Mechanism-Anchored Optimization of Adjunctive Intravenous Bacteriophage Therapy for Complicated MRSA Bacteremia

Project Summary / Abstract

Methicillin-resistant *Staphylococcus aureus* (MRSA) bacteremia remains among the deadliest common bloodstream infections, with 30-day mortality of roughly 20–30% despite optimal antibiotics. Failure is driven less by initial drug susceptibility than by *durable clearance*: metastatic seeding (endocarditis, vertebral osteomyelitis, prosthetic devices), persistent or relapsing bacteremia, biofilm on catheters and implants, and rising tolerance to vancomycin and daptomycin. Lytic bacteriophages are mechanistically suited as an adjunct: they self-amplify at the infection site, kill by a route orthogonal to antibiotics, penetrate biofilm, can act on metabolically dormant persisters, and can be delivered intravenously as a defined cocktail that broadens host range and raises the genetic barrier to resistance. This rationale is no longer purely hypothetical. First-in-human data from the Westmead single-arm study of IV AB-SA01 showed no phage-attributable adverse reactions and no detected in vivo resistance in critically ill *S. aureus* bacteremia patients, and Armata's randomized, placebo-controlled diSArm Phase 2a study of IV AP-SA02 plus best available antibiotic therapy (BAT) reported a day-12 clinical response of 88% versus 58% (p=0.047) in a small cohort. This proposal does **not** duplicate that efficacy signal; it dissects *why and when* adjunctive phage works so the approach can be deployed rationally. We will (1) define the in vitro determinants of phage–antibiotic synergy (PAS) and resistance suppression against contemporary MRSA bloodstream isolates; (2) characterize intra-cocktail variant adaptation and biofilm/persister killing on device-relevant surfaces; and (3) link these mechanistic readouts to outcomes in a small adjunctive-phage cohort treated under an FDA expanded-access / emergency IND (eIND) pathway with IRB oversight. All key biological resources will be authenticated, and analyses are powered for the precision they require. The expected outcome is a validated, mechanism-anchored framework for phage-susceptibility matching and PAS-informed dosing that de-risks pivotal trials and supports rational use of phage as the first new mechanistic class for *S. aureus* bacteremia in decades.

Specific Aims

Complicated MRSA bacteremia frequently relapses because antibiotics fail to clear biofilm-associated and antibiotic-tolerant persister populations. Adjunctive lytic phage therapy is now supported by an early randomized signal (diSArm Phase 2a: 88% vs 58% day-12 response, p=0.047, small cohort), but the mechanistic and pharmacodynamic rules that govern *which* patients benefit, *which* phage–antibiotic pairings synergize, and *how* a fixed cocktail adapts to an individual isolate remain undefined. Without those rules, phage will continue to be used empirically. We will close this gap.

Aim 1. Define the in vitro determinants of phage–antibiotic synergy and resistance suppression against contemporary MRSA bloodstream isolates. Using an authenticated panel of clinical MRSA bacteremia isolates, we will quantify host range of a *Staphylococcus* phage K–related myovirus cocktail (modeled on AB-SA01/AP-SA02), map PAS with standard-of-care agents (vancomycin, daptomycin, sub-lethal beta-lactams), and measure the frequency and fitness cost of phage-resistant escape mutants under mono- versus combination pressure. *Go/no-go:* cocktail covers ≥70% of the panel and at least one antibiotic pairing reproducibly lowers escape-mutant frequency ≥10-fold.

Aim 2. Characterize intra-cocktail variant adaptation, biofilm penetration, and persister killing on device-relevant surfaces. We will test whether a minor defined phage variant within the cocktail can expand to dominance against a given isolate (strain self-tuning), and quantify killing of biofilm and antibiotic-tolerant persisters on catheter- and prosthetic-relevant substrates, with and without antibiotics.

Aim 3. Link mechanistic readouts to clinical outcomes in an adjunctive-phage cohort under an expanded-access / emergency IND. In a small prospective cohort receiving IV phage plus BAT under eIND with IRB oversight, we will correlate baseline phage-susceptibility matching and PAS profiles with microbiologic clearance, relapse, and safety, prospectively testing the predictors derived in Aims 1–2.

Impact. By converting an early efficacy signal into validated, mechanism-anchored decision rules for phage matching and PAS-informed dosing, this work provides the scientific foundation to deploy adjunctive phage rationally and to design the next generation of pivotal MRSA bacteremia trials.

Significance

MRSA bacteremia carries 30-day mortality of roughly 20–30% even with optimal antibiotic therapy, and it is a named priority pathogen for antibacterial-resistance therapeutic development squarely within NIAID's mission. The clinical problem is not primarily initial bacterial susceptibility but *durable clearance*: metastatic seeding to heart valves, vertebrae, and prosthetic devices; persistent and

recurrent bacteremia; biofilm on indwelling hardware; and rising tolerance to vancomycin and daptomycin. These are precisely the niches where antibiotics stall, because biofilm-embedded and metabolically dormant persister cells survive drug exposure that would kill replicating organisms.

Lytic bacteriophages address this failure mode through an orthogonal mechanism. They self-amplify at the site of infection in an auto-dosing fashion, retain activity against multidrug-resistant and antibiotic-tolerant cells, can penetrate biofilm, and can be formulated as fixed cocktails to broaden host range and suppress resistance. The field has matured from compassionate-use case reports to early randomized evidence: the Westmead first-in-human study established IV safety of AB-SA01 in severe *S. aureus* infection including bacteremia, endocarditis, and septic shock, with no phage-attributable adverse reactions and no detected in vivo resistance; and the diSArm Phase 2a study provided the first randomized controlled signal of efficacy in complicated *S. aureus* bacteremia. AP-SA02 carries FDA Fast Track designation for MSSA/MRSA bacteremia, and a larger confirmatory program has been announced.

What the field lacks is the mechanistic and pharmacodynamic understanding to use phage *rationally* rather than empirically: which isolates are best matched, which antibiotic pairings synergize, and how cocktail composition behaves against an individual strain. The diSArm signal, while encouraging, comes from a small cohort and a fragile p-value; translating it responsibly requires understanding the mechanism beneath it. Filling this gap is the rate-limiting science for converting a promising signal into a durable, broadly deployable treatment for one of infectious disease's most lethal everyday problems.

Innovation

This proposal is innovative in three respects. First, rather than re-testing efficacy, it isolates the *mechanistic predictors* of adjunctive phage success — PAS profiles, escape-mutant frequency, and biofilm/persister killing — and prospectively validates them against clinical outcomes, an approach not yet undertaken for *S. aureus* bacteremia. Second, it interrogates a striking observation reported from the AP-SA02 program — that a minor (~2%) defined phage variant may expand to dominance when challenged with a patient's isolate — and asks, under controlled conditions, whether this intra-cocktail adaptation is a measurable, exploitable correlate of response; we treat this as a hypothesis to be tested rather than an established mechanism. Third, it builds a translational bridge from bench pharmacodynamics to bedside via the FDA expanded-access / emergency IND route for investigational phage, generating mechanism-linked human data without the cost and timeline of a registrational trial. Together these elements move phage therapy for *S. aureus* bacteremia from "it worked in a trial" toward "we know why, for whom, and with which antibiotic."

Approach

Rigor of prior research and key-resource authentication

The rationale rests on two human datasets (Westmead AB-SA01; diSArm AP-SA02) and one preclinical product-development report (AB-SA01), which we treat as hypothesis-generating, not confirmatory — diSArm in particular is a small Phase 2a cohort. All key biological resources will be authenticated: MRSA isolates by species confirmation (MALDI-TOF), methicillin-resistance genotyping (*mecA/mecC*), and short-read whole-genome sequencing with multilocus sequence typing; phage stocks by genome sequencing and identity/purity (endotoxin, titer) at each passage. Cocktail constituent proportions will be confirmed by deep sequencing before each experiment. Assays will be run with biological and technical replicates, blinded scoring where feasible, and pre-registered analysis plans. Where MRSA strain background or host sex of the source patient is recorded, both will be tracked as covariates (sex as a biological variable) in outcome models.

Aim 1 — In vitro determinants of phage–antibiotic synergy and resistance suppression

Rationale. Anti-staphylococcal therapeutic phages are obligately lytic myoviruses related to *Staphylococcus* phage K that adsorb to wall teichoic acid / cell-wall receptors; host range is strain-specific, motivating fixed three-phage cocktails. AB-SA01's three myoviruses lysed ~94.5% of 401 clinical *S. aureus* isolates in vitro, and sub-lethal beta-lactams and other agents can enhance phage replication/lysis while combination pressure suppresses resistance. We will quantify these properties against contemporary MRSA bacteremia isolates.

Phage source. A *Staphylococcus* phage K–related myovirus set modeled on AB-SA01/AP-SA02 will be obtained for research use through a curated therapeutic phage bank collaborator and/or the product developer under a material-transfer agreement [ILLUSTRATIVE]; GMP-grade product for Aim 3 is addressed separately (see Feasibility). Securing research-grade phage with documented genomes is a Year-1 milestone and a precondition for Aims 1–3.

Experimental design. Against an authenticated panel of clinical MRSA bloodstream isolates [ILLUSTRATIVE: n≈150, sized to estimate cocktail coverage to roughly ±8% at 95% confidence and to sample sequence-type and *agr*-type diversity], we will determine per-phage and cocktail host range by efficiency-of-plating and liquid lysis kinetics. PAS will be measured in checkerboard and time-kill formats pairing the cocktail with vancomycin, daptomycin, and sub-lethal beta-lactams. Resistance suppression will be assessed by quantifying the frequency of phage-resistant escape mutants under phage-alone versus phage-plus-antibiotic pressure, with whole-genome sequencing of escapees to identify receptor-pathway changes and growth-rate assays to measure fitness cost.

Expected outcomes. A quantitative map of cocktail host-range coverage of modern MRSA, identification of antibiotic pairings that maximize synergy, and evidence that combination pressure lowers escape-mutant frequency and selects costly, less-fit resistance.

Potential pitfalls & alternatives. Some isolates may be poorly covered by the fixed cocktail; we will catalog non-susceptibility patterns to inform host-range gaps rather than overstate coverage. If checkerboard synergy proves assay-sensitive, time-kill and replication-based readouts serve as orthogonal confirmation. If research-grade cocktail access is delayed, Aim 1 proceeds with the best-characterized available anti-staphylococcal myovirus set while MTA negotiations complete.

Aim 2 — Intra-cocktail variant adaptation, biofilm penetration, and persister killing

Rationale. A mechanistic observation reported from the AP-SA02 program is that a minor defined phage variant (~2% of product) can expand to dominance against a given patient's isolate, effectively self-tuning the cocktail. Combined with the capacity of phage to penetrate biofilm and act on persisters that tolerate antibiotics, this behavior may underlie durable clearance on devices and valves. We test it directly rather than assume it.

Experimental design. We will challenge the cocktail against individual MRSA isolates in serial passage and use amplicon/deep sequencing to track shifts in the relative abundance of defined cocktail constituents, testing whether minor variants expand in an isolate-specific manner. Biofilm and persister killing will be quantified on catheter- and prosthetic-relevant substrates [ILLUSTRATIVE materials] using established static and flow biofilm models, comparing phage alone, antibiotic alone, and the combination, with viable-count and metabolic readouts. Each condition will be run in replicate with pre-specified effect-size thresholds for "meaningful" biofilm/persister reduction.

Expected outcomes. Demonstration (or principled exclusion) of measurable, strain-dependent intra-cocktail adaptation; quantification of biofilm biomass reduction and persister killing by phage and by PAS beyond antibiotic alone; and identification of which mechanistic readouts most strongly distinguish effective from ineffective isolate–cocktail pairings.

Potential pitfalls & alternatives. Variant expansion may be modest or isolate-restricted; we will report the full distribution rather than assume universality, and a null result here is itself informative for cocktail design. If flow models are low-throughput, static microtiter biofilm assays provide screening-scale data with flow confirmation on a representative subset.

Aim 3 — Linking mechanism to outcomes under an expanded-access / emergency IND

Rationale. The diSArm Phase 2a signal (88% vs 58% day-12 response; small cohort) establishes plausibility but not mechanism. Prospectively testing whether bench predictors forecast clearance is the translational crux.

Experimental design. In a prospective adjunctive-phage cohort [ILLUSTRATIVE: n≈20–30, an explicitly hypothesis-generating correlative sample, not a powered efficacy comparison] of complicated MRSA bacteremia receiving IV phage plus BAT under an FDA expanded-access / emergency IND with IRB oversight, baseline isolates will undergo Aim 1–2 profiling (host-range match, PAS, predicted biofilm/persister killing). Pre-specified analyses will correlate these mechanistic predictors with microbiologic clearance, relapse through follow-up, hospital length of stay, and phage-attributable safety, mirroring endpoints used in the prior randomized and single-arm trials. Predictor–outcome associations will be reported with effect sizes and confidence intervals; given the sample, inference is estimation-focused rather than hypothesis-rejecting.

Expected outcomes. Preliminary evidence on whether mechanism-anchored matching predicts response, plus added safety experience to compare against the no-phage-attributable-toxicity profile of prior IV studies, yielding effect-size estimates and decision rules to power a future trial.

Potential pitfalls & alternatives. Expanded-access enrollment is opportunistic and small; this cohort is explicitly for predictor estimation, not powered efficacy. If enrollment lags, primary inference rests on Aims 1–2 and the cohort is reported descriptively. GMP-grade phage availability gates this aim (see Feasibility); the eIND timeline is built to follow, not precede, secured product.

Feasibility, Phage Sourcing, and Milestones

A common failure mode for translational phage proposals is unaddressed product access; we address it explicitly. **Research-grade phage** for Aims 1–2 will be secured under MTA from a curated therapeutic phage bank and/or the AB-SA01/AP-SA02 developer (Year-1 milestone). **GMP-grade phage** for the Aim 3 eIND cohort will be sourced through the same developer or an academic phage-therapy center with eIND experience; absent a GMP supply agreement, Aim 3 will not open, and the project will deliver its mechanistic core (Aims 1–2) plus a fully prepared regulatory package for later activation. Go/no-go milestones: (Y1) authenticated isolate panel assembled and research phage in hand; (Y2) Aim 1 coverage and resistance-suppression thresholds met; (Y3) Aim 2 readouts and GMP/eIND approvals secured; (Y4–5) Aim 3 correlative analyses and integrated predictor model.

Timeline

[ILLUSTRATIVE] Year 1: assemble and authenticate MRSA isolate panel, secure research-grade phage, establish host-range and PAS assays (Aim 1). Years 1–2: complete Aim 1 synergy and resistance mapping; begin Aim 2 adaptation and biofilm work. Years 2–4: complete Aim 2; secure GMP supply, eIND, and IRB approvals; initiate Aim 3 enrollment. Years 4–5: complete Aim 3 correlation analyses and integrate the mechanistic-predictor model.

Budget Justification

Modular R01 sketch [ILLUSTRATIVE], requested at [ILLUSTRATIVE: $250,000] direct costs/year for [ILLUSTRATIVE: 5 years]. **Personnel:** PI (microbiology/ID; [ILLUSTRATIVE: 2.4 calendar months]); Co-I infectious-diseases clinician for eIND/IRB and Aim 3 ([ILLUSTRATIVE: 1.2 months]); a phage biologist and a research technician for Aims 1–2 ([ILLUSTRATIVE: 12 months each]); a biostatistician ([ILLUSTRATIVE: 0.6 months]) for power/precision planning and analysis; and a study coordinator for Aim 3 ([ILLUSTRATIVE: part-time]). **Other costs:** clinical microbiology consumables; deep/whole-genome sequencing for isolate and escape-mutant authentication and cocktail-variant tracking; biofilm/flow-model supplies; MTA/GMP-grade phage product for cohort use; and regulatory/IRB submission costs. Equipment is assumed available through institutional cores. A precise modular budget will accompany the full application.

Vertebrate Animals

Not applicable. The proposed work uses clinical bacterial isolates, in vitro pharmacodynamic and biofilm models, and a human expanded-access cohort; no vertebrate animal studies are proposed.

Human Subjects / Clinical Trial

Aim 3 involves human subjects. Investigational phage will be administered under the FDA expanded-access / emergency IND (eIND) pathway — the established US route for case-by-case use of unapproved phage in serious, refractory infection — with prospective IRB approval, informed consent, and data-safety monitoring. Patients with complicated MRSA bacteremia will receive IV phage as an adjunct to best available antibiotic therapy, consistent with the dosing concept evaluated in prior IV studies (twice-daily IV phage in the Westmead study; IV AP-SA02 plus BAT in diSArm). Enrollment will not restrict by sex, race, or ethnicity; sex and relevant demographics will be recorded and examined as covariates. A pre-specified safety-monitoring plan will define phage-attributable adverse-event criteria and individual stopping rules, with periodic review by an independent monitor.

Endpoints — microbiologic clearance, relapse, length of stay, and phage-attributable safety — mirror those of the cited trials. This cohort is designed to estimate mechanistic predictors and add safety experience, not to serve as a registrational efficacy study; pivotal efficacy testing remains the domain of the announced larger program.

Team & Environment

[TEMPLATE — fill with real names/institutions.] **Contact PI:** [Name], molecular microbiology / phage biology, responsible for Aims 1–2 and overall integration. **Clinical Co-I / site PI:** [Name, ID physician] (model role: diSArm-style site leadership at an academic medical center with a high-volume *S. aureus* bacteremia population and prior eIND experience), responsible for eIND/IRB and Aim 3. **Co-I, phage product / regulatory:** [Name], experience modeled on the AB-SA01/AP-SA02 developer and an academic center for innovative phage applications, supporting MTA/GMP sourcing and eIND filings. **Biostatistician:** [Name], precision/power planning and correlative analysis. **Microbiology / sequencing core:** [Institution]. **Phage-bank collaborator:** [curated therapeutic phage bank] for research-grade material and genomes. Environment includes BSL-2 microbiology, biofilm/flow-model capacity, genomics cores, and an academic medical center with active *S. aureus* bacteremia enrollment and established eIND/IRB infrastructure.

References

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