Personalized Anti-*Staphylococcus aureus* Bacteriophage Cocktails as a Limb-Salvage Adjunct in Diabetic-Foot Osteomyelitis: From Characterized Cocktail to Biomarker-Informed Confirmatory Trial

Project Summary / Abstract

Diabetic-foot osteomyelitis (DFO) is a limb- and life-threatening complication of diabetic foot ulcers in which infection penetrates to bone, and it is a leading antecedent of nontraumatic lower-extremity amputation. *Staphylococcus aureus*, including methicillin-resistant strains (MRSA), is the single most common pathogen. Even with prolonged systemic antibiotics, DFO frequently fails to resolve because of poor vascular penetration to ischemic bone, dense biofilm on bone and necrotic tissue, intracellular and small-colony-variant (SCV) persistence, and rising antibiotic resistance — leaving amputation as a common fallback (Plumet et al., *J Virol* 2025). Bacteriophages are mechanistically suited to this problem: they self-amplify at the infection site, encode depolymerases and lysins that degrade biofilm matrix, and kill antibiotic-resistant and metabolically dormant *S. aureus* by mechanisms independent of antibiotics (Plumet et al., 2025). Clinical evidence has matured from compassionate use (Young et al., *Clin Ther* 2023) to a completed randomized controlled trial: the BX211 DANCE Phase 2b trial (NCT05177107) reported that personalized anti-*S. aureus* phage therapy plus standard of care was safe and produced a statistically significant, sustained reduction in ulcer surface area (percent area reduction p=0.046 at week 12), with the effect reported as most pronounced in patients whose ulcers extended to bone (BiomX press release, 31 March 2025).

This proposal converts that single positive Phase 2b signal into a reproducible, U.S.-based platform plus confirmatory efficacy data. We will (1) construct and characterize a sequenced, safety-screened, strictly lytic *Kayvirus*/*Silviavirus* cocktail and a rapid phage-susceptibility matching workflow against contemporary U.S. DFO isolates; (2) define phage–antibiotic interactions to rationally pair the cocktail with the antibiotics actually used in DFO; and (3) conduct a biomarker-informed, randomized, double-blind, placebo-controlled confirmatory trial of topical cocktail-plus-standard-of-care as a limb-salvage adjunct, prospectively enriched for bone-deep ulcers. The work is directly

responsive to NIDDK's diabetes-complications priorities and is designed to advance personalized phage therapy from proof-of-concept toward standard-of-care adjunctive use.

Specific Aims

DFO remains a leading cause of nontraumatic lower-extremity amputation. Antibiotics alone are insufficient against bone-deep, biofilm-laden, frequently resistant *S. aureus*, and the field lacks a transparent, reproducible, U.S.-based phage platform and confirmatory efficacy data. The DANCE Phase 2b trial established proof of concept (BiomX, 2025; NCT05177107). The **central hypothesis** of this proposal is that a well-characterized, biofilm-active anti-*S. aureus* phage cocktail — matched to each patient's isolate and rationally paired with standard antibiotics — will improve ulcer healing and limb salvage in bone-deep DFO.

Aim 1. Build and characterize a U.S. anti-*S. aureus* phage cocktail and rapid matching workflow. We will assemble a biobank of strictly lytic *Kayvirus* and *Silviavirus* phages, whole-genome sequence them to confirm lytic lifestyle and absence of toxin, virulence, and antibiotic-resistance genes, and quantify host range, biofilm penetration/degradation, and activity against SCVs across a contemporary panel of U.S. DFO *S. aureus* isolates (MSSA and MRSA). We will validate a phage-susceptibility test that returns an active cocktail within days of isolate recovery.

Aim 2. Define phage–antibiotic interactions relevant to DFO. Because phage–antibiotic synergy is real but not universal (Plumet et al., 2025), we will systematically test the cocktail against the antibiotics actually used in DFO (e.g., vancomycin, linezolid) in planktonic, biofilm, and osteomyelitis-relevant models to classify synergistic, neutral, and antagonistic pairings and define the preferred companion regimen and exposure sequence.

Aim 3. Conduct a biomarker-informed confirmatory trial of cocktail-plus-standard-of-care in bone-deep DFO. Under an FDA investigational pathway and IRB oversight, we will run a randomized, double-blind, placebo-controlled trial of topical personalized phage cocktail plus standard of care, prospectively enriched for ulcers extending to bone, with percent ulcer-area reduction (primary) and limb salvage (key secondary) as endpoints, governed by a pre-specified Go/No-Go decision rule.

Impact. Confirming and operationalizing the DANCE signal would establish personalized anti-*S. aureus* phage cocktails as a reproducible U.S. limb-salvage adjunct, converting many bone-deep diabetic foot infections from amputation candidates into salvage successes.

Significance

DFO is a defining complication of diabetes and a major driver of lower-extremity amputation, disability, cost, and premature mortality among the hundreds of millions of people worldwide living with diabetes. Reducing the burden of diabetes complications — including the diabetic foot — is squarely within NIDDK's mission. The therapeutic gap here is **mechanistic, not merely incremental**: standard systemic antibiotics are undermined in DFO by poor vascular penetration to ischemic bone, dense biofilm on bone and necrotic tissue, intracellular and small-colony-variant persistence, and escalating resistance including MRSA (Plumet et al., 2025). When antibiotics fail, amputation becomes the fallback, with its attendant loss of function and elevated downstream mortality.

Phage therapy addresses each of these failure modes through orthogonal biology. Phages self-amplify at the infection site, so effective dose rises where bacteria persist; *Kayvirus* and *Silviavirus* myoviruses encode depolymerases and lysins that disrupt the biofilm extracellular matrix and enable penetration into the shielded community on bone; and phage killing is independent of antibiotic-resistance status, reaching strains that defeat conventional drugs (Plumet et al., 2025).

The clinical evidence has matured from compassionate use to a completed RCT. A 2023 case series of high-amputation-risk diabetic-foot-infection patients given adjunctive topical anti-staphylococcal phage reported clinical benefit in most patients and limb salvage in a majority (Young et al., *Clin Ther* 2023). Most importantly, the BX211 DANCE Phase 2b trial (NCT05177107) randomized 41 patients and reported a statistically significant, sustained reduction in ulcer surface area (percent area reduction p=0.046 at week 12), with the effect reported as most pronounced in bone-deep ulcers (BiomX press release, 31 March 2025); the sponsor has reported planning a later-phase program pending FDA feedback.

A single positive Phase 2b, however, is not yet practice. The field needs (a) a transparent, U.S.-based, well-characterized cocktail and matching workflow that any qualified center could deploy; (b) rigorous data on which antibiotic pairings help versus do not; and (c) confirmatory efficacy in the bone-deep population where the signal was strongest. This proposal targets exactly those gaps, complementing rather than duplicating the sponsor program and ongoing European phage-therapy trials in this space.

Innovation

This work is innovative in four respects. First, it is **depth-/biomarker-informed by design**: rather than treating all DFO alike, we prospectively enrich for the bone-deep ulcers in which DANCE reported the strongest effect, aligning enrollment with the mechanism (biofilm on bone) that phages are best suited to attack. Second, it delivers a **transparent, characterized, U.S.-based cocktail and**

rapid susceptibility-matching workflow built on strictly lytic *Kayvirus*/*Silviavirus* genera, with genomes screened to exclude toxin, virulence, lysogeny, and resistance determinants — an academically reproducible counterpart to proprietary programs. Third, it treats **phage–antibiotic pairing as an empirical question**, systematically mapping synergy versus antagonism against the drugs actually used in DFO so the companion regimen is chosen by data, not assumption (Plumet et al., 2025). Fourth, it advances **topical, wound- and bone-directed delivery** as a practical limb-salvage adjunct layered onto standard care. CRISPR-armed and engineered/lysin-only phages are an active preclinical direction but are explicitly **out of scope**; we hold the clinical work to current evidence and natural lytic phages.

Approach

Rigor and reproducibility (cross-cutting). All assays are pre-registered with defined acceptance criteria, run with biological and technical replicates and blinded readout where feasible, and use authenticated, sequence-confirmed phage and bacterial stocks. *S. aureus* isolates are characterized for sex of the source patient where available, and Aim 3 is designed and analyzed to evaluate sex as a biological variable. Data, genome sequences, and protocols will be deposited in public repositories.

Aim 1 — Build and characterize a U.S. anti-*S. aureus* phage cocktail and rapid matching workflow

Rationale. Personalized phage therapy depends on matching active phages to each patient's isolate. *Kayvirus* and *Silviavirus* phages are favored for DFO because they are large, strictly lytic, and encode biofilm-disrupting depolymerases and lysins (Plumet et al., 2025). A U.S. biobank plus a fast, validated susceptibility test is the foundation for Aims 2–3.

Experimental design. We will (i) acquire/isolate strictly lytic anti-*S. aureus* phages of the target genera and whole-genome sequence each to confirm lytic lifestyle and absence of toxin, virulence, lysogeny, and antibiotic-resistance genes; (ii) assemble a contemporary panel of U.S. DFO *S. aureus* clinical isolates spanning MSSA, MRSA, and characteristic resistance phenotypes; (iii) quantify host range by spot and efficiency-of-plaquing assays to design a multi-phage cocktail that maximizes coverage and suppresses resistant mutants by requiring multiple receptors for escape; and (iv) measure biofilm penetration/degradation and SCV killing in established in vitro biofilm assays. We will lock a candidate cocktail composition and validate a phage-susceptibility-testing turnaround target of a few days from isolate recovery.

Expected outcomes. A sequenced, safety-screened phage bank; a defined cocktail with broad coverage of U.S. DFO isolates including MRSA; and a validated rapid matching assay supporting Aim 3.

Pitfalls & alternatives. Some isolates may be insensitive to the initial bank; we pre-specify a minimum coverage threshold and will expand phage diversity and propagation hosts to meet it. If resistant mutants emerge in vitro, cocktail composition will be re-optimized for multi-receptor escape. Endotoxin and purity standards for any in-human material will follow the investigational-product requirements established in Aim 3.

Aim 2 — Define phage–antibiotic interactions relevant to DFO

Rationale. Phage–antibiotic synergy is real but not universal: cocktails plus some antibiotics can outperform either agent alone, whereas other combinations show no added benefit (Plumet et al., 2025). Because all DFO patients receive standard antibiotics, the companion regimen must be chosen empirically.

Experimental design. Using the Aim 1 cocktail and isolate panel, we will quantify interactions with the antibiotics used in DFO (including vancomycin and linezolid) across (i) planktonic checkerboard/time-kill assays, (ii) biofilm-eradication assays on relevant substrates, and (iii) an osteomyelitis-relevant model incorporating bone or bone-mimetic matrix. Outcomes include bacterial-load reduction, biofilm biomass, resistance emergence, and order-of-exposure effects (phage-first vs concurrent). Interactions will be classified as synergistic, neutral, or antagonistic to nominate a preferred pairing and sequence.

Expected outcomes. A pairing map identifying which standard DFO antibiotics augment the cocktail and a recommended companion regimen and dosing sequence for the trial.

Pitfalls & alternatives. In vitro synergy may not predict in-bone behavior; the osteomyelitis-relevant model and conservative interpretation mitigate over-extrapolation. If antagonism is found with a clinically required antibiotic, the trial protocol will time phage administration to avoid the antagonistic window rather than excluding necessary therapy.

Vertebrate animals: if an in vivo osteomyelitis model is required to resolve in-bone pairing effects, it is addressed in the Vertebrate Animals section; the program relies on in vitro and ex vivo systems wherever they are sufficient.

Aim 3 — Biomarker-informed confirmatory trial of cocktail-plus-standard-of-care in bone-deep DFO

Rationale. DANCE reported safety and a significant, sustained ulcer-area reduction, most pronounced in bone-deep ulcers (BiomX, 2025; NCT05177107). A confirmatory, enrichment-designed trial is the logical next step toward practice.

Design. Randomized, double-blind, placebo-controlled, multi-site trial of personalized anti-*S. aureus* phage cocktail plus standard of care versus placebo plus standard of care — all patients receiving guideline-based standard-of-care antibiotics — prospectively enriched for ulcers extending to bone. Each enrolled isolate is matched to an active cocktail via the Aim 1 assay; the Aim 2 companion regimen guides antibiotic pairing. Delivery is topical into the wound and adjacent bone with weekly dosing through the treatment window, consistent with the topical adjunct route supported by the case-series and DANCE evidence (Young et al., 2023; BiomX, 2025).

Endpoints and analysis. A single confirmatory **primary endpoint** — percent ulcer-area reduction at end of treatment — is tested at two-sided $\alpha=0.05$ in the modified intention-to-treat population, with **limb salvage (avoidance of major amputation)** as the key secondary endpoint controlled for multiplicity in a pre-specified hierarchy; additional secondaries include wound closure, microbiologic response, and safety/tolerability. [ILLUSTRATIVE] Sample size is powered (≥80%, two-sided $\alpha=0.05$) to detect a DANCE-magnitude between-group difference in percent area reduction within the bone-deep stratum, yielding an estimated [ILLUSTRATIVE] N of ~120–160 randomized participants across [ILLUSTRATIVE] 6–10 sites after allowance for ~15% attrition; the final number and assumptions will be fixed with biostatistics and FDA input. A pre-specified interim analysis with an independent DSMB supports a **Go/No-Go** decision (futility/efficacy). Sex as a biological variable and MSSA-vs-MRSA status are pre-planned subgroup analyses.

Expected outcomes. Confirmation of a clinically meaningful ulcer-area reduction and a limb-salvage signal in bone-deep DFO, with a safety profile consistent with DANCE, supporting transition to a registrational program.

Pitfalls & alternatives. Enrollment of bone-deep DFO is challenging; multi-site recruitment, pragmatic eligibility, and a realistic accrual ramp protect the timeline. If a patient's isolate lacks a matching cocktail, they are managed per standard of care and analyzed accordingly (pre-specified). Manufacturing/regulatory timelines for personalized product are mitigated by the IND/expanded-access route below and early FDA engagement.

Timeline

[ILLUSTRATIVE] **Year 1:** Aim 1 phage banking, sequencing, isolate panel, assay validation.
[ILLUSTRATIVE] **Years 1–2:** Aim 2 pairing studies; lock cocktail and companion regimen.
[ILLUSTRATIVE] **Year 2:** regulatory submissions, protocol finalization, site activation.
[ILLUSTRATIVE] **Years 3–5:** Aim 3 enrollment, treatment, follow-up, analysis, dissemination.
Milestones gate progression: a coverage-validated cocktail (end Yr 1) precedes trial activation; a locked pairing recommendation (mid Yr 2) precedes site activation; the interim Go/No-Go (≈Yr 4) governs full enrollment.

Budget Justification (modular R01-style sketch)

[ILLUSTRATIVE] Modular request of [ILLUSTRATIVE] $500,000 direct costs per year for [ILLUSTRATIVE] 5 years. **Personnel:** PD/PI (phage microbiology/infectious disease); Co-I podiatric/vascular surgeon and Co-I clinical trialist; microbiologists/research staff for Aims 1–2; clinical research coordinators and a data manager for Aim 3; and a biostatistician — totaling roughly [ILLUSTRATIVE] 4.0 FTE across the team. **Other:** sequencing and phage characterization; isolate biobanking; investigational-product manufacturing/QC for personalized dosing; clinical per-patient costs and monitoring; and regulatory support. Animal costs are included only if the Aim 2 in vivo model is activated [ILLUSTRATIVE]. A detailed categorical budget will accompany submission.

Vertebrate Animals

Animal work is proposed only as a contingency in Aim 2: an in vivo *S. aureus* osteomyelitis model to resolve in-bone phage–antibiotic pairing effects if in vitro/ex vivo systems prove insufficient. If activated, the application will provide the required description (justification and species/numbers; procedures minimizing pain/distress with appropriate anesthesia/analgesia; veterinary care; humane endpoints/euthanasia per AVMA guidelines), with IACUC approval before any animal work and adherence to the 3Rs. If in vitro and ex vivo models are sufficient, this section is **Not applicable**.

Human Subjects / Clinical Trial

Aim 3 is a randomized, double-blind, placebo-controlled clinical trial in adults with *S. aureus* DFO, enriched for bone-deep ulcers, all receiving standard-of-care antibiotics. Because the personalized phage cocktail is investigational and patient-matched, administration will proceed under an appropriate FDA investigational pathway — a study IND, with the expanded-access/emergency IND route available for individual patients whose specific matched cocktail falls outside the standing protocol (the mechanism by which investigational phage has been provided in urgent limb-threatening cases). All sites will operate under IRB approval with written informed consent, an independent DSMB given the limb-threatening population, prospective registration on ClinicalTrials.gov, and predefined stopping rules. Standard of care (surgical debridement and guideline-based antibiotics) is never withheld; phage is strictly adjunctive. Eligibility is inclusive and consistent with the diabetic-foot population, with planned analysis of sex as a biological variable. The final enrollment number is [ILLUSTRATIVE] and will be set by powered sample-size calculation and FDA feedback.

Team & Environment

- **Program Director/Principal Investigator — [Name, Institution]:** phage biology and infectious diseases; overall scientific and regulatory leadership.
- **Co-Investigator, Limb Salvage — [Name, Institution]:** podiatric/vascular or orthopedic surgery; DFO debridement and topical/intraosseous delivery.
- **Co-Investigator, Clinical Trials — [Name, Institution]:** infectious-diseases trialist; protocol, IND/expanded access, DSMB interface.
- **Phage Core Lead — [Name, Institution]:** isolation, sequencing, host-range and biofilm assays, cocktail formulation (Aims 1–2).
- **Biostatistician — [Name, Institution]:** design, sample size, analysis plan, interim/Go-No-Go rules.
- **Regulatory/Manufacturing Lead — [Name, Institution]:** investigational-product QC, IND maintenance.
- **Environment:** an academic medical center with a multidisciplinary diabetic-foot/limb-salvage service, a phage laboratory with biobanking and sequencing, GMP-capable or partnered investigational-product manufacturing, and an experienced clinical-trials unit. Complementary funders to approach for adjacent aims include NIAID (antimicrobial resistance and phage therapeutics) and the DoD CDMRP (limb salvage, wound care, antimicrobial resistance).

References

(All factual claims are sourced to the four references below; no other citations are used.)

1. BiomX Inc. *BiomX Announces Positive Topline Results from Phase 2 Trial Evaluating BX211 for the Treatment of Diabetic Foot Osteomyelitis (DFO).* Press release, 31 March 2025. (DANCE Phase 2b; NCT05177107; 41 patients; percent area reduction p=0.046 at week 12.) https://www.globenewswire.com/news-release/2025/03/31/3052117/0/en/BiomX-Announces-Positive-Topline-Results-from-Phase-2-Trial-Evaluating-BX211-for-the-Treatment-of-Diabetic-Foot-Osteomyelitis-DFO.html
2. *Phase 2b Randomized, Parallel, Double-blind, Placebo-Controlled, Repeat Dose, Multi-Site Study for Safety, Tolerability, and Efficacy of Personalized Phage Treatment and Standard of Care for Subjects With Diabetic Foot Osteomyelitis Due to S. aureus (DANCE).* Sponsor: Adaptive Phage Therapeutics / BiomX. ClinicalTrials.gov NCT05177107. https://clinicaltrials.gov/study/NCT05177107
3. Plumet L, et al. *Phage therapy: a promising approach for Staphylococcus aureus diabetic foot infections.* Journal of Virology. 2025;99. doi:10.1128/jvi.00458-25 (PMC12172479). https://doi.org/10.1128/jvi.00458-25

4. Young MJ, Hall LML, Merabishvili M, Pirnay JP, Clark JR, Jones JD. *Phage Therapy for Diabetic Foot Infection: A Case Series.* Clinical Therapeutics. 2023;45(8):797-801. PMID 37442654. doi:10.1016/j.clinthera.2023.06.009 https://pubmed.ncbi.nlm.nih.gov/37442654/