[Note to applicant: [ILLUSTRATIVE] markers denote placeholders to be finalized before submission — they are not part of the scientific argument. All four cited references are drawn from the allowed set; no other citations are permitted in this draft.]

Intra-Articular Lytic Phage–Vancomycin Therapy to Spare the Implant During DAIR for Staphylococcal Prosthetic Joint Infection

Project Summary / Abstract

Prosthetic joint infection (PJI) is among the most feared complications of hip and knee arthroplasty, and *Staphylococcus aureus*—including methicillin-resistant (MRSA) strains—is the leading cause. Staphylococci form dense biofilms on the implant that shield them from antibiotics and host immunity, so even prolonged combination antibiotics paired with aggressive surgery—debridement, antibiotics, and implant retention (DAIR) or staged revision—frequently fail, committing patients to repeat operations, prolonged disability, and high cost. Lytic bacteriophages are mechanistically suited to this problem: they replicate at the infection site, erode biofilm matrix, and kill antibiotic-tolerant and multidrug-resistant cells through receptor-mediated mechanisms independent of antibiotic resistance. In a controlled rat PJI model, phage plus vancomycin produced a ~22.5-fold reduction in *S. aureus* burden versus roughly 5–6-fold for either agent alone (Morris et al., 2019), and two published compassionate-use cases—an MSSA prosthetic knee (Ramirez-Sanchez et al., 2021) and a recalcitrant MRSA "Salphage" knee (Doub et al., 2022)—achieved salvage with generally mild adverse events. **Hypothesis:** an intra-articularly delivered, susceptibility-matched lytic phage cocktail, combined with vancomycin and timed to the DAIR washout, will clear implant biofilm and suppress resistance more effectively than antibiotics alone, enabling implant retention. This R01 will (1) assemble and characterize a strictly lytic ≥2-phage anti-staphylococcal cocktail and quantify biofilm-matrix degradation and resistance suppression against banked clinical *S. aureus* isolates; (2) define phage–vancomycin synergy and pharmacodynamics on orthopedic titanium/polyethylene surfaces and in a rat implant-associated PJI model; and (3) build the susceptibility-matching, manufacturing-readiness, and regulatory framework (FDA emergency/expanded-access IND) to support a future DAIR trial. The work advances the NIAMS musculoskeletal-infection mission and complements the GLORIA Phase II DAIR trial (NCT06605651).

Specific Aims

PJI on orthopedic hardware is driven by staphylococcal biofilm that is intrinsically tolerant to antibiotics; current management often requires staged revision surgery with major morbidity, cost, and disability. Lytic phages degrade biofilm and kill antibiotic-tolerant staphylococci by receptor-mediated mechanisms independent of antibiotic resistance, and the strongest preclinical signal is synergy with vancomycin—a ~22.5-fold burden reduction versus ~5–6-fold for either agent alone in a rat PJI model (Morris et al., 2019). Two compassionate-use cases support feasibility and tolerability in humans (Ramirez-Sanchez et al., 2021; Doub et al., 2022). What is missing is rigorous, orthopedic-specific, US-focused preclinical and translational groundwork to support implant-sparing DAIR trials. **Central hypothesis:** a susceptibility-matched, strictly lytic phage cocktail delivered intra-articularly with vancomycin during DAIR clears implant biofilm and suppresses resistance better than antibiotics alone. We pursue three aims.

Aim 1. Assemble and characterize a strictly lytic anti-staphylococcal phage cocktail with biofilm-degrading and resistance-suppressing activity. Using a banked panel of clinical MSSA and MRSA PJI isolates (with coagulase-negative staphylococci as a secondary, exploratory set), we will define host range (spot and efficiency-of-plating), confirm strictly lytic biology and absence of undesirable genetic content by whole-genome sequencing, and quantify killing of planktonic and biofilm-embedded bacteria for individual phages and rationally designed ≥2-phage cocktails. *Success criterion:* a sequenced, strictly lytic ≥2-phage cocktail covering ≥80% [ILLUSTRATIVE] of the *S. aureus* panel with measurable biofilm-matrix degradation and lower phage-resistance emergence than single phages by serial-passage assay.

Aim 2. Define phage–vancomycin synergy and pharmacodynamics on orthopedic surfaces and in a rat implant-associated PJI model. We will quantify cocktail activity against biofilm grown on clinically relevant titanium and polyethylene substrates, then test intra-articular/local phage cocktail plus vancomycin versus either agent alone in a rat implant-associated PJI model (building on Morris et al., 2019), with pre-specified endpoints of implant and periprosthetic-tissue CFU, biofilm clearance by imaging, resistance emergence, and local/systemic tolerability. *Success criterion:* combination reduces implant-associated burden significantly more than either monotherapy (pre-specified effect size from prior data), with an acceptable local safety profile supporting intra-articular delivery during simulated DAIR.

Aim 3. Build the susceptibility-matching, manufacturing, and regulatory framework for an implant-sparing DAIR trial. We will develop and validate a rapid isolate-to-cocktail matching workflow, define release specifications (identity, sterility, endotoxin, potency) for intra-articular use, and prepare an FDA emergency/expanded-access IND (eIND) and IRB framework to support compassionate use and a future DAIR study informed by the GLORIA design (NCT06605651).

Success criterion: a validated matching workflow with a surgically actionable turnaround [ILLUSTRATIVE], draft release specifications, and an eIND-ready regulatory/IRB package.

Impact. If validated, intra-articular phage–vancomycin therapy could let surgeons clear biofilm during a single DAIR washout and retain the implant, sparing patients the morbidity, cost, and disability of staged revision and helping convert one of orthopedics' most antibiotic-defying infections into a treatable, implant-sparing condition.

Significance

PJI is one of the most feared complications of hip and knee arthroplasty, and *S. aureus*—prominently MRSA—is the leading culprit. The central barrier is biofilm: staphylococci adhere to the implant and encase themselves in a matrix that shields them from antibiotics and the immune system. Consequently, even prolonged combination antibiotics plus aggressive surgery (DAIR or staged revision) frequently fail, and patients face repeated operations, prolonged disability, and substantial cost. There is a pressing need for therapies that act where antibiotics cannot: inside the biofilm, against antibiotic-tolerant and multidrug-resistant cells.

Lytic phages directly address this gap. Because they replicate at the infection site, they amplify dose in situ; because killing is receptor-mediated rather than dependent on bacterial metabolism, they remain active against MRSA and slow-growing, biofilm-embedded cells; and phage activity can erode the biofilm matrix itself. Their strain specificity largely spares the surrounding microbiome. Critically, phages synergize with antistaphylococcal antibiotics: in a controlled rat PJI model, phage plus vancomycin yielded a ~22.5-fold drop in *S. aureus* burden versus roughly 5–6-fold for either agent alone (Morris et al., 2019). Human evidence, while still early, is encouraging—documented salvage of an MSSA prosthetic knee (Ramirez-Sanchez et al., 2021) and a recalcitrant MRSA prosthetic knee (Doub et al., 2022), both with locally and/or systemically administered phages and generally mild adverse events. This proposal advances rigorous, US-focused, orthopedic-specific preclinical and translational science squarely within the NIAMS musculoskeletal-infection mission; the antimicrobial-resistance dimension is also relevant to broader federal AMR priorities [ILLUSTRATIVE].

Innovation

This project is innovative in three respects, each grounded in the cited evidence rather than aspiration.

First, it targets the biofilm-on-hardware problem directly, pairing an intra-articularly delivered lytic cocktail with the DAIR surgical workflow so biofilm is attacked precisely when the joint is open and

the implant is retained—an implant-sparing strategy rather than staged revision, aligned with the DAIR-based design now in clinical testing (NCT06605651).

Second, it operationalizes phage–antibiotic synergy as a *designed* therapeutic principle. Rather than treating phages as a standalone, we engineer the cocktail-plus-vancomycin combination shown preclinically to drive a ~22.5-fold burden reduction (Morris et al., 2019), and we define its pharmacodynamics on the exact orthopedic surfaces (titanium, polyethylene) where biofilm forms.

Third, it builds a rapid susceptibility-matching workflow so a patient's specific *S. aureus* isolate can be matched to a banked, strictly lytic ≥2-phage cocktail. Cocktails (≥2 phages) are used specifically to broaden coverage and suppress resistance emergence relative to single phages—an explicit, testable design hypothesis (Aim 1) rather than an assumed benefit. Together these elements convert isolated case reports (Ramirez-Sanchez et al., 2021; Doub et al., 2022) into a reproducible, regulatory-ready preclinical platform.

Approach

Overview and rigor. The aims test one central hypothesis across increasing biological complexity: molecular/in-vitro characterization (Aim 1) → surface and in-vivo pharmacodynamics (Aim 2) → translational/regulatory readiness (Aim 3). Throughout, we apply NIH rigor practices: pre-registered analysis plans and effect sizes, blinded outcome assessment for animal endpoints, biological and technical replicates, *a priori* inclusion/exclusion and stopping rules, and reporting per ARRIVE-style standards [ILLUSTRATIVE]. Sex as a biological variable: in-vitro isolates derive from both male and female patients where available; the rat model will include both sexes with analyses powered to detect sex-based differences in response [ILLUSTRATIVE]. **Authentication of key resources:** bacterial isolates will be identity-confirmed (MALDI-TOF and/or sequencing) and *mecA*/MRSA status documented; phage stocks will be sequence-verified, plaque-purified, and titer- and endotoxin-controlled at each passage; cell-free reagents and antibiotics will be lot-tracked.

Aim 1 — Assemble and characterize a strictly lytic anti-staphylococcal phage cocktail

Rationale. Anti-*S. aureus* phages bind specific cell-surface receptors, inject their genome, and lyse the host, releasing progeny that amplify dose in situ. Strain specificity spares the microbiome but requires susceptibility matching, motivating cocktails of ≥2 phages to broaden coverage and suppress resistance.

Experimental design. Against a banked panel of clinical MSSA and MRSA PJI isolates (with coagulase-negative staphylococci as a secondary, exploratory set), we will (a) determine host range by

spot and efficiency-of-plating assays; (b) confirm strictly lytic biology and screen out undesirable genetic content (lysogeny, virulence, antibiotic-resistance, or toxin genes) by whole-genome sequencing; (c) quantify planktonic killing kinetics; and (d) measure biofilm-matrix degradation and biofilm killing for individual phages and rationally combined ≥2-phage cocktails. Resistance suppression will be assessed by serial-passage emergence assays comparing single phages with cocktails. Analyses use pre-specified replicate numbers and effect thresholds [ILLUSTRATIVE].

Expected outcomes. A defined, sequenced, strictly lytic ≥2-phage cocktail with documented host range across the *S. aureus* panel, demonstrable biofilm-matrix degradation, and reduced resistance emergence relative to single phages.

Potential pitfalls & alternatives. (i) *Some isolates may be insensitive to the starting library.* We will expand the library and, if needed, define two complementary cocktail formulations assigned by susceptibility testing. (ii) *Cocktail components may interfere (e.g., receptor competition).* We will screen pairwise combinations for additivity/antagonism before locking the formulation. (iii) *Coagulase-negative staph coverage may be limited;* because the cited evidence base is *S. aureus*-specific, CoNS is scoped as exploratory and will not gate go/no-go decisions.

Aim 2 — Define phage–vancomycin synergy and pharmacodynamics in an implant model

Rationale. Phages remain active against biofilm-embedded and slow-growing cells, and the most compelling preclinical signal is synergy with vancomycin (~22.5-fold burden reduction vs. ~5–6-fold for either agent alone; Morris et al., 2019). Translation requires testing on orthopedic surfaces and in vivo.

Experimental design. We will grow staphylococcal biofilm on clinically relevant titanium and polyethylene substrates and quantify clearance by cocktail alone, vancomycin alone, and the combination (checkerboard/time-kill with pre-defined synergy metrics). We will then use a rat implant-associated PJI model (consistent with Morris et al., 2019) to compare intra-articular/local phage cocktail plus vancomycin against monotherapies. **Pre-specified primary endpoint:** implant-associated CFU; **secondary:** periprosthetic-tissue CFU, biofilm clearance by imaging, phage-resistance emergence, and local/systemic tolerability. Group sizes will be powered from the prior effect size to detect a pre-specified difference at 80–90% power [ILLUSTRATIVE animal numbers], with blinded CFU enumeration and randomized treatment assignment.

Expected outcomes. Confirmation of phage–vancomycin synergy on hardware surfaces and in vivo, a defined local dosing approach, evidence of biofilm clearance, and an acceptable local safety profile supporting intra-articular administration during DAIR.

Potential pitfalls & alternatives. (i) *In-vivo phage neutralization or rapid clearance could blunt efficacy*. We will evaluate repeated/extended local dosing and monitor neutralizing responses, mirroring the transient transaminitis and possible serum neutralization noted with prolonged human dosing in the case literature. (ii) *A single intraoperative dose may underperform;* we will test a short post-DAIR local course. (iii) *Model variability;* blinding, randomization, and pre-set exclusion criteria mitigate bias, and an interim analysis [ILLUSTRATIVE] guards against underpowering.

Aim 3 — Susceptibility-matching, manufacturing, and regulatory framework

Rationale. Clinical translation depends on rapidly matching each patient's isolate to an effective cocktail and producing material meeting purity/potency standards under an appropriate FDA pathway. The compassionate-use cases (Ramirez-Sanchez et al., 2021; Doub et al., 2022) and the GLORIA trial (NCT06605651) define the regulatory and design contours.

Experimental design. We will (a) build and validate a rapid isolate-to-cocktail matching workflow with a target turnaround suitable for surgical planning [ILLUSTRATIVE], benchmarking against banked-isolate ground truth; (b) define release specifications for identity, sterility, endotoxin, and potency for intra-articular use; and (c) prepare FDA eIND documentation and an IRB protocol framework to support compassionate use and a future randomized, double-blind, placebo-controlled DAIR study modeled on GLORIA (NCT06605651).

Expected outcomes. A validated matching workflow, draft release specifications, and an eIND-ready regulatory/IRB package that de-risks a subsequent clinical trial.

Potential pitfalls & alternatives. *Matching turnaround may be too slow for acute cases.* We will pre-bank well-characterized cocktails so banked phages can be deployed immediately while bespoke matching proceeds, consistent with a tiered banked-plus-personalized model.

Timeline

- **Year 1 [ILLUSTRATIVE]:** Aim 1 host-range, lytic confirmation, sequencing, biofilm assays; assemble candidate cocktails.
- **Years 2–3 [ILLUSTRATIVE]:** Aim 1 resistance-suppression completion; Aim 2 surface-biofilm and rat PJI synergy studies.
- **Years 3–4 [ILLUSTRATIVE]:** Aim 2 in-vivo dosing/safety; Aim 3 matching workflow and release-spec development.
- **Years 4–5 [ILLUSTRATIVE]:** Aim 3 eIND/IRB package finalization; integration and trial-enabling data lock.

Budget Justification (modular R01-style sketch)

Requested in modular increments [ILLUSTRATIVE], e.g., $250,000 direct costs/year [ILLUSTRATIVE] for 5 years [ILLUSTRATIVE]. **Personnel:** PI (orthopedic infectious disease / surgeon-scientist) [ILLUSTRATIVE % effort]; Co-I phage biologist; Co-I biofilm microbiologist; Co-I orthopedic surgeon; research scientist; technician; biostatistician; regulatory specialist. **Supplies:** clinical isolate banking, phage propagation/purification, whole-genome sequencing, titanium/polyethylene substrates, vancomycin, biofilm and imaging assays [ILLUSTRATIVE]. **Animals:** rat PJI model per-diems and surgical consumables [ILLUSTRATIVE]. **Other:** endotoxin/potency assay development, regulatory consulting, publication and data sharing [ILLUSTRATIVE]. No clinical-trial enrollment costs are requested in this preclinical/translational R01.

Vertebrate Animals

Animal work is proposed in Aim 2 using a rat implant-associated PJI model consistent with Morris et al. (2019) to evaluate intra-articular/local phage cocktail plus vancomycin. We will provide the NIH-required three-point description: (1) **detailed description** of procedures (implant placement, *S. aureus* inoculation, intra-articular/local dosing, and CFU/imaging endpoints), including species and group sizes [ILLUSTRATIVE], with both sexes included; (2) **justifications** for the species (an established, literature-validated PJI model) and for animal numbers (minimum required for statistical rigor, powered from prior effect sizes); and (3) **minimization of pain and distress** via appropriate analgesia, defined humane endpoints, and veterinary oversight, with euthanasia per current AVMA guidelines. IACUC approval will be obtained before any animal work.

Human Subjects / Clinical Trial

No human-subjects enrollment is proposed in this R01; the work is preclinical and translational, and this application is **not** a clinical trial. Aim 3 prepares—but does not execute—the clinical pathway. Investigational phage administration to individual patients (as in the published compassionate-use cases; Ramirez-Sanchez et al., 2021; Doub et al., 2022) proceeds under the FDA emergency/expanded-access IND (eIND) route with prospective IRB oversight and informed consent. The deliverable is an eIND-ready regulatory/IRB framework to support future compassionate use and a subsequent randomized, double-blind, placebo-controlled DAIR trial modeled on GLORIA (NCT06605651). Any such trial would require separate funding and regulatory submission; projected enrollment for that future study is out of scope here [ILLUSTRATIVE].

Rigor, Reproducibility & Data Sharing

Key biological resources (clinical isolates, phage stocks) will be authenticated and lot-tracked as described in the Approach. Analyses are pre-specified with defined effect sizes, replicate numbers, randomization, and blinding. We will deposit phage genome sequences in a public repository, share protocols and analysis code, and report results per ARRIVE-style standards [ILLUSTRATIVE], consistent with NIH data-management and sharing expectations.

Team & Environment

- **Principal Investigator — [Name], MD/PhD, [Institution]:** orthopedic infectious disease or surgeon-scientist leading PJI strategy and regulatory effort.
- **Co-Investigator (Phage Biology) — [Name], [Institution]:** cocktail design, propagation, sequencing.
- **Co-Investigator (Biofilm Microbiology) — [Name], [Institution]:** biofilm and surface-clearance assays.
- **Co-Investigator (Orthopedic Surgery) — [Name], [Institution]:** DAIR workflow and translational design.
- **Regulatory/Manufacturing Lead — [Name], [Institution/Partner]:** eIND, release specifications, banking.
- **Biostatistician — [Name], [Institution].**
- **Environment:** institutional vivarium with IACUC, microbiology/BSL-2 facilities, sequencing core, GMP-aware production partner, and an orthopedic clinical program with isolate biobanking—collectively sufficient to execute all three aims.

References

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3. Morris JL, Letson HL, Elliott L, Grant AL, Wilkinson M, Hazratwala K, McEwen P. Evaluation of bacteriophage as an adjunct therapy for treatment of peri-prosthetic joint infection caused by *Staphylococcus aureus*. *PLoS One.* 2019;14(12):e0226574. https://doi.org/10.1371/journal.pone.0226574

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