Precision Bacteriophage Therapy for Recurrent Uncomplicated *Escherichia coli* Urinary Tract Infection: Isolate-Matched Cocktails, Resistance Steering, and a Microbiome-Sparing Path to Durable Cure

Project Summary / Abstract

Recurrent uncomplicated urinary tract infection (rUTI) is among the most common reasons women seek outpatient care and is overwhelmingly driven by uropathogenic *Escherichia coli* (UPEC). Each recurrence is typically met with another antibiotic course; repeated courses select for multidrug-resistant strains, disrupt the protective commensal microbiome, and fail to clear the intracellular bacterial reservoirs and biofilms that seed relapse. Lytic bacteriophages offer a mechanistically distinct option: they self-amplify at the site of infection, are highly specific to *E. coli* (sparing commensal flora), can be delivered intravesically, intraurethrally, or intravenously, and frequently retain activity against antibiotic-resistant isolates. The bladder is anatomically accessible, locally samplable, and relatively contained, making rUTI a tractable indication for precision phage therapy. This proposal builds directly on the LBP-EC01 program — whose ELIMINATE Part 1 results (Kim et al., *Lancet Infect Dis* 2024) demonstrated safety, dose-dependent urinary and systemic pharmacokinetics, and rapid urinary *E. coli* reduction with symptom resolution in all evaluable participants using an intraurethral-plus-intravenous regimen — and on the Leitner et al. (2021) double-blind intravesical trial, which found phage non-inferior to antibiotics with a favorable safety profile but underscored that delivery, dosing, and isolate-matching require optimization. Building on the translational roadmap of Morgan et al. (2025), we will (1) build and characterize a UPEC-matched lytic phage cocktail with quantitative host-range, biofilm, and persister activity; (2) define resistance-steering dynamics and phage–antibiotic synergy against patient-derived isolates; and (3) conduct an IND-enabled, isolate-matched pilot clinical evaluation in women with rUTI. The work aims to convert a frustrating cycle of repeat antibiotics into a single targeted, microbiome-sparing course for one of the largest real-world drivers of community antimicrobial resistance.

Specific Aims

Recurrent uncomplicated UTI caused by UPEC remains poorly served by antibiotics, which select for resistance, damage the microbiome, and leave intracellular reservoirs and biofilms intact. Lytic phages kill *E. coli* in a strain-specific manner, often retain activity against antibiotic-resistant isolates, and have shown safety and favorable pharmacokinetics in humans (Kim et al. 2024; Leitner et al. 2021). However, cocktail composition, dosing, delivery, and isolate-matching remain unstandardized, and no phage susceptibility-testing standards yet exist (Morgan et al. 2025). We propose a bench-to-bedside program with three aims.

Aim 1 — Build and characterize an isolate-matched anti-UPEC phage cocktail. We will assemble a contemporary clinical UPEC biobank and a panel of lytic phages targeting distinct UPEC surface receptors (LPS O-antigen, outer-membrane proteins, fimbriae), quantify host range, and measure biofilm degradation and activity against dormant persister cells. We will deliberately screen against the "passenger-phage" problem in which one component dominates and the rest contribute little (Morgan et al. 2025). *Outcome:* a defined, broad-coverage cocktail with a validated, rapid susceptibility assay.

Aim 2 — Define resistance steering and phage–antibiotic synergy. Using patient-derived isolates, we will characterize how phage-resistant escape mutants arise (e.g., LPS or efflux-pump alterations), test whether escape restores antibiotic susceptibility, and quantify whether sub-inhibitory antibiotics enhance phage replication. *Outcome:* quantitative synergy parameters and genotype-conditioned "resistance-steering" rules to guide combination regimens.

Aim 3 — IND-enabled, isolate-matched pilot clinical evaluation in women with rUTI. Under an FDA investigational new drug (IND) authorization with IRB oversight, we will conduct a small single-arm pilot (antibiotic-referenced) testing safety, tolerability, pharmacokinetics/pharmacodynamics, microbiologic clearance, symptom resolution, and microbiome preservation of the matched cocktail. *Outcome:* feasibility, safety, and preliminary efficacy data to power a definitive registrational trial.

Impact: Success would establish recurrent *E. coli* UTI as an early indication for precision, microbiome-sparing phage therapy and validate isolate-matching, phage–antibiotic synergy, and resistance steering as durable, generalizable tools against antimicrobial resistance.

Significance

Recurrent uncomplicated UTI is a core NIDDK benign-urology indication and a major driver of outpatient antibiotic use. Beyond resistance, recurrence imposes a substantial quality-of-life and

healthcare-utilization burden on women, who experience repeated symptomatic episodes, urgent care visits, and cumulative antibiotic exposure. UPEC accounts for the overwhelming majority of cases, and the spread of multidrug-resistant and ESBL-producing strains erodes the reliability of first-line agents such as TMP-SMX, nitrofurantoin, and fluoroquinolones. Critically, the biology of recurrence is not fully addressed by systemic antibiotics: UPEC forms intracellular bacterial reservoirs and biofilms in association with the bladder epithelium that survive conventional therapy and reseed infection, while repeated broad-spectrum courses deplete protective commensal flora and select for resistance.

Phage therapy targets these failure modes directly. Because lytic phages are highly *E. coli*-specific, they spare the commensal microbiome that broad-spectrum antibiotics destroy. Because phage susceptibility is frequently retained in antibiotic-resistant isolates, phages can act precisely where conventional drugs fail. Phages that replicate within, or kill, metabolically dormant persister cells may reach subpopulations that antibiotics cannot, with potential advantage in biofilm-associated disease — though this has not yet been demonstrated directly in UTI (Morgan et al. 2025). The urinary tract is anatomically accessible and locally samplable, supporting intravesical, intraurethral, or intravenous delivery with direct microbiologic monitoring; notably, intravesical administration may elicit lower neutralizing-antibody responses than intravenous dosing (Morgan et al. 2025).

The clinical evidence base, while early, is accelerating and points to feasibility. In ELIMINATE Part 1, an intraurethral-plus-intravenous LBP-EC01 regimen (with oral TMP-SMX background) was safe and well tolerated, produced dose-dependent urinary and systemic phage concentrations, and achieved rapid urinary *E. coli* reduction sustained to day 10 with complete symptom resolution in all 16 evaluable participants; non-serious tachycardia and chills occurred mainly at the highest intravenous doses (Kim et al. 2024). The blinded, ~288-participant Part 2 — comparing LBP-EC01 against placebo plus oral TMP-SMX — is the efficacy stage (NCT05488340). The Leitner et al. (2021) double-blind intravesical Pyophage RCT in men undergoing transurethral resection of the prostate found phage non-inferior to standard-of-care antibiotics with a favorable safety profile, but with modest microbiologic response across all arms and no superiority over placebo bladder irrigation — underscoring that delivery, dosing, and isolate-matching still require optimization. This proposal addresses precisely those gaps, positioning rUTI in women as a natural early indication for precision phage therapy.

Innovation

This program advances the field along three innovative axes, each grounded in current evidence. **First**, it operationalizes *isolate-matching* as a rigorous, prospectively validated diagnostic–therapeutic pairing rather than an empirical add-on, and builds the rapid susceptibility assay whose absence Morgan et al. (2025) identify as a core translational gap. We further address the "passenger-phage"

failure mode by selecting cocktail members for complementary, non-redundant killing rather than nominal breadth. **Second**, it treats *resistance steering* as a designed therapeutic feature: by mapping whether phage-escape mutations (altering LPS or multidrug efflux-pump components) trade phage resistance for renewed antibiotic susceptibility, we aim to convert bacterial evolution from a liability into a regimen-design lever, paired with phage–antibiotic synergy in which sub-inhibitory antibiotic exposure can boost phage replication. **Third**, the framework is explicitly compatible with the most advanced engineered approach in the field — CRISPR-Cas3-enhanced cocktails such as LBP-EC01, in which natural lytic phages carry a Cas3 payload that targets the *E. coli* genome to add a second, sequence-directed killing mechanism atop native lysis (Kim et al. 2024) — so that our matching and steering methods apply to both natural and engineered cocktails. Together these elements move beyond "phage as antibiotic substitute" toward a precision, microbiome-sparing platform.

Approach

Aim 1 — Build and characterize an isolate-matched anti-UPEC phage cocktail

Rationale. Strain-specific killing requires matching the cocktail to the patient's isolate; multi-phage cocktails can broaden coverage and suppress resistance, but composition and susceptibility-testing criteria remain unstandardized, and component phages may act as passengers rather than contributors (Morgan et al. 2025).

Experimental design. We will assemble a contemporary clinical UPEC biobank from rUTI patients [ILLUSTRATIVE: ~200 isolates], including ESBL/MDR strains, and curate a panel of well-characterized lytic phages targeting distinct UPEC receptors (LPS O-antigen, outer-membrane proteins, pili/fimbriae). Each phage will be whole-genome sequenced and screened to exclude lysogeny, toxin, and antibiotic-resistance genes, then profiled for host range by quantitative plaque assay and liquid-killing kinetics. To avoid passenger phages, candidate cocktails will be tested for *additive* coverage and independent activity of each member against representative isolates. Biofilm degradation (including depolymerase activity) will be measured on static and catheter-associated UPEC biofilms; persister killing will be assessed in stationary-phase and antibiotic-tolerant subpopulations. Cocktails will be down-selected for maximal coverage, complementary receptor targeting, and minimal cross-resistance.

Expected outcomes. A defined 3–5-phage cocktail [ILLUSTRATIVE] covering the majority of contemporary UPEC isolates, with a validated, rapid susceptibility assay suitable for clinical matching.

Potential pitfalls & alternative approaches. If single-cocktail coverage is insufficient, we will adopt a modular, patient-tailored matching scheme drawn from a larger phage library. If biofilm penetration

is inadequate, we will prioritize depolymerase-encoding phages or add purified enzymes. If passenger dynamics persist, we will compose smaller, fully complementary cocktails or sequence phages serially.

Aim 2 — Define resistance steering and phage–antibiotic synergy

Rationale. Phage resistance frequently arises via altered LPS or efflux-pump components, changes that can collaterally restore antibiotic susceptibility; sub-inhibitory antibiotics can in turn enhance phage replication. These dynamics could be engineered into regimens but must first be quantified in patient-derived isolates.

Experimental design. Using representative biobank isolates, we will select phage-resistant escape mutants in vitro, genotype resistance determinants (LPS biosynthesis, outer-membrane proteins, efflux systems) by whole-genome sequencing, and re-test antibiotic MICs to quantify any collateral re-sensitization. Checkerboard and time-kill assays will define phage–antibiotic interactions across clinically relevant agents (e.g., TMP-SMX, nitrofurantoin). Resistance evolution will be tracked under phage-alone, antibiotic-alone, and combination pressure to derive genotype-conditioned steering rules. These rules will directly inform the regimen and combination logic for Aim 3.

Expected outcomes. Quantitative maps of escape pathways and collateral antibiotic re-sensitization, plus synergy parameters defining combination regimens and dosing for the pilot trial.

Potential pitfalls & alternative approaches. If steering effects are isolate-dependent, we will define genotype-conditioned rules rather than a universal regimen. If a given phage–antibiotic pair is antagonistic, we will exclude it and prioritize sequential dosing. In vitro escape frequencies may not predict in vivo dynamics; we will therefore treat steering rules as hypotheses to be monitored prospectively in Aim 3 rather than as established clinical effects.

Aim 3 — IND-enabled, isolate-matched pilot clinical evaluation in women with rUTI

Rationale. Human safety and favorable, dose-dependent pharmacokinetics are established for anti-*E. coli* phage regimens (Kim et al. 2024; Leitner et al. 2021), but isolate-matched efficacy and microbiome preservation in rUTI require prospective testing.

Experimental design. Under an FDA IND with IRB oversight, we will enroll adult women with rUTI and culture-confirmed UPEC [ILLUSTRATIVE: ~30 participants], match each isolate to the Aim 1 cocktail using the validated susceptibility assay, and administer the cocktail by a route informed by the LBP-EC01 program (intraurethral and/or intravenous) and by intravesical experience (Leitner et al. 2021), with the route and dose pre-specified from Aim 1–2 data. Primary endpoints are safety and

tolerability; secondary endpoints include pharmacokinetics/pharmacodynamics (urinary and systemic phage titers), microbiologic clearance, symptom resolution, emergence of phage resistance, and 16S/metagenomic assessment of microbiome preservation versus an antibiotic-treated reference. The staged structure mirrors ELIMINATE (NCT05488340) to enable a subsequent registrational efficacy trial.

Expected outcomes. Demonstrated feasibility of overnight isolate-matching, confirmed safety, preliminary microbiologic and symptomatic benefit, and evidence of microbiome sparing — sufficient to power a definitive randomized controlled trial.

Potential pitfalls & alternative approaches. Because Leitner et al. (2021) showed non-inferiority but not superiority to placebo irrigation and modest microbiologic response, we will emphasize rigorous isolate-matching and adequate dosing, and will pre-specify phage–antibiotic combination arms (informed by Aim 2) if monotherapy clearance is suboptimal. Higher intravenous doses produced transient tachycardia/chills in ELIMINATE Part 1 (Kim et al. 2024); our dose-escalation and monitoring plan will account for this. If enrollment lags, we will add sites and adjust eligibility within the approved protocol.

Timeline

[ILLUSTRATIVE] **Years 1–2:** Aim 1 biobank assembly, phage characterization, cocktail down-selection, and susceptibility-assay validation; initiate Aim 2. **Years 2–3:** Complete Aim 2 resistance-steering and synergy studies; finalize regimen, route, and dose; prepare and submit IND (timeline contingent on GMP manufacturing and FDA feedback). **Years 3–5:** Aim 3 pilot enrollment, follow-up, microbiome and PK/PD analyses, and design of a registrational trial. Go/no-go milestones gate progression (validated cocktail and assay → IND clearance → first-patient-in).

Budget Justification (modular R01-style sketch)

[ILLUSTRATIVE] We request [ILLUSTRATIVE: $250,000] direct costs per year in modular increments for [ILLUSTRATIVE: 5 years]. **Personnel:** PI (microbiology/infectious disease), co-investigator urologist, clinical microbiologist, two postdocs/technicians for phage characterization and resistance studies, a clinical research coordinator, and biostatistics/bioinformatics support [ILLUSTRATIVE percent efforts]. **Supplies:** bacterial culture and phage propagation/purification reagents, whole-genome sequencing, biofilm and persister assays, and microbiome metagenomics. **Clinical (Aim 3):** GMP-grade cocktail production, IND regulatory support, IRB fees, participant costs, and laboratory monitoring [ILLUSTRATIVE]. **Other:** isolate biobanking, data management, and publication costs. A subaward [ILLUSTRATIVE] supports the clinical site and GMP

manufacturing partner. Because GMP manufacturing and the IND-enabled pilot are cost drivers, modular caps may require phasing or supplemental support; the bench aims (1–2) are fully executable within the requested budget.

Vertebrate Animals

Not applicable. The program comprises in vitro/ex vivo bacteriology (Aims 1–2) and human clinical research (Aim 3); no vertebrate animal work is proposed. If preclinical in vivo pharmacokinetic or efficacy modeling becomes necessary to support the IND, a Vertebrate Animals Section and an approved IACUC protocol will be added by amendment before any animal work begins.

Human Subjects / Clinical Trial

Aim 3 is a prospective clinical study in adult women with recurrent uncomplicated UTI and culture-confirmed UPEC. Investigational phage will be administered under an FDA IND authorization. All activities will have IRB approval, written informed consent, a data and safety monitoring plan with pre-specified stopping rules, and prospective registration on ClinicalTrials.gov. The design is referenced to the staged ELIMINATE trial (NCT05488340) and to the human safety and pharmacokinetic experience of Kim et al. (2024) and Leitner et al. (2021); the dose-escalation plan explicitly accounts for the transient tachycardia and chills observed at the highest intravenous doses in ELIMINATE Part 1. Enrollment follows NIH inclusion policies across the lifespan and demographic subgroups. Because rUTI predominantly affects women, the study population is appropriately weighted to women while remaining open to all eligible participants; sex/gender and race/ethnicity will be reported. This study meets the NIH definition of a clinical trial, and a full Clinical Trial information form (PHS Human Subjects and Clinical Trial Information) will be completed.

Team & Environment

[Template — to be completed with real names/institutions.] **Principal Investigator:** [Name], [Institution] — infectious disease/microbiology and phage biology; [ILLUSTRATIVE: prior bacteriophage characterization and translational experience]. **Co-Investigator (Clinical Lead):** [Name], Department of Urology, [Institution] — NIDDK-relevant rUTI clinical trials and recurrence biology. **Co-Investigator (Clinical Microbiology):** [Name] — UPEC isolate characterization and susceptibility-assay validation. **Bioinformatics/Genomics Core:** [Name] — phage and resistance-determinant sequencing and microbiome metagenomics. **Regulatory/GMP Partner:** [Organization] — IND support and GMP cocktail manufacturing. **Biostatistics:** [Name] — pilot design and analysis. A brief track-record statement [ILLUSTRATIVE] will document the team's relevant publications,

prior IND submissions, and phage-handling expertise to establish feasibility. **Environment:** [Institution] provides BSL-2 phage laboratories, a clinical microbiology lab, an NIDDK-aligned urology clinical research unit, an IND-experienced regulatory office, and an academic GMP facility, with potential benchmarking against established phage-therapy programs.

References

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