Funder target: NIDDK · R01 (Research Project Grant). Modular budget; 5-year project period. All bracketed [ILLUSTRATIVE] values are placeholders to be finalized with institutional data.

Subtractive Microbiome Therapy for IBD: A Strain-Resolved Oral Phage Cocktail Targeting an Inflammation-Driving *Klebsiella pneumoniae* Pathobiont

Project Summary / Abstract

Inflammatory bowel disease (IBD) is driven, in part, by discrete gut pathobionts rather than by uniform dysbiosis. A specific clade of *Klebsiella pneumoniae* (Kp) is enriched in the gut of IBD patients and associated with disease severity; on colonization of colitis-prone, germ-free, and conventionally colonized mice, these strains aggravate intestinal inflammation (Federici et al., *Cell* 2022). Because the offending agent is a single, definable pathobiont, broad-spectrum antibiotics are a poor fit: they collaterally deplete protective commensals and select for resistance in an organism already prone to carbapenem resistance. Lytic bacteriophages are uniquely matched to this problem because they are strain-specific—a rationally assembled cocktail can *subtract* the target Kp from a complex community while leaving the surrounding microbiome intact. A lytic five-phage combination has been shown to lower Kp burden and attenuate colitis in mice, to survive transit through an artificial human gut in a gastric-acid-dependent manner, and to be evaluated in healthy volunteers (Federici et al., *Cell* 2022). The same therapeutic logic generalizes within NIDDK's mission: a Kp-targeting phage cocktail reduced fecal Kp and ameliorated hepatobiliary injury in models of primary sclerosing cholangitis (PSC), the IBD-comorbid liver disease (Ichikawa, Nakamoto, Kredo-Russo et al., *Nat Commun* 2023). The indication thus rests on robust mechanism plus animal efficacy plus early human safety/pharmacokinetics—**not** on demonstrated efficacy in IBD patients (Sausset & De Sordi, *Cell Host & Microbe* 2022). This R01 addresses that specific gap. We will (1) build a strain-resolved Kp panel and a depolymerase-aware phage cocktail with quantified resistance dynamics; (2) test efficacy, microbiome selectivity, and host-immune endpoints in complementary colitis and humanized-microbiota mouse models; and (3) define a gastric-acid-protected oral formulation with the pharmacokinetic/pharmacodynamic (PK/PD) and biomarker package needed to design a future Phase 2 IBD trial. The work is responsive to NIDDK's digestive-

disease mission and lays rigorous preclinical groundwork for a precision-microbiome alternative to lifelong immunosuppression.

Specific Aims

IBD pathogenesis involves identifiable pathobionts, exemplified by an IBD-associated *Klebsiella pneumoniae* clade that aggravates murine colitis (Federici et al., *Cell* 2022). Lytic phage cocktails can deplete such a strain selectively, yet efficacy in the IBD setting has not been demonstrated, and the field has flagged phage resistance and the absence of clinical-efficacy data as the key open questions (Sausset & De Sordi, *Cell Host & Microbe* 2022). We propose to close the preclinical-to-trial gap with three aims.

Aim 1 — Assemble and characterize a resistance-robust, depolymerase-aware anti-Kp phage cocktail. We will curate a strain-resolved panel of IBD-associated Kp, map host range and capsule/LPS receptor usage, and rationally assemble a multi-phage cocktail (modeled on the validated five-phage combination concept) covering both phage-sensitive and escape-mutant Kp. We will quantify resistance emergence, characterize capsule-depolymerase activity against biofilm/adherent Kp, and confirm strictly lytic lifestyle and absence of integrase, toxin, and antimicrobial-resistance (AMR) genes by whole-genome sequencing. *Success criterion:* a sequence-validated cocktail covering ≥[ILLUSTRATIVE]% of panel isolates with resistance-emergence rates below a pre-specified threshold.

Aim 2 — Establish efficacy, microbiome selectivity, and host-immune endpoints in colitis and humanized-microbiota mouse models. In colitis-prone and gnotobiotic/humanized-microbiota mice colonized with target Kp, we will test whether the oral cocktail lowers fecal Kp burden, attenuates colitis, and reduces pathogenic T-cell and epithelial inflammatory readouts (including Th17/Th1 and inflammasome-associated markers such as caspase-11/IL-18), while sparing non-target commensals by shotgun metagenomics. *Success criterion:* significant Kp reduction and colitis attenuation **with** metagenomic preservation of non-target taxa, versus antibiotic-driven collateral loss.

Aim 3 — Define a gastric-protected oral formulation, PK/PD, and a trial-enabling biomarker package. We will optimize an acid-protected oral formulation, measure gut phage delivery and Kp pharmacodynamics, and assemble the safety/biomarker dossier required to design a future Phase 2 IBD efficacy study under an investigational pathway. *Success criterion:* a stable formulation delivering viable phage to the distal gut with a defined PK/PD relationship and a pre-specified go/no-go biomarker set.

Impact. Success would provide the rigorous, strain-resolved efficacy and translational package needed to advance the first oral phage cocktail aimed at subtracting an inflammation-driving Kp strain

in IBD—precision microbiome editing as an alternative to broad immunosuppression.

Significance

IBD affects millions and is managed largely with immunosuppressants that act broadly and carry infection and malignancy risks. A mechanistic shift is underway: rather than treating IBD as undifferentiated dysbiosis, specific pathobionts are now implicated as tractable drivers. Federici et al. (*Cell* 2022) analyzed four geographically distinct IBD cohorts (n = 537), identified an IBD-associated Kp clade linked to disease severity, and showed that colonization of colitis-prone, germ-free, and colonized mice with these strains aggravates intestinal inflammation. This reframes a subset of IBD as, in part, a single-organism problem—and a single-organism problem invites a single-organism solution.

Antibiotics are the wrong tool here. Broad-spectrum agents collaterally damage the protective commensals whose loss is itself implicated in IBD, and they drive resistance in an organism already notorious for carbapenem resistance. Phages are well matched precisely because they are strain-specific, can be delivered orally to the site of disease, and—because many Kp phages carry capsule depolymerases—can penetrate the adherent, capsule-shielded Kp that characterize gut colonization. The therapeutic relevance extends beyond the bowel: a Kp-targeting phage cocktail reduced fecal Kp and lessened hepatobiliary injury in mouse models of PSC, the IBD-comorbid liver disease (Ichikawa, Nakamoto, Kredo-Russo et al., *Nat Commun* 2023), suggesting one gut-directed intervention could address linked digestive-disease phenotypes within NIDDK's mission.

Critically, the field has strong mechanism, animal efficacy, and early human safety/PK—but **no** published efficacy readout in IBD patients, and commentary on this work explicitly identifies phage resistance and the need for clinical-efficacy data as the decisive next questions (Sausset & De Sordi, *Cell Host & Microbe* 2022). This is the precise, fundable gap: rigorous, reproducible preclinical efficacy with strain resolution, microbiome selectivity, and a trial-enabling translational package. Filling it would de-risk a precision-medicine alternative to lifelong immunosuppression and establish a generalizable "subtract-one-pathobiont" playbook for digestive disease.

Innovation

This proposal is innovative in concept and execution. Conceptually, it advances **subtractive** microbiome medicine: instead of adding drugs that suppress host immunity, it removes a defined causal pathobiont and leaves the protective community intact—an inversion of the standard IBD treatment logic. Technically, we move beyond a fixed consortium to a **resistance-aware, receptor-rational** cocktail design: host-range and capsule/LPS receptor mapping guide phage selection so the

cocktail covers both phage-sensitive and capsule-escape-mutant Kp, exploiting the fitness trade-off whereby capsule-loss escape mutants are frequently less virulent. We prioritize **capsule-depolymerase–armed** phages to degrade the polysaccharide capsule and biofilm matrix that shield gut-adherent Kp. Methodologically, pairing colitis-prone models with humanized-microbiota mice lets us measure, in the same study, both efficacy (colitis, Th17/Th1, inflammasome markers) and selectivity (metagenomic sparing of non-target commensals)—the two endpoints a precision therapeutic must satisfy. Finally, by front-loading a gastric-protected oral formulation and a PK/PD-plus-biomarker package, the project is explicitly trial-enabling, directly answering the resistance and clinical-efficacy questions the field has raised (Sausset & De Sordi, *Cell Host & Microbe* 2022).

Approach

Overall rigor. All in vivo studies will use predefined, blinded, randomized designs with a priori power analysis [ILLUSTRATIVE], pre-registered analysis plans, and authenticated reagents (Kp isolates confirmed by sequencing; phage stocks confirmed by genome and titer). **Sex as a biological variable:** both sexes will be included in all animal cohorts and analyzed for sex-based differences; cohorts will be sized to detect major sex effects [ILLUSTRATIVE], with follow-up powered studies if interactions emerge. Key reagents, isolates, and analysis code will be deposited to enable reproducibility.

Aim 1 — Assemble and characterize a resistance-robust, depolymerase-aware anti-Kp phage cocktail

Rationale. Single phages have narrow host range, and Kp readily mutates its capsule to escape; the validated solution is a rationally assembled cocktail combining phages active against phage-sensitive and escape-mutant Kp (Federici et al., *Cell* 2022, using a lytic five-phage combination). Capsule depolymerases improve penetration of biofilm-embedded Kp, and resistance dynamics are the field's leading concern (Sausset & De Sordi, *Cell Host & Microbe* 2022).

Experimental design. We will assemble a strain-resolved panel of IBD-associated Kp (clinical isolates plus reference strains spanning relevant capsule types). For each phage—characterized anti-Kp phages plus newly isolated environmental phages—we will determine host range, efficiency-of-plating, and receptor usage (capsule/LPS O-antigen) using capsule and LPS mutants. Whole-genome sequencing will confirm strictly lytic lifestyle and absence of integrase, toxin, and AMR genes. We will quantify depolymerase activity (halo assays; biofilm degradation). Candidate cocktails will be evaluated in vitro for breadth and for suppression of resistance emergence in serial-passage and time-kill assays, and we will assess phage-antibiotic synergy (PAS) as a potential adjunct against multidrug-resistant Kp.

Expected outcomes. A defined, sequence-validated cocktail covering the target clade and its escape mutants, with documented depolymerase activity and measured, low resistance-emergence rates—a development candidate carried into Aims 2–3.

Potential pitfalls & alternative approaches. Kp may escape via capsule switching; we mitigate with multi-receptor cocktails and characterize escape mutants for reduced virulence (capsule loss). If natural phages give insufficient breadth, we will broaden environmental isolation, add PAS, or evaluate engineered/sequence-specific approaches to re-sensitize or more narrowly target resistant strains—while keeping a natural lytic cocktail as the lead, consistent with the orally delivered, gastric-acid-resilient strategy validated in prior work (Federici et al., *Cell* 2022).

Aim 2 — Establish efficacy, microbiome selectivity, and host-immune endpoints in colitis and humanized-microbiota mouse models

Rationale. Target Kp aggravates inflammation in colitis-prone, germ-free, and colonized mice, and a phage cocktail attenuated this in prior work (Federici et al., *Cell* 2022). Efficacy must be paired with proof that non-target commensals are spared.

Experimental design. Colitis-prone and gnotobiotic/humanized-microbiota mice (both sexes) will be colonized with target Kp and randomized to oral cocktail versus vehicle (and, where informative, an antibiotic comparator). *Primary endpoint:* fecal Kp burden (qPCR/CFU). *Inflammation endpoints:* colitis severity (blinded histology, weight, colon length), Th17/Th1 readouts, and inflammasome-associated markers (caspase-11/IL-18). *Selectivity endpoint:* shotgun metagenomics to confirm sparing of non-target taxa versus antibiotic-driven collateral loss. Group sizes will be set by power analysis [ILLUSTRATIVE], with blinded scoring and pre-registered analysis plans.

Expected outcomes. Oral phage cocktail lowers Kp burden and attenuates colitis and Th17/Th1/inflammasome readouts while preserving the surrounding microbiome—distinguishing precision phage therapy from antibiotics.

Potential pitfalls & alternative approaches. In vivo phage resistance or insufficient gut titers may blunt efficacy; we will adjust dosing/regimen, deploy depolymerase-armed members, and test PAS. If colonization is unstable, we will use defined gnotobiotic colonization to standardize engraftment, complementing humanized-microbiota cohorts. If sex differences emerge in colonization or response, downstream studies will be powered accordingly.

Aim 3 — Define a gastric-protected oral formulation, PK/PD, and a trial-enabling biomarker package

Rationale. Orally delivered Kp-phage cocktails can survive gastric transit in a gastric-acid-dependent

manner and reach the gut, as shown in an artificial human gut and in healthy volunteers (Federici et al., *Cell* 2022). Translation requires a defined formulation and PK/PD relationship.

Experimental design. We will optimize an acid-protected oral formulation and measure phage stability through a simulated/artificial gut and in vivo gut delivery (luminal/fecal viable titers). PK/PD analyses will relate delivered phage to Kp suppression kinetics and resistance over time. We will assemble the translational dossier—formulation stability, dose-ranging, tolerability signals, and candidate response biomarkers (Kp burden, inflammatory readouts)—needed to design a future Phase 2 IBD efficacy trial, including regulatory framing (investigational phage delivered under an FDA IND; the emergency/expanded-access eIND route noted for individual use) and a clade-typing diagnostic-to-treatment concept.

Expected outcomes. A stable, gastric-protected oral formulation with defined gut delivery and PK/PD, plus a biomarker and regulatory package that operationalizes a go/no-go decision and a Phase 2 IBD trial design.

Potential pitfalls & alternative approaches. Acid lability or low gut titers will be addressed by encapsulation/enteric coating and dose escalation; if single-dose delivery is insufficient, repeated dosing and adjunctive PAS will be evaluated. Heterogeneity in patient Kp clades motivates the upfront sequencing/diagnostic step to match cocktail to strain.

Timeline

[ILLUSTRATIVE] Years 1–2: Aim 1 panel/cocktail assembly, sequencing, depolymerase and resistance characterization. Years 2–4: Aim 2 efficacy and selectivity studies across both mouse models. Years 3–5: Aim 3 formulation, PK/PD, and trial-enabling package; data integration and Phase 2 design. Milestones and go/no-go decisions [ILLUSTRATIVE] at the end of Years 2 and 4.

Budget Justification (modular R01-style sketch)

[ILLUSTRATIVE] Modular budget at $250,000 direct costs/year for 5 years. **Personnel:** PI (effort, leadership), Co-I microbiologist/phage biologist, Co-I gastroenterologist-immunologist, postdoctoral fellow(s), and a research technician supporting in vitro and animal work [ILLUSTRATIVE effort levels]. **Other Personnel:** bioinformatician for metagenomics and genome safety screening. **Equipment/Supplies:** phage production and purification, Kp culture, sequencing, gnotobiotic/humanized-microbiota mouse husbandry, histology, immune assays (Th17/Th1, caspase-11/IL-18), and formulation reagents. **Animal costs:** per-diem and gnotobiotic isolator use [ILLUSTRATIVE]. **Other:** publication, core-facility fees, and travel [ILLUSTRATIVE]. Final

modules and personnel effort to be set per institutional rates.

Vertebrate Animals

Animal work is proposed. **(1) Description.** Studies use colitis-prone and gnotobiotic/humanized-microbiota mice (both sexes) colonized with target Kp to test phage efficacy, microbiome selectivity, and host-immune endpoints (Aim 2; formulation/PK in Aim 3); species, strains, and numbers will be finalized with the IACUC. **(2) Justification.** In vitro systems cannot reproduce gut colonization, mucosal immunity (Th17/Th1, caspase-11/IL-18), or microbiome-sparing readouts central to a precision therapeutic. **(3) Minimization & rigor.** Power-based group sizes [ILLUSTRATIVE], blinded/randomized design, inclusion of both sexes, and shared cohorts for efficacy and selectivity endpoints minimize animal use. **(4) Welfare.** Humane, predefined endpoints for colitis severity; analgesia per veterinary guidance; IACUC-approved protocols. Euthanasia will follow AVMA Guidelines.

Human Subjects / Clinical Trial

No patient dosing occurs within this R01; human-derived material is limited to Kp clinical isolates and microbiota samples used to build strain panels and humanized models, handled under IRB oversight with appropriate consent and biosafety review. The project is explicitly trial-enabling: it produces the formulation, PK/PD, safety, and biomarker package to design a **future** Phase 2 IBD efficacy study. For that future study, investigational phage would be administered under an FDA Investigational New Drug (IND) application; the emergency/expanded-access IND (eIND) route is noted for individual compassionate use, and any clinical protocol would proceed under IRB approval with prospective registration. Prior oral Kp-phage work delivered viable phage to the gut and was evaluated in healthy volunteers (Federici et al., *Cell* 2022), informing this translational framing.

Team & Environment

[Template—fill with real names/institutions.] **Contact PI [Name, Institution]:** microbiome/phage therapeutics lead. **Co-Investigator [Name]:** Kp microbiology, phage isolation/genomics, depolymerase characterization. **Co-Investigator [Name]:** gastroenterology/mucosal immunology, colitis models and Th17/Th1/inflammasome endpoints. **Co-Investigator/Consultant [Name]:** gnotobiotic/humanized-microbiota mouse core. **Bioinformatics [Name]:** metagenomics and genome safety screening. **Regulatory/CMC consultant [Name]:** phage formulation and IND/eIND strategy. **Environment:** institutional gnotobiotic facility, BSL-2 phage/Kp laboratories, sequencing and histology cores, and IACUC/IRB/biosafety infrastructure. **Complementary funding:** adjacent aims

(e.g., AMR-focused phage work, patient-cohort biobanking) may be supported by NIAID or the Crohn's & Colitis Foundation; this R01 is scoped to the NIDDK digestive-disease question and stands alone.

References

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2. Ichikawa M, Nakamoto N, Kredo-Russo S, et al. Bacteriophage therapy against pathological *Klebsiella pneumoniae* ameliorates the course of primary sclerosing cholangitis. *Nature Communications.* 2023;14:3261. https://doi.org/10.1038/s41467-023-39029-9
3. Sausset R, De Sordi L. Host happy hour: Phage cocktail targets IBD-associated microbes. *Cell Host & Microbe.* 2022 Aug 10;30(8):1066-1068. https://www.cell.com/cell-host-microbe/fulltext/S1931-3128(22)00466-8