination) versus antibiotic on the primary clinical-score endpoint.

Aim 3 — Define resistance, re-sensitization, and the ocular safety/manufacturing package for translation. We will quantify phage-resistance frequency and receptor-loss mutants, test whether resistance trades off with antibiotic re-sensitization, assess ocular tolerability and innate/adaptive immune responses to topical phage, and establish ocular-grade purification (endotoxin control), formulation stability, and release assays to support a future eIND. As an exploratory milestone, we will pilot intravitreal dosing to assess tolerability and bacterial-burden reduction in an endophthalmitis model, informing a future dedicated study.

Impact. Success delivers the first rigorously de-risked, biofilm-targeted phage cocktail for antibiotic-refractory *P. aeruginosa* keratitis — a personalized, sight-sparing therapy for fluoroquinolone-resistant ulcers — with a credible regulatory on-ramp and pilot data for endophthalmitis.

Significance

P. aeruginosa is the leading cause of contact-lens–associated bacterial keratitis and a frequent cause of severe, vision-threatening corneal ulceration. These are not indolent infections: corneal melting can perforate the eye within 24–48 hours, and the organism's secreted toxins and biofilm accelerate tissue loss while degrading the performance of topical antibiotics. As multidrug resistance rises, fluoroquinolone and fortified-antibiotic regimens increasingly fail, leaving clinicians few options short of corneal transplantation or enucleation. There is a clear, unmet need for a therapy whose killing mechanism does not depend on the same molecular targets antibiotics use and that can engage bacteria embedded in biofilm.

Lytic phages address this gap mechanistically. They adsorb to specific surface receptors (LPS, type IV pili, exopolysaccharides), inject their genome, replicate, and lyse the cell, releasing progeny that propagate killing — so a small inoculum self-amplifies precisely where the bacteria are, then clears as the target is eliminated. Critically, phages kill antibiotic-resistant cells through resistance-independent mechanisms and can penetrate biofilm. The cornea is an attractive compartment: topical eye-drops are a routine ophthalmic route, the eye is accessible for direct microbiologic and clinical monitoring, and phages have shown minimal host toxicity. Preclinical proof-of-concept is converging from independent laboratories: KPP12 eye-drops enhanced clearance and preserved corneal transparency while suppressing neutrophil-mediated damage (Fukuda 2012); a two-phage cocktail (ΦR18 + ΦR26) suppressed *P. aeruginosa* in a mouse keratitis model (Furusawa 2016); and the biofilm-tropic Clew-1 reduced corneal burden by exploiting the very Psl matrix that protects the organism (Walton 2025). Human anti-pseudomonal phage therapy at other body sites is increasingly reported under compassionate use, including phage–antibiotic synergy that can enhance eradication while mitigating resistance (Elfadadny 2025), supporting translatability. No registered trial yet targets phage therapy specifically for keratitis — the central translational gap this proposal is designed to narrow.

Innovation

This program advances four innovations. **First**, it engineers a *defined cocktail* purpose-built for ocular *Pseudomonas* disease by deliberately pairing planktonic-active phages with complementary receptors (a Myoviridae phage plus a phage using a distinct receptor) with a *biofilm-tropic* phage. Because *P.*

aeruginosa is genetically heterogeneous and each phage has a narrow host range, complementary-receptor cocktails broaden coverage and suppress resistant mutants. **Second**, it operationalizes biofilm tropism as a therapeutic principle: rather than treating biofilm as an obstacle, Clew-1–type phages use the Psl exopolysaccharide as their receptor and replicate preferentially within biofilm (Walton 2025) — directly relevant to corneal ulcers and contaminated contact lenses. **Third**, it builds phage–antibiotic synergy into the design from the outset, exploiting evidence that phages plus sub-lethal antibiotics combine for greater killing, lower resistance, and potential antibiotic re-sensitization (Elfadadny 2025) — complementing rather than replacing ophthalmic standard of care. **Fourth**, it pairs a near-term lead indication (topical keratitis) with an exploratory intravitreal extension (endophthalmitis) inside one mechanistic framework, anchoring a realistic emergency-care workflow in which a corneal scraping is matched to a personalized cocktail. Engineered and enzyme-based adjuncts (depolymerases, endolysins) are noted as a forward-looking extension but are not required for the core aims.

Approach

Rigor and Reproducibility (applies to all aims)

All in vivo studies will be randomized, performed and scored by masked observers, and powered (≥80%, $\alpha=0.05$) from effect sizes in prior keratitis models (Fukuda 2012; Furusawa 2016; Walton 2025), with group sizes set by a biostatistician. Sex is treated as a biological variable: both male and female animals are included and analyzed. Bacterial isolates, phage stocks, and titers are authenticated and lot-controlled; key in vitro assays are run in independent triplicate. Reporting follows ARRIVE 2.0. Phage genomes are deposited; isolate panels and protocols are shared on request.

Aim 1 — Assemble and characterize a complementary-receptor cocktail with biofilm tropism

Rationale. Narrow host range and phage resistance are the principal liabilities of single-phage therapy. Complementary-receptor cocktails broaden coverage and suppress resistant mutants, and a biofilm-tropic component addresses the matrix that shields corneal *Pseudomonas* (Walton 2025).

Design. We will work with characterized lytic phages — a KPP12-type Myoviridae (Fukuda 2012), a second planktonic phage with a distinct receptor (e.g., a ΦR18-type Podoviridae; Furusawa 2016), and a Psl-targeting, biofilm-tropic Clew-1–type phage (Walton 2025). Against a panel (≥40) of contemporary keratitis clinical isolates we will quantify host range (spot and efficiency-of-plating assays), map receptor usage (LPS, type IV pili, Psl) with defined isogenic mutants, and confirm genome safety by whole-genome sequencing (excluding toxin, antibiotic-resistance, and lysogeny genes). Biofilm activity is measured in static and flow assays, including on contact-lens materials. Cocktail formulations are optimized in vitro for combined coverage and suppression of resistant outgrowth.

Expected outcomes. A locked 2–3 phage cocktail with documented broad coverage, complementary receptors, confirmed lytic genomes, and demonstrated biofilm penetration.

Pitfalls & alternatives. A Psl-tropic phage may not plaque on planktonic wild-type bacteria (as reported for Clew-1), complicating titering; we will titer under Psl-expressing/biofilm conditions and enumerate by qPCR. If host-range gaps remain, we will isolate or substitute phages with distinct receptors to close coverage before the go/no-go.

Aim 2 — Topical efficacy and phage–antibiotic synergy in the mouse keratitis model

Rationale. Independent groups show topical phage efficacy in mouse *P. aeruginosa* keratitis (Fukuda 2012; Furusawa 2016) and biofilm-burden reduction by a Psl-tropic phage (Walton 2025). A standardized, masked efficacy comparison against fortified-antibiotic standard of care is the key translational step.

Design. Scarified-cornea mouse infection is followed by topical cocktail drops, benchmarked against fortified/fluoroquinolone antibiotic and vehicle, with dose, timing, and multiplicity informed by prior models. *Endpoints:* corneal CFU, masked clinical/transparency score, and neutrophil infiltration (myeloperoxidase). *Synergy:* cocktail combined with sub-inhibitory antibiotic, evaluated against each monotherapy. Treatment begins at a clinically realistic post-establishment window, not only prophylactically.

Expected outcomes. The cocktail achieves ≥1-log CFU reduction versus vehicle and is non-inferior to antibiotic on clinical score, with combination therapy yielding greater killing and lower resistance than either alone.

Pitfalls & alternatives. Single dosing may be insufficient for established disease; we will test repeat dosing and earlier windows. If the planktonic phages dominate efficacy and the biofilm-tropic component adds little in acute infection, we will weight the cocktail toward biofilm contexts (lens-associated, chronic) and report component contributions transparently.

Aim 3 — Resistance, re-sensitization, and the ocular safety/manufacturing package

Rationale. Durable benefit and a credible regulatory path require characterizing resistance, exploiting antibiotic re-sensitization (Elfadadny 2025), and generating ocular safety and manufacturing data.

Design. We will passage isolates under cocktail pressure to measure resistance frequency and identify receptor-loss mutants, testing whether resistance trades off with antibiotic re-sensitization. Ocular tolerability and innate/adaptive immune responses to topical phage are assessed (corneal histology, cytokines, neutralizing-antibody development). We will establish purification with endotoxin control, eye-drop formulation stability, and release assays — the foundation for a future eIND. *Exploratory milestone:* a small intravitreal pilot in an endophthalmitis model to assess tolerability and vitreous bacterial-burden reduction, explicitly framed as feasibility data (no allowed preclinical precedent exists for intravitreal phage; this aim is hypothesis-generating).

Expected outcomes. A resistance/re-sensitization profile supporting cocktail-plus-antibiotic use, an ocular-safety dataset, a quality/manufacturing package suitable for regulatory pre-submission, and go/no-go intravitreal feasibility data.

Pitfalls & alternatives. Neutralizing antibodies could limit repeat dosing; because acute keratitis is a short-course indication, this is likely tolerable, and cocktail rotation provides mitigation. If a phage cannot be purified to ocular-grade endotoxin limits, it is replaced from the Aim 1 candidate pool. If the intravitreal pilot reveals inflammation or poor clearance, the topical indication remains the lead and the intravitreal route is deferred to a dedicated future study.

Milestones & Go/No-Go Criteria

- **End of Yr 1 (Aim 1):** Locked cocktail, ≥80% panel coverage, ≥2 receptors, lytic genomes. *No-go if coverage <80% or no biofilm activity.*
- **End of Yr 3 (Aim 2):** ≥1-log CFU reduction vs vehicle and non-inferiority vs antibiotic on clinical score; documented synergy. *No-go for combination claim if synergy absent.*
- **End of Yr 5 (Aim 3):** Ocular-safety dataset, ocular-grade purity/stability package, resistance/re-sensitization profile, intravitreal feasibility readout.

Timeline

[ILLUSTRATIVE] **Year 1:** Aim 1 — assemble panel, host-range/receptor mapping, genome safety, lock cocktail. [ILLUSTRATIVE] **Years 2–3:** Aim 2 — topical keratitis efficacy and phage–antibiotic synergy. [ILLUSTRATIVE] **Years 3–4:** Aim 3 — resistance/re-sensitization and ocular safety; intravitreal feasibility pilot. [ILLUSTRATIVE] **Years 4–5:** manufacturing/stability package, data integration, and eIND-enabling pre-submission. (Total project period [ILLUSTRATIVE] 5 years.)

Budget Justification (modular R01-style sketch)

[ILLUSTRATIVE] Requested at [ILLUSTRATIVE] $250,000 direct costs/year for [ILLUSTRATIVE] 5 years. **Personnel:** PI (microbiology/phage biology), Co-I ophthalmologist–scientist, Co-I in ocular immunology, [ILLUSTRATIVE] 2 postdocs/technicians, and biostatistician effort. **Animals:** [ILLUSTRATIVE] mouse keratitis cohorts and a small exploratory endophthalmitis pilot (per-diem, procurement, veterinary). **Supplies:** phage propagation/purification, endotoxin assays, sequencing, biofilm and lens-material assays, antibiotics, histology, immunoassays. **Other:** core facility fees (imaging, genomics), publication, travel. Modules and effort to be finalized with institutional budgeting; all figures above are [ILLUSTRATIVE] placeholders.

Vertebrate Animals

Animal work is proposed. The mouse *P. aeruginosa* keratitis model (consistent with Fukuda 2012; Furusawa 2016; Walton 2025) is the core in vivo system; a small exploratory endophthalmitis pilot (intravitreal) is included in Aim 3. Animals are required because corneal transparency, neutrophil-mediated tissue damage, and intraocular pharmacology cannot be recapitulated in vitro. Procedures (corneal scarification, topical dosing, intravitreal injection) follow established protocols. Analgesia, anesthesia, humane endpoints, randomization, masking, inclusion of both sexes, and group sizes ([ILLUSTRATIVE], set with biostatistician input to minimize animal numbers) operate under IACUC oversight with ARRIVE 2.0–aligned reporting.

Human Subjects / Clinical Trial

No human subjects research is proposed in this project period; the work is preclinical and eIND-enabling. We outline the translational path: investigational phage for a sight-threatening, antibiotic-refractory corneal infection would proceed initially under the FDA emergency/expanded-access IND (eIND) route, consistent with current compassionate-use anti-pseudomonal phage experience (Elfadadny 2025), with full IRB oversight, informed consent, and predefined microbiologic and visual-outcome endpoints. A future stand-alone clinical protocol and IND would be required before any registered trial.

Team & Environment

- **Principal Investigator** — [NAME], [INSTITUTION]; phage biology / anti-pseudomonal cocktail development.
- **Co-Investigator (Ophthalmology)** — [NAME], [INSTITUTION]; corneal infection models

and clinical translation.

- **Co-Investigator (Microbiology/Biofilm)** — [NAME], [INSTITUTION]; Psl/biofilm phage biology.
- **Co-Investigator (Ocular Immunology)** — [NAME], [INSTITUTION].
- **Manufacturing/Regulatory Consultant** — [NAME/ORG]; phage purification and eIND strategy.
- **Biostatistician** — [NAME], [INSTITUTION].
- **Environment** — academic medical center with vivarium/IACUC, ocular imaging, genomics, and BSL-2 phage facilities. **Alignment with NEI mission** to protect and prolong vision; secondary relevance to ocular-trauma–associated infection (e.g., DoD CDMRP) and antimicrobial-resistance portfolios (e.g., NIAID) is noted as a complementary, not primary, fit.

References

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3. Walton B, Abbondante S, Marshall ME, et al. A biofilm-tropic *Pseudomonas aeruginosa* bacteriophage uses the exopolysaccharide Psl as receptor. *eLife.* 2025;13:e102352. https://pubmed.ncbi.nlm.nih.gov/40788302/
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