Microbiome-Sparing Phage Decolonization of Multidrug-Resistant *E. coli* and *Enterococcus* Before Hematopoietic Cell Transplantation

Project Summary / Abstract

Patients entering intensive chemotherapy or allogeneic hematopoietic stem cell transplantation (allo-HSCT) develop profound neutropenia and mucosal barrier injury that convert the gut into the principal launchpad for life-threatening bloodstream infections (BSIs). Intestinal domination by multidrug-resistant (MDR) *Escherichia coli*—notably ESBL/carbapenemase-producing ST131 clones—and by *Enterococcus* (vancomycin-resistant *Enterococcus*, VRE, and biofilm-forming, cytolytic *E. faecalis*) directly precedes bacteremia; for *Enterococcus*, gut domination also worsens acute graft-versus-host disease (GVHD), a leading cause of non-relapse mortality. Antibiotic decolonization neither durably clears these organisms nor preserves the protective commensal microbiota that confers colonization resistance—and often deepens the dysbiosis that widens the pathogen niche. Lytic bacteriophages are mechanistically matched to this gap: they kill target strains with strain-level specificity, self-amplify on their host, penetrate biofilms, and spare commensals. Yet preclinical proof-of-concept remains incomplete—phage monotherapy is transient and selects escape mutants, and anti-phage immunity limits efficacy. We will develop **personalized lytic phage cocktails paired with rationally chosen synergy partners**—a microcin-producing probiotic for *E. coli*; a phage-derived lytic enzyme (endolysin) for biofilm-forming *E. faecalis*—to durably shrink these reservoirs below a pre-specified threshold before conditioning. We will (1) assemble patient-matched cocktails and quantify durability and resistance in human-feces continuous culture, (2) test synergy and microbiome-sparing decolonization in gnotobiotic/humanized mice while measuring the anti-phage immune barrier, and (3) conduct a small first-in-population safety/feasibility decolonization study in pre-HSCT carriers under an FDA expanded-access IND. This targets a leading, addressable driver of transplant-related mortality and would establish a generalizable, microbiome-sparing decolonization platform for immunocompromised oncology patients.

Specific Aims

The gut is the dominant source of BSI in neutropenic cancer and allo-HSCT patients, and intestinal

domination by MDR *E. coli* ST131 and by VRE/cytolytic *E. faecalis* precedes bacteremia and, for *Enterococcus*, aggravates acute GVHD. Antibiotic decolonization is non-durable and microbiome-toxic. Lytic phages offer specificity, self-amplification, biofilm penetration, and commensal sparing, but durability, escape mutants, and anti-phage immunity are unsolved. We test the central hypothesis that **personalized phage cocktails plus rational synergy partners durably reduce the resistant *E. coli* and *Enterococcus* gut reservoir while sparing protective commensals.** We define *decolonization success* a priori as a ≥3-log reduction in target-strain fecal burden sustained for the pre-conditioning window, without depletion of commensal alpha-diversity beyond a pre-specified margin.

Aim 1 — Assemble personalized lytic phage cocktails against carrier-resident MDR *E. coli* ST131 and *Enterococcus*, and quantify durability and resistance in human-feces continuous culture. We will phenotype patient gut isolates, match curated lytic phages to resident surface receptors, and broaden host range to raise the genetic barrier to escape. We will quantify log-reduction, resistance-mutant emergence (whole-genome sequencing of survivors), and commensal impact—directly addressing the escape mutant that persisted when the INTESTI cocktail was applied to CTX-M-15 ST131 *E. coli* in fecal continuous culture (Bernasconi 2020).

Aim 2 — Establish synergy strategies and microbiome-sparing decolonization in vivo, and define the anti-phage immune barrier. In gnotobiotic/humanized mice colonized with patient isolates, we will test phage + microcin-producing *E. coli* Nissle-1917 for ST131 (Porter 2022) and a phage cocktail ± an *E. faecalis*-specific endolysin for cytolytic *E. faecalis* (Fujimoto 2024), measuring decolonization durability, commensal preservation, GVHD-relevant and survival readouts, and the magnitude of phage-specific neutralizing immunity as a pre-specified efficacy determinant (Cheng 2024).

Aim 3 — Conduct a first-in-population safety/feasibility decolonization study in pre-HSCT MDR carriers under an FDA expanded-access IND. In screened ST131 *E. coli* and/or VRE/*E. faecalis* carriers awaiting allo-HSCT, we will administer a personalized oral cocktail (± synergy partner) to assess safety, the feasibility of a sequence-to-cocktail workflow within the pre-transplant window, target-strain reduction, and microbiome preservation. A pre-specified go/no-go gate governs progression to a future controlled trial.

Impact: Success delivers a microbiome-sparing "phage prep" for the pre-transplant checklist—shrinking the reservoir from which post-transplant bacteremia and *Enterococcus*-driven GVHD arise—and a generalizable decolonization platform for immunocompromised oncology patients.

Significance

For patients undergoing intensive chemotherapy or allo-HSCT, neutropenia and mucosal barrier injury make the gut the principal source of BSI, and the intestinal abundance of a resistant organism is a direct antecedent of invasive disease. MDR *E. coli*—especially ESBL/carbapenemase ST131 clones—and *Enterococcus* (VRE and cytolytic *E. faecalis*) are among the most consequential dominators. Beyond bacteremia, gut domination by biofilm-forming *E. faecalis* has been mechanistically tied to acute GVHD after allo-HCT, and an *E. faecalis*-targeted phage-derived enzyme suppressed GVHD and improved survival in gnotobiotic and humanized mice (Fujimoto 2024). This places gut decolonization squarely within NCI's supportive-care mission: reducing the treatment-related infectious and immune complications that limit cancer survival and drive non-relapse mortality.

Current decolonization is inadequate. Antibiotic regimens cannot durably clear these organisms and further deplete the commensal flora that provides colonization resistance, paradoxically widening the niche for resistant dominators. Lytic phages address this mismatch by killing target strains with surgical specificity, self-amplifying on their host, penetrating biofilms, and sparing commensals—precisely the profile needed to shrink a pathogen reservoir without collateral dysbiosis before conditioning. The opportunity is sharpened by an evidence gap: although CRE/VRE gut-decolonization is an active clinical target, current registered programs largely test microbial-consortium/FMT-type products rather than rigorously developed, personalized phage cocktails—leaving a clear, fundable niche.

Innovation

This proposal advances four innovations, each anchored in current preclinical evidence. **(1) Personalization over fixed product:** we sequence and phenotype a carrier's resident clone and assemble a receptor-matched cocktail, broadening host range to raise the barrier to escape—directly motivated by the resistant mutant that persisted when the INTESTI cocktail was applied to ST131 *E. coli* in fecal continuous culture (Bernasconi 2020). **(2) Rational synergy over phage monotherapy:** for *E. coli*, we pair phage with a microcin-producing *E. coli* Nissle-1917 probiotic—a combination that achieved a ~3.3-log reduction in ST131 gut colonization versus ~0.5-log for phage alone in mice (Porter 2022); for biofilm-forming cytolytic *E. faecalis*, we incorporate a narrow-spectrum *E. faecalis*-specific endolysin that decolonized the gut and improved survival in humanized gnotobiotic mice (Fujimoto 2024). **(3) Explicit engagement of the anti-phage immune barrier:** we treat phage-specific neutralizing immunity as a pre-specified efficacy determinant—given evidence that two treatment rounds induce neutralizing antibodies that reduce anti-VRE efficacy, with myophages more immunogenic than siphophages (Cheng 2024)—and we use this to inform cocktail composition and dosing schedule. **(4) Microbiome-sparing decolonization as the endpoint,** not pathogen kill alone—preserving colonization resistance is the feature that distinguishes phage from antibiotic

decolonization and is essential before transplant.

Approach

Rigor & reproducibility (all aims): isolates, phages, and consortia are authenticated and biobanked; analyses are pre-registered with predefined endpoints, blinding of outcome assessment, and biological replication across multiple independent donor microbiotas/mouse cohorts. Sex is included as a biological variable in all in vivo work, with both sexes balanced across arms.

Aim 1 — Personalized cocktail assembly; durability/resistance in human-feces continuous culture

Rationale. A patient-matched cocktail must be demonstrably lytic and durable against escape. The human-feces continuous-culture model is the validated bridge: the INTESTI cocktail drove CTX-M-15 ST131 *E. coli* to near-undetectable levels in one donor microbiota, yet a phage-resistant mutant persisted in a second—defining both the assay and the central durability problem (Bernasconi 2020).

Design. We will bank gut *E. coli* ST131 and *Enterococcus* (VRE, cytolytic *E. faecalis*) isolates; characterize surface receptors (LPS O-antigen, capsule, pili for *E. coli*; cell-wall/EPS for *Enterococcus*); screen curated lytic phages for host range; and assemble multi-phage cocktails maximizing receptor-class diversity. In anaerobic continuous-culture vessels seeded with human fecal communities spiked with the target strain, we will measure target log-reduction, resistance-mutant emergence (WGS of survivors), and 16S/metagenomic effects on commensals across [ILLUSTRATIVE] multiple independent donor microbiotas.

Expected outcomes. Patient-matched cocktails achieving multi-log target reduction with delayed/blunted resistance versus single phages, and a receptor-diversity rule predicting durability.

Pitfalls & alternatives. Resistant mutants may still emerge; we will counter with cocktail re-formulation, receptor-class rotation, and pre-positioning the Aim 2 synergy partners. If a clone lacks matched phages, we will prioritize broad-host-range phages and endolysin-based killing.

Aim 2 — In vivo synergy, microbiome sparing, and the anti-phage immune barrier

Rationale. Phage monotherapy is transient in vivo and limited by GI transit and anti-phage immunity. Synergy restores durability: phage + microcin-Nissle suppressed ST131 colonization far beyond phage alone (Porter 2022), and an *E. faecalis*-specific endolysin decolonized gut and improved survival (Fujimoto 2024). Neutralizing immunity impaired anti-VRE efficacy and must be quantified

(Cheng 2024).

Design. In gnotobiotic/humanized mice colonized with patient isolates, arms compare vehicle, phage cocktail alone, synergy partner alone, and combination—phage + microcin-Nissle for ST131; phage cocktail ± *E. faecalis* endolysin for cytolytic *E. faecalis*. Readouts: longitudinal fecal target burden, commensal preservation (metagenomics), and—for *E. faecalis*—GVHD-relevant and survival endpoints in transplant-relevant models. Anti-phage antibody/neutralization titers are measured serially and related to efficacy. Group sizes [ILLUSTRATIVE] will be fixed by an a priori power calculation (predefined effect size, two-sided alpha 0.05, 80% power) from preliminary variance estimates, both sexes balanced.

Expected outcomes. Combination therapy yields greater, more durable decolonization than monotherapy while sparing commensals; quantified immune-barrier thresholds inform dosing/rotation.

Pitfalls & alternatives. Humanized models are variable; we standardize donor consortia and pre-register endpoints. If neutralizing immunity dominates, we test cocktail rotation and front-loaded dosing before titers rise.

Aim 3 — Expanded-access decolonization study in pre-HSCT MDR carriers

Rationale. No controlled phage decolonization study exists in pre-transplant patients; active registered programs use consortium/FMT products, not personalized phage. A small, safety-focused window-of-opportunity study is the responsible first step.

Design. Pre-allo-HSCT patients screened positive for ST131 *E. coli* and/or VRE/cytolytic *E. faecalis* receive an oral personalized cocktail (± synergy partner) before conditioning. **Primary endpoints:** safety/tolerability and feasibility of the sequence-to-cocktail workflow within the pre-transplant window. **Secondary:** target-strain reduction against the pre-specified ≥3-log threshold, commensal preservation, and resistance/immune monitoring, benchmarked against contemporaneous untreated carrier controls. Enrollment is [ILLUSTRATIVE] a small initial safety/feasibility cohort. A pre-specified **go/no-go gate** (acceptable safety plus a minimum target-reduction signal) governs progression to a future controlled trial.

Expected outcomes. Demonstrated safety/feasibility and early signals of target reduction with microbiome preservation, de-risking a controlled trial.

Pitfalls & alternatives. Transient or partial decolonization is possible; the study is explicitly hypothesis-generating, with synergy partners and repeat dosing available. Manufacturing/regulatory timelines are mitigated by early FDA engagement.

Timeline

[ILLUSTRATIVE] Years 1–2: Aim 1 isolate banking, cocktail assembly, continuous-culture durability/resistance. [ILLUSTRATIVE] Years 2–4: Aim 2 in vivo synergy, microbiome, and immune-barrier studies. [ILLUSTRATIVE] Years 3–5: Aim 3 expanded-access IND filing, IRB approval, and enrollment, overlapping later in vivo work.

Budget Justification (modular R01-style sketch)

[ILLUSTRATIVE] Requested at [ILLUSTRATIVE] $250,000 direct costs/year over [ILLUSTRATIVE] 5 years. **Personnel:** PI (microbiology/phage biology), Co-I transplant infectious-disease physician, Co-I bioinformatician, [ILLUSTRATIVE] 2 postdocs, [ILLUSTRATIVE] 1 research technician, and a clinical research coordinator for Aim 3. **Other costs:** phage isolation/characterization and GMP-grade cocktail preparation; whole-genome and metagenomic sequencing; gnotobiotic/humanized mouse husbandry; continuous-culture consumables; regulatory/IND and IRB submission support; immunoassay reagents. **Equipment:** anaerobic continuous-culture systems as needed. A subaward to the clinical site supports Aim 3 monitoring.

Vertebrate Animals

Animal work is proposed in Aim 2. Gnotobiotic and humanized mice will be colonized with patient-derived MDR *E. coli* and *Enterococcus* to test decolonization, synergy, microbiome preservation, GVHD-relevant endpoints, and anti-phage immunity, consistent with published models (Porter 2022; Fujimoto 2024; Cheng 2024). Group sizes will be the minimum to achieve statistical power [ILLUSTRATIVE], both sexes balanced, with humane endpoints, analgesia where applicable, and IACUC approval. Endpoints emphasizing fecal burden and survival minimize distress.

Human Subjects / Clinical Trial

Aim 3 involves human subjects: pre-allo-HSCT carriers of MDR *E. coli* and/or *Enterococcus*. Because the personalized phage cocktails are investigational, administration proceeds under the FDA expanded-access (emergency) IND route appropriate for personalized investigational phage, with full IRB oversight, informed consent, and a data-safety monitoring plan. Eligibility, dosing, and pre-specified stopping/go-no-go rules are defined a priori; target-strain reduction, commensal preservation, resistance, and anti-phage immunity are monitored. Enrollment is [ILLUSTRATIVE] a small initial safety/feasibility cohort, balanced for sex where feasible. The study is designed to inform, not substitute for, a future controlled decolonization trial.

Team & Environment

Principal Investigator [Name, Institution] — phage biology/microbiology. **Co-Investigators:** [Name], transplant infectious-diseases physician (Aim 3 clinical lead); [Name], computational microbiologist (host-range and metagenomic analysis); [Name], anaerobic/continuous-culture microbiologist (Aim 1). **Collaborators/consultants** with directly relevant expertise span enterococcal phage-derived enzymes, INTESTI-based *E. coli* decolonization, phage + microcin probiotic strategies, VRE/gut-reservoir AMR programs, and an established phage source/biobank. The environment provides BSL-2 microbiology, anaerobic culture, gnotobiotic/humanized mouse facilities, genomics cores, and a transplant unit with regulatory/IRB infrastructure. *Alternate funding:* if positioned outside NCI's supportive-care scope, NIAID is an appropriate alternate home given the AMR and phage-therapy focus.

References

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