Phage-Driven Daptomycin Resensitization to Cure Vancomycin-Resistant *Enterococcus faecium* Bacteremia in Immunocompromised Hosts

Project Summary / Abstract

Vancomycin-resistant *Enterococcus faecium* (VRE) bacteremia is a leading hospital-acquired bloodstream infection in neutropenic, transplant, and critically ill patients, and it carries high mortality because therapeutic options are narrow. The frontline agent daptomycin is increasingly undermined by daptomycin-nonsusceptible strains, and VRE forms catheter and endovascular biofilms that shield it from antibiotics. Once daptomycin resistance emerges in an endovascular or line-associated infection, no reliably bactericidal regimen remains.

Lytic bacteriophages kill *E. faecium* independently of its antibiotic-resistance mechanisms, self-amplify at the infection site, penetrate biofilm, and—critically—impose selective pressure for loss of surface receptors that can simultaneously restore daptomycin susceptibility ("collateral resensitization"). In a recent ex vivo simulated endocardial-vegetation model, a defined phage cocktail combined with daptomycin and ceftaroline resensitized daptomycin-nonsusceptible *E. faecium* and prevented phage resistance (Kunz Coyne et al., 2023). A 2024 clinical case of recurrent VRE bacteremia showed human feasibility but also exposed the principal limitation—neutralizing anti-phage antibody that coincided with relapse after several months (Stellfox et al., 2024).

We will convert these observations into a defined, mechanism-anchored preclinical regimen. We will (1) assemble and characterize a multi-phage cocktail against a contemporary U.S. VRE bloodstream-isolate panel and define the receptor-loss genetics that drive daptomycin resensitization; (2) optimize phage–antibiotic synergy and resistance suppression in high-inoculum, biofilm, and simulated endocardial-vegetation PK/PD models; and (3) establish in vivo efficacy and the kinetics of neutralizing anti-phage antibody in a neutropenic murine VRE bacteremia model to define a time-limited, rotation-ready dosing strategy. The work builds directly on a 2002 murine proof of concept (Biswas et al., 2002), 2023 ex vivo synergy data, and the 2024 human case, and is designed to generate the rigorous preclinical package required for a future expanded-access/IND clinical

evaluation.

Specific Aims

VRE bacteremia in immunocompromised hosts is frequently caused by daptomycin-nonsusceptible *E. faecium* for which no reliably bactericidal regimen exists. Three independent lines of evidence motivate a phage-based solution. First, a single intraperitoneal dose of phage ENB6 rescued 100% of mice from lethal VRE bacteremia, and ~50% even when treatment was delayed to a moribund state (Biswas et al., 2002). Second, defined phage cocktails plus daptomycin and ceftaroline restore daptomycin susceptibility and achieve bactericidal killing of daptomycin-nonsusceptible *E. faecium* in a simulated endocardial-vegetation PK/PD model, while preventing phage resistance (Kunz Coyne et al., 2023). Third, a 2024 case of recurrent VRE bacteremia tracked over seven years demonstrated human feasibility—several months of clinical improvement and reduced intestinal VRE burden—until a neutralizing anti-phage antibody response coincided with relapse (Stellfox et al., 2024). Our central hypothesis is that a defined enterococcal phage cocktail, paired with daptomycin (± ceftaroline) and dosed within the pre-immune window, converts daptomycin-resistant VRE bacteremia from an untreatable to a curable infection while suppressing the emergence of both phage and antibiotic resistance.

Aim 1. Build and characterize a defined enterococcal phage cocktail against contemporary U.S. VRE bloodstream isolates, and define the genetics of daptomycin resensitization. We will screen lytic enterococcal phages (e.g., ENB6, the NV-497/NV-503-01 lineage, and clinical siphoviruses including Φ9184 and ΦHi3) against a banked panel of VRE bacteremia isolates, quantify host range and receptor usage, and select a complementary cocktail. We will whole-genome-sequence phage-resistant escape mutants to map surface-receptor/capsule changes and test the prediction that these changes restore daptomycin susceptibility. *Milestone/go-no-go:* a ≥2-phage cocktail covering a majority of the panel with confirmed daptomycin resensitization gates entry into in vivo work.

Aim 2. Optimize phage–antibiotic synergy and resistance suppression in PK/PD models. Using high-inoculum time-kill, catheter-biofilm, and 96-h simulated endocardial-vegetation ex vivo models with humanized antibiotic exposures, we will identify cocktail-plus-daptomycin (± ceftaroline) regimens that maximize bactericidal killing and minimize emergence of phage-resistant and daptomycin-resistant subpopulations relative to monotherapies. *Milestone:* ≥2-$\log_{10}$ killing advantage over the best monotherapy with suppressed resistant subpopulations in the vegetation model.

Aim 3. Establish in vivo efficacy and define anti-phage immunity kinetics. In a neutropenic murine VRE bacteremia model we will test the lead cocktail with and without daptomycin/ceftaroline for survival and bacterial clearance, and serially quantify neutralizing anti-phage antibody and phage pharmacokinetics to define the effective window and test whether phage rotation preserves activity.

Milestone: survival/clearance superiority of the combination over monotherapy, and a quantitative antibody-onset timeline informing dosing.

Impact: Success would provide the rigorous preclinical foundation for deploying phages as a daptomycin resensitizer—turning untreatable daptomycin-resistant VRE bacteremia, endocarditis, and line infections back into curable disease while blocking further resistance evolution.

Significance

VRE *E. faecium* bacteremia disproportionately strikes hematology-oncology, transplant, and ICU patients, in whom intestinal VRE domination seeds the bloodstream and mortality is high because the therapeutic margin is thin. Daptomycin and linezolid are the mainstays, but daptomycin-nonsusceptible strains are increasingly encountered, and biofilm on catheters, valves, and prosthetic material further protects the organism. There is no reliably curative regimen once daptomycin resistance emerges in an endovascular or line-associated infection—a gap made vivid by the 2024 case of recurrent VRE bacteremia that persisted across seven years and multiple antibiotic regimens before phage therapy was attempted (Stellfox et al., 2024).

Phages address this gap through a mechanism orthogonal to antibiotic resistance. Lytic enterococcal phages adsorb to surface receptors (the enterococcal polysaccharide antigen or wall teichoic acids) and lyse the cell regardless of vancomycin or daptomycin resistance; they self-amplify at the infection site and degrade biofilm matrix to reach embedded cells. The strain specificity of phages, which spares the gut microbiome, is a further advantage in precisely the patients whose VRE arises from intestinal domination (Stellfox et al., 2024). The unmet need is underscored by the therapeutic pipeline: the only registered enterococcal phage trial to date targets a chronic periprosthetic joint infection (NCT06942624), not the far deadlier problem of bacteremia. Establishing a defined, reproducible cocktail and its resensitization genetics would move enterococcal phage therapy from one-off compassionate use toward a generalizable, evidence-based treatment.

Innovation

This proposal advances three innovations beyond the current literature.

- **Phages as daptomycin resensitizers, not just lytic agents.** We exploit collateral sensitivity—the receptor/capsule loss that confers phage escape simultaneously restoring daptomycin susceptibility—to design a combination that is mutually resistance-suppressing. This mechanism has been observed ex vivo (Kunz Coyne et al., 2023) but has not been developed into a defined regimen with mapped, isolate-level genetics.

- **A contemporary, niche-faithful preclinical bridge.** We pair a panel of contemporary U.S. VRE bloodstream isolates with high-inoculum, biofilm, and simulated endocardial-vegetation PK/PD models, bridging the 2002 murine proof of concept (Biswas et al., 2002) and the 2024 human case (Stellfox et al., 2024) that the field currently lacks.
- **Anti-phage immunity treated as a design variable.** Rather than treating neutralizing antibody as an afterthought, we quantify its kinetics in vivo to engineer earlier, time-limited courses and pre-staged phage rotation—directly targeting the failure mode that ended the 2024 human response.

Engineered and CRISPR-Cas phage approaches, while promising for broadening host range, remain preclinical for *Enterococcus* and are out of scope; we deliberately prioritize a defined natural-phage cocktail realistic to advance toward the clinic.

Approach

Rigor and reproducibility (applies to all aims). Key biological resources (phage stocks, bacterial isolates) will be authenticated by whole-genome sequencing and periodically re-verified; phage preparations will be purified and endotoxin-tested to translationally relevant limits. Experiments will use ≥3 independent biological replicates, pre-specified endpoints, randomized treatment assignment, and blinded enumeration where feasible. In vivo studies will include both sexes and analyze sex as a biological variable; group sizes are set by a priori power analysis. Statistical plans (survival by log-rank; longitudinal burden by mixed-effects models; resistant-subpopulation frequencies by appropriate count models) are developed with the biostatistics core.

Aim 1 — Cocktail assembly, host range, and resensitization genetics

Rationale. Phage adsorption is strain-specific, so durable coverage requires a complementary multi-phage cocktail; and the central therapeutic hypothesis—that receptor/capsule loss restores daptomycin susceptibility—must be established genetically in contemporary isolates.

Experimental design. We will characterize well-defined lytic enterococcal phages (ENB6; the NV-497/NV-503-01 lineage; clinical siphoviruses including Φ9184 and ΦHi3) against a banked collection of U.S. VRE bacteremia isolates spanning daptomycin-susceptible and daptomycin-nonsusceptible phenotypes. Host range will be quantified by efficiency-of-plating and planktonic growth-kinetic killing assays. Phage-resistant escape mutants will be selected and whole-genome-sequenced to identify surface-receptor and capsule changes; matched parental/escape pairs will be tested for daptomycin (and ceftaroline) MIC shifts to confirm resensitization. Cocktails will be composed to maximize panel coverage and receptor complementarity (limiting shared escape routes).

Expected outcomes. A defined 2–4-phage cocktail covering the majority of the isolate panel, a catalogue of escape mutations, and direct genetic evidence that phage escape carries a daptomycin-susceptibility trade-off.

Potential pitfalls & alternatives. Some isolates may resist all available phages; we will expand sourcing through established phage banks and enrich for new phages against non-covered strains. If resensitization is inconsistent, we will prioritize phages whose receptors are mechanistically linked to daptomycin susceptibility and stratify analyses by receptor class. If escape mutants are unfit, we will confirm trade-offs in competition assays rather than over-interpreting MIC alone.

Aim 2 — PK/PD optimization of phage-antibiotic synergy

Rationale. Bacteremia involves high inoculum and biofilm-protected, often endovascular, foci; efficacy and resistance suppression must be demonstrated under conditions that mimic these niches, consistent with prior ex vivo synergy findings (Kunz Coyne et al., 2023).

Experimental design. The lead cocktail will be tested alone and combined with daptomycin (± ceftaroline) in high-inoculum time-kill assays, static and dynamic catheter-biofilm models, and a 96-h simulated endocardial-vegetation ex vivo model using humanized antibiotic exposures. Primary endpoints are the magnitude and durability of bactericidal killing and the emergence of phage-resistant and daptomycin-resistant subpopulations, benchmarked against each monotherapy. Surviving populations will be sequenced to link the resistance/resensitization genotypes from Aim 1 to PK/PD outcomes.

Expected outcomes. Cocktail-plus-daptomycin (± ceftaroline) regimens that achieve sustained bactericidal killing in biofilm and vegetation models while suppressing resistance more effectively than monotherapy.

Potential pitfalls & alternatives. Phage–antibiotic ordering/timing may matter; we will test sequential versus simultaneous exposures. If ceftaroline adds little in a given isolate class, daptomycin-plus-phage will be advanced for that class. If dynamic-model phage titers fall through adsorption/decay, we will adjust dosing intervals informed by Aim 3 pharmacokinetics.

Aim 3 — In vivo efficacy and anti-phage immunity kinetics

Rationale. A single phage dose previously rescued 100% of mice from lethal VRE bacteremia (Biswas et al., 2002), but the 2024 human case showed neutralizing anti-phage IgG curtails benefit after several months (Stellfox et al., 2024); because our target population is immunocompromised, defining this window in a neutropenic host is essential to dosing strategy.

Experimental design. Using a neutropenic (e.g., cyclophosphamide-conditioned) murine VRE bacteremia model that reflects the at-risk population, we will compare vehicle, cocktail alone, daptomycin/ceftaroline alone, and the combination, with survival and quantitative blood/tissue bacterial burden as endpoints. In parallel immunocompetent and repeat-dosing arms, we will serially quantify neutralizing anti-phage antibody and phage pharmacokinetics to define the effective window and test whether pre-staged phage rotation (from Aim 1) preserves activity. Both sexes are included; enumeration is blinded; sample sizes follow power analysis.

Expected outcomes. Demonstration that the combination improves survival and clearance over monotherapy, and a quantitative timeline of neutralizing-antibody onset that informs earlier, time-limited, rotating regimens.

Potential pitfalls & alternatives. Murine immune kinetics may not fully predict humans; results will be framed as relative timing to motivate early dosing rather than absolute thresholds. Neutropenic conditioning may blunt antibody responses—an informative comparison against immunocompetent arms rather than a confound. If single-cocktail efficacy wanes, pre-staged rotation cocktails will be evaluated.

Timeline

[ILLUSTRATIVE] **Year 1:** Aim 1 host-range screening, cocktail assembly, escape-mutant sequencing and resensitization mapping. **Go/no-go (end of Year 1):** a defined cocktail covering a majority of the panel with confirmed daptomycin resensitization gates entry into in vivo work. [ILLUSTRATIVE] **Years 2–3:** Aim 2 PK/PD, biofilm, and vegetation optimization; begin Aim 3 efficacy studies. [ILLUSTRATIVE] **Years 4–5:** complete Aim 3 immunity-kinetics and rotation studies; integrate datasets into a preclinical package for an expanded-access/IND pathway.

Budget Justification (modular R01-style sketch)

[ILLUSTRATIVE] Request of [ILLUSTRATIVE] $250,000 direct costs per year for [ILLUSTRATIVE] 5 years. Personnel: PI ([ILLUSTRATIVE] 2.4 calendar months), co-investigators in clinical microbiology and PK/PD, [ILLUSTRATIVE] 2 postdoctoral scientists, [ILLUSTRATIVE] 1 research technician, and biostatistics support. Other: whole-genome sequencing, daptomycin/ceftaroline and media, biofilm/simulated-vegetation model consumables, endotoxin testing and phage purification, vertebrate-animal per-diems and immunoassays, and phage-bank/isolate access fees. Equipment is largely in place. A standard NIH modular justification will accompany the budget pages.

Vertebrate Animals

Animal work is proposed (Aim 3). A neutropenic murine VRE bacteremia model will assess survival, bacterial clearance, and anti-phage antibody kinetics, building on the established bacteremia model of Biswas et al. (2002). The application will address the required vertebrate-animal points: (1) description and justification of species (mouse) and proposed numbers, set by power analysis to minimize animal use ([ILLUSTRATIVE] numbers to be finalized); (2) scientific justification for use, including why in vitro/ex vivo models in Aims 1–2 cannot substitute for host immunity and pharmacokinetics; (3) minimization of pain and distress via humane endpoints (moribundity-triggered euthanasia), appropriate analgesia/anesthesia, and monitoring; and (4) euthanasia consistent with current AVMA Guidelines. Both sexes will be studied. All procedures will undergo IACUC review and approval at an AAALAC-accredited facility.

Human Subjects / Clinical Trial

Not applicable to the funded scope—this R01 is preclinical and involves no human-subjects intervention. Banked, de-identified VRE clinical isolates will be used under appropriate institutional human-subjects determination (anticipated non-human-subjects / exempt). We note the intended translational path: investigational phage therapy for VRE bacteremia currently proceeds in the U.S. as personalized/compassionate use, and a future first-in-patient evaluation would be pursued under an FDA emergency/expanded-access investigational new drug (eIND) route with full IRB oversight and informed consent. The only registered enterococcal phage trial to date targets a chronic *E. faecium* periprosthetic joint infection (NCT06942624), not bacteremia—underscoring the unmet need this preclinical package is designed to address.

Team & Environment

- **Principal Investigator [NAME, INSTITUTION]** — enterococcal phage biology and clinical phage characterization (model role: Van Tyne Lab, University of Pittsburgh).
- **Co-Investigator, PK/PD [NAME, INSTITUTION]** — phage–antibiotic synergy and simulated endocardial-vegetation modeling (model role: Anti-Infective Research Laboratory, Wayne State University).
- **Co-Investigator, Phage Biology [NAME, INSTITUTION]** — enterococcal phage receptor biology (model role: Duerkop Lab, University of Colorado).
- **Phage Bank / Compassionate-Use Liaison [NAME, INSTITUTION]** — phage sourcing and translational coordination (model role: IPATH, UC San Diego; U.S. Naval Medical Research Center).
- **Biostatistician and Clinical Microbiology Core [NAMES]** — study design, power analysis,

and isolate banking.

Environment: BSL-2 microbiology and molecular facilities, genomics/sequencing core, dynamic PK/PD modeling capability, and an AAALAC-accredited animal facility. **Alternate funders:** NCI (hematology-oncology VRE population) and BARDA (antibacterial-resistance/medical-countermeasure development).

References

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2. Biswas B, Adhya S, Washart P, Paul B, Trostel AN, Powell B, Carlton R, Merril CR. Bacteriophage therapy rescues mice bacteremic from a clinical isolate of vancomycin-resistant *Enterococcus faecium*. *Infection and Immunity.* 2002;70(1):204-210. PMID: 11748184. https://doi.org/10.1128/IAI.70.1.204-210.2002

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