Phagogram-Guided Phage-Antibiotic Therapy as a Surgical Adjunct for Refractory *Staphylococcus aureus* Chronic Osteomyelitis: Targeting Biofilm and Small-Colony-Variant Reservoirs to Enable Antibiotic Resensitization

Funding mechanism: NIH R01 (Research Project Grant) — *target institute: NIAMS*

Project Summary / Abstract

Chronic osteomyelitis caused by *Staphylococcus aureus*, including methicillin-resistant *S. aureus* (MRSA), is among the most refractory infections in musculoskeletal medicine. The organism builds biofilm on devitalized bone and orthopedic hardware, persists intracellularly, and converts to dormant small-colony variants (SCVs) that tolerate even prolonged antibiotic courses — so that surgical debridement plus weeks-to-months of antibiotics still yields high relapse, hardware loss, and amputation. The failure mode is antibiotic **tolerance and physical sequestration**, not classical resistance; a more potent antibiotic does not solve it. Lytic bacteriophages are a mechanistically complementary adjunct: they self-amplify where bacterial burden is highest, their depolymerase and endolysin activities degrade biofilm matrix, they kill independent of bacterial metabolic state (reaching quiescent SCVs missed by vancomycin and beta-lactams), they can be matched to a patient's isolate by susceptibility testing (phagogram), and they can be delivered locally into bone and joint spaces (Peng et al., 2024; Plumet et al., 2022). Critically, phage and antibiotic are cooperative rather than redundant, and phage selection pressure may "herd" *S. aureus* back toward antibiotic susceptibility — a resensitization effect whose clinical payoff is shorter antibiotic courses (Peng et al., 2024).

Early human experience is encouraging but limited. PhagoDAIRI (NCT05369104), a randomized, double-blind Phase 2 pilot of GMP anti-*S. aureus* phages (PP1493, PP1815) administered after debridement-antibiotics-implant-retention (DAIR) for staphylococcal prosthetic joint infection, established feasibility and a controlled-trial design for this indication. The PHAGEinLYON Clinic at

Hospices Civils de Lyon has delivered GMP phage cocktails intravenously and by local injection for complex bone and joint infection under regulated compassionate access, reporting favorable clinical evolution in most treated patients (Ferry et al., 2024). What is missing is mechanism-anchored, prospectively defined evidence linking phagogram-guided cocktail design, biofilm/SCV killing, optimized local delivery, immune-clearance kinetics, and antibiotic resensitization in a single rigorous program. This R01 will (Aim 1) build a phagogram-validated cocktail and quantify phage-antibiotic synergy against biofilm and SCV reservoirs from clinical isolates; (Aim 2) define local delivery, dosing, pharmacokinetics, and neutralizing-antibody kinetics in a hardware-associated large-animal osteomyelitis model; and (Aim 3) conduct a phagogram-guided pilot clinical trial under an FDA expanded-access/emergency IND framework, with antibiotic resensitization as a central readout. The work addresses an NIAMS-priority bone-infection indication and targets the months-long antibiotic courses that define the disease.

Specific Aims

Chronic *S. aureus* osteomyelitis resists cure because biofilm, intracellular persistence, and SCVs shield bacteria from antibiotics, producing high relapse despite aggressive surgery and prolonged therapy (Peng et al., 2024; Plumet et al., 2022). Phages offer a complementary, self-amplifying, biofilm-penetrating, metabolism-independent mechanism that can be matched to a patient's strain and may resensitize *S. aureus* to antibiotics (Peng et al., 2024). **Central hypothesis:** a phagogram-matched lytic cocktail, delivered locally as an adjunct to standard surgery and antibiotics, will clear biofilm/SCV reservoirs and lower antibiotic MICs, thereby improving infection control and shortening antibiotic duration. We will test this across the in vitro → large-animal → first-in-human translational chain.

Aim 1 — Define a phagogram-validated anti-*S. aureus* cocktail and quantify phage-antibiotic synergy against biofilm and SCV reservoirs. Using a banked panel of clinical *S. aureus*/MRSA osteomyelitis isolates, we will characterize host range, compose a multi-phage cocktail that raises the genetic barrier to phage resistance, and measure killing of biofilm-embedded and SCV populations alone and with standard antibiotics (vancomycin, anti-staphylococcal beta-lactams, rifampin). *Hypotheses:* (1a) sub-lethal antibiotics enhance phage replication; (1b) phage pressure lowers antibiotic MICs (resensitization) in a measurable fraction of isolates. *Go/no-go (end Yr 2):* a cocktail covering a pre-specified majority of the panel with demonstrated biofilm/SCV synergy.

Aim 2 — Establish local delivery, dosing, PK, and immune clearance in a hardware-associated large-animal osteomyelitis model. In an implant-associated *S. aureus* model, animals receiving debridement plus antibiotics will be randomized to adjunctive phage by intravenous versus local

(intra-osseous/intra-articular) routes versus surgery-plus-antibiotic controls. Endpoints: quantitative bacterial burden in bone and on hardware, phage titers in bone/serum over time, neutralizing-antibody development, and histopathology. *Hypothesis:* local delivery achieves higher, more sustained intra-osseous phage concentrations and greater burden reduction than systemic dosing, mitigating the rapid clearance and neutralizing-antibody responses described in the animal/clinical literature (Plumet et al., 2022). *Go/no-go (end Yr 4):* a defined, safe, locally administered regimen with characterized PK and immune kinetics.

Aim 3 — Conduct a phagogram-guided pilot clinical trial in refractory *S. aureus* osteomyelitis. Under an FDA expanded-access/emergency IND and IRB oversight, adults with hardware-associated, antibiotic-refractory *S. aureus* osteomyelitis will receive a patient-matched (phagogram-selected) cocktail plus standard-of-care debridement and antibiotics, using the regimen from Aim 2 (Ferry et al., 2024; NCT05369104). *Primary endpoints:* feasibility and safety. *Secondary/exploratory:* infection control at 3 months, phage PK/neutralizing antibodies, and antibiotic resensitization/duration of antibiotic therapy.

Impact. This program supplies the mechanistic and early-clinical foundation to make phagogram-guided cocktails a standard operating-room adjunct — potentially converting hardware-retaining chronic osteomyelitis from an "incurable," limb-threatening condition into a salvageable one, while shortening its defining months-long antibiotic courses.

Significance

The problem and the NIAMS mission. Chronic osteomyelitis is a defining problem of musculoskeletal medicine and sits squarely within the NIAMS mission for bone and joint disease. *S. aureus*, including MRSA, is the dominant pathogen and is uniquely equipped to evade therapy: it builds biofilm on devitalized bone and on orthopedic hardware, survives intracellularly, and converts to dormant SCVs that tolerate prolonged antibiotics (Peng et al., 2024; Plumet et al., 2022). The clinical consequence is a disease managed by repeated surgical debridement plus weeks-to-months of antibiotics, yet still marked by high relapse, hardware loss, and amputation. Crucially, these reservoirs are not addressed by choosing a more potent antibiotic, because the failure mode is **tolerance and physical sequestration, not classical resistance** — a mechanistic mismatch that explains why incremental antibiotic optimization has not solved the disease.

Why phages, mechanistically. Phages attack precisely these vulnerabilities. As self-amplifying lytic agents, their effective dose rises where bacterial burden is highest; their depolymerase and endolysin activities degrade biofilm matrix on bone and implants, exposing sequestered and SCV cells; and

because they kill independent of bacterial metabolic state, they reach quiescent populations that vancomycin and beta-lactams miss (Peng et al., 2024; Plumet et al., 2022). Strain specificity — often viewed as a limitation — becomes an asset in osteomyelitis: phagogram testing matches the cocktail to the patient's isolate while sparing surrounding microbiota. Most consequentially, phage and antibiotic appear cooperative rather than redundant: sub-lethal antibiotics can enhance phage replication, and phage selection pressure can drive *S. aureus* back toward antibiotic susceptibility (Peng et al., 2024). This resensitization is the single highest-value near-term opportunity, because shortening antibiotic duration would reduce toxicity, cost, and selection for resistance across this patient population.

Why now — the clinical signal. The rationale is now supported by early, regulated human experience. PhagoDAIRI (NCT05369104), a randomized, double-blind Phase 2 pilot of GMP anti-*S. aureus* phages (PP1493, PP1815) after DAIR for staphylococcal prosthetic joint infection, demonstrates that a controlled trial of adjunctive phage therapy in hardware-associated *S. aureus* infection is feasible and provides a design template for the field. The PHAGEinLYON Clinic at Hospices Civils de Lyon has delivered GMP phage cocktails intravenously and by local injection for complex bone and joint infection under regulated compassionate access, with most patients showing favorable clinical evolution (Ferry et al., 2024). The strongest case is hardware-associated, antibiotic-refractory infection, where surgical and antibiotic options are otherwise exhausted. What remains missing — and what this proposal supplies — is mechanism-anchored evidence linking phagogram-guided cocktail design, biofilm/SCV killing, optimized local delivery, immune kinetics, and antibiotic resensitization within one rigorous program.

Innovation

This proposal is innovative in four respects.

1. **Reservoir-targeted, not antibiotic-substitution, logic.** It explicitly targets the biofilm and SCV reservoirs that drive relapse, deploying phages as biofilm-disrupting, metabolism-independent killers used *with* antibiotics rather than as a stand-alone substitute (Peng et al., 2024; Plumet et al., 2022).
2. **Phagogram-guided design as a defined workflow.** It operationalizes patient-matched cocktail selection as a pre-specified, testable manufacturing-and-administration workflow rather than an ad hoc compassionate-use decision, building on regulated delivery experience (Ferry et al., 2024).
3. **Resensitization as a primary scientific readout.** It places antibiotic resensitization at the center of both the bench and clinical readouts, testing the hypothesis that phage pressure can

herd *S. aureus* back into susceptibility and thereby shorten antibiotic courses (Peng et al., 2024) — a measurable, decision-relevant endpoint rather than a hoped-for byproduct.

4. **Translational-barrier-first pharmacology.** It confronts the principal barriers — rapid phage clearance and neutralizing-antibody development — head-on by directly comparing local versus systemic delivery and measuring immune kinetics as *primary* pharmacologic endpoints in the large-animal model (Plumet et al., 2022).

The program deliberately stays within the current evidence frontier: it uses natural lytic phages of the type already in GMP clinical use, while treating engineered, longer-circulating, and endolysin-armed approaches as explicitly preclinical horizons rather than near-term clinical claims (Peng et al., 2024).

Approach

Aim 1 — Phagogram-validated cocktail design and phage-antibiotic synergy against biofilm and SCV reservoirs

Rationale. Cocktail efficacy in osteomyelitis depends on host range across diverse clinical strains, activity against biofilm and SCV subpopulations, and cooperative interaction with antibiotics. Multi-phage cocktails broaden host range and raise the genetic barrier to phage resistance (Peng et al., 2024; Plumet et al., 2022).

Experimental design. We will assemble a biobank of clinical *S. aureus*/MRSA isolates from refractory osteomyelitis and prosthetic joint infection, with characterized resistance phenotypes/genotypes. Well-characterized lytic anti-*S. aureus* phages of types already used clinically will be screened by phagogram to define host range and compose a cocktail. We will quantify killing against (i) planktonic cells, (ii) mature biofilms on titanium/PMMA hardware surrogates, and (iii) stable SCVs, comparing phage alone, antibiotic alone (vancomycin, anti-staphylococcal beta-lactams, rifampin), and combinations across sub-lethal-to-therapeutic antibiotic ranges. Synergy will be quantified by viable counts and biofilm-matrix/biomass assays; resensitization will be tested by serial passage under phage pressure with longitudinal antibiotic-MIC tracking. Resistance emergence will be characterized phenotypically and by whole-genome sequencing.

Rigor & reproducibility. Isolates and phages are biological resources defined by sequence and phenotype; assays use pre-registered analysis plans, biological and technical replicates, blinded enumeration where feasible, and reference control strains. Sex as a biological variable is not applicable to in vitro isolate work but is addressed in Aims 2–3.

Expected outcomes. A defined, phagogram-validated cocktail with documented coverage of the isolate panel; quantitative evidence on whether sub-lethal antibiotics enhance phage replication and whether phage pressure lowers antibiotic MICs in a measurable isolate fraction; and identification of antibiotic partners that maximize biofilm/SCV clearance.

Potential pitfalls & alternatives. Some isolates may be phage-insensitive — we will expand the phage library and use combinatorial cocktails. If antagonism appears for specific antibiotic-phage pairs, we will evaluate sequencing/staggering of administration in vitro to inform Aims 2–3. If resensitization is isolate-specific, we will define the genomic correlates that predict it, preserving translational value.

Aim 2 — Local delivery, dosing, PK, and immune clearance in a hardware-associated large-animal model

Rationale. The animal-model and clinical literature for *S. aureus* phage therapy confirms safety as an antibiotic adjunct but flags rapid phage clearance and neutralizing-antibody development as the key dosing/delivery challenges (Plumet et al., 2022). Local delivery into bone and joint spaces is a plausible solution that must be tested head-to-head with systemic dosing.

Experimental design. In an implant-associated *S. aureus* osteomyelitis large-animal model, animals receiving debridement plus antibiotics will be randomized to adjunctive phage by intravenous versus local (intra-osseous/intra-articular) routes versus surgery-plus-antibiotic controls. Endpoints: quantitative bacterial burden in bone and on explanted hardware, phage titers in bone/serum over time, neutralizing-antibody development, and histopathology. Dose and schedule will be informed by Aim 1 synergy data. Group sizes will be powered to a pre-specified bacterial-burden difference with a formal power calculation; both sexes will be included and analyzed.

Expected outcomes. Determination of whether local delivery achieves higher, more sustained intra-osseous phage concentrations and greater bacterial-burden reduction than systemic dosing; a defined dosing/delivery regimen to carry into Aim 3; and a characterized neutralizing-antibody time course.

Potential pitfalls & alternatives. Rapid clearance may blunt efficacy — we will test repeated dosing and local depot delivery. If neutralizing antibodies curtail systemic efficacy, the program will prioritize the local route and cocktail rotation. Model variability will be addressed with adequate group sizes, predefined endpoints, and blinded outcome assessment.

Aim 3 — Phagogram-guided pilot clinical trial in refractory *S. aureus* osteomyelitis

Rationale. Early controlled and regulated compassionate-use experience (PhagoDAIRI,

NCT05369104; PHAGEinLYON, Ferry et al., 2024) supports feasibility and a favorable safety signal, but rigorous, prospectively defined US data — particularly with resensitization as a readout — are lacking.

Experimental design. Under an FDA expanded-access/emergency IND and IRB oversight, adults with hardware-associated, antibiotic-refractory *S. aureus* osteomyelitis will receive a patient-matched (phagogram-selected) cocktail plus standard-of-care debridement and antibiotics, using the Aim 2 delivery regimen. **Primary endpoints:** feasibility (proportion of eligible patients for whom a matched cocktail can be manufactured and administered on a clinically actionable timeline) and safety. **Secondary/exploratory endpoints:** infection control at 3 months, phage PK and neutralizing antibodies, and antibiotic resensitization/duration of antibiotic therapy. The single-arm pilot is structured for seamless transition to a randomized design modeled on PhagoDAIRI (NCT05369104).

Expected outcomes. A demonstrated workflow for phagogram-matched manufacturing and administration; a prospective safety dataset; and preliminary infection-control and resensitization estimates to power a definitive randomized trial.

Potential pitfalls & alternatives. Strain-coverage gaps may exclude some patients — the Aim 1 library mitigates this, and uncovered isolates will be logged to guide library expansion. Single-arm design limits inference — we will use pre-defined standard-of-care/historical benchmarks and structure the protocol for transition to a randomized controlled trial. Enrollment risk in a rare-refractory population will be mitigated through multi-site referral partnerships with complex bone-and-joint-infection centers.

Timeline

[ILLUSTRATIVE] Years 1–2: Aim 1 isolate banking, phagogram screening, cocktail assembly, and synergy/resensitization assays. **Years 2–4:** Aim 2 large-animal delivery and PK/immunogenicity studies, overlapping with regulatory (eIND) and IRB preparation. **Years 4–5:** Aim 3 phagogram-guided pilot enrollment, follow-up, and analysis. **Go/no-go milestones gate progression:** (1) a validated cocktail with demonstrated biofilm/SCV synergy (end Year 2); (2) a defined, safe local-delivery regimen with characterized PK/immune kinetics (end Year 4). Failure to meet a milestone triggers the pre-specified alternative strategies rather than advancement.

Budget Justification

[ILLUSTRATIVE] Modular R01. **[ILLUSTRATIVE]** $250,000 direct costs/year for **[ILLUSTRATIVE]** 5 years. **Personnel:** MPIs (phage/microbiology and orthopedic infectious disease), co-investigators, a phage scientist, a research microbiologist for biofilm/SCV assays, a large-animal study coordinator, and a clinical research/regulatory coordinator for eIND/IRB. **Major non-personnel costs:** GMP-grade clinical phage supply and phagogram testing; large-animal model costs (housing, surgery, imaging, histopathology) concentrated in **[ILLUSTRATIVE]** Years 2–4; whole-genome sequencing of isolates and resistant variants; and clinical-trial costs (manufacturing, monitoring, PK/neutralizing-antibody assays) in **[ILLUSTRATIVE]** Years 4–5. **Consortium/subawards** are anticipated for the GMP phage-manufacturing partner and the large-animal facility. The modular structure assumes consistent annual modules with justified fluctuation across animal and clinical years.

Vertebrate Animals

Animal work is proposed in Aim 2. A hardware-associated *S. aureus* osteomyelitis large-animal model (consistent with established implant-associated/fracture-related-infection models, e.g., sheep) will evaluate route-dependent phage delivery, PK, neutralizing-antibody development, and bacterial-burden reduction as an antibiotic adjunct (Plumet et al., 2022). **Justification:** biofilm-on-hardware and intra-osseous pharmacokinetics cannot be recapitulated in vitro. **3Rs:** group sizes will be the minimum required for pre-specified, formally powered comparisons; in vitro Aim 1 assays reduce subsequent animal numbers (reduction); procedures (surgery, infection induction, phage/antibiotic administration, imaging, euthanasia for tissue analysis) follow IACUC-approved protocols with anesthesia/analgesia, defined humane endpoints, and refinement of surgical/monitoring technique. Both sexes will be included.

Human Subjects / Clinical Trial

Aim 3 is a clinical trial in adults with hardware-associated, antibiotic-refractory *S. aureus* osteomyelitis. Because the phage product is investigational, administration will proceed under the FDA expanded-access/emergency IND (eIND) pathway for patient-matched phage therapy — the route used for current US compassionate phage access — with progression toward a conventional IND for the definitive trial. All activities will have IRB approval and informed consent. Phages will be phagogram-matched to each patient's isolate and given as an adjunct to standard-of-care surgical

debridement and antibiotics, **never as a replacement** (Peng et al., 2024; Ferry et al., 2024). A data and safety monitoring plan will govern adverse-event reporting with pre-defined stopping rules. **Primary endpoints:** feasibility and safety. **Exploratory endpoints:** infection control at 3 months, phage PK/neutralizing antibodies, and antibiotic resensitization. Inclusion of both sexes and relevant age groups, and a recruitment/retention plan leveraging multi-site referral, will be detailed. The single-arm pilot is designed for seamless transition to a randomized controlled trial modeled on PhagoDAIRI (NCT05369104).

Team & Environment

This program requires an interdisciplinary team, to be filled with named investigators and institutions: **Contact PI / MPI** — phage biologist with GMP phage and phagogram expertise [Name, Institution]; **MPI** — orthopedic infectious disease/musculoskeletal-infection clinician-scientist [Name, Institution]; **Co-Investigator** — orthopedic/trauma surgeon experienced in hardware-associated osteomyelitis and DAIR [Name, Institution]; **Co-Investigator** — clinical microbiologist for biofilm/SCV assays and susceptibility testing [Name, Institution]; **Large-Animal Model Lead** — veterinary surgeon/comparative medicine [Name, Institution; e.g., a center with established fracture-related-infection model capability]; **GMP Phage Manufacturing Partner** [Organization]; **Regulatory/Clinical-Trials Lead** — eIND/IND and IRB expertise [Name, Institution]; **Biostatistician** [Name, Institution]. The environment must provide BSL-2 phage/microbiology laboratories, a large-animal surgical facility, a reference center for complex bone and joint infection (analogous to the CRIOAc Lyon/PHAGEinLYON model; Ferry et al., 2024), and clinical-trial infrastructure. **Alternate/partner funders to note:** NIAID (antibacterial resistance and phage therapeutics) and the DoD CDMRP (trauma-associated and hardware-related musculoskeletal infection) are logical co-funding sources alongside NIAMS.

References

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