Project Title

Strain-Matched Bacteriophage Cocktails Against Gut *Klebsiella pneumoniae* to Halt Primary Sclerosing Cholangitis

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Project Summary / Abstract

Primary sclerosing cholangitis (PSC) is a chronic, progressive cholestatic liver disease of biliary inflammation and fibrosis that frequently progresses to cirrhosis and is a leading indication for liver transplantation. No medical therapy alters its natural history. Recent work reframes PSC, in part, as a disease of the gut-liver axis: gut-derived *Klebsiella pneumoniae* (Kp) isolated from PSC patients disrupts the intestinal epithelial barrier, translocates to mesenteric lymph nodes (MLNs), and drives a hepatic T helper 17 (Th17) response that aggravates hepatobiliary injury (Nakamoto et al., *Nat Microbiol* 2019). Because this pathobiont is a specific, culturable organism acting through a defined mechanism, it is an unusually clean target for precision antibacterial therapy. Lytic bacteriophages can selectively deplete PSC-associated Kp without the broad collateral dysbiosis caused by antibiotics. An anti-Kp phage cocktail lowered Kp burden and attenuated liver inflammation in hepatobiliary injury-prone mice (Ichikawa et al., *Nat Commun* 2023), and an orally delivered anti-Kp cocktail (BX002-A) demonstrated safety and high-titer fecal delivery in healthy volunteers (NCT04737876).

Three barriers block translation, and each defines an aim. First, phage activity against PSC Kp is strictly strain-specific, and a cocktail raised against one PSC Kp strain did not protect against a second strain (Ichikawa et al., 2023); no cocktail has been matched to contemporary US PSC isolates. Second, single phages are rapidly defeated by resistance, so durable suppression must be engineered. Third, no diagnostic exists to match a patient's resident Kp to a cocktail. We will (1) build a strain-matched, resistance-aware anti-Kp cocktail against US PSC-derived Kp and define its host range and resistance dynamics in vitro; (2) test oral, systemic, and combined dosing in hepatobiliary injury mouse models to determine whether covering both the gut reservoir and the translocated MLN pool reduces Kp burden and liver injury beyond either route alone; and (3) establish a stool-based companion-diagnostic workflow that identifies a patient's resident Kp strain and predicts cocktail susceptibility, de-risking a future eIND-enabled study. This work targets the upstream microbial trigger of PSC rather than its downstream fibrosis and provides the preclinical and translational foundation for precision phage therapy in a transplant-bound disease.

Specific Aims

PSC has no disease-modifying therapy and progresses, in many patients, to cirrhosis and transplant. The identification of gut Kp as a causal pathobiont that translocates and drives hepatic Th17-mediated injury (Nakamoto et al., 2019) creates a precision-medicine opportunity: selectively eliminate the culprit strain. Lytic phages can do this without the off-target dysbiosis of antibiotics; an anti-Kp cocktail has shown efficacy in mice (Ichikawa et al., 2023), and oral anti-Kp phage proved safe and gut-deliverable in humans (NCT04737876). Yet three barriers block translation, and we address each with a falsifiable aim and predefined go/no-go criteria.

Aim 1. Build and characterize a strain-matched, resistance-aware anti-Kp phage cocktail against contemporary US PSC isolates. *Hypothesis:* a multi-phage cocktail incorporating phages active against escape mutants suppresses US PSC Kp markedly longer in vitro than any single phage. We will isolate and genomically characterize lytic phages against a panel of Kp strains cultured from US PSC patients, iteratively add phages targeting escape mutants to extend liquid-culture suppression, map host range across the panel by efficiency-of-plating (EOP), and benchmark against the cocktail reported by Ichikawa et al. (2023). *Success criterion:* a formulation that suppresses ≥80% of panel isolates and prolongs suppression versus the best single phage by a prespecified margin.

Aim 2. Determine whether combined oral plus systemic phage delivery reduces gut and translocated Kp and attenuates liver injury in mouse models. *Hypothesis:* because oral phage lowers fecal Kp while systemic phage reduces Kp already translocated to MLNs (Ichikawa et al., 2023), combined dosing reduces both compartments and liver injury more than either route alone. In hepatobiliary injury-prone mice colonized with PSC Kp (both sexes), we will compare vehicle, oral, systemic, and combined dosing on Kp burden (stool, MLN), hepatic Th17 responses, liver biochemistries and histology, and off-target microbiota effects, under blinded, randomized, power-justified designs.

Aim 3. Establish a companion-diagnostic workflow matching a patient's resident Kp strain to cocktail susceptibility. *Hypothesis:* in vitro phage susceptibility of a patient's resident Kp predicts ex vivo Kp suppression. We will detect, culture, and phage-type resident Kp from PSC patient stool, then quantify how well in vitro susceptibility predicts ex vivo suppression, producing the patient-stratification framework and addressable-fraction estimate needed for an eIND-enabled trial.

Impact. If a strain-matched, resistance-aware cocktail durably suppresses PSC-associated Kp and attenuates liver injury, phage therapy could become the first treatment acting on the upstream cause of PSC, with a companion-diagnostic model that generalizes to other gut-liver-axis diseases.

Significance

The problem and the unmet need. PSC is a rare but devastating cholestatic liver disease that progresses to biliary cirrhosis and is among the leading indications for liver transplantation, with no medical therapy proven to alter its natural history and no approved disease-modifying drug. This sits squarely within NIDDK's mission to reduce the burden of digestive and liver diseases. Current management is supportive; ursodeoxycholic acid does not improve transplant-free survival, leaving transplantation as the only definitive intervention. A therapy that acts on a defined upstream driver would represent a categorical advance.

The enabling discovery. The conceptual breakthrough is the demonstration that PSC is driven, at least in part, by a specific gut pathobiont. Nakamoto et al. (*Nat Microbiol* 2019) showed, using PSC patient samples, gnotobiotic colonization, and a bacterial-organoid co-culture system, that PSC-derived Kp disrupts the gut epithelial barrier, translocates to MLNs, and induces a hepatic Th17 response that increases susceptibility to hepatobiliary injury. This establishes Kp as causal rather than a bystander and identifies a discrete, druggable node in the gut-liver axis — a level of mechanistic specificity rarely available in chronic liver disease.

Why phage, and why now. The standard tool for bacterial depletion — broad-spectrum antibiotics — is poorly suited to a chronic disease requiring sustained, selective microbial control: it causes collateral dysbiosis and selects for resistance. Lytic phages offer strain-level precision. In hepatobiliary injury-prone mice, an anti-Kp cocktail lowered Kp and attenuated liver inflammation and disease severity, and oral dosing depleted Kp while largely sparing the surrounding microbiota (Ichikawa et al., *Nat Commun* 2023). Oral anti-Kp phage has a human safety and fecal-PK precedent (NCT04737876). Gut-microbiota-directed strategies, including phages against Kp in PSC, are increasingly recognized as a rational direction for chronic liver disease (Liu et al., *FASEB J* 2021).

The gap this proposal closes. Existing evidence is mouse-efficacy plus human-safety, but no cocktail is matched to contemporary US PSC isolates, durable suppression is unproven, and no diagnostic links a patient to a cocktail. By targeting the upstream trigger and supplying the missing translational scaffold, this work could shift PSC care from managing downstream inflammation and fibrosis to interrupting its microbial cause.

Innovation

This proposal is innovative in both concept and execution.

Conceptually, it treats PSC as a targetable, infection-like disease of a single translocating pathobiont rather than an idiopathic fibrotic process, pursuing cause-directed rather than symptom-directed therapy — a reframing made actionable only by the recent causal mechanism (Nakamoto et al., 2019).

Technically, it advances three features beyond the current state:

- ***Strain-matched design.*** Rather than a fixed cocktail, we build against contemporary US PSC isolates and directly confront the documented liability that a cocktail raised against one PSC Kp strain failed to protect against a different strain (Ichikawa et al., 2023).
- ***Resistance-aware, sustained suppression.*** We extend the iterative escape-mutant approach reported to prolong in vitro suppression (Ichikawa et al., 2023), attacking head-on the rapid resistance that defeats single phages — with prespecified suppression-duration endpoints rather than a static formulation.
- ***Dual-compartment delivery.*** We test combined oral-plus-systemic dosing to cover both the luminal gut reservoir and the translocated MLN pool — a mechanistic distinction documented but not yet therapeutically optimized.

Translationally, the **companion-diagnostic workflow** — stool-screen the patient, then deploy a matched cocktail — operationalizes precision phage therapy and supplies the missing link between existing mouse efficacy and a credible PSC trial.

Approach

Overall rigor. Across aims, in vitro assays use biological and technical replicates with prespecified acceptance criteria; in vivo studies are randomized, investigator-blinded for outcome assessment, powered on prior effect sizes, and include both sexes (sex as a biological variable analyzed, not merely reported). Key reagents (phages, Kp isolates) are authenticated by sequencing and EOP, and all isolates, phages, and analysis pipelines are version-controlled and archived for reproducibility.

Aim 1 — Build and characterize a strain-matched, resistance-aware anti-Kp phage cocktail

Rationale. Phage activity against PSC Kp is strictly strain-specific, and a non-matched PSC strain escaped a cocktail in prior work (Ichikawa et al., 2023). A US-focused program requires phages

active against contemporary domestic PSC isolates plus a resistance-aware design.

Design. We assemble a biobank of Kp isolates cultured from stool of US PSC patients (Aim 3 pipeline plus collaborating IBD/hepatology biorepositories). Against this panel we isolate lytic phages from environmental and wastewater sources, plaque-purify them, sequence genomes to confirm lytic lifestyle and absence of toxin/antibiotic-resistance/lysogeny genes, and characterize host range by spot and EOP assays. We then perform iterative escape-mutant selection — propagating Kp under phage pressure, isolating resistant mutants, and adding phages active against them — to extend liquid-culture suppression, following the strategy reported to markedly prolong suppression of PSC Kp (Ichikawa et al., 2023). The published anti-Kp cocktail serves as a benchmark comparator.

Quantitative milestones / success criteria. (i) ≥10 genomically defined lytic phages; (ii) a formulation suppressing ≥80% of panel isolates by EOP; (iii) liquid-culture suppression prolonged versus the best single phage by a prespecified margin. **Go/no-go (end Yr 2):** a benchmark-beating cocktail before in vivo scale-up.

Expected outcomes. A genomically defined multi-phage cocktail with broad coverage across US PSC Kp and prolonged in vitro suppression, plus a host-range map linking Kp genotype/serotype to phage susceptibility.

Pitfalls & alternatives. If no single fixed cocktail covers the panel, we define 2–3 formulations matched to dominant susceptibility clusters (consistent with the companion-diagnostic concept). If wild phages are insufficiently broad, we incorporate host-range-expanded phages as a contingency.

Aim 2 — Determine whether combined oral plus systemic phage delivery reduces gut and translocated Kp and attenuates liver injury

Rationale. Oral phage lowers fecal Kp, whereas systemic phage reduces Kp already translocated to MLNs (Ichikawa et al., 2023); covering both compartments may be required to interrupt the gut-liver axis driving hepatic Th17 injury (Nakamoto et al., 2019).

Design. Hepatobiliary injury-prone mice (both sexes) are colonized with characterized PSC Kp and randomized to four arms: vehicle, oral cocktail, systemic cocktail, or combined. **Primary endpoint:** Kp burden in stool and MLNs. **Secondary endpoints:** hepatic Th17 responses (flow cytometry/IL-17), serum liver biochemistries, and blinded histologic biliary/hepatic injury scoring. To test the central advantage over antibiotics, we profile the surrounding microbiota (16S/shotgun metagenomics) to verify oral phage depletes Kp without broad dysbiosis. **Statistics:** group sizes set by a priori power analysis on prior effect sizes (≥80% power, two-sided α=0.05) [ILLUSTRATIVE n≈10–12/group]; primary comparisons by mixed-effects models with multiplicity control; sex included as a factor.

Go/no-go (end Yr 4): a positive combined-dosing signal (greater dual-compartment Kp reduction and reduced liver injury than either monotherapy) before finalizing the translational package.

Expected outcomes. Combined dosing reduces fecal and MLN Kp more than either route alone and attenuates hepatic Th17 responses and liver injury, while oral phage leaves the surrounding microbiota largely intact — replicating and extending Ichikawa et al. (2023).

Pitfalls & alternatives. Emergent in vivo resistance is the principal risk; we serially re-isolate Kp, track susceptibility, and deploy updated escape-mutant-targeting phages from Aim 1. If systemic phage is rapidly cleared or immunogenic on repeat dosing, we test dose timing/frequency and consider that oral monotherapy may suffice for the luminal reservoir, reserving systemic dosing for translocation-dominant disease.

Aim 3 — Establish a companion-diagnostic workflow matching patient Kp to cocktail susceptibility

Rationale. Because efficacy is strain-dependent, a clinical program needs a way to identify each patient's resident Kp and predict whether a given cocktail will suppress it — stool-screen, then match.

Design. From PSC patient stool, we detect Kp (selective culture plus molecular methods), isolate and genotype strains, and phage-type them against the Aim 1 cocktail(s) by EOP and liquid-suppression assays. We then quantify, in ex vivo cultures of patient stool/isolates, how well in vitro susceptibility predicts actual Kp suppression, reporting predictive performance (sensitivity/specificity, predictive values) with confidence intervals. We codify a turnaround-time and decision framework (susceptible → matched cocktail; non-susceptible → cocktail assembly/expansion) suitable for an eIND pathway. Enrollment numbers and timelines are [ILLUSTRATIVE].

Expected outcomes. A validated stool-to-susceptibility pipeline with defined predictive accuracy, the patient-stratification basis for a future trial, and an estimate of the addressable fraction of US PSC patients carrying targetable Kp.

Pitfalls & alternatives. Not all PSC patients carry detectable Kp; we quantify the addressable fraction and define screening criteria rather than assume universal applicability. If culture is low-yield, we lean on molecular detection plus targeted enrichment. This aim is diagnostic/translational and does not administer phage to patients.

Timeline

[ILLUSTRATIVE] 5-year R01. **Years 1–2:** Aim 1 phage isolation, sequencing, host-range mapping, and sustained-suppression cocktail assembly; Aim 3 stool-screening pipeline build-out and Kp biobanking. **Years 2–4:** Aim 2 mouse dosing studies (sequential route-comparison, then combined-dosing cohorts). **Years 3–5:** Aim 3 predictive-accuracy validation and eIND-readiness package (CMC-aware phage characterization, FDA pre-submission). **Milestones gate progression:** benchmark-beating cocktail (end Yr 2) before in vivo scale-up; positive combined-dosing signal (end Yr 4) before finalizing the translational package.

Budget Justification (modular R01-style sketch)

[ILLUSTRATIVE] Modular budget at approximately $250,000 direct costs/year for 5 years. **Personnel:** PI (3 calendar months), a microbiologist/phage biologist, a mouse-model scientist, a bioinformatician (partial effort), and a research coordinator for stool collection. **Animals:** hepatobiliary injury-prone mouse cohorts, gnotobiotic/SPF housing, and colonization services (Aim 2). **Other:** high-throughput sequencing (phage genomes, Kp genotyping, microbiota profiling), phage purification/endotoxin removal, EOP/liquid-suppression assays, and regulatory consulting for eIND/CMC readiness. **Equipment:** no major instrumentation requested; institutional cores used. All figures [ILLUSTRATIVE] and to be finalized with institutional budgeting.

Vertebrate Animals

Animal work is proposed in Aim 2. Hepatobiliary injury-prone mouse models colonized with PSC-derived Kp will test oral, systemic, and combined phage dosing, consistent with the models in which an anti-Kp cocktail attenuated liver injury (Ichikawa et al., 2023) and the gnotobiotic models that established Kp causality (Nakamoto et al., 2019). **Justification:** the gut-to-MLN-to-liver axis cannot be recapitulated in vitro. **Minimization (3Rs):** group sizes [ILLUSTRATIVE] are set by power analysis to the minimum needed; pilot data and shared control arms reduce totals; both sexes are included to avoid duplicative single-sex studies. Procedures (oral gavage, systemic dosing, tissue/MLN harvest, euthanasia) follow IACUC-approved protocols with appropriate anesthesia/analgesia and humane endpoints. Final details to be completed with the institutional IACUC.

Human Subjects / Clinical Trial Determination

Aim 3 uses human biospecimens (PSC patient stool) for Kp detection, isolation, and phage-typing. It is diagnostic/laboratory-based and does **not** administer any intervention to participants; by the NIH definition it is **not a clinical trial**. Collection proceeds under IRB-approved protocols with informed consent, and isolates are biobanked per institutional policy. This proposal includes **no in-human phage dosing**; it builds the strain-matching and characterization foundation for a future study. Any subsequent investigational administration of phage to PSC patients would require a separate IND/emergency-or-expanded-access IND (eIND) — the route by which US phage therapy has proceeded — plus full IRB oversight and FDA engagement. Oral anti-Kp phage has an existing human safety and fecal-PK precedent (BX002-A; NCT04737876). Enrollment figures are [ILLUSTRATIVE].

Investigators & Environment

NIH weighs investigator track record and environment heavily; the assembled team is specified by the capabilities each role must demonstrate (template roles to fill with named investigators and documented productivity).

- **Principal Investigator** — hepatologist or gut-microbiome physician-scientist with a publication and funding record in PSC / the gut-liver axis; responsible for overall direction (Aims 1–3).
- **Co-Investigator, Phage Biology** — demonstrated bacteriophage isolation, genomics, and cocktail design, ideally with anti-Kp experience (Aim 1).
- **Co-Investigator, Microbiome / Animal Models** — gnotobiotic/SPF hepatobiliary injury models and microbiota profiling (Aim 2).
- **Co-Investigator, Clinical/Translational** — established PSC patient cohort access, stool biobanking, and IRB experience (Aim 3).
- **Bioinformatics Core** — phage/Kp comparative genomics and microbiota analysis.
- **Regulatory/CMC Advisor** — eIND/IND strategy and phage manufacturing characterization.
- **Environment.** An academic medical center with an established PSC/IBD clinic and biorepository, gnotobiotic mouse facility, genomics core, and GMP-capable or GMP-partnered phage production; potential collaboration with groups holding anti-Kp phage and PSC-pathobiont expertise.

Alternate funder note. NIDDK is the primary target given the liver-disease focus; the antibacterial/phage and infectious-disease dimensions also align with NIAID should a co-funding or

alternate route be advantageous.

References

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