Nebulized Phage-Antibiotic Therapy for Post-Viral Secondary MRSA Pneumonia: Translating Systemic Proof-of-Concept to the Injured Airway

Project Summary / Abstract

Secondary bacterial pneumonia after a viral respiratory infection — classically post-influenza, and increasingly recognized after COVID-19 and RSV — is a leading driver of excess mortality during respiratory viral epidemics and pandemics, and *Staphylococcus aureus*, including methicillin-resistant *S. aureus* (MRSA), is among the dominant culprits. Viral injury to the airway epithelium, impaired mucociliary clearance, and dysregulated immunity create a lower-airway niche in which *S. aureus* establishes drug-resistant, biofilm-associated, and intracellular populations that respond poorly to standard antibiotics. Lytic bacteriophages are mechanistically suited to this problem: they are self-amplifying, strain-targeted agents that kill through a route independent of antibiotic-resistance determinants, can be delivered directly to the lung by nebulization, and act in concert with antibiotics. A defined multi-phage cocktail broadens host-range coverage across heterogeneous MSSA/MRSA strains and raises the genetic barrier to phage resistance. The translational rationale is now concrete but incomplete: Armata Pharmaceuticals' AP-SA02, a fixed intravenous multi-phage *S. aureus* cocktail, reported positive Phase 1b/2a results in complicated *S. aureus* bacteremia (diSArm, NCT05184764). Whether a comparable benefit can be achieved by **nebulized** phage in the **virally injured lung** — a different delivery route, anatomic compartment, and pathophysiology — is untested. Using influenza-primed murine and confirmatory rat models of secondary MRSA pneumonia, we will (1) define the efficacy and lung pharmacodynamics of a nebulized anti-staphylococcal phage cocktail; (2) test phage–antibiotic synergy and suppression of resistance in the inflamed, virally injured airway; and (3) establish stockpile-relevant formulation, host-range coverage, and dosing to de-risk an eventual expanded-access IND. Each aim carries quantitative go/no-go milestones. The work directly serves NIAID's antibacterial-resistance and pandemic-preparedness missions and is complementary to BARDA medical-countermeasure stockpiling and NHLBI acute-lung-injury interests.

Specific Aims

Secondary bacterial pneumonia after viral infection contributes disproportionately to mortality during respiratory viral pandemics, and the responsible *S. aureus* populations are increasingly drug-resistant and biofilm-protected. Nebulized lytic phage cocktails offer a resistance-independent, lung-targeted, antibiotic-synergistic adjunct, but no completed trial has tested them in this specific indication, and the strongest human data (AP-SA02; diSArm, NCT05184764) come from intravenous treatment of bacteremia rather than from the airway. **Central hypothesis:** a defined, nebulized, multi-phage anti-staphylococcal cocktail will self-amplify in the virally injured lung and, combined with standard antibiotics, will clear secondary MRSA pneumonia more effectively than antibiotics alone while suppressing the emergence of resistance. This project rigorously bridges the systemic-to-airway gap preclinically.

Aim 1 — Define efficacy and lung pharmacodynamics of a nebulized anti-staphylococcal phage cocktail in post-viral secondary pneumonia. Building on the demonstration that nebulized phages reduced lung MRSA burden and improved outcomes in a rat ventilator-associated pneumonia model (Prazak et al., 2020), we will test a defined multi-phage *S. aureus* cocktail in influenza-primed murine and confirmatory rat models of secondary MRSA pneumonia. We will quantify survival, lung bacterial burden (CFU), and phage amplification kinetics (PFU) at the infection site. *Go/no-go [ILLUSTRATIVE]:* advance only if nebulized phage produces a prespecified reduction in lung CFU versus vehicle with evidence of in-situ phage amplification.

Aim 2 — Test phage–antibiotic synergy and suppression of phage/antibiotic resistance in the inflamed airway. Because phages can act synergistically with antibiotics rather than competing with them (Fernández et al., 2021), we will compare phage-plus-antibiotic against each agent alone, measuring bacterial clearance, survival, and the longitudinal emergence of phage-resistant or antibiotic-resistant variants in recovered isolates, including beta-lactam MIC shifts. *Go/no-go [ILLUSTRATIVE]:* advance only if the combination is non-inferior to, and trends superior to, antibiotic monotherapy in clearance while not increasing antibiotic-resistant variant emergence.

Aim 3 — Establish stockpile-relevant formulation, host-range coverage, and dosing to de-risk translation. We will characterize nebulizer-compatible cocktail stability, titer retention after aerosolization, and host-range coverage against a curated panel of contemporary MSSA/MRSA isolates, defining a candidate dosing regimen and draft release criteria suitable for an eventual expanded-access (emergency) IND. *Go/no-go [ILLUSTRATIVE]:* a cocktail meeting prespecified post-nebulization titer-retention and strain-coverage thresholds.

Impact: Success would convert phage therapy for post-viral staphylococcal pneumonia from compassionate-use, case-by-case heroics into a defined, stockpile-ready, antibiotic-sparing adjunct — among the first respiratory indications positioned to follow the AP-SA02 systemic signal into the airway.

Significance

Secondary bacterial pneumonia after viral respiratory infection is a recurring engine of pandemic mortality. The historical record — in which bacterial coinfection is widely held to account for the bulk of excess deaths in the 1918 influenza pandemic — and contemporary experience with influenza, COVID-19, and RSV converge on the same vulnerability, and *S. aureus* (including MRSA) is a dominant pathogen. The pathophysiology is distinctive and clinically consequential: viral destruction of the airway epithelium, impaired mucociliary clearance, and dysregulated host immunity open a lower-airway niche where *S. aureus* invades and persists as antibiotic-resistant, biofilm-associated, and intracellular populations that respond poorly to conventional antibiotics (Fernández et al., 2021; Speck et al., 2021). This is precisely the setting in which antibiotic monotherapy underperforms and in which a mechanistically orthogonal agent is most valuable.

Bacteriophages address several of these failure modes simultaneously. They are self-amplifying and strain-targeted, killing through receptor binding, genome injection, and lysis — a mechanism independent of the resistance determinants that defeat beta-lactams. Their depolymerases and endolysins can penetrate and disrupt biofilm matrix where antibiotics struggle (Fernández et al., 2021). Critically, phages can be delivered directly to the lung by nebulization and can act in concert with — rather than in competition with — antibiotics. The case for phage therapy in *S. aureus* pneumonia complicating influenza A has been explicitly articulated (Speck et al., 2021), and nebulized phages have shown efficacy in a rodent MRSA pneumonia model (Prazak et al., 2020), yet no completed trial has tested phage in post-viral secondary pneumonia. Closing that gap is squarely within NIAID's antibacterial-resistance and pandemic-preparedness mission, is complementary to BARDA's interest in stockpilable medical countermeasures, and intersects NHLBI's focus on acute lung injury.

Innovation

This proposal is innovative in four respects. First, **indication**: rather than treating *S. aureus* infection generically, it targets the specific, high-mortality, pandemic-relevant context of post-viral secondary pneumonia, modeling viral priming explicitly rather than infecting naïve lungs. Second, **translational bridge**: it directly tests whether the positive systemic signal from AP-SA02 in complicated *S. aureus* bacteremia (diSArm, NCT05184764) can be reproduced in the **airway** via **nebulized** delivery — a change of route, compartment, and pathophysiology that the existing intravenous clinical program does not address. Third, **mechanistic interrogation of phage–antibiotic interplay in vivo**: we move beyond co-administration to ask, in the virally injured lung, whether the combination suppresses resistance emergence and whether sub-lytic phage pressure shifts MRSA beta-lactam susceptibility (Fernández et al., 2021). Fourth, **stockpile orientation**: formulation, aerosol stability, and host-range coverage are treated as primary endpoints from the outset, designing toward a fixed, deployable cocktail compatible with an expanded-access IND rather than bespoke per-patient matching.

Approach

Rigor, Reproducibility, and General Design

All in vivo studies will use prospective randomization to treatment arm, blinded outcome assessment (CFU, PFU, clinical scoring, survival), and group sizes set by a priori power analysis to the primary endpoint [ILLUSTRATIVE]. **Sex as a biological variable:** both male and female animals will be enrolled in balanced numbers and sex will be included as a covariate; any sex-dependent effects will be reported and, if detected, powered for follow-up. **Authentication of key resources:** phage stocks will be genome-sequenced and verified free of lysogeny/toxin and antibiotic-resistance genes and characterized for titer and purity (e.g., endotoxin) before use; bacterial strains will be confirmed by species identification, *mec* genotyping, and susceptibility profiling; viral stocks will be titered and sequence-confirmed. Analysis plans, primary endpoints, and the go/no-go thresholds below will be pre-registered internally before unblinding. Biological and technical replicates and exact statistical tests will be specified per experiment [ILLUSTRATIVE].

Aim 1 — Efficacy and lung pharmacodynamics of a nebulized anti-staphylococcal phage cocktail

Rationale. Nebulized bacteriophages improved outcomes and reduced lung MRSA burden in a rat ventilator-associated pneumonia model (Prazak et al., 2020), and inhalable phage formulations have shown efficacy in rodent MRSA pneumonia (Fernández et al., 2021). Whether self-amplifying phages achieve therapeutic kinetics in the *virally injured* lung — with altered surfactant, mucus, and inflammatory milieu — is untested and is the foundational question here.

Experimental design. Mice (and confirmatory rats) will receive sublethal influenza A, followed after a defined interval by intratracheal MRSA challenge to reproduce secondary pneumonia. Animals will be randomized to nebulized phage cocktail, nebulized vehicle, or untreated control. Primary endpoints are survival and quantitative lung bacterial burden (CFU); pharmacodynamic endpoints are phage titer (PFU) in lung homogenate and bronchoalveolar lavage over time to distinguish active in-situ amplification from passive deposition. Group sizes are set by power analysis to the primary endpoint [ILLUSTRATIVE].

Expected outcomes. We anticipate that nebulized phage will reduce lung CFU and improve survival relative to vehicle, with measurable in-lung phage amplification confirming active replication.

Go/no-go milestone [ILLUSTRATIVE]. Proceed to Aim 2 only if nebulized phage achieves a prespecified log-reduction in lung CFU versus vehicle with PFU kinetics indicating amplification.

Potential pitfalls & alternatives. Viral-induced changes in mucus and surfactant could impair phage diffusion or inactivate virions; if amplification is blunted, we will test higher multiplicity, repeat dosing, and depolymerase-bearing phages with better matrix penetration (Fernández et al., 2021). If the influenza-priming window proves variable, we will calibrate the viral-to-bacterial interval to standardize disease severity, and validate findings across two host species (mouse, rat) to guard against model-specific artifact.

Aim 2 — Phage–antibiotic synergy and resistance suppression in the inflamed airway

Rationale. Phages can act synergistically with antibiotics, and the AP-SA02 program reported a positive Phase 1b/2a signal when phage was added to standard-of-care antibiotics in *S. aureus* bacteremia (NCT05184764). Demonstrating additive clearance and resistance suppression in the post-viral lung would establish nebulized phage as a rational antibiotic adjunct, not a replacement.

Experimental design. Using the Aim 1 model, animals will be randomized to antibiotic alone, phage cocktail alone, or the combination. Endpoints include lung CFU, survival, and longitudinal recovery of isolates for blinded phenotyping: phage susceptibility, antibiotic MICs (especially beta-lactam), and resistance-variant frequency. A multi-phage cocktail is used specifically to raise the barrier to phage resistance across heterogeneous strains.

Expected outcomes. We expect the combination to outperform either monotherapy in clearance and survival, with reduced emergence of resistant variants and, in a detectable fraction of recovered isolates, lowered beta-lactam MICs consistent with phage-driven susceptibility shifts (Fernández et al., 2021).

Go/no-go milestone [ILLUSTRATIVE]. Advance the combination to Aim 3 dosing only if it is at least non-inferior to antibiotic monotherapy in clearance and does not increase antibiotic-resistant variant emergence.

Potential pitfalls & alternatives. Beta-lactam re-sensitization observed in vitro may not manifest in vivo; if it is not detected, the combination's value can still rest on additive clearance and resistance suppression, which are the primary endpoints. If phage-resistant mutants emerge, cocktail composition will be rebalanced; as an orthogonal Gram-positive contingency for future indications, endolysin-based agents are noted as a fallback strategy (Fernández et al., 2021), though this proposal remains focused on *S. aureus*.

Aim 3 — Stockpile-relevant formulation, host-range coverage, and dosing

Rationale. A pandemic countermeasure must be a stable, fixed, deployable product. Aerosolization can shear phage particles and reduce viable titer, and host range must cover contemporary strain diversity in post-viral pneumonia.

Experimental design. We will measure cocktail titer retention through the nebulizer, physical/biological stability under defined storage conditions, and host-range coverage against a curated panel of contemporary MSSA/MRSA isolates by quantitative susceptibility testing. These data will define a candidate dosing regimen, a coverage threshold, and draft release criteria. No human dosing occurs in this aim.

Expected outcomes. A defined cocktail with documented post-nebulization viability, characterized stability, and a coverage profile across the strain panel, yielding a regimen and release-criteria package to support a future expanded-access IND.

Go/no-go milestone [ILLUSTRATIVE]. A cocktail meeting prespecified post-nebulization titer-retention and strain-coverage thresholds is required to declare translational readiness.

Potential pitfalls & alternatives. If aerosolization substantially reduces titer, we will optimize formulation and nebulizer type; if coverage gaps appear, additional phages will be incorporated and, where banking is feasible, curated genomic matching can supplement the fixed cocktail.

Timeline

Years 1–2 [ILLUSTRATIVE]: establish and validate the influenza-priming secondary-pneumonia models and complete Aim 1 efficacy/pharmacodynamic studies. Years 2–3 [ILLUSTRATIVE]: Aim 2 combination and resistance studies. Years 3–4 [ILLUSTRATIVE]: Aim 3 formulation, stability, and host-range characterization, with regulatory-readiness package assembly. Year 5 [ILLUSTRATIVE]: confirmatory studies, data integration, and pre-IND interactions. Aims overlap to allow iterative cocktail refinement; each transition is gated by the go/no-go milestone above.

Budget Justification (modular R01-style sketch)

Requested at the standard modular level of $250,000 direct costs per year for 5 years [ILLUSTRATIVE]. **Personnel:** multi-PI structure (a phage biologist and a pulmonary/critical-care investigator), a microbiology postdoctoral fellow, a research technician for animal models, and a parttime biostatistician [ILLUSTRATIVE effort levels]. **Animals & per-diems:** influenza-primed murine and confirmatory rat cohorts across three aims [ILLUSTRATIVE]. **Supplies:** phage propagation and purification, nebulization hardware, antibiotics, microbiology consumables, and sequencing for isolate phenotyping and resource authentication. **Other:** publication and dissemination costs. No equipment module above the modular cap is requested. Final allocations [ILLUSTRATIVE] will follow institutionally negotiated rates.

Vertebrate Animals

Influenza-primed murine and confirmatory rat models of secondary MRSA pneumonia will reproduce the post-viral airway niche that cannot be modeled in vitro, consistent with prior nebulized-phage pneumonia studies (Prazak et al., 2020). All procedures will follow an IACUC-approved protocol with humane endpoints, anesthesia/analgesia for invasive procedures (intratracheal challenge, nebulized dosing), balanced enrollment of both sexes, the minimum group sizes required for statistical power [ILLUSTRATIVE], and adherence to the 3Rs (replacement, reduction, refinement). Survival, clinical scoring, and predefined humane-euthanasia criteria will be specified to minimize distress.

Human Subjects / Clinical Trial

No human subjects are enrolled in this preclinical R01; therefore this is not an NIH-defined clinical trial. The program is designed to de-risk eventual human use. For any future administration of investigational phage in critically ill post-viral pneumonia patients, the anticipated regulatory route is an FDA emergency/expanded-access IND for individual or intermediate-size use — as has supported prior compassionate-use respiratory phage cases — with full IRB oversight, informed consent (or emergency-use provisions), and FDA coordination. The AP-SA02 clinical program in complicated *S. aureus* bacteremia (NCT05184764) provides a referenceable manufacturing and regulatory precedent for an eventual airway program.

Team & Environment

This project requires a multidisciplinary team; roles below are templates to be filled with named investigators and institutions:

- **Contact PI / Phage biology lead** — [Name, Institution]: cocktail design, propagation, host-range and resistance assays, resource authentication.
- **Multi-PI / Pulmonary & critical care lead** — [Name, Institution]: post-viral pneumonia models, nebulized delivery, translational endpoints.

- **Co-Investigator, Medical microbiology / ID** — [Name, Institution]: MRSA strain panels, antibiotic susceptibility, synergy interpretation.
- **Co-Investigator, Aerosol/formulation science** — [Name, Institution]: nebulizer compatibility, stability, release criteria.
- **Biostatistician** — [Name, Institution]: randomization, power, and survival analysis.
- **Regulatory advisor** — [Name]: expanded-access IND strategy and pre-IND interactions.

The environment will provide BSL-2 microbiology, an approved animal facility with influenza and aerosol-challenge capability, nebulization and aerosol-characterization equipment, and sequencing-core access. Reference programs in this space include Armata Pharmaceuticals (AP-SA02; NCT05184764), academic phage centers with compassionate-use experience, Gram-positive pneumonia phage groups (Fernández et al., 2021), and nebulized-phage VAP modeling groups (Prazak et al., 2020).

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