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Personalized Lytic Bacteriophage Cocktails as a Device-Sparing Adjunct for Staphylococcal LVAD Infection: Resolving the Benefit-or-Harm Question (PHAGE-LVAD)

Project Summary / Abstract

Left ventricular assist devices (LVADs) sustain life in advanced heart failure, but infection of the driveline, pump pocket, or outflow graft is among their most common and lethal complications, affecting a substantial minority of recipients (commonly cited as roughly 15–30% within two years [ILLUSTRATIVE — to be anchored to the candidate site's registry data]). Most of these infections are staphylococcal: *Staphylococcus aureus* and biofilm-forming *Staphylococcus epidermidis* entrench on synthetic surfaces, resist antibiotics, and frequently cannot be cured short of device exchange or transplantation — options constrained by surgical risk and donor scarcity, and largely unavailable to the growing destination-therapy population. For these patients, care collapses to lifelong suppressive antibiotics with recurrent relapse and high mortality.

Obligately lytic bacteriophages are mechanistically suited to this failure mode: their activity is independent of antibiotic-resistance status, they express depolymerases and endolysins that degrade biofilm matrix and lyse embedded cells, they self-amplify at the infection focus, and they can be host-matched strain-by-strain to the patient's own isolate. Yet the evidence is genuinely mixed. Patient-derived phages reduced *S. epidermidis* biofilm on explanted driveline material by ~1.9 log when combined with rifampicin (Pitton 2025), and single-patient compassionate use has been reported (Aslam 2019; Rojas 2022) — but a rigorous in vitro study found that phage combinations added **no benefit, and sometimes antagonism, against *S. aureus* driveline isolates, with worsened bacterial growth when phage was mistimed** (Molendijk 2025). The decisive question for the field is therefore not whether phages *can* kill these organisms, but **under what strain, dose, and sequencing conditions they help rather than harm** — and whether that knowledge can be translated safely.

This R01 proposes a US-focused, IND-enabling program built around that question. **Aim 1** builds a patient-derived anti-staphylococcal phage bank and a turnaround-time–defined host-matching pipeline. **Aim 2** uses explanted driveline biofilm to map, strain by strain, where phage–antibiotic combinations are synergistic, additive, or antagonistic — directly reconciling the Pitton and Molendijk results and converting dose/sequence/timing from a hazard into a controlled variable. **Aim 3** assembles a complete IND-enabling package and conducts a small, prospectively designed feasibility cohort under a single protocol-level study IND in transplant-ineligible, surgically high-risk patients, with predefined safety, microbiologic, and device-retention endpoints. **Central hypothesis:** for staphylococcal LVAD infection, a host-matched lytic cocktail delivered under empirically defined

dose/sequence rules, as an adjunct to standard surgical and antibiotic care, can safely reduce biofilm burden and buy device-sparing time. Success would give destination-therapy and high-surgical-risk patients a rational salvage option and generate the safety and effect-size data required to justify a future randomized trial.

Specific Aims

Staphylococcal LVAD infection is frequent and frequently incurable without device exchange or transplant — interventions many recipients cannot receive. Lytic phages offer resistance-independent, biofilm-penetrating, host-matchable killing, but the current evidence is limited to ex vivo proof-of-concept and single-patient reports, and at least one rigorous study found phage combinations gave **no benefit and possible harm** against *S. aureus* driveline isolates (Molendijk 2025). The field's bottleneck is not enthusiasm but **reproducible, mechanism-anchored rules for when these agents help versus harm.** We will supply them.

Central hypothesis. For staphylococcal LVAD infection in patients with no curative option, a host-matched, obligately lytic phage cocktail — delivered locally as an adjunct to standard surgical debridement and antibiotics, under empirically derived dose/sequence/timing rules — can safely reduce device biofilm burden and preserve device function, whereas mistimed or strain-mismatched phage can be inert or antagonistic.

Aim 1 — Build and characterize a patient-derived anti-staphylococcal phage bank with a rapid host-matching pipeline. Against banked and prospectively collected *S. aureus* and *S. epidermidis* device isolates, we will isolate and whole-genome–sequence obligately lytic phages, confirm strictly lytic lifestyle, exclude integrase/toxin/AMR cargo, and define an efficiency-of-plating host-range panel with a target receipt-to-recommendation turnaround time [ILLUSTRATIVE]. This extends the patient-derived strategy of Pitton 2025 into a standardized US bank. *Milestone/go-no-go:* a sequenced, host-characterized bank covering a prespecified fraction of circulating strains.

Aim 2 — Map phage–antibiotic synergy, additivity, and antagonism on explanted driveline biofilm. Using explanted LVAD driveline segments as the ex vivo substrate (after Pitton 2025), we will quantify viable biofilm burden under phage alone, rifampicin alone, and phage + rifampicin (co-administered and in both sequences), plus other clinically relevant antibiotics — systematically varying phage dose, antibiotic concentration, and relative timing. We will **directly reconcile** the ~1.9-log *S. epidermidis* + rifampicin reduction (Pitton 2025) with the negative/antagonistic *S. aureus* result and mistiming hazard (Molendijk 2025), and track resistant-escape emergence. *Go/no-go:* strain-resolved dose/sequence rules that achieve a prespecified log-reduction threshold without antagonism advance to Aim 3.

Aim 3 — Assemble an IND-enabling package and conduct a small prospective feasibility cohort under a single study IND. In transplant-ineligible, surgically high-risk adults with confirmed staphylococcal LVAD infection, we will deliver an Aim 1 host-matched cocktail under Aim 2 dose/sequence rules — including a viscous/slow-release local galenic modeled on Rojas 2022 and the local adjunctive approach of Aslam 2019 — as an adjunct to standard surgery and systemic antibiotics. Unlike a series of single-patient emergency INDs, this operates under **one prospectively designed, IRB-approved protocol** with predefined safety, microbiologic, and device-retention endpoints [ILLUSTRATIVE]. *Go/no-go:* a safety/feasibility package adequate to power a future randomized trial.

Impact. The program would establish, for the first time in a US setting, the strain-resolved dosing, sequencing, and safety parameters that determine whether personalized phage therapy helps or harms in staphylococcal LVAD infection — converting conflicting early signals into a decision-ready evidence base for a high-mortality NHLBI device population.

Significance

LVADs are life-sustaining cardiovascular technologies squarely within NHLBI's mission, yet device infection remains one of their most common and lethal complications. A substantial minority of recipients develop infection (commonly ~15–30% within two years [ILLUSTRATIVE — to be anchored to candidate-site registry data]), most often staphylococcal, at the driveline exit site, pump pocket, or outflow graft. Once staphylococci establish biofilm on synthetic surfaces, antibiotics penetrate poorly and rarely sterilize the device; definitive cure typically requires device exchange or heart transplantation. Both carry substantial surgical risk and depend on donor availability, and neither is realistic for destination-therapy patients implanted with no transplant pathway. For them, the standard of care collapses to lifelong suppressive antibiotics with recurrent relapse and mortality — a genuine therapeutic dead end that this proposal addresses.

Lytic phages target the specific failure modes of this disease. Their killing does not depend on antibiotic-resistance phenotype, so they remain active against multidrug-resistant staphylococci. They penetrate and disrupt biofilm via depolymerases and endolysins, reaching matrix-embedded organisms on driveline tubing that antibiotics access poorly — an effect demonstrated ex vivo on explanted LVAD driveline material (Pitton 2025). They self-amplify where host bacteria are densest, and a cocktail host-matched to the patient's own *S. aureus* or *S. epidermidis* clone broadens coverage and suppresses resistant escape.

Crucially, the significance of this work lies not in asserting that phages work, but in **resolving a documented contradiction**. The strongest anti-biofilm signal pairs phage with rifampicin against *S. epidermidis* (Pitton 2025), yet phage combinations gave no benefit — and sometimes antagonism —

against *S. aureus* driveline isolates, with worsened growth when phage was mistimed (Molendijk 2025). A therapy that is beneficial for one organism and inert or harmful for another, depending on timing, cannot be deployed safely until those boundaries are mapped. The realistic near-term value is a **personalized, device-sparing adjunct** that buys biofilm control and time — turning a forced pump exchange into a potential rescue — not a stand-alone replacement for surgery and antibiotics. Establishing this in a US setting directly serves a high-mortality NHLBI cardiovascular-device population while advancing antimicrobial-resistance goals of cross-institute relevance. (Scope note: the present evidence base, and therefore this program, is LVAD-specific; extension to lead- and pocket-based cardiac implantable electronic device infection is a logical future direction but is **not** claimed here, as no in-set evidence supports it.)

Innovation

This proposal is innovative in four respects, each anchored to the actual evidence rather than to phage-therapy enthusiasm.

- **The conflicting biofilm literature is the central scientific question, not a footnote.** We power the ex vivo work specifically to reconcile the positive phage + rifampicin signal against *S. epidermidis* (Pitton 2025) with the negative/antagonistic *S. aureus* result and mistiming hazard (Molendijk 2025), making strain × dose × sequence × timing the primary experimental variable. Few phage programs are designed around a falsification rather than a hoped-for confirmation.

- **Patient-derived, host-matched phage banking purpose-built for cardiac-device staphylococci.** We operationalize the LVAD-patient isolation strategy of Pitton 2025 into a defined, turnaround-time–driven US pipeline suitable for time-critical clinical decisions.

- **An honest, study-IND clinical design.** Rather than accumulating uncontrolled single-patient emergency INDs (as in prior compassionate cases; Aslam 2019; Rojas 2022), we build a complete IND-enabling package and run one prospectively designed, uniformly monitored feasibility protocol — the structure required to produce interpretable safety and effect-size data.

- **Local-delivery galenic translation.** We adapt the viscous slow-release galenic used by Rojas 2022 and the local adjunctive route of Aslam 2019 to deliver host-matched cocktails to the pocket or driveline tract under a defined US regulatory pathway.

A note on engineering: where promising staphylococcal phages prove temperate, CRISPR-Cas removal of integrase genes to lock a lysogeny-free lytic state is a recognized strategy. We treat this as

a **secondary, higher-risk path** behind isolation of naturally obligately lytic phages, not as a headline deliverable, because its clinical maturity for staphylococci is limited.

Approach

Rigor, reproducibility, and biological variables

All phages and isolates are authenticated by whole-genome sequencing and archived as a versioned, re-orderable bank (key-resource authentication). Ex vivo assays are pre-registered with defined replicate counts, blinded enumeration, and prespecified log-reduction thresholds and antagonism definitions. Sex is recorded for every isolate-source patient and treated as a biological variable in analysis; the clinical cohort will not exclude by sex and will report outcomes disaggregated where numbers permit. Negative and antagonistic results (the Molendijk-type outcome) are reported, not suppressed.

Aim 1 — Patient-derived anti-staphylococcal phage bank and rapid host-matching pipeline

Rationale. Effective personalized therapy requires a library of well-characterized lytic phages covering the staphylococcal strains that actually infect LVADs, plus a fast, reproducible way to match a phage or cocktail to a new isolate. Pitton 2025 established that phages active against device staphylococci can be isolated in the LVAD-patient setting; we standardize this into a US bank around banked clinical isolates.

Experimental design. We will assemble a biorepository of *S. aureus* and *S. epidermidis* isolates from explanted/infected devices and exit-site cultures. Against these we will isolate lytic phages from environmental and existing-library sources, purify them, and perform whole-genome sequencing to confirm obligately lytic lifestyle and to exclude integrase/lysogeny, toxin, and AMR genes. Temperate-but-promising candidates may be engineered using CRISPR-Cas to remove integrase genes (secondary path). We will define a host-range/efficiency-of-plating panel and a target receipt-to-recommendation turnaround time [ILLUSTRATIVE].

Expected outcomes. A sequenced, host-characterized anti-staphylococcal phage bank; a validated rapid host-matching assay; and a documented set of integrase-free lytic candidates.

Potential pitfalls & alternatives. Some isolates may lack a matching phage; we mitigate with cocktail design, continued library expansion, and sourcing from established libraries used in compassionate cardiac-device cases. If CRISPR engineering reduces fitness, we prioritize naturally lytic phages.

Aim 2 — Phage–antibiotic optimization on explanted driveline biofilm

Rationale. The strongest staphylococcal anti-biofilm signal pairs phage with rifampicin, but synergy is strain- and sequence-dependent and not universal — Molendijk 2025 found no benefit and possible antagonism against *S. aureus*, with worsened growth when phage was mistimed. Determining **when combinations help versus harm** is decisive for safe clinical use and is the program's pivotal experiment.

Experimental design. Using explanted LVAD driveline material as the ex vivo biofilm substrate (after Pitton 2025), we will grow patient-strain biofilms and quantify viable biofilm burden (log reduction) under phage alone, rifampicin alone, phage + rifampicin (co-administered and in both sequences), and additional clinically relevant antibiotics. We will systematically vary phage dose, antibiotic concentration, and relative timing/sequence, benchmarking against the ~1.9-log reduction reported for vB_SepS_BE22 + rifampicin against *S. epidermidis* (Pitton 2025) and against the antagonism conditions reported by Molendijk 2025. Resistant/escape-mutant emergence is tracked and sequenced.

Expected outcomes. A strain-resolved map of synergy, additivity, and antagonism; explicit dose/sequencing rules that maximize biofilm killing while avoiding the antagonism and mistiming hazards; and go/no-go criteria for advancing specific cocktail–antibiotic pairings to Aim 3.

Potential pitfalls & alternatives. Ex vivo biofilm is heterogeneous; we standardize segment handling and replicate across isolates. If phage + rifampicin is antagonistic for a strain, that pairing is excluded and alternative anti-biofilm regimens or phage-only local therapy are evaluated. A predominantly antagonistic landscape is itself a publishable, field-shaping result and would redirect, not end, the program.

Aim 3 — IND-enabling package and prospective feasibility cohort under a single study IND

Rationale. Human experience to date is limited to single-patient compassionate use of local adjunctive phage plus antibiotics (Aslam 2019) and a phage-cocktail galenic alongside surgery (Rojas 2022). These motivate but cannot substitute for a prospectively designed, uniformly monitored cohort under one protocol — the necessary next US step and the structure NHLBI/FDA expect before a randomized trial.

Experimental design. A small number [ILLUSTRATIVE] of consenting transplant-ineligible, surgically high-risk adults with confirmed staphylococcal LVAD infection will receive an Aim 1 host-matched lytic cocktail paired with an Aim 2–optimized antibiotic regimen, delivered locally (including a viscous/slow-release galenic to the pocket or driveline tract, modeled on Rojas 2022 and

Aslam 2019) as an adjunct to standard surgical debridement and systemic antibiotics. The work includes assembly of a full IND-enabling package (CMC/purity, endotoxin, stability, preclinical safety bridging from Aims 1–2) supporting **a single protocol-level study IND**, not a series of emergency single-patient INDs. Predefined endpoints [ILLUSTRATIVE] include safety/tolerability, microbiologic response, infection-related and device-retention outcomes, with optional microbiome tracking.

Expected outcomes. Demonstrated feasibility and a structured safety/microbiologic dataset to power a future randomized trial; refined local-delivery and dosing protocols.

Potential pitfalls & alternatives. This rare, high-acuity population enrolls slowly; we engage multiple implanting centers and prespecify a realistic accrual window. Phage neutralization or non-response may occur; cocktail re-matching and dose adjustment are prespecified. The study is explicitly adjunctive and is not designed or powered to replace surgery or antibiotics.

Timeline

[ILLUSTRATIVE] **Years 1–2:** Aim 1 isolate biobanking, phage isolation/sequencing, optional engineering, host-matching assay validation. **Years 2–4:** Aim 2 ex vivo driveline biofilm mapping (overlapping late Aim 1). **Years 3–5:** Aim 3 IND-enabling package and study-IND/IRB submissions beginning Year 3, with prospective adjunctive treatments and follow-up in Years 4–5. Annual go/no-go reviews gate progression bank → ex vivo → clinical; an antagonism-dominated Aim 2 redirects rather than advances.

Budget Justification (modular R01-style sketch)

[ILLUSTRATIVE] A standard modular R01 budget is requested. **Personnel:** PI (cardiology/infectious disease/cardiac surgery), co-investigator phage biologist, microbiology/biofilm technologists, a regulatory/clinical research coordinator for the study IND and IRB, and biostatistician effort. **Aim 1:** isolation reagents, whole-genome sequencing, optional CRISPR engineering, GMP-oriented purification consultation. **Aim 2:** explanted driveline acquisition/handling, biofilm assays, rifampicin and comparator antibiotics, escape-mutant sequencing. **Aim 3:** clinical-grade cocktail preparation, galenic formulation, IND-enabling CMC/safety studies, regulatory costs, monitoring, and microbiologic/microbiome assays. All dollar figures, module counts, and effort percentages are [ILLUSTRATIVE] and to be finalized with institutional budgeting.

Vertebrate Animals

Not applicable as a primary program. The proposed work uses banked bacterial isolates, bacteriophages, explanted human LVAD driveline material, and consenting human subjects. Should IND-enabling safety bridging require a limited in vivo study, a full vertebrate-animals section and IACUC approval will be added by amendment; no vertebrate animal work is proposed in the current scope.

Human Subjects / Clinical Trial

Aims 1 and 3 involve human-derived material and human subjects. Aim 3 will proceed under **a single protocol-level FDA Investigational New Drug (study IND)** for the investigational phage product, with institutional IRB approval, documented informed consent, predefined safety monitoring and stopping rules, and adverse-event reporting; this design is deliberately distinct from the single-patient emergency-IND route used in prior compassionate cardiac-device cases, in order to yield interpretable prospective data. Enrollment and endpoints are [ILLUSTRATIVE] and limited to a feasibility scale appropriate to this rare, high-acuity population; the intervention is adjunctive to standard surgical and antibiotic care. The cohort will not exclude by sex and will report sex-disaggregated outcomes where numbers permit. Explanted driveline material and clinical isolates used in Aims 1–2 will be obtained under appropriate human-tissue consent/IRB provisions.

Team & Environment

This program requires a multidisciplinary team [names/institutions to be supplied]: a **Contact PI** with combined expertise in mechanical circulatory support and cardiovascular-device infection; a **Co-Investigator in phage biology/microbiology** (isolation, sequencing, optional engineering); a **Cardiac surgeon / LVAD program director** providing explanted driveline material and surgical co-management; an **Infectious-diseases physician** experienced in device infection and antibiotic optimization; a **Regulatory/clinical-trials lead** to manage the study IND and IRB; a **Biostatistician**; and a **clinical-grade phage manufacturing collaborator**. The environment must include an active LVAD implant program with sufficient infected-device volume, a BSL-appropriate phage laboratory with sequencing access, and an established expanded-access/clinical phage pathway. Established programs in this space (e.g., UC San Diego IPATH; Inselspital/University Hospital Bern; Erasmus MC Rotterdam) illustrate the collaborative, host-matching model this team would engage and emulate.

References

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