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Resistance-Aware, Biomarker-Guided Inhaled Bacteriophage Therapy for Chronic *Pseudomonas aeruginosa* Infection in Non-Cystic Fibrosis Bronchiectasis

Project Summary / Abstract

Non-cystic fibrosis (non-CF) bronchiectasis is a chronic, irreversible suppurative lung disease in which permanently dilated, mucus-laden airways become a lifelong reservoir for *Pseudomonas aeruginosa*. Chronic *P. aeruginosa* infection is the dominant microbiological predictor of frequent exacerbations, accelerated lung-function decline, hospitalization, and death, yet no therapy durably reduces its airway burden. The standard of care — prolonged inhaled and systemic anti-pseudomonal antibiotics — achieves only partial, temporary suppression of antibiotic-tolerant airway biofilms while selecting for multidrug resistance, accruing toxicity, and disrupting the airway microbiome (Singh et al., 2025). Lytic bacteriophages are a mechanistically distinct option: self-amplifying, strain-specific predators that can be aerosolized directly to the infected compartment, penetrate biofilm, remain active against pan-resistant strains, and spare the broader microbiome (Singh et al., 2025).

The field has advanced from case reports to a completed, registered randomized controlled trial: Armata's Phase 2 Tailwind study (NCT05616221) of inhaled multi-phage AP-PA02 in adults with non-CF bronchiectasis and chronic pulmonary *P. aeruginosa*. Tailwind reported good tolerability and a reduction in sputum *P. aeruginosa* density that was most evident in post-hoc pooled analysis, establishing clinical plausibility while leaving the underlying pharmacology, host–pathogen dynamics, and resistance management undefined.

We propose a mechanistic, biomarker-guided R01 that converts this clinical signal into durable, generalizable knowledge. **Aim 1** builds a curated, sequence-verified anti-pseudomonal phage library and validates a rapid, reproducible isolate-to-cocktail susceptibility-matching workflow against

banked non-CF bronchiectasis airway isolates, with prespecified coverage and turnaround targets. **Aim 2** defines, in human-sputum and biofilm models, the pharmacodynamics of phage and phage–antibiotic combinations and maps the resistance and antibiotic re-sensitization trajectories of escape mutants. **Aim 3** is an eIND-enabled, single-arm pilot of nebulized personalized phage cocktails in adults with chronic pulmonary *P. aeruginosa*, with dense phage-kinetic, microbiological, and host-response sampling and prespecified, blinded endpoints. Together these aims address **why** inhaled phage works, **for whom**, and **how to keep it working** — the mechanistic questions that must be answered alongside industry efficacy trials to make inhaled phage a reliable new therapeutic class for chronic *Pseudomonas* airway infection, directly advancing the NHLBI mission in chronic lung disease.

Specific Aims

Chronic *P. aeruginosa* infection defines the highest-risk non-CF bronchiectasis phenotype, yet no therapy reliably lowers its burden without driving resistance (Singh et al., 2025). The Tailwind Phase 2 trial (NCT05616221) showed that inhaled multi-phage AP-PA02 was well tolerated and reduced sputum *P. aeruginosa* density — most clearly in post-hoc pooled analysis — establishing clinical plausibility but leaving the governing pharmacology, host–pathogen dynamics, and resistance management largely undefined. Critically, Tailwind included both a phage-alone cohort and a phage-plus-inhaled-antibiotic cohort but was not designed or powered to resolve whether combination therapy adds benefit. Our **overarching hypothesis** is that inhaled phage efficacy in this setting is governed by definable, measurable parameters — cocktail–isolate match, in-airway phage amplification ("auto-dosing"), and the fitness/re-sensitization cost of resistance — and that characterizing these parameters yields a deployable, resistance-aware treatment strategy.

Aim 1 — Build and validate a rapid isolate-to-cocktail susceptibility-matching pipeline. *Hypothesis:* A receptor-diverse lytic phage library plus a standardized assay can generate a patient-matched cocktail covering the majority of circulating non-CF bronchiectasis *P. aeruginosa* strains within a clinically actionable window. We will assemble a sequence-verified library of lytic phages spanning complementary receptors (LPS, type IV pili, flagella), quantify host range against a banked isolate collection, and lock a reproducible matching assay. *Success criteria [ILLUSTRATIVE]:* ≥80% of isolates covered by ≥2 complementary phages; cross-operator concordance ≥90%; turnaround suitable for Aim 3.

Aim 2 — Define phage–antibiotic pharmacodynamics and resistance trajectories in airway-relevant models. *Hypothesis:* Receptor-targeted cocktails and rationally chosen phage–antibiotic combinations suppress *P. aeruginosa* more durably than monotherapy, and phage-resistant escape mutants frequently pay fitness costs and/or re-sensitize to antibiotics. Using sputum-supplemented and biofilm systems, we will measure killing by single phages, cocktails, and cocktail-plus-inhaled-

antibiotic combinations, and characterize escape mutants for fitness, virulence, receptor/efflux changes, and antibiotic susceptibility shifts. *Success criteria [ILLUSTRATIVE]:* ≥1 combination achieving prespecified additivity/synergy and ≥1 rotation/combination strategy that measurably delays resistance versus single-phage exposure.

Aim 3 — Conduct an eIND-enabled pilot of personalized nebulized phage cocktails. *Hypothesis:* Matched nebulized cocktails are feasible and well tolerated, achieve detectable in-airway phage amplification, and reduce sputum *P. aeruginosa* density. In a single-arm, open-label study [ILLUSTRATIVE: n≈12–15] of adults with non-CF bronchiectasis and chronic pulmonary *P. aeruginosa*, each participant (own pre/post control) receives an Aim 1–matched cocktail under FDA emergency/expanded-access IND oversight, with dense sampling of phage kinetics, bacterial density, emergent resistance, and host response. *Success criteria [ILLUSTRATIVE]:* prespecified feasibility (match generated and treatment delivered for a defined fraction of enrollees), safety consistent with prior inhaled-phage experience, and paired kinetic/density data adequate to model auto-dosing.

Impact. By linking a deployable matching pipeline, mechanistic pharmacodynamics, and a carefully monitored human pilot, this work supplies the resistance-aware, biomarker-guided foundation needed to translate a positive but early Phase 2 phage signal into a durable new therapeutic class for chronic *Pseudomonas* airway infection.

Significance

The problem and the NHLBI-relevant burden. Non-CF bronchiectasis is a chronic, irreversible suppurative lung disease whose natural history is dominated by *P. aeruginosa*. Once chronic infection is established, patients suffer more frequent exacerbations, faster lung-function decline, more hospitalizations, and increased mortality; *P. aeruginosa* status is the dominant microbiological prognostic marker in this population (Singh et al., 2025). This is squarely within the NHLBI mission: a progressive structural lung disease with high morbidity, recurrent acute care utilization, and no disease-modifying antimicrobial strategy.

Why current therapy fails. *P. aeruginosa* is intrinsically hard to clear: it forms antibiotic-tolerant biofilms in dilated airways and accumulates multidrug resistance, so prolonged inhaled and systemic anti-pseudomonal antibiotics achieve suppression at best while promoting resistance, toxicity, and airway-microbiome disruption (Singh et al., 2025). No approved therapy durably lowers *P. aeruginosa* burden in this setting, and the conventional antibiotic pipeline is thin.

Why phage is mechanistically matched to this niche. The target is a single, persistently colonizing pathogen in an accessible, inhalable compartment, making aerosolized delivery directly to the airway reservoir feasible (Singh et al., 2025). The clinical foundation now extends beyond anecdote.

Tailwind (NCT05616221) — a completed double-blind, placebo-controlled Phase 2 RCT of inhaled AP-PA02 in adults with non-CF bronchiectasis and chronic pulmonary *P. aeruginosa* — demonstrated tolerability and a reduction in sputum bacterial density that was most apparent in post-hoc pooled analysis. Real-world experience is consistent: a 2025 case report describes successful induced phage-cocktail treatment of a chronic bronchiectasis patient carrying *P. aeruginosa* among mixed pathogens (Jernigan & Hentish, 2025), and early US expanded-access experience includes inhaled/IV anti-*Pseudomonas* phage (AB-PA01) in lung-transplant and bronchiectasis recipients (Aslam et al., 2020).

The gap this R01 fills. What remains missing — and what a mechanistic, NHLBI-aligned R01 is positioned to supply — is rigorous understanding of phage pharmacodynamics in human airway secretions, the determinants of response across diverse patient isolates, and the resistance-management strategies that will decide whether phage delivers *durable* benefit. Registration trials such as Tailwind are not designed to produce these data. Resolving them would accelerate a fundamentally new, microbiome-sparing approach to a leading driver of morbidity and mortality in chronic lung disease, and would generate transferable principles (cocktail design, auto-dosing pharmacodynamics, resistance steering) applicable across inhaled phage development.

Innovation

This proposal is innovative in four respects.

1. **Reframing the question.** It treats inhaled phage for bronchiectasis as a *pharmacology and resistance-management* problem rather than a one-off efficacy question, generating the mechanistic data that registration trials such as Tailwind (NCT05616221) are not built to produce.
2. **Operationalized personalization.** Rather than a fixed product, we develop and validate a rapid isolate-to-cocktail matching pipeline that selects complementary phages by receptor target to broaden coverage and raise the genetic barrier to resistance (Singh et al., 2025).
3. **Resistance as an exploitable feature.** We systematically map how escape mutants pay fitness costs and re-sensitize to antibiotics or lose virulence — the rational basis for phage–antibiotic combinations and cocktail rotation (Singh et al., 2025).
4. **Mechanism embedded in the clinic.** These readouts are embedded inside an eIND-enabled human pilot, so bacterial-density, phage-kinetic, and host-response endpoints are interpreted against the same models used to design the cocktails. The approach is deliberately matched to the current evidence base — a positive but early Phase 2 signal — and is designed to de-risk next-generation inhaled phage strategies, including depolymerase-optimized and CRISPR-Cas3–armed cocktails in development for respiratory *P. aeruginosa* (Singh et al., 2025).

Approach

Rigor, Reproducibility, and Authentication of Key Resources

All bacterial isolates and phage stocks will be authenticated (whole-genome sequencing of phages to confirm lytic-only lifestyle and absence of toxin/AMR genes; species/strain confirmation and susceptibility profiling of isolates) and biobanked with documented provenance. Assays will use prespecified, version-controlled protocols; in vitro experiments will be performed with biological and technical replicates and predefined statistical analysis. Clinical microbiology readouts in Aim 3 (CFU density, resistance emergence) will be performed by operators blinded to phage-kinetic results, with a prespecified analysis plan and an independent biostatistician. Sex as a biological variable will be incorporated throughout (isolate-source sex recorded; analyses examine sex-associated differences as data permit).

Aim 1 — Build and validate a rapid isolate-to-cocktail susceptibility-matching pipeline

Rationale. Phages are narrowly strain-specific, so effective products are cocktails of complementary phages ideally matched to a patient's isolate (Singh et al., 2025). A clinically deployable workflow requires both a receptor-diverse library and a fast, standardized susceptibility assay.

Experimental design. We will assemble a panel of well-characterized lytic *P. aeruginosa* phages chosen for complementary receptor usage (LPS, type IV pili, flagella) and confirm lytic-only lifestyle by genome sequencing. Against a biobank of non-CF bronchiectasis airway isolates [ILLUSTRATIVE: ~150 isolates], we will quantify host range, efficiency-of-plating, and quantitative liquid-culture suppression. We will lock a standardized susceptibility-testing protocol that outputs a ranked, patient-matched cocktail, and assess turnaround time and cross-operator reproducibility.

Expected outcomes. A curated, sequence-verified phage library; quantified coverage across circulating strains; and a validated matching assay with defined sensitivity and turnaround suitable for Aim 3 ([ILLUSTRATIVE] targets in Specific Aims).

Pitfalls & alternatives. If library coverage is inadequate for some isolates, we will expand the panel via additional environmental/clinical phage isolation, prioritizing broad–host-range phages. If plaque assays are variable on mucoid strains, quantitative liquid-culture kinetics will serve as the primary matching readout. If receptor classes are unevenly represented, targeted isolation against pili/flagella-dependent strains will rebalance coverage.

Aim 2 — Define phage–antibiotic pharmacodynamics and resistance trajectories in airway-relevant models

Rationale. Phages diffuse through biofilm water channels and, via depolymerases/lysins, degrade the polysaccharide matrix to reach dormant cells that antibiotics miss; sub-inhibitory antibiotics can in turn enhance phage activity, and escape mutants frequently re-sensitize to antibiotics or lose virulence (Singh et al., 2025). Because Tailwind included a phage-plus-inhaled-antibiotic cohort but was not powered to resolve combination benefit (NCT05616221), defining the mechanistic basis of combination effects is a priority.

Experimental design. In sputum-supplemented planktonic cultures and established *P. aeruginosa* biofilms, we will measure time-kill for single phages, matched cocktails, and cocktail-plus-inhaled-antibiotic (e.g., aminoglycoside- or polymyxin-class) combinations across clinically relevant concentrations, scoring additivity/synergy by prespecified criteria. We will isolate phage-resistant mutants and characterize growth fitness, virulence-associated phenotypes, receptor/LPS and efflux changes, and antibiotic susceptibility shifts to test the re-sensitization hypothesis. Cocktail-rotation schedules will be modeled to suppress resistance emergence.

Expected outcomes. Quantitative pharmacodynamic profiles identifying additive/synergistic phage–antibiotic combinations, and a resistance/re-sensitization map defining rotation and combination strategies for Aim 3.

Pitfalls & alternatives. If sputum components neutralize phage activity, we will titrate phage dose and test mucolytic pre-treatment. If escape mutants do not consistently re-sensitize, receptor-diverse multi-phage cocktails will be used to raise the genetic barrier to resistance, and steering toward virulence-attenuating mutations will be prioritized as the alternative objective.

Aim 3 — Conduct an eIND-enabled pilot of personalized nebulized phage cocktails

Rationale. Tailwind established that inhaled phage is tolerable and can reduce sputum *P. aeruginosa* density (NCT05616221); a mechanistically instrumented pilot is needed to connect in-airway phage kinetics to bacterial-density change and host response in individual patients.

Experimental design. In a single-arm, open-label feasibility study [ILLUSTRATIVE: n≈12–15] of adults with non-CF bronchiectasis and chronic pulmonary *P. aeruginosa*, each participant's isolate will be matched (Aim 1) to a personalized nebulized cocktail delivered via home nebulizer [ILLUSTRATIVE: twice daily for ~10 days, informed by the Tailwind treatment course]. Each participant serves as their own pre/post control. We will densely sample sputum for phage concentration (kinetics/auto-dosing), *P. aeruginosa* CFU density, emergent phage resistance, and host

inflammatory markers, plus safety and symptom measures, with the primary microbiological readout assessed ~1 week after end of treatment [ILLUSTRATIVE: ~Day 17, mirroring Tailwind] and follow-up thereafter [ILLUSTRATIVE: through ~Day 24]. CFU and resistance assays will be run blinded to kinetic data under a prespecified analysis plan. Investigational phage will be administered under an FDA emergency/expanded-access IND (eIND) with full IRB oversight and independent DSMB monitoring.

Expected outcomes. Demonstrated feasibility of rapid matching-to-treatment; a safety/tolerability profile consistent with prior inhaled-phage experience; and paired phage-kinetic and bacterial-density data linking in-airway auto-dosing to microbiological response and on-treatment resistance.

Pitfalls & alternatives. If recruitment is slow, we will broaden inclusion across collaborating bronchiectasis clinics. If a matched cocktail cannot be generated for a candidate, the Aim 1 library will be expanded; participants without a qualifying match will not be dosed. If monotherapy responses are modest, the eIND framework permits adding an inhaled anti-pseudomonal antibiotic informed by Aim 2. A predefined interim feasibility/safety review governs continuation.

Timeline

[ILLUSTRATIVE] **Years 1–2:** Assemble and sequence phage library; build isolate biobank; lock and validate matching assay (Aim 1). Begin sputum/biofilm pharmacodynamics (Aim 2). Prepare eIND and IRB submissions. **Years 2–4:** Complete phage–antibiotic and resistance/re-sensitization studies (Aim 2); finalize regulatory approvals; manufacture and qualify clinical-grade phage stocks. **Years 3–5:** Enroll and treat pilot participants with dense sampling (Aim 3); integrated analysis linking in vitro pharmacodynamics to in vivo kinetics, bacterial-density change, and resistance. A go/no-go at the end of Year 2 (assay validated, ≥1 synergistic combination identified, eIND active) gates initiation of dosing.

Budget Justification (modular R01-style sketch)

[ILLUSTRATIVE] We request [ILLUSTRATIVE: $500,000] direct costs/year for [ILLUSTRATIVE: 5 years] (modular). **Personnel:** PD/PI (microbiology/phage biology), clinical Co-I (pulmonology), Co-I in infectious diseases/phage therapy, study coordinator, two research technicians, biostatistician, and a regulatory/quality specialist for eIND and clinical-grade stock release [ILLUSTRATIVE: ~60% of direct costs]. **Phage production & characterization:** sequencing, host-range and pharmacodynamic assays, endotoxin removal, and clinical-grade stock qualification [ILLUSTRATIVE: ~20%]. **Clinical pilot:** participant costs, nebulizers, dense sputum sampling, microbiology, phage-kinetic and host-response assays, and DSMB [ILLUSTRATIVE: ~15%]. **Other:**

isolate biobanking, data management, publication, and dissemination [ILLUSTRATIVE: ~5%]. Costs scale with the resistance-mapping and clinical-monitoring scope rather than participant numbers.

Vertebrate Animals

Not applicable. The proposed work uses bacterial isolates, in vitro airway-relevant culture systems, and a human clinical pilot; no vertebrate animal studies are proposed. Should an inhalation-toxicology bridging study be required by the eIND, it would be added by amendment with a full Vertebrate Animal Section and IACUC approval.

Human Subjects / Clinical Trial

Aim 3 is a single-arm, open-label clinical study and constitutes an NIH-defined clinical trial. Investigational phage cocktails will be administered under an **FDA emergency/expanded-access Investigational New Drug (eIND)** application — the established US regulatory route for patient-specific phage products, consistent with prior US compassionate-use anti-*Pseudomonas* phage experience (Aslam et al., 2020). All activities will proceed under **IRB approval** with written informed consent and independent **DSMB** safety oversight; the study will be registered on ClinicalTrials.gov. Eligible participants are adults (≥18 years) with non-CF bronchiectasis and documented chronic pulmonary *P. aeruginosa* infection. Inclusion/exclusion criteria, prespecified stopping rules, and adverse-event reporting will follow FDA/IRB requirements. **Inclusion across the lifespan, sex/gender, and race/ethnicity:** enrollment will reflect the non-CF bronchiectasis population, which is enriched for older adults and women; recruitment and analysis plans address equitable inclusion, and sex will be examined as a biological variable. Dense biospecimen collection (sputum, blood) supports the mechanistic endpoints; risks are minimized by prior inhaled-phage tolerability data (NCT05616221; Jernigan & Hentish, 2025) and active monitoring. A **data-sharing and resource-sharing plan** will provide de-identified clinical data, phage genome sequences, and isolate/phage metadata to the community consistent with NIH policy.

Team & Environment

This program requires an integrated phage-microbiology and pulmonary-clinical team at an institution with bronchiectasis expertise, a CLIA/clinical microbiology laboratory, phage production/characterization capability, and regulatory/quality infrastructure for eIND submissions. (Named personnel and letters of support to be provided; roles below.)

- **Program Director / Principal Investigator — [NAME, INSTITUTION]:** phage biology,

host-range and pharmacodynamic assays, overall scientific direction.

- **Clinical Co-Investigator (Pulmonology) — [NAME, INSTITUTION]:** non-CF bronchiectasis cohort, clinical conduct of Aim 3.
- **Co-Investigator (Infectious Diseases / Phage Therapy) — [NAME, INSTITUTION]:** eIND-based phage administration and compassionate-use experience (modeled on established US academic phage-therapy programs).
- **Microbiology Core Lead — [NAME, INSTITUTION]:** isolate biobank, susceptibility matching, resistance characterization.
- **Phage Production / Quality & Regulatory — [NAME, ORGANIZATION]:** clinical-grade stock qualification and eIND support (modeled on existing inhaled-phage manufacturing precedent).
- **Biostatistician — [NAME, INSTITUTION]:** pharmacodynamic modeling and pilot-study analysis.

Environment. Facilities will include BSL-2 microbiology, phage production/endotoxin-removal capacity, a clinical research unit for nebulized dosing and dense sampling, and institutional IRB/IND regulatory support.

References

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