Nebulized Anti-*Pseudomonas* Bacteriophage Cocktail as an Antibiotic-Sparing Adjunct for Ventilator-Associated Pneumonia: Preclinical Optimization and IND-Enabling De-Risking (PHAGE-VAP)

Project Summary / Abstract

Ventilator-associated pneumonia (VAP) is among the most common and lethal ICU-acquired infections, and *Pseudomonas aeruginosa* is a leading cause—associated with high attributable mortality, prolonged mechanical ventilation, and frequent treatment failure. Intrinsic and acquired multidrug resistance (MDR), tenacious biofilm on endotracheal tubes and the airway surface, and a near-empty antibacterial pipeline make *P. aeruginosa* VAP a paradigmatic target for alternatives to small-molecule antibiotics—the core problem NIAID's antimicrobial-resistance (AMR) program seeks to solve. Lytic bacteriophages offer an orthogonal mechanism: they self-amplify where bacteria are densest, kill only the target species (sparing lung and gut microbiota), penetrate and degrade biofilm via depolymerases, and remain active against pan-resistant strains. Critically, the ventilated airway is directly accessible for nebulized delivery, enabling high local titers without systemic exposure.

We hypothesize that a rationally designed nebulized phage cocktail, delivered as an adjunct to standard antipseudomonal antibiotics, will accelerate bacterial clearance, lower the effective antibiotic dose, and suppress resistance in *P. aeruginosa* VAP. The strongest mechanistic precedent is Weissfuss et al. (*Nature Communications* 2025), who showed in a mechanically ventilated mouse model that adjunctive phage plus meropenem outperformed either monotherapy, reduced the minimum effective meropenem concentration, prevented resistance to both agents, and limited epithelial damage. Human inhaled-phage feasibility is supported by Armata's Phase 2 Tailwind topline (December 2024), and PhagoBurn (Jault et al., 2019) provides the field's defining cautionary lesson: an anti-*Pseudomonas* cocktail proved tolerable but failed on efficacy because delivered titer collapsed by orders of magnitude—making dose, stability, and delivery the decisive translational variables our program places at its center.

We will (1) assemble and characterize a broadly covering, depolymerase-enabled cocktail against contemporary US ICU *P. aeruginosa* isolates and define phage–antibiotic synergy in vitro; (2) optimize nebulized delivery and adjunctive efficacy in a ventilated murine VAP model, quantifying dosing, antibiotic-sparing, and resistance dynamics; and (3) generate IND-enabling product, stability, and inhalation-safety data to support an FDA IND, with expanded-access use as a near-term bridge. The first dedicated VAP RCT (NCT07202234) will not read out until ~2028, leaving a clear, fundable preclinical and IND-enabling gap that a US R01 can fill now.

Specific Aims

Ventilator-associated pneumonia caused by MDR *P. aeruginosa* kills a large fraction of those it infects, prolongs ventilation, and increasingly defeats every available antibiotic—yet the ventilated airway is uniquely accessible to inhaled therapeutics. Lytic bacteriophages self-amplify at the infection site, spare the lung and gut microbiome, degrade biofilm, and remain active against pan-resistant strains. The pivotal barrier is no longer biological plausibility but **translational execution**: a delivery-optimized, resistance-aware, IND-ready product matched to the strains circulating in US ICUs. We will close that gap.

Central hypothesis. A rationally composed, nebulized lytic phage cocktail acts synergistically with standard antibiotics to accelerate clearance, reduce effective antibiotic exposure, and suppress resistance in *P. aeruginosa* VAP.

Aim 1. Assemble and characterize a broadly covering anti-*P. aeruginosa* phage cocktail and define phage–antibiotic synergy in vitro. We will host-range–screen a curated lytic phage panel against a contemporary US ICU *P. aeruginosa* biobank, select 3–5 phages with complementary receptor specificities (LPS, type IV pili, flagella) and confirmed depolymerase activity, and quantify coverage, biofilm disruption on endotracheal-tube material, and synergy with meropenem and tobramycin. Composition will be locked using checkerboard, time-kill, and resistance-emergence assays against pre-specified thresholds. *Go/no-go: ≥80% biobank coverage and demonstrated synergy with ≥1 antibiotic before advancing.*

Aim 2. Optimize nebulized delivery and adjunctive efficacy in a ventilated murine VAP model. Building on the Weissfuss et al. paradigm, we will characterize aerosol deposition and post-nebulization phage viability, then test vehicle, phage alone, antibiotic alone, and the combination on lung CFU (primary), survival, oxygenation/ventilation surrogates, lung-injury and inflammatory markers, and airway/gut microbiome. Dedicated sub-studies will test whether adjunctive phage achieves efficacy at reduced antibiotic dose and suppresses resistant-clone outgrowth. *Go/no-go: combination superiority on lung CFU and ≥1-log dose-sparing before advancing.*

Aim 3. Generate IND-enabling manufacturing, stability, and inhalation-safety data. We will produce endotoxin-reduced, GMP-representative lots with release assays, define nebulizer compatibility and shelf-life, and complete repeat-dose inhalation safety and immunogenicity studies to assemble a pre-IND regulatory package—supporting a traditional IND while enabling expanded-access (single-patient eIND) use as a near-term bridge for treatment-limited patients, paired with a same-day "phagogram" companion-susceptibility workflow.

Impact. By converting strong but early evidence into a delivery-optimized, antibiotic-sparing, IND-ready product matched to US ICU strains, this project moves *P. aeruginosa* VAP from heroic last-resort compassionate use toward a standardized, microbiome-sparing adjunct that could shorten ventilation and curb resistance—advancing NIAID's AMR mission and seeding a first-in-human ICU trial.

Significance

The problem. VAP develops in a substantial fraction of mechanically ventilated patients and is among the deadliest ICU-acquired infections; *P. aeruginosa* is a leading and especially lethal cause, driving excess mortality, prolonged ventilation, and major cost. *P. aeruginosa* VAP combines three features that defeat conventional therapy: intrinsic and acquired MDR, robust biofilm on the endotracheal tube and airway surface, and a near-empty small-molecule pipeline. Even when an active antibiotic exists, monotherapy can fail through selection of resistant subpopulations and can paradoxically worsen lung injury by triggering release of epithelium-damaging virulence factors. This is precisely the unmet need NIAID's AMR portfolio prioritizes.

Why phages, why this route. Lytic phages provide an orthogonal mechanism: they self-amplify where bacteria are densest, are exquisitely species-specific (sparing the lung and gut microbiome that broad-spectrum antibiotics disrupt), penetrate and—via depolymerases—degrade biofilm matrix, and retain activity against strains resistant to all available antibiotics. The ventilated airway is directly cannulated, making it an ideal compartment for nebulized delivery that achieves high local titer without systemic exposure.

The decisive evidence. Weissfuss et al. (*Nature Communications* 2025) demonstrated in a mechanically ventilated mouse model that adjunctive phage plus meropenem outperformed either monotherapy, reduced the minimum effective meropenem concentration, prevented resistance to both agents, and limited epithelial damage—directly motivating an antibiotic-sparing, resistance-suppressing adjunctive strategy. Inhaled-phage delivery to the human lung is increasingly validated: Armata's Phase 2 Tailwind study of inhaled AP-PA02 reported significant lung *P. aeruginosa* CFU reductions in non-CF bronchiectasis (December 2024).

The gap we fill. The first dedicated VAP RCT (NCT07202234, nebulized cocktail vs. saline for MDR Gram-negative VAP including *P. aeruginosa*) is enrolling but will not read out until ~2028, leaving a clear preclinical and IND-enabling window. Crucially, the PhagoBurn RCT (Jault et al., 2019) established tolerability of an anti-*Pseudomonas* cocktail but failed on efficacy because the delivered titer fell by orders of magnitude below intended—an instructive cautionary precedent that makes dose, stability, and delivery optimization (our central focus) the make-or-break translational variables. A US R01 that delivers a delivery-optimized, US-strain-matched, IND-ready product now is the logical bridge to first-in-human ICU testing.

Innovation

This proposal advances the field in four respects.

1. **Indication.** It targets *P. aeruginosa* VAP specifically—an indication with compelling mechanistic data but no optimized, IND-ready nebulized product—rather than the better-studied chronic bronchiectasis/CF setting.
2. **Antibiotic-sparing by design.** It operationalizes phage–antibiotic synergy as a deliberate dose-reduction and resistance-suppression strategy, prospectively quantifying the effect Weissfuss et al. observed and exploiting phage-resistant escape mutants that pay a fitness/virulence cost and may re-sensitize to antibiotics (e.g., loss of LPS- or efflux-linked receptors).
3. **Delivery as a first-class variable.** It treats delivery—the failure mode that sank PhagoBurn—as a core efficacy readout, integrating nebulizer engineering, aerosol deposition, and post-nebulization viability into the in vivo design rather than as an afterthought.
4. **Bedside-ready translation.** It embeds a same-day phagogram companion-susceptibility concept and a depolymerase-enabled, biofilm-active cocktail, anticipating precision, isolate-matched use and a defined IND/expanded-access regulatory route for investigational phage.

Approach

Rigor of prior data and general design. The program builds directly on a published, peer-reviewed ventilated-VAP efficacy model (Weissfuss et al., 2025) and on positive human inhaled-phage data (Tailwind, 2024). All in vivo studies will be randomized and blinded at outcome assessment, powered from pilot variance, and will include both sexes (sex analyzed as a biological variable). Key biological resources—phage stocks (identity by genome sequence; purity; titer), bacterial isolates (species/strain confirmation, resistance genotype), and antibiotics—will be authenticated and lot-tracked per NIH rigor expectations.

Aim 1 — Cocktail assembly, biofilm activity, and phage–antibiotic synergy

Rationale. Phages bind strain-specific surface receptors (LPS, type IV pili, flagella); cocktails of complementary specificities broaden coverage and raise the genetic barrier to resistance, and depolymerase-bearing phages degrade the exopolysaccharide matrix shielding organisms on endotracheal tubes. Coverage and synergy against contemporary US ICU isolates must be established before in vivo testing.

Experimental design. We will compile a biobank of de-identified US ICU *P. aeruginosa* isolates spanning prevalent MDR lineages and serotypes. A curated lytic phage panel will be host-range–screened; 3–5 phages with complementary receptors and confirmed depolymerase activity will be selected. We will quantify planktonic killing (time-kill), biofilm disruption on endotracheal-tube–relevant substrates (crystal violet, viable counts, confocal microscopy), and synergy with meropenem and tobramycin (checkerboard FIC index, time-kill). Resistance-emergence assays will compare phage, antibiotic, and combination arms; whole-genome sequencing of escape mutants will map receptor changes and antibiotic re-sensitization. Composition and ratios will be locked against pre-specified coverage and synergy thresholds.

Expected outcomes. A locked, broadly covering, biofilm-active cocktail with documented synergy and a resistance profile showing combination-driven suppression and antibiotic re-sensitization in a substantial fraction of escape mutants.

Pitfalls & alternatives. If coverage is insufficient, we will expand the panel, add receptor classes, or pursue host-range–expanded constructs. If antagonism arises with an antibiotic, we will prioritize the synergistic partner and test staggered exposure timing.

Aim 2 — Nebulized delivery and adjunctive efficacy in ventilated murine VAP

Rationale. The airway is directly accessible, but nebulization can shear phages and reduce titer—the failure mode that crippled PhagoBurn. Delivery and dose must be optimized in a ventilated model that recapitulates VAP.

Experimental design. Using the Weissfuss et al. mechanically ventilated *P. aeruginosa* pneumonia paradigm, we will first characterize nebulizer output, particle size, and post-nebulization phage viability, selecting a device/formulation that preserves titer. In vivo arms will compare vehicle, nebulized phage alone, antibiotic alone, and combination, with **pre-specified primary endpoint lung CFU** and secondary endpoints of survival, oxygenation/ventilation surrogates, lung-injury and inflammatory markers, and airway/gut microbiome impact. A dose-sparing sub-study will test efficacy at reduced antibiotic concentrations; a resistance sub-study will track resistant-clone outgrowth across arms by sequencing. Group sizes will be powered from pilot variance with

randomization, blinded outcome assessment, and both sexes.

Expected outcomes. Evidence that the optimized nebulized cocktail deposits viable phage in the lung, that the combination outperforms monotherapies on clearance and lung protection, and that adjunctive phage enables antibiotic dose reduction while suppressing resistance—extending Weissfuss et al. with a delivery-optimized, US-isolate-matched cocktail.

Pitfalls & alternatives. If aerosol delivery underperforms, we will use intratracheal instillation as a bridging route and test alternative stabilizing excipients. If murine pharmacokinetics limit phage persistence, repeat dosing (modeled on the twice-daily NCT07202234 schedule) will be evaluated.

Aim 3 — IND-enabling manufacturing, stability, and inhalation safety

Rationale. Translation requires endotoxin-controlled product, defined stability and nebulizer compatibility, and inhalation safety/immunogenicity data—the package that converts efficacy into a fileable program.

Experimental design. We will produce endotoxin-reduced, GMP-representative cocktail lots with release assays (titer, purity, endotoxin, identity by sequence). Stability and nebulizer-compatibility studies will define shelf-life and in-use titer retention. Repeat-dose inhalation safety, local airway tolerability, and anti-phage neutralizing-antibody responses will be assessed in an appropriate species. We will assemble a pre-IND data package and operationalize a same-day phagogram for matched-isolate selection.

Expected outcomes. A documented, stable, low-endotoxin product with acceptable inhalation safety and a regulatory dossier supporting an FDA IND and near-term expanded-access (single-patient eIND) use, plus a future first-in-human ICU study.

Pitfalls & alternatives. If endotoxin or stability targets are unmet, we will refine purification (additional chromatography) and test lyophilized formulations. If neutralizing-antibody responses are detected, dosing-interval and cocktail-rotation strategies will be evaluated.

Timeline

[ILLUSTRATIVE] Aim 1 in Years 1–2; Aim 2 in Years 2–4; Aim 3 in Years 3–5, with FDA pre-IND interaction in Year 4. Total project period: 5 years [ILLUSTRATIVE]. Quantitative annual go/no-go milestones gate progression: **(M1)** locked cocktail with ≥80% biobank coverage and demonstrated antibiotic synergy; **(M2)** in vivo combination superiority on lung CFU with ≥1-log antibiotic dose-sparing; **(M3)** GMP-representative lot meeting endotoxin/stability specs with acceptable inhalation

safety.

Budget Justification (modular R01-style sketch)

[ILLUSTRATIVE] Requested at $250,000 direct costs/year for 5 years [ILLUSTRATIVE]. **Personnel:** PI (2.4 calendar months [ILLUSTRATIVE]); Co-Investigators in pulmonary/critical-care medicine and phage biology; a clinical microbiologist; an aerosol/inhalation scientist; and a research associate for in vivo work. **Other costs:** *P. aeruginosa* isolate biobanking and sequencing; phage propagation, purification, and endotoxin reduction; nebulizer hardware and aerosol characterization; ventilated murine model per-diems and histology; GMP-representative lot production and stability testing. Modules and effort to be finalized with institutional budgeting; all figures here are [ILLUSTRATIVE] placeholders.

Vertebrate Animals

Animal work is proposed in Aim 2 (efficacy) and Aim 3 (inhalation safety). We will use a mechanically ventilated murine model of *P. aeruginosa* pneumonia adapted from the published Weissfuss et al. paradigm. **Justification:** no in vitro or computational system reproduces the ventilated airway, mucociliary clearance, aerosol deposition, and host immune response required to evaluate nebulized phage efficacy, antibiotic-sparing, and resistance dynamics. **Minimization:** numbers will be set by power analysis from pilot variance [ILLUSTRATIVE group sizes], with shared control cohorts and both sexes to avoid duplicate studies. **Welfare:** humane endpoints, analgesia/anesthesia, and veterinary oversight will follow IACUC-approved protocols; the 3Rs will guide design, including blinded, randomized outcome assessment.

Human Subjects / Clinical Trial

No human-subjects clinical trial is conducted within this R01; the work is preclinical and IND-enabling. We will prepare for clinical translation by (1) assembling a pre-IND regulatory package supporting a traditional FDA IND, with expanded-access (single-patient eIND) use as a near-term bridge for critically ill, treatment-limited *P. aeruginosa* VAP patients—the established US route for investigational phage—and (2) defining a same-day phagogram companion-susceptibility workflow for isolate-matched dosing. De-identified clinical *P. aeruginosa* isolates used for biobanking will be obtained under appropriate IRB review and material-transfer agreements. Any future first-in-human study (separate funding) will proceed under IND with full IRB oversight and informed consent.

Team & Environment

This project requires multidisciplinary expertise; roles below are templates to be filled with named investigators and institutions. **Contact PI [NAME, INSTITUTION]:** bacteriophage biology and cocktail design. **MPI/Co-I [NAME]:** pulmonary and critical-care medicine, ventilated infection models. **Co-I [NAME]:** clinical microbiology and *P. aeruginosa* MDR epidemiology, isolate biobank. **Co-I [NAME]:** aerosol science/inhalation pharmacology and nebulizer engineering. **Co-I [NAME]:** GMP-representative manufacturing, purification, and endotoxin control. **Regulatory consultant [NAME]:** phage IND/expanded-access strategy. **Environment:** BSL-2 microbiology and phage production suites, an established ventilated small-animal facility, aerosol characterization instrumentation, an ICU clinical-isolate pipeline, and institutional cores for sequencing, histology, and immunoassays. US-based compassionate-use phage experience (e.g., academic personalized-phage programs) will inform the regulatory pathway. **Future partner funders** for clinical-stage and product development—engaged only after this R01—include NHLBI (pulmonary endpoints), BARDA, and DoD CDMRP.

References

1. Weissfuss C, Kneib N, Bischoff P, et al. Adjunctive phage therapy improves antibiotic treatment of ventilator-associated-pneumonia with *Pseudomonas aeruginosa*. *Nature Communications*. 2025;16:4500. PMID: 40368965.
2. 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 Infectious Diseases*. 2019;19(1):35-45. PMID: 30292481.
3. Chinese PLA General Hospital. A Randomized Controlled Trial of Bacteriophage Cocktail Therapy for Multidrug-Resistant Gram-Negative Ventilator-Associated Pneumonia. ClinicalTrials.gov identifier NCT07202234; registered 2025, est. completion 2028.
4. Armata Pharmaceuticals. Phase 2 Tailwind Study of Inhaled AP-PA02 in Non-Cystic Fibrosis Bronchiectasis Subjects with Chronic Pulmonary *Pseudomonas aeruginosa* Infection — positive topline results announced December 2024.