Strictly-Lytic, Commensal-Sparing Phage Cocktail Editing of *Fusobacterium nucleatum* to De-Repress Antitumor Immunity in Checkpoint-Inhibitor Non-Responders

Funder / mechanism: NCI · U01 (Research Project Cooperative Agreement) **Indication:** Microbiome conditioning to convert immune-checkpoint-inhibitor (ICI) non-responders in colorectal cancer (CRC) **Modality:** Oral, GMP-grade, strictly lytic anti–*Fusobacterium nucleatum* bacteriophage cocktail, given as companion conditioning alongside anti–PD-1 **Status:** Concept for discussion — illustrative figures flagged [ILLUSTRATIVE]; not a submitted application

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Project Summary / Abstract

Response to immune checkpoint inhibitors (ICIs) is shaped by the gut microbiome, and *Fusobacterium nucleatum* (*Fn*) is a mechanistically central "response-blunting" taxon: it recruits myeloid-derived suppressor cells (MDSCs), induces PD-L1, and dampens $CD8^+$ T-cell infiltration, sustaining an immunosuppressive tumor microenvironment (TME). The tools available to "condition" the microbiome before immunotherapy cut the wrong way: broad-spectrum antibiotics indiscriminately deplete the very commensals ICI efficacy depends on, while fecal microbiota transplant (FMT) and diet are nonspecific and, for FMT, donor-dependent and transfer-risk-bearing. Bacteriophages are uniquely suited to this problem: they are receptor-specific predators that can subtract a single deleterious lineage while sparing the immunostimulatory community.

This U01 will develop and rigorously test an orally delivered, **defined, strictly lytic** anti-*Fn* phage cocktail as a precision **companion conditioning** agent given alongside checkpoint blockade. The mechanistic premise rests on two complementary preclinical proofs-of-concept from the same platform: (i) *Fn*-targeting phages that inhibit *Fn* growth and, conjugated to drug-loaded nanoparticles, augment first-line CRC chemotherapy in orthotopic and spontaneous mouse tumors, with a clean piglet safety profile [Zheng 2019]; and (ii) a non-lytic *Fn*-binding M13 silver-nanoparticle hybrid (M13@Ag) that scavenged *Fn*, reduced intratumoral MDSCs, and — combined with anti–PD-1 — significantly slowed tumor growth and prolonged survival in orthotopic CT26 CRC [Dong 2020].

M13@Ag establishes the *immune mechanism* (subtracting *Fn* de-represses anti-PD-1 efficacy); our innovation is to deliver that subtraction with a **strictly lytic, self-amplifying, commensal-sparing oral cocktail** suitable for GMP manufacture and outpatient use.

We will (1) assemble and characterize a clinical-grade lytic cocktail with quantified *Fn* coverage and measured commensal sparing; (2) define how *Fn* depletion remodels the TME and potentiates anti–PD-1 in orthotopic CRC; and (3) complete IND-enabling safety, biodistribution, and manufacturing (CMC) to position a biomarker-selected first-in-human conditioning trial. As an NCI U01, endpoints, immune-monitoring assays, and microbiome-sequencing standards will be harmonized with NCI immuno-oncology programs under shared go/no-go milestones. The outcome is a translational, GMP-trackable phage-conditioning candidate that turns the gut from an unpredictable variable into a tunable lever on cancer-immunity outcomes.

Specific Aims

Gut-microbiome composition is a determinant of anti–PD-1 response, yet the only tools to condition it before immunotherapy — antibiotics, FMT, diet — cannot subtract a single harmful taxon without collateral damage to the commensals ICIs require. Phages offer receptor-mediated strain specificity: a defined, strictly lytic cocktail can deplete *Fn* while sparing beneficial Ruminococcaceae/*Faecalibacterium* and short-chain-fatty-acid (SCFA) producers, "de-repressing" antitumor immunity so checkpoint blockade can work. We propose:

Aim 1. Assemble and characterize a defined, strictly lytic, commensal-sparing anti-*Fn* phage cocktail. We will compile **strictly lytic** phages against a panel of clinical *Fn* strains, quantify host range and lytic efficiency, and demonstrate selective depletion of *Fn* without reducing beneficial SCFA-producing commensals in defined consortia and complex stool-derived communities. We will pre-empt resistance via complementary-receptor cocktail design and depolymerase-bearing, biofilm-penetrating components. *Go/no-go:* a cocktail meeting pre-specified coverage and commensal-sparing thresholds.

Aim 2. Define how phage-mediated *Fn* depletion remodels the TME and potentiates anti–PD-1. In orthotopic CRC mouse models colonized with *Fn*, we will test whether oral cocktail dosing reduces intratumoral MDSC recruitment, lowers PD-L1, restores $CD8^+$ T-cell infiltration, and — combined with anti–PD-1 — inhibits tumor growth and extends survival, benchmarked against the published M13@Ag *Fn*-scavenging result [Dong 2020]. *Go/no-go:* combination superiority over either agent alone on pre-specified immune and survival endpoints.

Aim 3. Complete IND-enabling safety, biodistribution, and CMC to enable a first-in-human

conditioning trial. We will conduct GLP-style repeat-dose oral safety/biodistribution studies, manufacture cocktail to quality standards for clinical phage products with endotoxin/purity release specifications, and prepare an FDA emergency/expanded-access IND (eIND) pathway and IRB-reviewed protocol for ICI-eligible CRC patients selected by stool sequencing. *Go/no-go:* an acceptable safety package plus a release-spec'd, GMP-trackable cocktail.

Impact: Success would deliver the first precision microbiome-editing agent designed to convert predicted checkpoint-inhibitor non-responders into responders, establishing strictly lytic phage conditioning as an oral, outpatient adjunct to immuno-oncology.

Significance

ICIs have transformed oncology, but a majority of patients still do not respond, and gut-microbiome composition is an established modulator of that response. The microbiome is causal and editable — responder-derived FMT can produce objective responses in ICI-refractory disease — but FMT is donor-dependent, variable, and carries transfer risk, and it cannot be reduced to a defined release-specified product. Among response-blunting taxa, *Fn* is mechanistically central: it recruits MDSCs, induces PD-L1, and dampens $CD8^+$ T-cell infiltration, building an immunosuppressive TME that blunts checkpoint blockade. The standard conditioning levers cut the wrong way — broad-spectrum antibiotics deplete exactly the Ruminococcaceae/*Faecalibacterium* and SCFA producers ICI efficacy depends on. There is therefore a clear unmet need for a **subtractive, strain-specific** tool.

Engineered and naturally lytic phages meet that need, and the preclinical evidence base is directly on point. *Fn*-targeting phages inhibit *Fn* growth and, as drug-conjugated nanoparticles, augment first-line CRC chemotherapy in orthotopic and spontaneous tumors, with negligible changes in piglet haemocyte counts, immunoglobulin/histamine levels, and liver/renal function [Zheng 2019] — establishing both target tractability and a favorable safety signal. Critically for immuno-oncology, the M13@Ag *Fn*-binding hybrid links *Fn* subtraction directly to reduced intratumoral MDSCs and improved anti–PD-1 efficacy with prolonged survival in orthotopic CT26 CRC [Dong 2020] — the exact causal chain a conditioning agent must exploit. The broader engineering toolkit (cocktail design, depolymerases/biofilm penetration, CRISPR-armed specificity, GMP manufacture, and documented synergy with checkpoint blockade) is reviewed in [Xu 2026].

An NCI U01 is the right vehicle: converting non-responders to responders requires harmonized immune-monitoring assays, microbiome-sequencing standards, and shared go/no-go criteria across a cooperative program — the infrastructure NCI immuno-oncology networks provide. (NIAID, given its phage-therapy and microbiome portfolios, is a logical alternate or partner home.)

Innovation

1. **Conditioning, not cytotoxic adjunctivity.** Prior phage work paired *Fn* depletion with *chemotherapy* [Zheng 2019] or used a metal-armed binding capsid [Dong 2020]. We reframe a **defined, strictly lytic, orally delivered cocktail** as pre-/co-immunotherapy microbiome editing whose explicit endpoint is conversion to ICI responsiveness.
2. **Strictly lytic and self-amplifying — distinct from the published constructs.** Unlike the non-lytic M13@Ag binding/scavenging hybrid (which depends on stoichiometric silver-nanoparticle payload) [Dong 2020], a replicating lytic cocktail amplifies at the target and carries no metal payload, simplifying CMC and the regulatory profile for repeat oral dosing.
3. **Commensal-sparing by design — as a release criterion.** Unlike antibiotics, the cocktail is validated to subtract *Fn* while quantitatively preserving SCFA producers, turning strain specificity into a *measurable* specification rather than an aspiration.
4. **Mechanism-anchored immune readouts.** We tie depletion to MDSC reduction, PD-L1 lowering, and $CD8^+$ restoration as the causal chain enabling checkpoint blockade — the relationship M13@Ag demonstrated [Dong 2020].
5. **Engineering headroom.** The platform is compatible with biofilm-penetrating depolymerases for mucosal/tumoral biofilms, with M13-display *Fn*-binders as an *orthogonal* fallback, and with a roadmap toward CRISPR-armed precision editing [Xu 2026].

Approach

Aim 1 — Assemble and characterize a defined, strictly lytic, commensal-sparing anti-*Fn* phage cocktail

Rationale. The therapeutic advantage of phages over antibiotics is receptor-mediated strain specificity. Realizing it for an oral conditioning product requires broad coverage of clinical *Fn* strains, low resistance liability, **strictly lytic** lifecycle (no lysogeny/toxin-transfer risk), and demonstrable sparing of beneficial commensals [Xu 2026].

Experimental design. We will isolate and genomically vet **strictly lytic** anti-*Fn* phages (screening out temperate phages, integrases, and known virulence/AMR genes) and screen them against a panel of *Fn* clinical isolates [ILLUSTRATIVE: ~20–30 strains spanning subspecies]. We will quantify host range, efficiency of plating, and killing kinetics; combine complementary-receptor phages into a defined cocktail to suppress resistance; and incorporate depolymerase-bearing, biofilm-penetrating

components. Selectivity will be assessed in defined consortia and in complex stool-derived communities by 16S/shotgun sequencing, with the pre-specified criterion that *Fn* is depleted while Ruminococcaceae/*Faecalibacterium* relative abundance and SCFA output are preserved. As an *orthogonal* binding modality (not part of the lytic release product), we will bank engineered M13-display *Fn*-binders [scaffold per Dong 2020] for use only if lytic coverage proves incomplete.

Expected outcomes. A characterized strictly lytic cocktail with documented coverage, defined resistance mitigation, and quantified commensal sparing — release-style specifications suitable for downstream CMC.

Pitfalls & alternatives. Phage resistance may emerge; we mitigate via multi-receptor cocktails and depolymerases and can iterate composition. If lytic coverage of some strains is incomplete, the banked M13-display *Fn*-binders provide an orthogonal target-binding mechanism [Dong 2020], explicitly flagged as a distinct (non-lytic) construct with its own CMC path.

Aim 2 — Define how phage-mediated *Fn* depletion remodels the TME and potentiates anti–PD-1

Rationale. Conditioning is valuable only if subtracting *Fn* causally de-represses antitumor immunity and improves checkpoint outcomes — the relationship M13@Ag demonstrated by scavenging *Fn*, reducing MDSCs, and enhancing anti–PD-1 [Dong 2020].

Experimental design. In orthotopic CRC mouse models colonized with *Fn* (e.g., CT26 orthotopic, as in [Dong 2020]), animals will receive oral cocktail, anti–PD-1, the combination, or vehicle/isotype controls [ILLUSTRATIVE: n per arm powered for survival and immune endpoints; pre-registered analysis plan]. Readouts: intratumoral MDSC frequency ($CD11b^{+}Gr\text{-}1^{+}$), PD-L1 expression, $CD8^{+}$ T-cell infiltration and function, tumor growth, and survival, with paired gut-community sequencing to link bacterial depletion to immune change. The published M13@Ag *Fn*-scavenging combination serves as the *qualitative* mechanistic benchmark [Dong 2020]; we do not assume its effect size transfers to a lytic cocktail.

Expected outcomes. Oral cocktail dosing reduces MDSC recruitment and PD-L1, restores $CD8^{+}$ infiltration, and — combined with anti–PD-1 — slows tumor growth and extends survival beyond either agent alone, establishing the causal conditioning chain.

Pitfalls & alternatives. Murine colonization and TME imperfectly model human disease; we will use orthotopic models and, where feasible, humanized-microbiome approaches. If lysis alone yields modest immune shifts, the orthogonal M13-display binders (with capsid-mediated APC engagement) offer a synergy-enhancing alternative [Dong 2020; Xu 2026]. Conditioning effect sizes are not yet established in this context; the program is powered as preclinical/hypothesis-testing, not confirmatory.

Aim 3 — Complete IND-enabling safety, biodistribution, and CMC to enable a first-in-human conditioning trial

Rationale. Translation requires GLP-style safety, manufacturing consistency, and a regulatory route. Prior piglet work indicates a favorable safety profile for *Fn*-targeting phage delivery [Zheng 2019], and the engineering literature defines GMP expectations for clinical phage products [Xu 2026].

Experimental design. We will perform repeat-dose oral safety and biodistribution studies; develop a manufacturing/QC process with endotoxin, purity, identity, and potency release specifications consistent with clinical phage-product quality expectations [Xu 2026]; and prepare an FDA eIND/expanded-access submission plus an IRB-reviewed protocol for ICI-eligible CRC patients, including stool-sequencing-based patient selection for *Fn* and response-blunting taxa.

Expected outcomes. A safety/biodistribution package, a GMP-trackable cocktail with release specs, and a regulatory/clinical dossier enabling a biomarker-selected first-in-human conditioning study.

Pitfalls & alternatives. Phage immunogenicity/neutralization could affect dosing; oral mucosal delivery and cocktail rotation mitigate this. If a full traditional IND is premature, the eIND/expanded-access route enables initial supervised human experience.

Timeline

[ILLUSTRATIVE] **Year 1:** strictly lytic cocktail assembly, genomic safety vetting, host-range/selectivity (Aim 1). [ILLUSTRATIVE] **Years 2–3:** mechanism and anti–PD-1 efficacy studies (Aim 2); initiate safety. [ILLUSTRATIVE] **Years 4–5:** IND-enabling GLP safety/biodistribution, CMC, and eIND/IRB package (Aim 3). Annual NCI cooperative-program milestone reviews with pre-specified go/no-go criteria at each Aim transition.

Budget Justification (modular sketch)

[ILLUSTRATIVE] Requested at [ILLUSTRATIVE: $X] direct costs/year in modular increments. **Personnel** [ILLUSTRATIVE]: contact PI, phage biologist, tumor immunologist, microbiome bioinformatician, GLP/regulatory-CMC lead, study coordinator. **Other costs:** phage isolation/genomic vetting and sequencing (Aim 1); orthotopic immuno-oncology studies with flow/IHC (Aim 2); GLP safety/biodistribution, GMP-style manufacturing/QC, and regulatory filing (Aim 3). **Animal costs** under Aims 2–3 [ILLUSTRATIVE]. **Cooperative-agreement travel** for NCI

program meetings [ILLUSTRATIVE]. All figures are placeholders pending institutional budgeting.

Vertebrate Animals

Animal work is proposed. Mouse orthotopic CRC models colonized with *Fn* will be used for mechanism and efficacy (Aim 2), and rodent (and, if warranted, large-animal) studies for GLP safety/biodistribution (Aim 3), consistent with prior piglet safety work [Zheng 2019]. **Justification:** in vitro systems cannot capture MDSC/$CD8^+$ TME remodeling or systemic safety. Group sizes [ILLUSTRATIVE] will be the minimum needed for rigorous, pre-registered endpoints; humane endpoints, analgesia, and IACUC oversight apply, with explicit attention to replacement, reduction, and refinement.

Human Subjects / Clinical Trial

No human dosing occurs in this award; Aim 3 culminates in a clinical-readiness package. The planned first-in-human conditioning study would enroll ICI-eligible CRC patients [ILLUSTRATIVE: enrollment to be determined] selected by stool sequencing for *Fn* and response-blunting taxa. Because the cocktail is investigational, initial human use is anticipated via the FDA emergency/expanded-access IND (eIND) route, under full IRB oversight, informed consent, and a data-safety monitoring plan, with manufacturing aligned to clinical phage-product quality expectations [Xu 2026].

Team & Environment

[Template — to be completed with real names/institutions.] **Contact PI:** [name/institution] (phage therapy / microbiome editing). **MPI/Co-I:** [name] tumor immunology/checkpoint blockade; [name] phage engineering (lytic-phage genomics, depolymerases, M13 display); [name] microbiome bioinformatics; [name] GLP/regulatory and CMC. **Collaborating/translational sites:** an academic phage-therapy center [ILLUSTRATIVE] for clinical translation, with platform expertise informed by published *Fn*-targeting phage-microbiota work [Zheng 2019; Dong 2020]. **Environment:** BSL-appropriate phage and anaerobe facilities, orthotopic immuno-oncology models, flow/IHC and sequencing cores, and GLP/GMP-capable partners. As a U01, the team will participate in NCI cooperative-program governance, shared assays, and milestone review.

References

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