Steering Chronic *Pseudomonas aeruginosa* into an Evolutionary Corner: A Biomarker-Guided, Trade-Off-Selected Nebulized Phage Cocktail for Cystic Fibrosis

Project Summary / Abstract

Chronic *Pseudomonas aeruginosa* (Pa) infection remains a leading driver of lung-function decline, exacerbations, and mortality in cystic fibrosis (CF). Even with highly effective CFTR modulators such as elexacaftor/tezacaftor/ivacaftor, a substantial subset of patients remains chronically infected with mucoid, biofilm-encased, progressively multidrug-resistant Pa and exhausts effective antibiotic options. Lytic bacteriophages are mechanistically orthogonal to antibiotics: they self-amplify at the infection site, can be matched to a patient's resistant strain, and many encode depolymerases that degrade the alginate/exopolysaccharide biofilm matrix. First-in-human and compassionate-use data are encouraging but immature. The double-blind, placebo-controlled BX004-A nebulized three-phage trial (n=9 adults) met its primary safety and tolerability endpoints and noted a potential reduction in Pa sputum burden, with efficacy conclusions limited by sample size (Weiner et al., *Nat Commun* 2025). A personalized "evolutionary trade-off" approach in nine adults reduced sputum Pa by a median of 10^4 CFU/mL (P=0.006) and improved predicted FEV1 by a median of 6% (P=0.004) without adverse events, and documented post-treatment isolates with reduced antibiotic resistance or virulence (Chan et al., *Nat Med* 2025). A 2026 systematic review (19 studies, 51 patients; Pa the predominant target) reported microbiological improvement in 35/51 (68.6%) and FEV1 improvement in 20/27 (74%), with phages generally safe but severe adverse events "rare but worthy of attention" (Terlizzi et al., *Int J Infect Dis* 2026). The field's central gap is not whether phages *can* help, but a disciplined, prospective definition of *which* selection strategy, dose, and biomarkers predict and explain durable benefit. We will (1) build and validate a curated, obligate-lytic, trade-off-steering anti-Pa phage bank with quantitative biofilm and resensitization assays; (2) define CF-relevant pharmacodynamics, host-range matching, and phage–antibiotic synergy; and (3) under a conventional investigational new drug (IND) application, conduct a biomarker-anchored, single-arm clinical study of personalized nebulized cocktails powered to confirm the trade-off mechanism *in vivo* and to deliver an explicit go/no-go decision for a subsequent NHLBI-scale controlled trial. The goal is to convert a relentless, resistance-driven infection into a controllable one, sparing lung function and delaying

transplant.

Specific Aims

Chronic Pa in CF progresses despite suppressive antibiotics and CFTR modulators, and patients run out of options as strains approach pan-resistance. Lytic phage cocktails offer a self-amplifying, biofilm-penetrating, antibiotic-orthogonal modality, but the human evidence base consists of small case series and single first-in-human trials with no licensed product and no validated rule for *how* to choose phages. We will rigorously define the phage-selection logic, pharmacodynamics, and biomarkers needed to advance personalized nebulized phage therapy, and we tie each aim to quantitative success milestones.

Aim 1. Build and validate a trade-off-steering anti-Pa phage bank. We will assemble and characterize a curated panel of obligate lytic anti-Pa phages, prioritizing those whose receptors (e.g., efflux-pump components, LPS, type IV pili, virulence-associated structures) make phage escape costly through antibiotic resensitization or reduced virulence. We will quantify host range across banked CF Pa isolates and confirm depolymerase-mediated biofilm degradation. *Milestone (go/no-go):* a characterized bank that lyses ≥80% of banked CF Pa isolates with ≥1 trade-off-enforcing cocktail per dominant receptor class [ILLUSTRATIVE thresholds].

Aim 2. Define CF-relevant pharmacodynamics and phage–antibiotic synergy (PAS). Using mucoid biofilm and CF airway-surface-liquid models, we will measure killing kinetics, phage self-amplification, time-to-emergence of phage resistance, and the resensitization/virulence phenotype of escapees, and we will map PAS to identify combinations that clear infection where neither agent does alone. *Milestone:* ≥1 cocktail with quantified amplification in biofilm and a ranked PAS combination demonstrating ≥2-log added kill over the best single agent [ILLUSTRATIVE].

Aim 3. Biomarker-anchored clinical confirmation of the trade-off mechanism. Under a conventional IND with IRB and DSMB oversight, we will treat chronically infected adults with strain-matched nebulized cocktails selected via Aim 1–2 logic, with safety/tolerability as the primary endpoint and a pre-specified mechanistic biomarker — in-airway phage amplification and emergence of antibiotic-resensitized Pa escapees — as the principal secondary endpoint. *Milestone (go/no-go to a controlled trial):* acceptable safety plus detection of the resensitization trade-off *in vivo* in a pre-specified fraction of treated participants [ILLUSTRATIVE].

Impact: Success would establish the selection rules, pharmacodynamics, and biomarkers that turn personalized phage therapy from anecdote into a reproducible precision modality — potentially the first genuinely new antibacterial approach CF clinics have added in a generation — and would hand NHLBI a de-risked, mechanism-validated candidate for a definitive efficacy trial.

Significance

Chronic Pa drives the accelerated decline in lung function that shapes CF prognosis. The organism establishes mucoid, biofilm-encased colonies deep in the airway and becomes progressively multidrug- or pan-drug-resistant after years of suppressive antibiotics. CFTR modulators improve airway clearance but do not eradicate established Pa, leaving a substantial chronically infected subset with dwindling options — a population this proposal directly targets. Phages address this gap through mechanisms entirely orthogonal to antibiotics, so resistance to one does not predict resistance to the other, and the CF airway is unusually accessible to nebulized delivery, allowing high local titers with minimal systemic exposure. The clinical signal is real but immature: a placebo-controlled nebulized cocktail trial met safety endpoints with a potential Pa-burden signal (Weiner et al., 2025); personalized trade-off phage produced statistically significant reductions in sputum Pa and improvements in predicted FEV1 (Chan et al., 2025); and a 19-study, 51-patient systematic review reported microbiological improvement in 68.6% and FEV1 improvement in 74% (20/27) with a generally favorable but not uniformly benign safety profile (Terlizzi et al., 2026). Pediatric protocols using obligate lytic personalized phage are underway (Singh et al., 2023). The unmet need is a disciplined, prospective definition of *which* selection strategy, dose, and biomarker predicts durable benefit and *why* — precisely the mechanism-to-early-clinical question an NHLBI R01 is positioned to answer, with the Cystic Fibrosis Foundation as a natural non-federal co-funder.

Innovation

Most reported phage use is reactive host-range matching. Our central innovation is to operationalize the **evolutionary trade-off ("steering") strategy** — validated *in vivo* by Chan et al. (2025) — as a quantifiable, prospectively testable design principle: deliberately selecting phages whose escape mutants pay a defined cost (antibiotic resensitization or attenuated virulence) rather than merely maximizing lytic breadth. Second, we pair this with **depolymerase-explicit biofilm targeting** to crack open the mucoid alginate matrix that shields Pa. Third, we embed a **pre-specified mechanistic biomarker** (in-airway amplification plus resensitization of escapees) as a primary scientific readout from the outset, so the clinical study *confirms a mechanism* rather than merely describing outcomes. Finally, we structure the program around a **curated, characterized obligate-lytic phage bank** suitable for rapid strain matching, aligning with the emerging "sequence-the-strain, pull-matched-phages, nebulize-a-personalized-cocktail" paradigm while remaining grounded in non-engineered phages consistent with current human CF evidence.

Approach

Aim 1 — Build and validate a trade-off-steering anti-Pa phage bank

Rationale. Receptor recognition is strain-specific, so cocktails of 3–4 complementary phages broaden host range and suppress phage-resistant mutants; targeting receptors tied to efflux or virulence makes escape costly. The Yale trade-off results (Chan et al., 2025) motivate making this selection logic systematic and reproducible. **Experimental design.** We will expand an obligate lytic anti-Pa phage collection and characterize each isolate by whole-genome sequencing (confirming lytic lifestyle, absence of integrase/toxin/AMR genes, and presence of depolymerase genes), receptor class, and host range across a bank of CF Pa clinical isolates spanning mucoid and multidrug-resistant phenotypes [ILLUSTRATIVE: ~60 isolates]. Candidate "steering" phages will be flagged where *in vitro* escape mutants show measurable antibiotic resensitization (MIC shift) or reduced virulence markers. Cocktails of 3–4 phages will be assembled for complementary receptor coverage and cross-suppression of resistance. **Expected outcomes.** A documented bank with host-range matrices and a prioritized set of trade-off-enforcing cocktails ready for Aims 2–3. **Potential pitfalls & alternatives.** Some isolates may resist all banked phages; we will iteratively enrich for new phages against gap isolates (environmental and clinical sourcing) and, if depolymerase activity is limiting, prioritize phages with demonstrated matrix-degrading phenotypes. If trade-off phenotypes are weaker than reported, we will broaden the resistance/virulence panel screened and select on the strongest observed cost.

Aim 2 — Define CF-relevant pharmacodynamics and phage–antibiotic synergy

Rationale. Clinical benefit depends on phages amplifying and penetrating biofilm in the airway milieu, and PAS is repeatedly observed where combinations clear infections neither agent clears alone. **Experimental design.** Using mucoid biofilm and CF airway-surface-liquid models, we will measure killing kinetics, phage amplification, time-to-emergence of phage resistance, and the resensitization/virulence phenotype of escapees. We will systematically test phage + standard anti-Pseudomonal antibiotic combinations (e.g., tobramycin, beta-lactams, fluoroquinolones) to map synergy and identify dosing/sequencing that maximizes clearance and suppresses resistance. Key findings will be cross-validated in *ex vivo* CF sputum where feasible. **Expected outcomes.** Quantitative pharmacodynamic parameters and a ranked PAS combination set, plus confirmation that steering phages drive escapees toward antibiotic resensitization under realistic conditions. **Potential pitfalls & alternatives.** *In vitro* models imperfectly capture CF sputum; if matrix components suppress amplification, we will adjust cocktail composition, add depolymerase-rich phages, or pre-treat with mucolytic-compatible conditions, and we will down-weight PAS pairs that fail *ex vivo* validation.

Aim 3 — Biomarker-anchored clinical confirmation of the trade-off mechanism

Rationale. First-in-human and compassionate-use data support safety and a Pa-burden/FEV1 signal, but no study has prospectively confirmed the trade-off *mechanism* as a pre-specified endpoint under a controlled regulatory framework. **Design and regulatory framework.** Under a conventional **IND** (not single-patient emergency use), with IRB approval, informed consent, and independent DSMB oversight, a single-arm cohort of chronically infected adults [ILLUSTRATIVE: n≈12–15] will receive strain-matched nebulized cocktails selected via Aim 1–2 logic. We deliberately power and scope this as a **mechanism-confirming, hypothesis-generating** study, not an efficacy trial. **Endpoints.** *Primary:* safety and tolerability. *Principal secondary (mechanistic):* in-airway phage amplification and emergence of antibiotic-resensitized Pa escapees (the trade-off biomarker). *Additional secondary:* lower-airway phage delivery, sputum Pa burden (CFU/mL), and predicted/measured FEV1, interpreted descriptively and consistent in direction with Weiner et al. (2025) and Chan et al. (2025). **Analysis and rigor.** Endpoints, the biomarker-positivity definition, and the go/no-go threshold for a future controlled trial are pre-specified. Sex is analyzed as a biological variable, with deliberate attention to balanced enrollment given the female predominance of prior cohorts (8/9 in Chan et al., 2025). Assays and cocktail lots are validated under the Authentication/Rigor plan below; methods are harmonized with multicenter efforts to enable future pooling. **Potential pitfalls & alternatives.** Small single-arm samples cannot support efficacy inference; we therefore frame efficacy readouts as descriptive and gate progression on the mechanistic biomarker and safety. If accrual of eligible chronically infected adults is slow, we will open a second CF-center site (subaward) and broaden eligibility within the chronically infected, option-limited population. If the *in vivo* trade-off is not detected at the pre-specified rate, the program stops or returns to Aim 1–2 rather than advancing to a controlled trial.

Timeline

[ILLUSTRATIVE] Years 1–2: Aim 1 bank assembly/characterization; begin Aim 2 pharmacodynamics. Years 2–3: complete Aim 2 PAS mapping; finalize cocktails; prepare and submit IND; secure IRB/DSMB. Years 3–5: Aim 3 clinical study, mechanistic-biomarker analysis, go/no-go determination, and reporting. Go/no-go milestones gate each transition (see Specific Aims).

Rigor, Reproducibility & Authentication of Key Resources

Phage identity and purity (sequence-confirmed obligate-lytic genome; endotoxin within release limits) and Pa isolate identity are authenticated at entry and before clinical use. Assays use defined positive/negative controls, biological and technical replicates, and pre-registered analysis plans for trade-off and PAS readouts. Sex is incorporated as a biological variable across *in vitro*, *ex vivo*, and

clinical components. Cocktail lots used clinically are produced under GMP-aligned conditions with documented release testing.

Budget Justification

Modular R01-style request [ILLUSTRATIVE]. Personnel: multiple PD/PI (clinical CF lead and phage/evolutionary-biology lead), microbiology/biofilm scientists, a clinical research coordinator, regulatory/IND support, and bioinformatics. Other costs: phage sequencing/characterization, GMP-aligned phage preparation for clinical use, nebulizer/delivery consumables, sputum microbiology and biomarker assays, and IND/regulatory and DSMB costs. Estimated [ILLUSTRATIVE] $250,000 direct costs/year across [ILLUSTRATIVE] 5 years, in two modules; subaward [ILLUSTRATIVE] to a partnering CF center for Aim 3 accrual. Final figures to be set with institutional budgeting and Cystic Fibrosis Foundation co-funding.

Vertebrate Animals

Not applicable. No vertebrate animal work is proposed; pharmacodynamics use *in vitro* biofilm, CF airway-surface-liquid, and *ex vivo* sputum systems.

Human Subjects / Clinical Trial

Aim 3 involves human subjects and constitutes an NIH-defined clinical trial. Investigational phage will be administered under a conventional **IND** to chronically infected adults with limited effective options, with full IRB oversight, informed consent, and independent DSMB safety monitoring. Enrollment [ILLUSTRATIVE: n≈12–15] adults at one to two partnering CF centers. The primary endpoint is safety/tolerability; the principal secondary endpoint is the mechanistic trade-off biomarker, with lower-airway delivery, sputum Pa burden, and FEV1 as additional secondary endpoints. Sex is analyzed as a biological variable with attention to balanced enrollment. The design extends the safety-first, delivery-confirming structure of prior first-in-human nebulized phage studies (Weiner et al., 2025) and personalized compassionate-use experience (Chan et al., 2025; Singh et al., 2023) by adding pre-specified mechanistic confirmation and a go/no-go gate to a controlled trial.

Team & Environment

[TEMPLATE — fill with real names/institutions.] **Contact PD/PI:** [CF pulmonologist–investigator with adult CF trial experience]. **MPI:** [phage/evolutionary microbiologist with trade-off-selection expertise]. **Co-Is:** [biofilm microbiologist], [clinical pharmacologist for PAS], [bioinformatician],

[regulatory/IND lead]. **Required environment/capabilities:** an academic CF Care Center with an adult CF clinic and sputum microbiology; a phage biology/therapy program with sequencing, host-range, and trade-off-assay capacity; GMP-aligned phage production access; and an established IND/DSMB and clinical-trials infrastructure. Field collaborators/comparators include Yale's Center for Phage Biology & Therapy, UC San Diego IPATH/CIPHER, the NIAID/ARLG multicenter program, BiomX, and Walter Reed/Naval Medical Research Command phage banks; non-federal co-funding via the Cystic Fibrosis Foundation (with alternates NIAID and BARDA).

References

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