Closing the Coverage Gap in Lytic Phage Therapy for Treatment-Refractory *Mycobacterium abscessus* Pulmonary Disease

Project Summary / Abstract

Mycobacterium abscessus is among the most treatment-refractory human pathogens: a rapidly growing nontuberculous mycobacterium (NTM) that causes chronic pulmonary, skin, and disseminated disease, disproportionately in people with cystic fibrosis (CF), bronchiectasis, or immunosuppression. It is intrinsically resistant to most antibiotic classes and to many macrolides via inducible *erm(41)*, so standard multidrug regimens last 12+ months, are poorly tolerated, and frequently fail to achieve sustained culture conversion. Lytic mycobacteriophages kill *M. abscessus* by a mechanism wholly independent of antibiotic-resistance determinants, and engineered lytic phages produced the first-ever therapeutic use of genetically engineered phages in a human — for disseminated drug-resistant *M. abscessus* (Dedrick et al., *Nature Medicine* 2019). Subsequent work documented detailed host and pathogen responses (Nick et al., *Cell* 2022) and, across 20 compassionate-use patients with drug-resistant mycobacterial disease, favorable clinical or microbiologic responses in slightly more than half, with no therapy-attributable adverse reactions (Dedrick et al., *Clinical Infectious Diseases* 2023). That same series exposes the field's decisive bottleneck: a productively lytic phage cannot be matched to many clinical isolates, so most patients who need this therapy cannot currently be treated. This project attacks that coverage gap. We will (1) expand and rigorously characterize the matchable lytic phage repertoire against a deliberately diverse *M. abscessus* isolate panel, using BRED/recombineering to convert temperate phages into obligately lytic derivatives and pairing them with the broadly active phage Muddy into defined 2–3 phage cocktails; (2) define phage–antibiotic synergy and intramacrophage killing in vitro to rationally design cocktails that suppress resistant-mutant emergence and reach the intracellular niche, with pre-specified quantitative success criteria; and (3) prospectively and uniformly characterize host and pathogen responses — including neutralizing-antibody kinetics and pathogen genetic stability — in matched compassionate-use patients treated under an FDA expanded-access IND, with data collection harmonized to the POSTSTAMP framework (Nick et al., *J Cyst Fibros* 2025). The expected outcome is a larger, better-characterized phage library plus the standardized synergy and host-response correlates needed to move matched phage therapy from single-patient rescue toward controlled

efficacy testing.

Specific Aims

Treatment-refractory *M. abscessus* pulmonary disease has no reliable cure. Engineered lytic phages are the most promising mechanism-independent option, yet a large fraction of clinical isolates currently have no matched lytic phage, and the correlates that predict clinical benefit are defined only from a handful of cases. We propose three aims to close this coverage gap and standardize the evidence base.

Aim 1. Expand and characterize the matchable lytic phage repertoire against a diverse *M. abscessus* isolate panel. We will screen a clinically representative collection of *M. abscessus* isolates (smooth and rough variants, multiple subspecies/genotypes) against an expanded phage library and use BRED/recombineering to delete repressor genes from temperate phages, converting them into obligately lytic, non-lysogenizing therapeutics. *Primary endpoint:* the fraction of isolates with at least one active lytic phage (the "matchable fraction"), benchmarked against the matching rate reported for the prior compassionate-use program. *Success criterion (go/no-go):* engineered + naturally lytic phages together yield a matchable fraction meaningfully greater than naturally lytic phages alone on the same panel.

Aim 2. Define phage–antibiotic synergy and intracellular killing to rationally design resistance-suppressing cocktails. We will assemble defined 2–3 phage cocktails (Muddy plus engineered derivatives selected for complementary host range) and test them — alone and with clinically relevant antibiotics — for (a) suppression of resistant-mutant emergence and (b) killing of *M. abscessus* residing inside human macrophages. *Success criterion:* identification of ≥2 cocktail compositions that reduce resistant-mutant outgrowth relative to single phages and show measurable intramacrophage killing, advanced as prioritized candidates for clinical matching.

Aim 3. Prospectively and uniformly characterize host and pathogen responses in matched compassionate-use patients. Under an FDA expanded-access IND and local IRB oversight, with data collection harmonized to the POSTSTAMP framework, we will collect serial specimens from treated patients to measure phage-induced lysis, neutralizing-antibody kinetics, pathogen genetic stability, and standardized clinical/microbiologic response. *Deliverable:* a uniformly collected correlate dataset, larger than any prior single report, defining which parameters track with benefit and feeding response-rate estimates into controlled-trial design.

Impact. By enlarging the matchable repertoire and defining the synergy and host-response parameters that govern success, this work provides the preclinical foundation and standardized correlates required to advance matched phage therapy toward controlled efficacy trials — and ultimately treatment

guidelines — for an otherwise intractable infection.

Significance

M. abscessus pulmonary disease is a therapeutic dead-end. Intrinsic resistance to multiple antibiotic classes, plus inducible macrolide resistance via *erm(41)*, forces 12+ month multidrug regimens that are poorly tolerated and frequently fail to achieve sustained culture conversion. The burden falls heavily on people with CF and bronchiectasis, in whom chronic *M. abscessus* infection can complicate or preclude lung transplantation. Lytic mycobacteriophages offer a fundamentally different therapeutic logic — adsorption, genome injection, and host-cell lysis at the end of a productive replication cycle — a route unaffected by the antibiotic-resistance determinants that make this organism so intractable.

The clinical precedent is unusually strong for a novel modality. The first therapeutic use of engineered phages in any human treated disseminated drug-resistant *M. abscessus* after bilateral lung transplant in a 15-year-old with CF, with clinical improvement and no serious treatment-related adverse events over months of IV dosing (Dedrick et al., 2019). A detailed second case documented phage-induced lysis, pathogen genetic stability, and rising serum neutralizing-antibody titers that nonetheless did not prevent clinical benefit, with the explanted lung culture-negative for *M. abscessus* at transplant (Nick et al., 2022). The largest experience to date treated 20 patients with drug-resistant mycobacterial disease on compassionate use (IV and/or aerosolized) with no therapy-attributable adverse reactions and favorable clinical or microbiologic responses in slightly more than half (Dedrick et al., 2023).

Yet that same body of work exposes the decisive bottleneck. *M. abscessus* phage susceptibility is exquisitely isolate-specific, and across the compassionate-use program a productively lytic phage could be matched to only a minority of screened isolates (Dedrick et al., 2023); many patients who need this therapy therefore cannot be treated. Closing the coverage gap, and standardizing the correlates of response, is the rate-limiting step between compelling anecdote and a deployable therapy. The need to move from single-patient use toward rigorous, IND-based evaluation is precisely what motivates the POSTSTAMP trial framework (Nick et al., 2025). This work sits squarely within NIAID's antibacterial-resistance and mycobacterial-infection priorities and is of direct relevance to NHLBI and the Cystic Fibrosis Foundation.

Innovation

This proposal is innovative in three respects. First, it **reframes the problem from individual rescue to coverage at the population level**: rather than reporting another successful case, we systematically attack the matching limitation by expanding and engineering the phage repertoire against a

deliberately diverse isolate panel that includes phage-resistant smooth (glycopeptidolipid-coated) variants alongside more-susceptible rough variants. Second, it deploys **phage engineering as a standardized pipeline rather than a one-off**: the BRED/recombineering conversion of temperate mycobacteriophages into obligately lytic, non-lysogenizing agents — the same engineering strategy that underpinned the landmark cases — is here executed prospectively, at panel scale, with the explicit objective of maximizing the matchable fraction. We are candid that the engineering method is established; the innovation is its systematic application to coverage as a measurable endpoint. Third, it **integrates two mechanisms that target niches antibiotics miss** — phage–antibiotic synergy against drug-resistant isolates and phage killing of intracellular *M. abscessus* — and uses both as rational cocktail-design criteria rather than post hoc observations. Combining systematic coverage expansion, scalable engineering, and intracellular/synergy-guided cocktail design, tied to pre-specified quantitative success criteria, distinguishes this program from prior single-patient efforts.

Approach

Rigor, reproducibility, and key-resource authentication (applies to all aims)

All isolates will be genotyped (subspecies assignment) and morphotype-confirmed (smooth vs rough); phage stocks will be sequence-verified and titered by independent operators. Each in vitro endpoint (host range, lytic efficiency, resistant-mutant suppression, intramacrophage killing) will be measured in independent biological replicates with pre-defined positive/negative controls and blinded plaque/enumeration scoring where feasible. Key biological resources (phage library, engineered derivatives, isolate panel, macrophage source) will be authenticated and catalogued, and protocols deposited to enable reproduction. As a relevant biological variable, donor sex will be recorded for primary human macrophages and patient sex will be recorded and reported in Aim 3 analyses; analyses will be examined for sex-associated differences where sample size permits.

Aim 1 — Expand and characterize the matchable lytic phage repertoire

Rationale. The dominant practical constraint on phage therapy for *M. abscessus* is its narrow, isolate-specific host range; across the compassionate-use program a productively lytic phage could be matched to only a minority of isolates (Dedrick et al., 2023). Increasing the matchable fraction is the single highest-value preclinical objective. Temperate phages are abundant in mycobacteriophage collections and can be engineered into obligately lytic therapeutics, providing a tractable route to broaden coverage (Dedrick et al., 2019; Nick et al., 2022).

Experimental design. We will assemble a clinically representative panel of *M. abscessus* isolates spanning smooth and rough morphotypes and multiple subspecies/genotypes. Each isolate will be screened against an expanded phage library for plaque formation and lytic efficiency (efficiency-of-

plaquing relative to a permissive host). Temperate candidates will be converted to obligately lytic, non-lysogenizing derivatives by repressor-gene deletion via BRED/recombineering, following the established engineering paradigm; the broadly active phage Muddy will anchor cocktail design. For every isolate we will record whether ≥1 active lytic phage exists and the breadth of phages active against it.

Quantitative endpoints & success criteria. *Primary:* matchable fraction (proportion of isolates with ≥1 active lytic phage), reported with confidence intervals and benchmarked against the prior program's matching rate. *Go/no-go:* engineered + naturally lytic phages together produce a matchable fraction meaningfully greater than naturally lytic phages alone on the identical panel; failing this, Aim 2/3 proceed using the naturally lytic subset and we re-prioritize scaffold discovery.

Potential pitfalls & alternatives. Smooth variants may remain refractory; we explicitly test rough/smooth pairs and prioritize phages active against smooth isolates. Some isolates will resist all available phages — an expected outcome consistent with prior data — which we report transparently as a coverage estimate rather than force. Where single phages are unavailable, we test engineered host-range variants and alternative temperate scaffolds.

Aim 2 — Phage–antibiotic synergy and intracellular killing for rational cocktail design

Rationale. Multi-phage cocktails are used to suppress emergence of phage resistance; phages can act against *M. abscessus* in the presence of antibiotics; and the intracellular (macrophage) niche is a plausible reservoir for relapse. Defining these effects systematically allows rational rather than ad hoc cocktail design (Dedrick et al., 2019; 2023; Nick et al., 2022).

Experimental design. Using phages from Aim 1, we will build defined 2–3 phage cocktails (Muddy plus engineered derivatives) selected for complementary host range. We will quantify (a) suppression of resistant-mutant emergence by cocktails versus single phages (frequency-of-resistance and time-to-outgrowth assays); (b) phage–antibiotic combinations against drug-resistant isolates across multiple clinically relevant antibiotics and concentrations (checkerboard/time-kill); and (c) intracellular killing in *M. abscessus*-infected human macrophages (intracellular CFU over time, phage vs control).

Quantitative endpoints & success criteria. *Success:* ≥2 cocktails that reduce resistant-mutant outgrowth relative to component single phages and produce measurable intramacrophage CFU reduction; these advance as prioritized clinical-matching candidates. Synergy is reported as isolate- and antibiotic-specific, not assumed universal.

Potential pitfalls & alternatives. Synergy may be antibiotic- and isolate-specific; we test multiple drug pairings and report combinations as isolate-dependent. If intracellular delivery is limiting, we

compare cocktail formulations for macrophage penetration. Inter-phage antagonism is possible and is screened for before any cocktail advances.

Aim 3 — Prospective, uniform characterization of host and pathogen responses in matched compassionate-use patients

Rationale. Single cases and the 20-patient series establish safety (no therapy-attributable adverse reactions) and document phage-induced lysis, pathogen genetic stability, and neutralizing-antibody titers that rose but did not prevent benefit (Dedrick et al., 2019; 2023; Nick et al., 2022). Collecting these correlates *uniformly and prospectively* across more patients is the bridge from anecdote toward controlled efficacy data — the explicit motivation for the POSTSTAMP framework (Nick et al., 2025).

Design and scope. This aim is an observational correlate study layered onto expanded-access treatment; it is explicitly hypothesis-generating and is **not** represented as a randomized efficacy trial. Under an FDA expanded-access IND and local IRB approval, and with specimen handling, assays, and safety surveillance harmonized to POSTSTAMP procedures, we will enroll treatment-refractory patients whose isolates are matched in Aims 1–2 (matched phages administered per the treating protocol; route per clinical indication). We will collect serial blood, sputum, and — where clinically available — tissue to measure phage-induced lysis, serum neutralizing-antibody kinetics, pathogen genetic stability by sequencing, and standardized clinical and microbiologic (culture-conversion) response on pre-specified schedules. Because matched phages will not exist for every referred patient, unmatched patients may serve as a non-randomized reference group for descriptive comparison, as contemplated in the POSTSTAMP design (Nick et al., 2025); we pre-specify that any such comparison is descriptive and confounded by indication.

Endpoints & analysis. *Co-primary correlates:* trajectory of microbiologic response and serum neutralizing-antibody kinetics; *secondary:* phage-induced lysis markers, pathogen genetic stability, standardized clinical response. Analyses are descriptive with effect estimates and intervals; correlate–outcome associations are reported as hypothesis-generating.

Potential pitfalls & alternatives. Enrollment is constrained by the matchable fraction; expanding coverage in Aim 1 directly mitigates this, and unmatched patients still contribute as a reference group. Neutralizing antibodies may attenuate IV efficacy; we track titers serially and, consistent with prior observations that benefit occurred despite rising titers (Nick et al., 2022), evaluate aerosolized delivery where clinically indicated. As compassionate use, this component cannot establish definitive efficacy and is framed accordingly.

Timeline

[ILLUSTRATIVE] **Years 1–2:** Aim 1 isolate-panel assembly, genotyping/morphotyping, phage screening, and BRED/recombineering engineering; matchable-fraction go/no-go at end of Year 2. [ILLUSTRATIVE] **Years 2–3:** Aim 2 cocktail assembly, resistance-suppression, synergy, and intramacrophage assays. [ILLUSTRATIVE] **Years 2–5:** Aim 3 expanded-access IND/IRB activation and rolling enrollment of matched patients with serial sampling. [ILLUSTRATIVE] **Year 5:** integrated analysis of coverage, synergy, and host-response correlates; design of a controlled efficacy protocol.

Budget Justification (modular R01-style sketch)

[ILLUSTRATIVE] This is a modular R01 request of [ILLUSTRATIVE] $250,000 direct costs per year for [ILLUSTRATIVE] 5 years. **Personnel:** [ILLUSTRATIVE] PI (3.0 calendar months) overseeing phage engineering and clinical correlates; [ILLUSTRATIVE] Co-Investigator(s) in CF/NTM pulmonary medicine and clinical microbiology; [ILLUSTRATIVE] 1–2 postdoctoral scientists and [ILLUSTRATIVE] 1 research technician for phage discovery, BRED/recombineering, and synergy/macrophage assays; [ILLUSTRATIVE] a part-time clinical research coordinator for Aim 3. **Other costs:** [ILLUSTRATIVE] mycobacteriology and BSL consumables, phage library expansion and sequencing, macrophage culture, neutralizing-antibody and pathogen-sequencing assays, and IND/IRB regulatory support. **Justification of scope:** the modular cap is appropriate because the engineering, synergy, and correlate assays leverage established mycobacteriophage methods and an existing manufacturing/matching pipeline rather than de novo platform development.

Vertebrate Animals

Not applicable. The proposed work uses bacterial isolates, bacteriophages, and human macrophage cultures in vitro (Aims 1–2) and human clinical specimens collected under compassionate use (Aim 3); no vertebrate animal experiments are proposed.

Human Subjects / Clinical Trial

Aim 3 involves human subjects. Investigational phages will be administered to individual treatment-refractory patients under an FDA expanded-access IND for compassionate use — the regulatory route used in prior cases and series (Dedrick et al., 2019; 2023) — and **not** as an investigator-initiated randomized trial; activities and data collection will be harmonized to the ongoing POSTSTAMP IND-based framework (Nick et al., 2025). All enrollment, consent, specimen collection, and data use will

occur under local IRB approval with FDA oversight, with informed consent (and assent where applicable, as in the index pediatric case). Prior compassionate-use experience reported no therapy-attributable adverse reactions (Dedrick et al., 2023); safety monitoring (including neutralizing-antibody surveillance) will follow the POSTSTAMP approach. Because matched phages may not exist for every referred isolate, unmatched patients may constitute a non-randomized reference group for descriptive comparison only. Patient sex will be recorded and reported. This component is hypothesis-generating and is not represented as providing definitive efficacy evidence. Whether this layered correlate study meets the NIH definition of a clinical trial will be confirmed with the program and the IRB, and the appropriate human-subjects/clinical-trial forms completed accordingly.

Team & Environment

To be completed with real names, institutions, and biosketches.

- **Contact PI [NAME, INSTITUTION]:** mycobacteriophage discovery, engineering (BRED/recombineering), and clinical-matching pipeline — the function exemplified by an established academic mycobacteriophage program.
- **Clinical Co-Investigator(s) [NAME, INSTITUTION]:** CF/NTM pulmonary medicine and compassionate-use phage administration — the function exemplified by an academic CF/NTM center engaged in IND-based phage therapy (POSTSTAMP).
- **Coordinating/regulatory partner [NAME]:** multi-site IND coordination and data management — function exemplified by an established CF/therapeutics clinical-trials network.
- **Phage library resource [NAME]:** the source mycobacteriophage collection underpinning *M. abscessus* phage matching.
- **Environment:** BSL-appropriate mycobacteriology and phage-engineering laboratories, human macrophage culture capability, a sequencing core, and established expanded-access IND/IRB regulatory infrastructure; potential partner/alternate funders to engage include NHLBI and the Cystic Fibrosis Foundation.

References

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2. Nick JA, Dedrick RM, Gray AL, et al. Host and pathogen response to bacteriophage

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