Personalized Lytic Mycobacteriophage Therapy for Refractory Pulmonary *Mycobacterium avium* Complex Disease: An Isolate-Matching Platform and Clinical-Readiness Framework

Project Summary / Abstract

Pulmonary *Mycobacterium avium* complex (MAC) disease is a chronic, progressive nontuberculous mycobacterial (NTM) infection that requires 12–18+ months of macrolide-based multidrug therapy yet frequently fails to achieve durable culture conversion. Outcomes are eroded by intrinsic drug resistance, emerging macrolide resistance, and poor antibiotic penetration into the granulomas, biofilms, and macrophages where *M. avium* persists. Patients with macrolide-resistant or refractory disease have few options. Mycobacteriophages—self-amplifying, strain-targeted lytic agents—are mechanistically attractive because they can reach mycobacteria in niches where antibiotics stall, and a clinical pipeline already exists for related NTM, most advanced for *M. abscessus* (Dedrick 2023; Nick 2022). For *M. avium*, however, the human evidence rests on a single well-documented compassionate-use case (intravenous phage Muddy, favorable response) plus early preclinical phage discovery (Golla 2024), so phages must be advanced as a rigorously studied experimental adjunct, not an established therapy. This proposal builds the missing translational foundation and a clinical-readiness package. **Aim 1** establishes a standardized, quantitative isolate-matching platform, screening a defined collection of clinical *M. avium* isolates against an expanding mycobacteriophage panel and dissecting the smooth-versus-rough morphotype barrier to lytic susceptibility. **Aim 2** evaluates phage and phage–antibiotic activity against intracellular and biofilm-resident *M. avium* and characterizes the neutralizing-antibody and innate responses that could blunt durability (a barrier directly documented in the anchor cohort; Dedrick 2023). **Aim 3** designs and pilots a personalized, cocktail-capable clinical framework for refractory MAC under an FDA expanded-access IND (eIND) pathway with IRB oversight, generating prospectively standardized pharmacodynamic, microbiologic, pulmonary, and immunologic endpoints to justify and power a future prospective trial. The work targets the two field-acknowledged rate-limiters—the thin repertoire of phages active against slow-growing, smooth-morphotype *M. avium*, and management of neutralizing-antibody responses (Bonacorsi 2024).

Successful completion delivers a validated matching pipeline, a defensible cocktail-design rationale, and a trial-ready clinical framework, positioning adjunctive phage therapy as a credible option for patients with refractory MAC. This work directly serves NIAID's mycobacterial-infection mission, with clear NHLBI co-interest in the pulmonary-function endpoint.

Specific Aims

Pulmonary MAC disease frequently resists prolonged macrolide-based regimens because *M. avium* persists in biofilms, granulomas, and macrophages and is increasingly macrolide-resistant. Mycobacteriophages offer a self-amplifying, strain-specific lytic mechanism that can act where antibiotics fail, but for *M. avium* the human evidence is limited to one favorable intravenous Muddy case within a 20-patient compassionate-use cohort (Dedrick 2023) plus preclinical discovery (Golla 2024). The field's own syntheses identify two rate-limiters: a thin repertoire of phages active against slow-growing, smooth-morphotype *M. avium*, and neutralizing-antibody responses that may limit durability (Bonacorsi 2024). **Our overarching hypothesis is that a standardized isolate-matching platform, paired with niche- and immune-aware activity testing, can identify lytic phage/cocktail regimens active against refractory *M. avium* and supply the prospectively standardized endpoints needed to convert single-patient experience into a fundable trial.** We will build this translational and clinical-readiness foundation across three Aims.

Aim 1. Build and validate a personalized *M. avium* phage isolate-matching platform. We will assemble a panel of candidate lytic mycobacteriophages—anchored on the broad-host-range lytic phage Muddy (the agent used in the *M. avium* case; Dedrick 2023), strictly lytic engineered derivatives of temperate phages (the deletion-of-repressor strategy deployed clinically in *M. abscessus*; Nick 2022), and MAP/NTM lytic phages with documented cross-species host range (Golla 2024)—and screen a defined collection of clinical *M. avium* isolates by plaque assay, efficiency-of-plating, and liquid-culture killing. We will test the hypothesis that smooth (glycopeptidolipid-associated) morphotypes are killed less efficiently than rough variants, mirroring *M. abscessus*. *Success criterion:* a reproducible workflow (assay coefficient of variation defined a priori) yielding a quantitative susceptibility map and empirical cocktail-composition rules. *Go/no-go:* a pre-specified minimum lytic-match rate across the isolate panel triggers panel expansion (Aim 1) versus advance to Aim 2.

Aim 2. Define phage and phage–antibiotic activity in disease-relevant niches and characterize immune barriers. Using macrophage-infection and biofilm models of *M. avium*, we will quantify killing by single phages and cocktails, test phage–antibiotic synergy (including macrolide pairings), and measure suppression of phage-resistant outgrowth. In parallel we will characterize neutralizing-antibody kinetics and innate cytokine responses to lead phages—an effect documented after intravenous dosing in the anchor cohort (Dedrick 2023)—to anticipate constraints on repeat or

prolonged dosing. *Success criterion:* quantified intracellular/biofilm log-kill for lead regimens and a neutralization risk profile informing route and dosing in Aim 3.

Aim 3. Design and pilot a personalized clinical framework for refractory MAC under an eIND pathway. We will operationalize an isolate-to-treatment protocol—screen, match, and administer a tailored single phage or cocktail—with prospectively standardized endpoints (serial AFB culture status and time-to-culture-positivity, FEV1 and pulmonary measures, serial neutralizing-antibody titers), executed as expanded-access cases under FDA eIND authorization with IRB oversight and informed consent. *Success criterion:* a reproducible, regulatory-vetted protocol and a multi-domain endpoint dataset sufficient to design and power a subsequent prospective trial.

Impact: By solving isolate-matching, the smooth-morphotype barrier, and immune characterization together, this project converts a single promising case into a reproducible, clinically actionable strategy for patients with refractory or macrolide-resistant MAC.

Significance

Pulmonary MAC disease imposes one of the longest, most burdensome regimens in infectious disease: 12–18 months or more of multidrug, macrolide-anchored therapy. Even with adherence, durable culture conversion is frequently not achieved, and the course is worsened by intrinsic drug resistance, rising macrolide resistance, and the inability of antibiotics to penetrate granulomas, biofilms, and macrophages where *M. avium* resides. The organism's biology—slow growth, an intracellular lifestyle, and biofilm formation—creates a durable reservoir that conventional pharmacology struggles to clear, leaving a large unmet need for adjunctive options, particularly in macrolide-resistant disease where few alternatives exist.

Mycobacteriophages address this gap on mechanistic grounds. As self-amplifying lytic agents that bind species- and strain-specific surface receptors, they can concentrate at the site of infection and act on bacilli in niches where antibiotics stall, including biofilms and potentially macrophage-resident organisms; phage–antibiotic interactions reported in NTM raise the possibility of shortening or rescuing failing regimens (Bonacorsi 2024). A clinical pipeline already exists for related NTM: compassionate-use experience and engineered-phage work have advanced furthest in *M. abscessus* (Dedrick 2023; Nick 2022), and review syntheses describe the field moving from novelty toward cautious optimism while explicitly flagging immunologic (neutralizing-antibody) and biological barriers (Bonacorsi 2024).

For *M. avium* specifically, the evidence is deliberately characterized here as early. The anchor human datapoint is a single pulmonary *M. avium* patient (a person with cystic fibrosis) within a 20-patient compassionate-use cohort, treated intravenously with the single phage Muddy, with a favorable

response including early FEV1 improvement, lengthening time-to-culture-positivity, and consecutive AFB cultures showing no growth on follow-up; notably, single-phage therapy was not associated with emergent phage resistance in that cohort (Dedrick 2023). Preclinically, lytic phages active against *M. avium* subsp. *paratuberculosis* with cross-species host range spanning rapid- and slow-growing pathogenic mycobacteria have been isolated (Golla 2024). This is a strong proof-of-concept, not a basis for clinical adoption. The significance of the proposed work is that it supplies precisely what is missing between a single case and a fundable trial: a validated matching platform, niche- and immune-aware activity data, and a standardized, regulatory-vetted clinical framework. This directly serves NIAID's mycobacterial-infection mission, with clear NHLBI co-interest in the pulmonary-function endpoint.

Innovation

This project is innovative in three respects. **First**, it is, to our knowledge, the first program to systematically build a personalized phage-matching platform specifically for slow-growing, smooth-morphotype *M. avium*, rather than extrapolating from the more advanced *M. abscessus* experience. **Second**, it integrates strictly lytic engineered phages—derived by deleting repressor/integration genes from temperate phages to force a non-lysogenic cycle, a strategy already deployed clinically in NTM (Nick 2022)—into a rational cocktail-design framework intended to broaden coverage and suppress resistance. **Third**, it couples laboratory matching with a clinical-readiness framework under an eIND/expanded-access pathway, embedding prospectively standardized microbiologic, pulmonary, and immunologic (neutralizing-antibody) endpoints so that individual expanded-access cases generate trial-grade, comparable evidence. By targeting the two field-acknowledged rate-limiters—the thin repertoire of phages active against smooth *M. avium*, and neutralizing-antibody responses (Bonacorsi 2024; Dedrick 2023)—the project is designed to convert cautious optimism into an actionable, reproducible strategy.

Approach

Rigor & reproducibility. All susceptibility and killing assays will use pre-registered protocols with defined positive/negative controls, biological and technical replicates, blinded enumeration where feasible, and a priori acceptance criteria (assay coefficient of variation, EOP thresholds). Isolates and phages will be authenticated by sequencing. Sex as a biological variable will be addressed in Aim 3 (balanced enrollment where feasible; sex reported and analyzed) and in immune assays where donor-derived cells are used.

Aim 1 — Build and validate a personalized *M. avium* phage isolate-matching platform

Rationale. Phage therapy for mycobacteria must be personalized: each clinical isolate is screened against a phage panel to find lytic matches, and active phages are combined into cocktails to broaden coverage and suppress resistance. Morphotype is a recurring constraint—smooth, glycopeptidolipid-associated variants are typically resistant or poorly killed in liquid culture, while rough variants are more susceptible (a pattern characterized in *M. abscessus*; Nick 2022). A reproducible matching platform is the prerequisite for everything downstream.

Experimental design. We will assemble a curated panel of candidate lytic mycobacteriophages, anchored on Muddy (Dedrick 2023), and including strictly lytic engineered derivatives of temperate phages (Nick 2022) and MAP/NTM lytic phages with documented cross-species host range (Golla 2024). A defined collection of clinical *M. avium* isolates [ILLUSTRATIVE: ~60–100 isolates, geographically diverse] will be characterized for morphotype (smooth vs. rough) and screened for phage susceptibility using plaque assays, efficiency-of-plating, and liquid-culture killing. Morphotype will be paired with susceptibility to quantify the smooth-morphotype barrier, and we will test whether rough-variant selection or alternative readouts (Aim 2 intracellular assays) recover detectable killing.

Expected outcomes. A standardized, time-efficient matching workflow with defined performance characteristics; a susceptibility map across isolates and phages; empirical cocktail-composition rules; and a quantified relationship between morphotype and lytic susceptibility in *M. avium*.

Milestones / go–no-go. [ILLUSTRATIVE] Year 1: validated assays meeting pre-specified CV. Year 2: susceptibility map complete. If the lytic-match rate falls below a pre-specified threshold, we trigger panel expansion (additional discovery, prioritizing strictly lytic engineered phages) before advancing isolates to Aim 2.

Potential pitfalls & alternatives. The repertoire of phages active against slow-growing, smooth *M. avium* is thin, so match rates may be low. Mitigations: expand the panel via additional discovery; prioritize strictly lytic engineered phages; and—where smooth-variant killing is poor in liquid culture—use rough-variant and intracellular readouts (Aim 2) that may better reflect in vivo susceptibility.

Aim 2 — Define phage and phage–antibiotic activity in disease-relevant niches and characterize immune barriers

Rationale. MAC persistence is driven by biofilm and intracellular/macrophage reservoirs. Mechanistically attractive features of phages for MAC include biofilm penetration and potential activity on intracellular bacilli, and phage–antibiotic interactions have been reported in NTM (Bonacorsi 2024). Because a single phage sufficed—without emergent resistance—in the one *M.*

avium case (Dedrick 2023), cocktails are expected to matter mainly where single-phage coverage or resistance is a concern; niche-level activity and resistance suppression must therefore be measured directly. Neutralizing-antibody responses, documented after intravenous dosing in the anchor cohort (Dedrick 2023), are a flagged barrier to durability (Bonacorsi 2024).

Experimental design. Lead phages and cocktails from Aim 1 will be tested against *M. avium* in (i) macrophage-infection models to assess intracellular killing and (ii) biofilm models to assess penetration and reduction of viable bacilli. We will evaluate phage–antibiotic combinations (including macrolide-based pairings) for synergy and for suppression of phage-resistant outgrowth. Immunologic characterization will include neutralizing-antibody development against lead phages and innate cytokine responses [ILLUSTRATIVE timepoints], to anticipate constraints on repeat or prolonged dosing and to inform route selection.

Expected outcomes. Quantitative single-phage versus cocktail activity (log-kill) in intracellular and biofilm niches; identification of phage–antibiotic pairings that potentiate killing and suppress resistance; and an immunologic risk profile (neutralization kinetics) informing dosing and route in Aim 3.

Potential pitfalls & alternatives. Smooth-morphotype resistance may persist intracellularly; if so, we will prioritize cocktails and synergistic antibiotic pairings and focus clinical translation on isolates/morphotypes with demonstrable killing. If neutralizing antibodies rapidly emerge (as in some cohort cases; Dedrick 2023), we will model route (e.g., inhaled vs. intravenous) and phage-rotation strategies to preserve activity.

Aim 3 — Design and pilot a personalized clinical framework for refractory MAC under an eIND pathway

Rationale. No phage product is FDA-approved for any NTM indication, and all human use to date has been compassionate/expanded-access; the single *M. avium* case was treated this way (Dedrick 2023). To move responsibly toward a prospective trial, the field needs a standardized isolate-to-treatment protocol with predefined endpoints, executed within the existing regulatory framework.

Experimental design. We will operationalize a protocol in which a refractory MAC patient's isolate is screened and matched (Aim 1), and a tailored single phage or cocktail is administered with prospectively standardized endpoints: serial AFB culture status and time-to-culture-positivity, FEV1 and pulmonary measures (NHLBI co-interest), and serial neutralizing-antibody titers (Aim 2). Treatment will be delivered to eligible refractory/macrolide-resistant patients [ILLUSTRATIVE: a small pilot series of ~5–10 patients] as expanded-access cases under FDA eIND authorization with IRB oversight and informed consent. Endpoints mirror the anchor case (FEV1 change, time-to-positivity, culture conversion) to enable cross-case comparability.

Expected outcomes. A reproducible isolate-to-treatment clinical framework; standardized multi-domain endpoint data from pilot expanded-access cases; and a clinical-readiness package (including immunologic monitoring) to justify, design, and power a future prospective NTM phage trial for MAC.

Potential pitfalls & alternatives. Eligible, matchable patients may be scarce given the thin phage repertoire and morphotype barrier; we will coordinate referrals through established NTM phage centers and registries. Because this is expanded-access rather than a controlled trial, causal inference is limited—so endpoints are standardized prospectively to maximize interpretability and to seed a subsequent randomized study; the pilot is explicitly hypothesis-generating.

Investigators

[Template roles to fill with real names/institutions.] The assembled team spans the three disciplines this project requires. The **PD/PI** ([Name/Institution]) provides translational mycobacterial infectious-disease leadership and overall scientific/regulatory direction. A **mycobacteriophage discovery/engineering co-investigator** contributes phage isolation, host-range screening, and engineering of strictly lytic derivatives (the approach used clinically in *M. abscessus*; Nick 2022). An **NTM pulmonary/clinical co-investigator** leads refractory-patient identification and clinical execution of Aim 3. An **immunology co-investigator** leads neutralizing-antibody and innate-response characterization (Aim 2). **Phage-therapeutics/IND expertise** supports eIND strategy and regulatory submissions. Collectively the team has the complementary expertise to execute laboratory matching, niche/immune assays, and regulated expanded-access cases.

Environment

The work will be performed in BSL-appropriate mycobacteriology and phage-production facilities with capacity for slow-growing NTM culture, plaque/EOP and liquid-killing assays, macrophage-infection and biofilm models, and immunologic (neutralizing-antibody, cytokine) assays, supported by sequencing for isolate and phage authentication. Aim 3 will be conducted at an academic medical center with an established NTM clinical program and mature regulatory infrastructure for eIND/IRB submissions, leveraging existing NTM phage referral networks and registries to identify eligible, matchable patients.

Timeline

[ILLUSTRATIVE] **Year 1:** Assemble phage panel and clinical *M. avium* isolate collection; validate

plaque/liquid susceptibility and morphotyping assays to pre-specified CV (Aim 1). **Year 2:** Complete susceptibility mapping and cocktail-composition rules (Aim 1); stand up macrophage and biofilm models (Aim 2). **Year 3:** Phage–antibiotic synergy, resistance-suppression, and neutralizing-antibody studies (Aim 2); finalize clinical protocol and obtain eIND/IRB approvals (Aim 3). **Year 4:** Initiate expanded-access pilot cases with standardized endpoints (Aim 3). **Year 5:** Complete pilot follow-up, immunologic analysis, and clinical-readiness package for a prospective trial.

Budget Justification (modular R01-style sketch)

[ILLUSTRATIVE] We request [ILLUSTRATIVE: $250,000] direct costs per year for [ILLUSTRATIVE: 5 years] in standard R01 modular increments. **Personnel:** PD/PI ([ILLUSTRATIVE: 2.4 calendar months]) for scientific and regulatory leadership; co-investigators in mycobacteriophage biology, NTM pulmonary medicine, and immunology ([ILLUSTRATIVE: 1–2 months each]); [ILLUSTRATIVE: 2] research scientists for phage propagation, susceptibility screening, and macrophage/biofilm assays; a clinical research coordinator for Aim 3 expanded-access cases and regulatory documentation. **Other expenses:** mycobacterial culture and BSL-appropriate consumables, phage production and characterization, immunologic assays (neutralizing-antibody titers), sequencing for isolate/phage authentication, and clinical monitoring/laboratory costs for pilot cases. **Justification of scope:** the modular request reflects the dual laboratory-plus-clinical structure; the clinical component is intentionally limited to a small expanded-access pilot rather than a powered trial, consistent with the current early evidence base.

Vertebrate Animals

Not applicable. The proposed work uses clinical bacterial isolates, in vitro susceptibility assays, macrophage-infection and biofilm models, and expanded-access human cases; no vertebrate animal studies are proposed. Should in vivo efficacy modeling become necessary, a vertebrate-animals section and IACUC approval would be added by amendment.

Human Subjects / Clinical Trial

Aim 3 involves human subjects. Investigational mycobacteriophages have no FDA approval for any NTM indication; all human use to date has been compassionate/expanded-access (Dedrick 2023). Pilot administration will therefore proceed under an FDA expanded-access Investigational New Drug (eIND) authorization for each case, with prospective IRB review and approval, informed consent, and predefined safety monitoring; the anchor cohort reported no adverse reactions attributed to therapy across routes and pathogens (Dedrick 2023), supporting feasibility while not establishing efficacy.

Eligibility will focus on refractory and/or macrolide-resistant pulmonary MAC with an isolate demonstrating a lytic phage match. Endpoints—serial AFB culture status and time-to-culture-positivity, FEV1 and pulmonary measures, and neutralizing-antibody titers—will be standardized prospectively. This pilot is hypothesis-generating and explicitly designed to inform, not substitute for, a future prospective trial. Sex/gender, pediatric, and inclusion considerations will follow NIH policy, recognizing that NTM phage compassionate use has spanned adult and pediatric settings (Dedrick 2023); sex will be reported and analyzed as a biological variable.

References

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