Dosing and Resistance Trade-offs of a Multi-Receptor Lytic Phage Cocktail for MDR *Pseudomonas aeruginosa* and *Acinetobacter baumannii* Burn-Wound Infection

Project Summary / Abstract

Thermal injury destroys the skin barrier and leaves devitalized eschar that is rapidly colonized by *Pseudomonas aeruginosa* and *Acinetobacter baumannii*, two high-priority multidrug-resistant (MDR) pathogens that form biofilm on the wound bed, drive sepsis and skin-graft failure, and increasingly resist carbapenems and colistin. Lytic bacteriophages are a mechanistically attractive adjunct: they self-amplify at the infection site, penetrate biofilm, are applied topically to an accessible surface, and spare commensal flora. Yet the field lacks a quantitative, mechanism-level understanding of **how delivered phage dose, receptor diversity, and the fitness cost of resistance jointly determine clearance on burned tissue** — the gap this R01 addresses. The one randomized trial to date, PhagoBurn (NCT02116010; Jault et al., *Lancet Infect Dis* 2019), is informative precisely because manufacturing instability dropped the delivered titer far below the intended dose, so the under-dosed phage arm cleared *P. aeruginosa* more slowly than standard care. This points to dose and delivery — not an absence of phage activity — as decisive variables, but the underlying dose–response and resistance dynamics on eschar have never been defined.

We test the central hypothesis that a deliberately **multi-receptor** lytic cocktail, governed by **measured input titer** and paired with standard antibiotics, reduces *P. aeruginosa* and *A. baumannii* burden and biofilm on burn wounds in a dose-dependent manner, and that phage selection drives **receptor-loss escape mutants whose fitness cost restores antibiotic susceptibility**. We will (1) assemble and genomically characterize a multi-receptor lytic cocktail against contemporary burn isolates, incorporating a capsule depolymerase shown to potentiate *A. baumannii* killing in a mouse burn model (Borzilov et al., *Viruses* 2025); (2) quantify dose–response, biofilm penetration, and phage–antibiotic synergy under strict titer control in vitro and in a murine burn-wound model; and (3) define the biophysical determinants of stable high-titer topical delivery and the durability of clearance, mapping the mechanism of titer loss that confounded prior work. Investigational phage has reached US patients through the FDA emergency-IND route used in landmark *A. baumannii* rescue

(Schooley et al., *AAC* 2017), and our quantitative findings will de-risk a future adequately dosed study. The work is mechanistic, US-focused, and aligned with the NIGMS sepsis, trauma, burn, and wound-healing portfolio.

Specific Aims

Burn-wound infection by MDR *P. aeruginosa* and *A. baumannii* is a leading cause of invasive, hard-to-treat sepsis, and a frequently proposed indication for phage therapy. The single randomized trial (PhagoBurn) delivered phage far below its intended titer because of product instability, and its under-dosed arm cleared *P. aeruginosa* more slowly than standard care — yet the field still lacks a quantitative, mechanistic account of how delivered dose, receptor coverage, and the fitness cost of resistance govern clearance on burned tissue. We test the **central hypothesis** that a multi-receptor lytic cocktail, governed by measured input titer and combined with standard antibiotics, reduces bacterial burden and biofilm on burn wounds in a dose-dependent manner, and that phage-driven receptor loss carries a fitness cost that re-sensitizes resistant bacteria to antibiotics.

Aim 1. Build and genomically characterize a multi-receptor lytic phage cocktail against contemporary burn isolates. We will phenotype a panel of *P. aeruginosa* and *A. baumannii* burn-wound clinical isolates for surface-receptor and capsule (K-type) diversity, screen banked and newly isolated lytic phages, and select components that collectively engage distinct receptors (LPS, type IV pili, and the *A. baumannii* capsule). We will add a capsule depolymerase of the class shown to potentiate killing of K-type *A. baumannii* (Borzilov et al., 2025). All components undergo whole-genome sequencing to confirm an obligately lytic lifecycle and exclude toxin, virulence, and antibiotic-resistance genes. *Milestone:* a sequenced cocktail covering ≥80% of panel isolates with non-overlapping receptors.

Aim 2. Quantify dose–response, biofilm penetration, and phage–antibiotic synergy under measured titer control. Using biofilm assays on eschar-mimicking substrates and a murine burn-wound infection model, we will establish dose–response relationships with verified PFU at every step, quantify phage–antibiotic synergy (PAS), and test whether phage pressure selects fitness-costly receptor-loss mutants that regain antibiotic susceptibility. *Milestone:* a defined input-titer threshold for ≥2-log burden reduction, with quantified PAS and resistance trade-offs.

Aim 3. Define the determinants of stable high-titer topical delivery and durable clearance. We will dissect the mechanism of titer loss during topical application and storage, identify formulation parameters (carrier, endotoxin control) that preserve infectivity, and relate recovered PFU to in vivo efficacy and relapse. A translational-readiness summary (identity, potency, endotoxin, stability) will be compiled to support a future adequately dosed study under the FDA emergency/expanded-access IND route (Schooley et al., 2017). *Milestone:* a formulation preserving ≥90% titer over a defined shelf

interval with confirmed in vivo activity.

Impact. By delivering a quantitative, mechanistic account of dose, receptor coverage, and resistance trade-offs on burned tissue, this project converts a costly null trial into actionable design rules and positions phage-augmented burn care for a definitive, adequately dosed efficacy trial.

Significance

Burn wounds are a leading portal for invasive, hard-to-treat infection. The destroyed barrier, devitalized eschar, and prolonged ICU stays favor colonization by *P. aeruginosa* and *A. baumannii*, which readily form biofilm and drive sepsis and graft failure. Because carbapenem and colistin resistance in these organisms is rising, antibiotic options have narrowed, and burn-wound infection has become one of the most frequently cited indications for bacteriophage therapy. The unmet need is acute for US civilian burn centers and for military mass-casualty care, where these same pathogens dominate combat and blast wounds. This work sits squarely within the NIGMS sepsis, trauma, burn, and wound-healing mission: its aims are mechanistic — the quantitative biology of host–phage interaction, biofilm penetration on devitalized tissue, and the evolutionary trade-offs of resistance — rather than disease-specific drug development.

Phages fit this problem mechanistically well. They are obligately lytic against specific bacteria, self-amplify at the infection site, penetrate biofilm, and are applied topically to an accessible surface while sparing commensal flora. The wound's accessibility makes it tractable for combining phages with the antibiotics and surgical debridement already standard in burn care. The clinical record supports promise and tolerability but not yet efficacy, and the reasons are instructive. A personalized cocktail rescued a patient with disseminated MDR *A. baumannii* (Schooley et al., 2017), establishing a US emergency-IND pathway and catalyzing dozens of subsequent compassionate-use cases. PhagoBurn (Jault et al., 2019) then showed that topical anti-*P. aeruginosa* phage is tolerable, but — because product instability left the delivered titer far below the intended dose — it underperformed standard care. PhagoBurn's limitations were multifactorial (small sample, narrow host range, single pathogen), with delivered dose the most consequential and most fixable. What the field still lacks is the quantitative dose–response, biofilm-penetration, and resistance-trade-off data needed to specify *how much* phage, against *which* receptors, achieves durable clearance on eschar. Supplying that mechanistic foundation is the purpose of this proposal.

Innovation

This project's innovation is conceptual and quantitative rather than a novel claim about phage existence. First, **titer as a measured independent variable**: every assay and animal experiment is

governed by verified input PFU, turning the dosing failure that confounded PhagoBurn into a controlled dose–response axis. Second, **multi-receptor cocktail logic with explicit escape mapping**: components are chosen to engage LPS, type IV pili, and the *A. baumannii* capsule, and resistance routes are mapped so that escape from one component does not confer escape from others. Third, **mechanistic exploitation of the resistance trade-off**: we test the hypothesis that phage-driven receptor loss imposes a fitness cost that restores antibiotic susceptibility, treating evolution as a therapeutic lever rather than only a liability. Fourth, **enzyme-augmented anti-biofilm strategy**: a capsule depolymerase of the class shown to enhance lytic killing of K-type *A. baumannii* in mouse sepsis and burn-skin models (Borzilov et al., 2025) is used to probe how matrix degradation alters phage access on eschar. Finally, **delivery treated as quantitative biology**: rather than assuming a formulation works, we dissect the biophysical mechanism of titer loss, linking recovered PFU directly to in vivo efficacy and relapse.

Approach

Aim 1 — Build and genomically characterize a multi-receptor lytic phage cocktail against contemporary burn isolates

Rationale. Lytic phages bind strain-specific surface receptors (LPS, type IV pili, and in *A. baumannii* the capsular polysaccharide), so a cocktail must engage multiple receptor classes to cover heterogeneous burn isolates and to limit shared escape routes. Depolymerases that degrade capsule/matrix can strip biofilm and expose otherwise protected bacteria.

Experimental design. From a contemporary panel of *P. aeruginosa* and *A. baumannii* burn-wound clinical isolates, we will characterize surface-receptor and capsule (K-type) diversity. We will screen banked and newly isolated lytic phages for plaque formation across the panel and select components that collectively engage distinct receptors. A capsule depolymerase of the class active against K-type *A. baumannii* (Borzilov et al., 2025) will be expressed and tested for matrix/capsule degradation. All phages and the depolymerase will undergo whole-genome sequencing to confirm an obligately lytic lifecycle and exclude toxin, virulence, and antibiotic-resistance genes. Host range and cross-resistance will be mapped to assemble a cocktail that maximizes coverage with minimal shared escape routes.

Expected outcomes. A defined, fully sequenced multi-receptor cocktail plus depolymerase, with documented host range across the panel and a strictly lytic, toxin-gene-free profile.

Potential pitfalls & alternatives. Some isolates may resist all available phages; we will expand isolation against gap strains and prioritize broad-receptor phages. If a depolymerase is poorly expressed or unstable, we will substitute an alternative capsule-degrading enzyme or rely on naturally matrix-degrading phages. Escape will be limited by selecting non-overlapping receptors so resistance

to one component does not confer resistance to others.

Aim 2 — Quantify dose–response, biofilm penetration, and phage–antibiotic synergy under measured titer control

Rationale. PhagoBurn's slow clearance tracked to a delivered titer far below the intended dose, so potency must be defined as a function of verified input PFU. PAS is repeatedly observed, and phage-driven receptor loss can restore antibiotic susceptibility at a fitness cost — a mechanism we test directly.

Experimental design. We will quantify cocktail and depolymerase activity against planktonic and biofilm cultures on eschar-mimicking substrates, establishing dose–response across a controlled titer range with measured PFU at every step. PAS will be tested by combining sub-inhibitory antibiotics with phage and scoring replication and killing. We will then evaluate the cocktail topically in a murine burn-wound infection model seeded with *P. aeruginosa* and with K-type *A. baumannii* (consistent with the burn-skin model of Borzilov et al., 2025), comparing phage alone, antibiotic alone, the combination, and standard topical care, with bacterial burden and biofilm as co-primary endpoints. Surviving bacteria will be characterized for receptor-loss escape and restored antibiotic susceptibility, and fitness cost quantified by competition assay.

Rigor. Animal studies use predefined group sizes from power analysis, randomized allocation, blinded outcome scoring, and both sexes (see *Sex as a Biological Variable*). Phage and bacterial stocks are authenticated by sequencing and titer before use.

Expected outcomes. Dose–response curves identifying the input titer needed for reliable burden and biofilm reduction; quantified PAS; and evidence that phage pressure re-sensitizes resistant isolates — together specifying the dosing target a future trial must meet.

Potential pitfalls & alternatives. If monotherapy plateaus due to escape, the combination and depolymerase arms provide the primary translational path. If the murine model under-represents eschar biofilm, we will extend biofilm exposure or adjust inoculum. Titer loss during application will be addressed mechanistically in Aim 3.

Aim 3 — Define the determinants of stable high-titer topical delivery and durable clearance

Rationale. Because the wound is an accessible surface, topical delivery is feasible, but only if the cocktail reaches the wound at high, verified titer with controlled endotoxin — exactly what prior product instability defeated. Understanding *why* titer is lost is a quantitative-biology problem upstream of any future manufacturing decision.

Experimental design. We will dissect the mechanism of titer loss by measuring PFU recovery across candidate carriers (e.g., aqueous and hydrogel) under storage and simulated use, identifying the biophysical drivers (adsorption, aggregation, matrix interaction) of infectivity loss. We will define stability and release-relevant readouts with PFU recovery as the key metric, and relate recovered titer to in vivo efficacy and relapse in the Aim 2 model. A concise translational-readiness summary — identity, potency, endotoxin limits, and titer-stability data, plus the personalization logic of matching a cocktail to a patient isolate within days — will be assembled to support a future adequately dosed study under the FDA emergency/expanded-access IND framework used in personalized *A. baumannii* therapy (Schooley et al., 2017).

Expected outcomes. A mechanistic account of titer loss, a formulation that preserves infectivity with defined stability, and a translational-readiness summary linking recovered PFU to durable in vivo clearance.

Potential pitfalls & alternatives. If a hydrogel reduces recovered titer, we will revert to a validated liquid carrier or smart-dressing alternative. If a single component limits stability, the Aim 1 composition will be rebalanced toward more robust phages without sacrificing receptor coverage.

Rigor, Reproducibility, and Authentication of Key Resources

All phages, the depolymerase, and bacterial isolates are authenticated by whole-genome sequencing and quantitative titration before use and re-verified at intervals. In vitro assays use biological and technical replicates with prespecified statistics; dose–response and synergy analyses are powered a priori. Animal experiments are randomized and outcome-blinded with predefined humane endpoints. Data, sequences, and protocols will be deposited in public repositories to support reproducibility.

Sex as a Biological Variable

[ILLUSTRATIVE] Both male and female mice will be studied in the burn-wound model, with sex included as a covariate and group sizes powered to detect sex-dependent differences in bacterial clearance and biofilm response. Any sex-based differences will be reported; no sex will be excluded without empirical justification.

Milestones & Go / No-Go Decisions

[ILLUSTRATIVE] **End of Aim 1 (Yr 2):** sequenced multi-receptor cocktail covering ≥80% of panel isolates with non-overlapping receptors and a confirmed lytic, toxin-gene-free profile — *go* to in vivo dosing. **Mid Aim 2 (Yr 3):** input-titer threshold achieving ≥2-log burden reduction in vivo; if not met, escalate dose/optimize cocktail before synergy studies. **End of Aim 2 (Yr 4):** quantified PAS and

resistance trade-off — *go* to delivery optimization. **End of Aim 3 (Yr 5):** formulation preserving ≥90% titer over the defined shelf interval with confirmed in vivo activity — *go* to translational-readiness package.

Timeline

[ILLUSTRATIVE] **5-year R01.** [ILLUSTRATIVE] Years 1–2: Aim 1 isolate panel, phage selection, sequencing, depolymerase production. [ILLUSTRATIVE] Years 2–4: Aim 2 biofilm potency, PAS, resistance trade-offs, and murine burn-model dose–response. [ILLUSTRATIVE] Years 3–5: Aim 3 mechanism of titer loss, formulation, stability, and translational-readiness summary, overlapping Aim 2 so dosing targets feed delivery work. [ILLUSTRATIVE] Year 5: integration into a readiness package for a future adequately dosed study.

Budget Justification (modular R01-style sketch)

[ILLUSTRATIVE] Modular budget of **$250,000 direct costs/year** for **5 years**. [ILLUSTRATIVE] **Personnel:** PI (microbiology/phage biology; ~2.4 calendar months), Co-Investigator (burn/infectious disease), one postdoctoral scientist, one research technician, and partial effort for a quality/regulatory consultant. [ILLUSTRATIVE] **Supplies:** bacterial culture and isolate banking, phage propagation and purification, depolymerase expression, sequencing, biofilm/eschar-mimic assays, and formulation/stability consumables. [ILLUSTRATIVE] **Animals:** murine burn-wound model per-diems and veterinary costs. [ILLUSTRATIVE] **Other:** endotoxin and potency assay services. Modular justification will accompany the standard NIH escalation and the facilities/administrative rate negotiated by the awardee institution.

Vertebrate Animals

Animal work is proposed. [ILLUSTRATIVE] We will use a murine burn-wound infection model (consistent with the burn-skin model reported by Borzilov et al., 2025) to evaluate topical cocktail efficacy against *P. aeruginosa* and K-type *A. baumannii*. Justification: bacterial burden, biofilm clearance, and phage–antibiotic synergy on devitalized burn tissue cannot be fully recapitulated in vitro. Procedures will follow approved IACUC protocols with appropriate analgesia/anesthesia for thermal-injury models, predefined humane endpoints, randomized allocation, blinded scoring, and group sizes minimized by power analysis under the 3Rs, including both sexes. Final species, strains, and numbers will be specified in the funded protocol.

Human Subjects / Clinical Trial

No human-subjects research is proposed in this R01; the work is preclinical and mechanistic. The program is nonetheless designed to inform future clinical use. Investigational phage therapy for these pathogens has been delivered to US patients through the FDA emergency/expanded-access investigational new drug (eIND) pathway, as in the landmark personalized *A. baumannii* case (Schooley et al., 2017). Aim 3 will assemble the supporting characterization and translational-readiness summary for a future adequately dosed study, which would proceed only under a separate FDA IND/eIND authorization and institutional IRB oversight, with informed consent, and is outside the scope and budget of this award.

Team & Environment

[ILLUSTRATIVE — to be completed with real names/institutions.] **Principal Investigator:** [Name, PhD], phage biology/microbiology, [US academic institution]. **Co-Investigator (Clinical):** [Name, MD], burn surgery/infectious disease, [US burn center/ABA-verified ICU]. **Co-Investigator (Quality):** [Name], pharmaceutical sciences/regulatory affairs. **Collaborators/Consultants:** [Name], clinical phage program (a US center such as an IPATH-model biobank); [Name], military burn/mass-casualty advisor. **Environment:** BSL-2 microbiology and phage purification suites, biofilm and AAALAC-accredited animal facilities, a sequencing core, and access to a verified burn center providing contemporary clinical isolates. Letters of support from a clinical phage center and a burn ICU will document isolate access and the translational pathway.

References

1. Jault P, Leclerc T, Jennes S, et al. Efficacy and tolerability of a cocktail of bacteriophages to treat burn wounds infected by *Pseudomonas aeruginosa* (PhagoBurn): a randomised, controlled, double-blind phase 1/2 trial. *Lancet Infect Dis.* 2019;19(1):35-45. https://pubmed.ncbi.nlm.nih.gov/30292481/
2. Schooley RT, Biswas B, Gill JJ, et al. Development and Use of Personalized Bacteriophage-Based Therapeutic Cocktails To Treat a Patient with a Disseminated Resistant *Acinetobacter baumannii* Infection. *Antimicrob Agents Chemother.* 2017;61(10):e00954-17. https://doi.org/10.1128/AAC.00954-17
3. Borzilov AI, Volozhantsev NV, Korobova OV, et al. Bacteriophage and Phage-Encoded Depolymerase Exhibit Antibacterial Activity Against K9-Type *Acinetobacter baumannii* in Mouse Sepsis and Burn Skin Infection Models. *Viruses.* 2025;17(1):70. https://doi.org/10.3390/v17010070
4. PhagoBurn: Evaluation of Phage Therapy for the Treatment of *Escherichia coli* and

Pseudomonas aeruginosa Wound Infections in Burned Patients (registered trial record). ClinicalTrials.gov identifier NCT02116010; EudraCT 2014-000714-65. https://clinicaltrials.gov/study/NCT02116010