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Review

Methylobacterium in Oncology: A Critical Analysis of Biological and Therapeutic Potential

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Abstract

Methylobacterium and taxonomically related *Methylorubrum* are environmentally widespread methylotrophs that may be detected in human tissues and disease-associated fluids, but their biological and clinical meaning in oncology remains uncertain. This focused critical review distinguishes sequencing associations from taxon-specific confirmation and experimental perturbation. Breast studies report discordant relative-abundance findings; notably, one apparent tumour enrichment was not accompanied by a statistically significant paired difference in species-targeted qPCR for *M. radiotolerans*. Gastric studies associate enrichment with fewer CD8+ tissue-resident memory T cells, while a mouse experiment supports changes in TGF-beta expression and TRM-cell features. Conversely, a mammary co-transplantation model supports an immune-dependent antitumour effect of a defined *M. radiotolerans* strain. These findings do not establish a shared mechanism across cancers. Urogenital, colorectal, pleural and lung studies remain predominantly exploratory, with limited independent microbial-assay validation. Taxonomic instability, short-read identification, compositional effects and environmental contamination restrict inference. Live organisms, supernatants, crude extracts and semi-purified enzymes require separate evaluation; product activity cannot establish the safety of live bacterial administration. Current evidence supports testable mechanistic hypotheses, not an established diagnostic, prognostic or predictive biomarker, a causal cancer organism, or a clinically supported bacterial therapy.

Keywords: *Methylobacterium*; *Methylorubrum*; tumour microbiome; contamination; immune microenvironment; biomarker validation; microbial products

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1. Introduction

Cancer remains a major global health burden [1]. Clinically useful biomarkers or microbiome-directed interventions require evidence that extends beyond detection of a taxon. The interest in *Methylobacterium* arises from a specific unresolved question: can closely related environmental bacteria exert different effects in distinct tumour habitats? Human abundance studies and experimental mammary and gastric models suggest this possibility [2–5]. However, recurrent detection alone does not make this genus cancer-specific or clinically useful. Its environmental ubiquity also makes it a plausible contaminant. We therefore examine which associations survive methodological scrutiny, which mechanisms have experimental support, and what remains hypothetical. The objective is a focused

critical appraisal of biological and therapeutic potential, rather than an exhaustive catalogue or an argument for immediate clinical translation.

2. Literature Search and Appraisal

A focused literature update was undertaken on 20 October 2026 using site-restricted web searches of PubMed-indexed records and PubMed Central, supplemented by journal publisher pages and reference checking. The core search combined (*Methylobacterium* OR *Methylorubrum*) with (cancer OR tumor OR tumour OR neoplasm OR carcinoma OR oncology). Targeted follow-up searches combined the taxon names or individual study titles with breast, gastric, prostate, bladder, colorectal, renal, lung, pleural, pancreatic, biomarker, CD8, TGF-β, supernatant, methioninase, taxonomy, contamination or catheter. No lower publication-date restriction was applied; reports available by the update date were considered. This search route was not a complete export from every bibliographic database.

Titles and abstracts were assessed for direct taxon-specific oncology relevance, followed by accessible full texts and methods. Eligible evidence comprised human cancer-associated specimens, experimental exposure to the organism or its products, and oncology-associated infection reports relevant to safety. Environmental studies without an oncology experiment, reports without a separable taxon-specific finding, duplicate records and reviews used as substitutes for original results were excluded from the primary evidence synthesis. Duplicate reports were reconciled by title and DOI. Infection and treatment-toxicity studies were interpreted separately from tumour-biology studies. Study selection was purposive; this is not a registered systematic review or meta-analysis, and no formal risk-of-bias score was assigned.

For each major study, the appraisal considered specimen and comparator, sample size, taxonomic resolution, assay, abundance measure, clinical endpoint, experimental support, contamination controls and validation (Tables 1 and 2). Evidence is described as sequencing association, taxon-specific confirmation, or experimental perturbation; these categories are not interchangeable or formal certainty grades. Numerical abundance is reproduced only when its scale and comparison are verifiable. A value not verified from accessible material is labelled NV, rather than estimated from a graph. Accessible reports and uneven methods reporting limit completeness and preclude a pooled abundance estimate.

Table 1. Major human studies and limits of their interpretation.

Study and Setting	Specimen and Sample Size	Taxon and Resolution	Detection Method	Direction and Quantitative Abundance	Clinical Endpoint	Mechanistic Support	Validation Status	Main Limitation
Wang 2017 [2] Breast	57 cases/21 controls enrolled; 39 case-associated and 24 control tissue specimens analysed	*Methylobacterium* genus	16S sequencing	Lower in cases; median proportion 0.10 vs. 0.24; $p = 0.03$	Case–control status; exploratory pathology associations	No taxon intervention	No external microbial-assay validation	Very low read depth; unadjusted comparisons
Xuan 2014 [3] Breast	20 paired tumour/adjacent specimens	*M. radiotolerans* species assignment	16S; β-actin-normalised species-targeted qPCR	Relative enrichment by 16S; β-actin-normalised species-targeted qPCR showed no significant paired difference ($p = 0.2508$).	Tissue contrast	Host immune-gene associations only	Within-cohort qPCR	Species-specific qPCR was β-actin-normalised, not tissue-mass-calibrated; no significant paired difference under that assay.
Sarkar 2022 [6] Prostate	FFPE: discovery 33 PCa/13 BPH; validation 16 PCa/15 BPH	*M. organophilum* species-targeted confirmation	16S; two qPCR primer sets	Higher in PCa; numerical relative abundance NV; qPCR normalized to GAPDH	PCa vs. BPH	No bacterial perturbation	Second-cohort relative qPCR	Not calibrated absolute burden or a clinical test
Al Shareef 2026 [7] Prostate	40 FFPE tissues: 23 PCa/17 BPH	*Methylobacterium* genus	16S; SILVA 138	Higher in PCa; quantitative abundance NV	Disease status; exploratory survival	PICRUSt2 inference only	No independent cohort	Small retrospective cohort; inferred function
Gonçalves 2023 [8] Prostate	Urine, glans and biopsies; 15 PCa/15 non-PCa participants	*Methylobacterium/Methylorubrum* merged genus label	16S sequencing	Higher in non-PCa urine; quantitative abundance NV	Case–control status	None established	No external assay validation	Small cohort; urine is not tumour tissue
Peng 2022 [5] Gastric	53 cancers/30 gastritis mucosae; mouse model	*Methylobacterium* human genus signal; intervention identity NV	16S; immune assays; intervention	Tumour enrichment; quantitative abundance NV	Survival; CD8+ TRM association	Mouse exposure: lower TGF-β/TRM features	Human–model triangulation; no clinical assay validation	Effector and mediation unresolved
Yang 2022 [9] Gastric	13 proximal/16 distal cancers with paired non-tumour tissue	*Methylobacterium/Methylorubrum* merged genus label	16S; untargeted metabolomics	Higher in distal tumour; quantitative abundance NV	Anatomical tissue contrast	Metabolite correlations	No independent validation	Correlation does not establish microbial metabolic flux
Wang 2020 [10] Colorectal	75 CRC patients sampled at three tissue sites; 26 healthy controls	*Methylobacterium* genus	16S; spatial comparisons	Higher off/adjacent than on tumour; quantitative abundance NV	Spatial disease association	Computational "driver" label	No intervention validation	No causal test of the proposed driver role

Table 1. *Cont.*

Study and Setting	Specimen and Sample Size	Taxon and Resolution	Detection Method	Direction and Quantitative Abundance	Clinical Endpoint	Mechanistic Support	Validation Status	Main Limitation
Ma 2026 [11] Colorectal	30 patients: tumour, adjacent tissue, stool; 30 healthy stool controls	*Methylobacterium/Methylorubrum* merged genus label	16S V3–V4	Comparator-dependent; quantitative abundance NV	Cross-sectional stage association	None established	No independent validation	Tissue/stool contrast is not a disease effect alone
Gao 2025 [12] rNET	Stool: 21 rNET/19 healthy participants	*M. iners* metagenomic species assignment	Shotgun metagenomics	Metastatic-status signal; quantitative abundance NV	Apparent AUC 0.971; 95% CI 0.901–1.000	None established	In-study assessment only	Small subgroup comparison; feature-selection optimism
Hrbáček 2023 [13] Bladder	Catheterized urine: 34 cases/29 benign-urology controls	*Methylobacterium/Methylorubrum* merged genus label	16S sequencing	Higher in cancer urine; quantitative abundance NV	Case–control status	None established	No external assay validation	Collection/device effects; limited outcome evidence
Gu 2026 [14] Bladder	Tumour/adjacent tissue; host transcriptomic cohorts; microbial *n* NV	*Methylobacterium* human genus signal; experimental species: *M. mesophilicum* (supplier Cat. No. YJ1026)	16S; host RNA analysis; cell assays	Higher in adjacent tissue; quantitative abundance NV	Host four-gene survival score	Supernatant inhibits cell growth	External host-score validation only	Not external validation of a bacterial assay
Leal 2026 [15] Renal	Urine: 28 treated patients/21 healthy men; serial sampling	*Methylobacterium/Methylorubrum* merged genus label	16S sequencing	Higher after treatment; quantitative abundance NV	Treatment-associated change	None established	No taxon-specific response validation	Community richness result is not taxon prognosis
Kwok 2023 [16] Pleural malignancy	165 effusions; lung-cancer MPE subgroup n = 57	*Methylobacterium* genus	16S sequencing	Group/outcome-associated signal; quantitative abundance NV	Exploratory mortality association	None established	No validated standalone assay	Outcome wording is directionally ambiguous
Zhang 2026 [17] SCLC	71 tumour/metastatic biopsy specimens	*Methylobacterium* genus	16S V3–V4; read-count positivity	Higher in non-responders; quantitative abundance NV	Response 45.5% positive vs. 85.0% negative	Experiments concerned other genera	Internal models for other genera	No treatment interaction; any-read threshold

Table 1. *Cont.*

Study and Setting	Specimen and Sample Size	Taxon and Resolution	Detection Method	Direction and Quantitative Abundance	Clinical Endpoint	Mechanistic Support	Validation Status	Main Limitation
Shouval 2020 [18] Allogeneic HSCT	Serial saliva; 184 recipients	*Methylobacterium* genus	Microbiome profiling	Enriched with severe mucositis; quantitative abundance NV	Treatment toxicity; no overall-survival association	None established	No validated tumour assay	Treatment-toxicity association in a serial salivary HSCT cohort; not a tumour-detection, biomarker-validation, or cancer-outcome study.
Gaiser 2019 [19] Pancreatic cysts	105 surgical patients; cyst fluid and paired plasma	*Methylobacterium* genus	16S microbiota analysis	Cyst-grade/comparator-dependent; quantitative abundance NV	Cyst histopathology	No taxon-specific mechanism	No validated circulating assay	Selected surgical population; plasma not proof of tumour origin

Abundance denotes relative sequence abundance unless explicitly specified. NV means not verified from accessible material; it does not mean zero or not reported in the original study. Values were not digitized from plots. Directions apply only to the listed specimen and comparator; no cross-study abundance ranking or pooled effect is implied. Species assignments from reads are not strain verification. BPH, benign prostatic hyperplasia; FFPE, formalin-fixed paraffin-embedded; HSCT, haematopoietic stem-cell transplantation; MPE, malignant pleural effusion; PCa, prostate cancer; rNET, rectal neuroendocrine tumour; SCLC, small-cell lung cancer; TRM, tissue-resident memory T cell.

Table 2. Reported contamination controls in selected studies.

Study	Collection or Environmental Controls	Extraction or Reagent Blanks	PCR Negatives	Technical Replication and Confirmation	Interpretive Consequence
Wang 2017 [2]	Operating-room water/glove and pathology transport/tool controls	Parallel extraction controls	No-template controls	Sequenced background comparisons; independent repeat extraction NV	Signal compared with several sources of background; biological causality unresolved
Xuan 2014 [3]	Detailed collection-control framework NV	Blank-level results NV	Detailed negative-control results NV	Beta-actin-normalised species-specific qPCR; duplicate measurements reported	Duplicate PCR does not exclude upstream contamination; β-actin-normalised species-specific qPCR does not provide a tissue-mass-calibrated burden.
Sarkar 2022 [6]	Environmental/device-control results NV	Detailed blank results NV	Detailed negative-control results NV	Two species-specific primer sets; second cohort	Confirmation improves taxonomic support but is relative qPCR, not proof of viable residence
Al Shareef 2026 [7]	Laboratory precautions described; dedicated environmental-control results NV	Extraction blanks explicitly reported	PCR negatives explicitly reported	Independent repeat extraction NV	Stage-specific blanks reported; quantitative background and strain validation still matter
Zhang 2026 [17]	Dedicated collection-control results NV	Stage-specific blank results NV	Negatives mentioned without full stage-specific breakdown	Independent extraction/strain confirmation NV	An any-read positive threshold is particularly sensitive to background
Gu 2026 [14]	Sampling controls described in indexed text; full results NV	Extraction controls described; full results NV	PCR controls described; full results NV	Accessible abstract confirms cell experiments; repeat-extraction detail NV	Host-score validation cannot establish contamination-free bacterial detection

This comparison records verifiable reporting, not a retrospective audit of laboratory practice. NV means that the specific control, its result or its scope could not be verified; it must not be interpreted as proven omission. Technical replicate PCR, a second primer pair and an independent patient cohort answer different questions from collection and extraction blanks. A sterile medium control for a cell experiment is also not a sequencing contamination control.

3. Evidence Synthesis

3.1. Breast Studies Show Why Relative and Absolute Abundance Must Be Separated

Wang et al. reported lower *Methylobacterium* relative abundance in breast specimens from cancer patients than from non-cancer participants [2]. The study enrolled 57 cancer and 21 non-cancer participants, but its final tissue analysis comprised 39 cancer-patient specimens and 24 non-cancer specimens, rather than 78 independent tissue observations. Sampling controls were unusually extensive, although shallow tissue sequencing, demographic imbalance and uncorrected multiple comparisons weaken inference (Tables 1 and 2). Xuan et al. reported relative enrichment of *M. radiotolerans* in 20 paired tumours, whereas the β-actin-normalised species-targeted qPCR signal did not differ significantly between paired samples ($p = 0.2508$) [3]. Although the original report used the term "absolute levels", the species-specific result was reported after β-actin normalisation and does not provide a tissue-mass-calibrated estimate of absolute *M. radiotolerans* burden. The GAPDH-normalised qPCR in Table 1 pertains to the separate Sarkar prostate study and is not part of the Xuan breast-tissue assay. Several methodological differences could contribute to the discordance with Wang et al.: the studies used different tissue comparators and participant groups, sequenced different 16S regions at different read depths, used distinct DNA-extraction and bioinformatic workflows, and reported different contamination-control frameworks. Community depletion in tumours can also increase relative abundance without bacterial expansion. These differences preclude a universal protective or harmful interpretation.

The mammary intervention study used *M. radiotolerans* ATCC 27329, providing strain-defined evidence distinct from the genus-level tissue signal [4]. Co-transplantation with Py230 cells delayed growth in immunocompetent mice, with loss of benefit in NSG mice. This supports dependence on the host immune setting but does not isolate a CD8-specific mechanism, prove endogenous human tumour colonization, or demonstrate treatment of established disease. Bacterial sonicate experiments must be evaluated separately from live-bacterial exposure (Table 3).

Table 3. Experimental modalities and the evidence each supports.

Modality and Source	Reported Evidence	What the Experiment Supports	What It Does Not Support	Required Next Validation
Live strain and separate sonicate experiments [4]	*M. radiotolerans* ATCC 27329; Py230 co-transplantation; immune-context comparison	Strain- and model-specific growth effect; immune dependence	Human endogenous colonisation, established-tumour efficacy, or a CD8-specific mediator	Separate viable/inactivated/sonicate arms; immune perturbation; established-tumour and infection studies
Culture supernatant [14]	Bladder-cell proliferation/colony inhibition; host gene-expression changes	In vitro activity of the tested mixture	A defined active molecule, tumour selectivity or live-bacterial efficacy	Matched medium, pH/nutrient and normal-cell controls; sterility/endotoxin tests; fractionation and rescue
Crude organic extract [20]	ERI-135 environmental isolate; ethyl-acetate extract tested in A549 cells	Natural-product screening activity	A therapeutic index, a purified anticancer drug or evidence for administering the isolate	Chemical identification, purity, solvent controls, reproducible dose–response and selectivity
Semi-purified enzyme preparation [21]	*Methylobacterium*-derived L-methioninase with tamoxifen; cell and nude-mouse experiments	Preclinical combination activity of that preparation	Activity of a fully purified enzyme or safety/efficacy of live bacteria	Purity and specific activity; inactive-enzyme and methionine-rescue controls; pharmacokinetics/immunogenicity

All modalities remain preclinical. Future fully purified or recombinant enzymes are proposed products, not the formulation established by the semi-purified study. Biological containment, local delivery and selective microbial depletion are future strategies, not validated clinical safeguards or therapies.

3.2. Gastric Studies Support a Different Immune Association

Peng et al. associated tumour *Methylobacterium* enrichment with adverse outcome and fewer CD8+ tissue-resident memory T cells (TRM cells), combining 53 gastric cancers, 30 gastritis controls and a mouse intervention [5]. Experimental exposure reduced tumour TGF-β expression and TRM-cell features. This is stronger evidence of a biological effect than sequencing alone, but it does not establish that this pathway fully mediates tumour progression. In a separate study of 13 proximal and 16 distal gastric cancers, the merged *Methylobacterium–Methylorubrum* label was enriched specifically in distal tumours and correlated with tissue metabolites [9]. Different anatomical sites and taxonomic labels should not be pooled as one exposure. Gastritis controls also differ from cancer-free normal mucosa in inflammation and microbial ecology.

3.3. Urogenital Signals Depend on the Sampled Compartment

Prostate studies reported *M. organophilum* enrichment confirmed by relative qPCR in a second cohort, and genus-level enrichment in an independent Arab FFPE cohort [6,7]. These results support further tissue investigation; neither establishes a clinically calibrated diagnostic assay. By contrast, a small urine study found the merged *Methylobacterium–Methylorubrum* signal enriched in participants without prostate cancer [8]. Bladder-cancer urine showed relative enrichment in a catheterized cohort, whereas a separate tissue study found enrichment in adjacent non-tumour tissue [13,14]. The latter externally validated a host four-gene survival score, not a microbial-abundance test, and supernatant experiments addressed cell growth rather than clinical efficacy. In renal cell carcinoma, the urinary signal increased after systemic treatment; community-richness survival findings cannot be assigned to *Methylobacterium* [15]. These are different exposures and endpoints, rather than interchangeable measurements of the same biomarker.

3.4. Other Tissues and Fluids Provide Exploratory Signals

Colorectal studies report spatial and comparator-dependent patterns: higher abundance in off-tumour or adjacent mucosa, and differences between tissue and stool [10,11]. A computational "driver" designation is not an initiation experiment, and a cross-sectional stage correlation does not demonstrate progression. A stool metagenomic study of 21 rectal neuroendocrine-tumour patients and 19 controls reported an apparent AUC of 0.971 for *M. iners* in a metastatic-status comparison [12]. Feature selection and evaluation within this small dataset make the estimate vulnerable to optimism; it is not an independently validated diagnostic or prognostic result.

Pleural-fluid analysis included 165 participants across five diagnostic groups, but only 57 had lung-cancer malignant effusion [16]. The source contains directionally ambiguous outcome wording; a stable favourable or adverse *Methylobacterium* survival effect should not be inferred without resolving the underlying contrast. In 71 small-cell lung cancers, an unadjusted response comparison associated *Methylobacterium* positivity with lower response (45.5% versus 85.0%) [17]. Positivity was defined post hoc as any detected read (read count > 0), rather than a prespecified quantitative threshold. The reported multivariable response model used Clostridium and Lactobacillus with clinical covariates; it did not provide a covariate-adjusted *Methylobacterium* effect or a treatment-by-taxon interaction. This observation therefore does not establish treatment-effect modification. Experimental metabolite work in that paper concerned other genera and cannot validate a *Methylobacterium* mechanism. Salivary enrichment during severe post-transplant mucositis concerns treatment toxicity [18]. A pancreatic cyst study also analysed paired plasma, illustrating why absence of a validated blood assay must not be misrepresented as absence of all blood-related research [19]. None establishes a clinically validated circulating *Methylobacterium* test.

4. Discussion

4.1. Taxonomy and Contamination Limit Cross-Study Interpretation

In 2018, 11 species were reassigned to *Methylorubrum*; later phylogenomic work challenged the split [22,23]. Consequently, a merged database label is not evidence that every organism shares a function, and historical names should not be silently converted into strain identities. Table S1 lists the reported species-level labels in the reviewed cancer studies and their nomenclature caveats. Tables preserve source labels and distinguish genus-level 16S findings, provisional species assignments and cultured strains. Short amplicons, variable regions, database releases, closely related reference sequences and rRNA copy-number variation can alter identification. Species names inferred from reads remain weaker evidence than a genome-characterized isolate. Comparative work should retain amplicon sequences, database version and assignment confidence, and use full-length markers or genome data where possible. Functional conclusions require testing the actual isolate rather than a taxonomic name.

Control reporting is heterogeneous (Table 2). Wang et al. included operating-room and pathology controls, extraction controls and no-template controls [2]. Al Shareef et al. explicitly reported extraction blanks and PCR negatives [7]. The small-cell lung-cancer study mentioned negatives, but did not establish an equally detailed stage-specific control framework in the accessible methods [17]. Duplicate qPCR measurements in the paired breast study address analytical repeatability, not contamination introduced before amplification [3]. Computational tools such as decontam and microDecon can prioritise sequence features enriched in blanks or inversely related to input DNA, but they depend on representative negative controls and their assumptions can remove genuine low-biomass signals [24,25]. They are therefore complements to, not substitutes for, collection, extraction, PCR and library controls. These differences prevent equal confidence in all sequencing associations. "Not verified" denotes unavailable detail, not proof that a control was omitted.

Biological interpretation requires more than a significant group comparison [26]. Samples should exceed matched collection, extraction, reagent and library backgrounds; batches should be randomised and independently replicated. Absolute load should be measured by calibrated taxon-specific qPCR or digital PCR, with spike-ins and a defined denominator such as tissue mass or urine volume. DNA detection does not establish viability; localization supports spatial presence, culture supports recovery of viable organisms, and RNA or metabolites support activity only with appropriate attribution and controls. Combining these measurements is preferable to treating any single assay as proof of colonisation. The breast qPCR result demonstrates directly why relative enrichment must not be equated with increased bacterial burden [3].

4.2. Mechanistic Support Is Narrow and Context-Dependent

The gastric findings connect *Methylobacterium* exposure, reduced TGF-β and fewer CD8+ TRM cells in one experimental setting [5]. TGF-β can support tissue-residency programmes in some T-cell contexts [27], while exerting other, sometimes suppressive effects in cancer. TRM cells may contribute to local antitumour surveillance, but their phenotypes and functions remain tissue-dependent [28]. Reduced TGF-β therefore cannot be assumed to improve antitumour immunity. Fewer TRM cells are compatible with impaired local immune surveillance; they do not, by themselves, demonstrate a spatially excluded T-cell phenotype. A causal mediation claim would require, for example, TGF-β restoration or receptor-specific perturbation, selective TRM manipulation and rescue of the tumour phenotype. The bacterial effector and its host receptor remain unresolved.

The tumour microenvironment extends beyond T cells. No reviewed *Methylobacterium* study establishes effects on myeloid cells, natural killer cells, B cells or stromal immune

crosstalk. These are mechanistic hypotheses, not inferred functions: future work should pair spatial localisation and absolute quantification with multiparameter immune profiling, defined-isolate exposure and cell-specific depletion or rescue experiments.

The mammary experiment supports an immune-dependent effect of a particular strain [4]. NSG mice have several immune defects, so comparison with immunocompetent hosts cannot identify adaptive immunity or CD8+ cells as the sole required component. CD8 depletion, adoptive transfer and host-pathway perturbations would help distinguish these possibilities. Carotenoid-pathway signals and tumour-versus-normal host transcriptomic differences nominate metabolic and inflammatory hypotheses; they are not evidence that a bacterial carotenoid or TLR–NF-κB pathway mediates the growth effect. The model also tests exposure at implantation, leaving efficacy against established tumours unresolved.

Metabolic evidence has different meanings across modalities. Gastric microbe–metabolite correlations do not identify bacterial synthesis, transport or metabolic flux [9]. Predicted prostate pathways from 16S data likewise do not measure tumour metabolism [7]. Methionine depletion is a plausible product mechanism for L-methioninase, but the cited anticancer preparation was semi-purified, so impurities and enzymatic specificity require control [21]. In bladder cells, supernatant-associated growth inhibition and changes in BCHE/CFL2 expression establish an in vitro response, not the necessity of these genes or a secreted antitumour molecule [14]. Medium-only, pH, nutrient and normal-cell controls, fractionation and molecular rescue are needed to exclude nonspecific growth effects. Figure 1 separates observed associations, model-supported effects and proposed causal links.

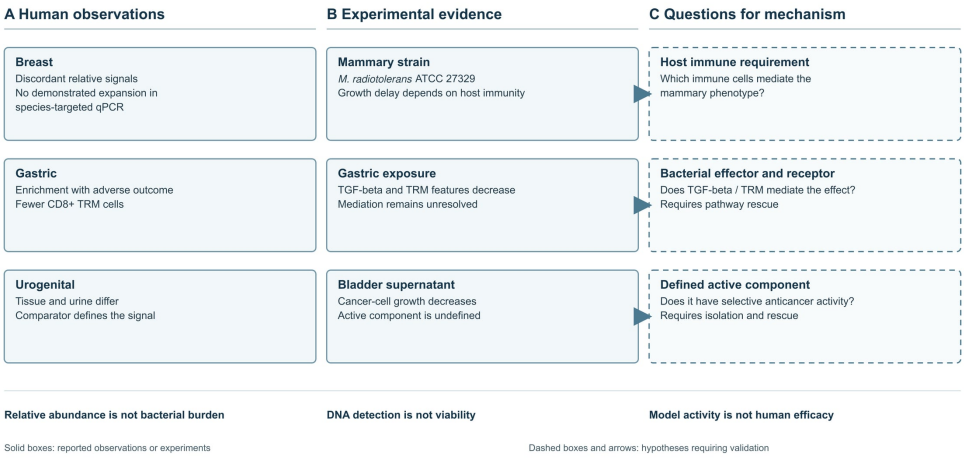

Figure 1. Evidence and hypotheses linking *Methylobacterium* to cancer. The revised schematic separates specimen-specific human observations (Panel (**A**)), perturbation findings (Panel (**B**)) and unanswered mechanistic tests (Panel (**C**)). Solid boxes denote reported observations or experiments; dashed boxes and arrows denote hypotheses requiring validation. The figure does not imply absolute expansion, viability, causal mediation, clinical biomarker performance or human anticancer efficacy [2–6,8,13,14].

4.3. Biomarker Claims Require Endpoint-Specific Validation

A diagnostic marker distinguishes disease states; a prognostic marker associates with a future outcome in a defined clinical setting; a predictive marker identifies differential benefit between treatments. Cancer-versus-control abundance is a diagnostic discovery association. A stage correlation is not longitudinal prognosis, and response differences within one treatment cohort do not establish a predictive biomarker. These distinctions are particularly important for the small-cell lung-cancer result [17], the exploratory rectal-

neuroendocrine AUC [12], and the bladder host-gene score, whose validation cannot be transferred to bacterial quantification [14].

Clinical testing should start with one intended use and a prespecified population. Candidate microbial measures should be compared with stage, grade, age, sex, treatment, antibiotic exposure and established disease-specific variables, including PSA or cystoscopy where relevant. Discovery and feature selection must be separated from model evaluation, using nested resampling when appropriate and an untouched external cohort. A prospective multicentre study should report confidence intervals, calibration, decision benefit and failures as well as AUC. Absolute quantification and batch robustness are necessary before deciding whether the genus adds information beyond community or host features. No reviewed microbial assay currently satisfies this full sequence of analytical, clinical and utility validation.

4.4. Therapeutic Modalities Cannot Be Treated as Equivalent

Table 3 separates four experimental modalities. Live *M. radiotolerans* ATCC 27329 has a strain-specific mammary model result [4]. Bladder supernatant is an undefined extracellular mixture [14]. The ERI-135 ethyl-acetate extract is an environmental natural-product screen against A549 cells [20]. The methioninase study used a semi-purified preparation with tamoxifen, reporting greater tumour inhibition in a small nude-mouse experiment [21]. None permits extrapolation to clinical efficacy of live *Methylobacterium*. Sonicates contain disrupted cellular material and should also be distinguished from secreted supernatants. Fully purified or recombinant enzymes are possible future products, not the formulation already demonstrated.

Manufacturing questions follow the material tested: viable identity and genetic stability for live strains; composition, residual medium and batch potency for supernatants; chemical identity and solvent residues for extracts; and enzyme purity, specific activity and stability for protein preparations. Reduced replication risk does not establish safety of a cell-free preparation, which may retain endotoxin or immunogenic components. Product classification also differs. FDA guidance for live biotherapeutic products does not apply to oncolytic bacteria or microbial gene-therapy vectors; the guidance notes that these products should be considered under the relevant CBER review framework and that prospective sponsors should seek product-specific regulatory advice [29]. Non-live mixtures and isolated enzymes likewise require assessment under the relevant product category.

4.5. Infection Risk Is a Prerequisite for Live-Product Development

Culture-confirmed catheter-associated *M. radiotolerans* infections in patients with leukaemia demonstrate a relevant hazard, rather than an incidence estimate for therapeutic dosing [30,31]. Device-associated infections make surface attachment and biofilm formation a clinically relevant risk to test directly for any proposed live product, even though the reviewed reports do not quantify biofilm formation by a therapeutic candidate. Proposed live products would require strain-level antimicrobial susceptibility, genome screening for virulence and transferable resistance, genetic stability and a feasible rescue treatment. Preclinical studies must measure biodistribution, persistence, translocation, shedding and clearance, including after immune suppression or barrier injury. Horizontal gene transfer, device biofilms, immunogenicity, cytokine responses and off-target organ effects also require evaluation. A normal short-term body-weight curve would not resolve these questions.

Local delivery, auxotrophy and kill switches are proposed containment approaches, not established safeguards for this genus in oncology. They would need tests of escape frequency, stability, dissemination and reversibility before clinical use. Gastric and mammary findings

also raise a separate concern: reducing infection risk would not exclude a context-dependent tumour-promoting effect. Blood detections should be assessed for infection, catheter sources and processing background before receiving an oncological interpretation. The retracted pan-cancer blood-and-tissue microbiome report further cautions against accepting classifier performance without rigorous analytical validation [32].

4.6. Limitations and Future Directions

The first priority is independent replication of one tissue-specific signal using standardised controls and a prespecified, calibrated estimate of absolute load. The next is a matched tissue-fluid study with longitudinal outcomes, to establish whether a readily collected specimen represents the local exposure. Mechanistic work should then test defined isolates or components in established-tumour models, with spatial localisation, immune-cell profiling, molecular target identification and pathway-specific rescue experiments. Supplementation and depletion remain opposing research hypotheses: a protective strain may merit component discovery, whereas an adverse gastric interaction may merit selective blockade. Neither strategy is presently supported as patient care.

This appraisal is limited by a small, heterogeneous literature, purposive retrieval, incomplete access to some methods and quantitative abundance values, and inconsistent reporting of negative controls. Sample types, comparators, populations and taxonomic pipelines differ too much for quantitative pooling. Publication bias and residual confounding by demographic, clinical and processing factors remain plausible. The concentration of mechanistic evidence in a few models restricts generalisation. These limitations favour a concise hypothesis-led interpretation and prevent claims that *Methylobacterium* is uniquely important relative to better-studied tumour-associated genera.

5. Conclusions

Methylobacterium is a candidate for context-specific investigation, not an established cancer biomarker or therapeutic target. The breast evidence illustrates why relative sequencing signals and species-targeted qPCR must be interpreted separately, and the contrasting mammary and gastric experiments justify testing strain–host interactions rather than genus-wide functions. Progress requires rigorous taxonomic assignment, contamination control, calibrated burden measurements and direct mechanistic experiments before clinical performance can be assessed. Therapeutic development must distinguish live organisms from non-live products and establish efficacy and safety independently. The present evidence supports focused, falsifiable experimental questions rather than clinical claims.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/microorganisms14122741/s1, Table S1. Reported species-level labels in cancer studies and nomenclature caveats.References [3,4,6,8,9,11–15,22,23] are cited in Table S1.

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