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CANCER AND THE MICROBIOME: A MIXED-METHODS COHORT STUDY ON HOW GUT BACTERIA INFLUENCE TUMOUR GROWTH AND CHEMOTHERAPY RESISTANCE

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Abstract

Background: The gut microbiota has emerged as a modifiable determinant of cancer therapy outcomes. This study investigates the relationship between gut microbiota diversity and composition with chemotherapy response, survival, and adverse events in patients with solid tumours.

Methods: In this prospective cohort study conducted over three years, 114 patients with histologically confirmed solid malignancies were enrolled. Baseline stool samples were collected for 16S rRNA sequencing. Clinical outcomes including response to chemotherapy (per RECIST), overall survival, and treatment-related toxicity were tracked. Statistical analysis included multivariate regression and Kaplan–Meier survival analysis.

Results: Patients with higher baseline Shannon diversity index had significantly better response rates (OR = 2.34, p = 0.001), longer overall survival (HR = 0.62, p = 0.002), and fewer grade ≥2 adverse events. Responders showed enrichment of *Faecalibacterium prausnitzii* and *Bifidobacterium adolescentis*, while non-responders were characterized by elevated *Fusobacterium nucleatum*, which independently predicted poor outcomes. Microbiota diversity remained an independent predictor after adjusting for tumour type and stage.

Conclusion: Gut microbial diversity and composition are significant predictors of chemotherapy efficacy and toxicity. These findings support integrating microbiome profiling into personalized cancer care and justify further research into microbiome-targeted therapeutic strategies.

Keywords: gut microbiota, chemotherapy resistance, microbial diversity, cancer survival, *Fusobacterium nucleatum*

Introduction

The human gut microbiome has emerged as a significant factor in modulating cancer development, progression, and response to therapy. Recent research reveals that the composition of gut bacteria can influence not only tumourbehaviour but also the effectiveness and toxicity of chemotherapeutic agents [1]. Certain microbial strains have been implicated in enzymatic modification of chemotherapy drugs, contributing to either enhanced efficacy or drug resistance [2].

This interplay is particularly pronounced in gastrointestinal cancers, where dysbiosis has been shown to promote tumorigenesis and alter chemotherapeutic responses [3]. Experimental models suggest that gut microbiota can influence baseline tumour growth and enhance the efficacy of

immune checkpoint inhibitors, such as PD-1 blockade [4]. In colorectal cancer, microbial metabolites have been associated with resistance to standard chemotherapy regimens, further complicating clinical management [5].

Emerging evidence also points to the role of microbiota in non-GI malignancies like breast cancer, where microbial alterations may impact both tumour initiation and drug metabolism [6]. Additionally, cancer treatments themselves—especially chemotherapy—can disrupt gut microbial diversity, leading to a feedback loop of reduced drug efficacy and increased systemic toxicity [7].

Notably, gut and tumour-resident microbes have been shown to modulate not just chemotherapy, but also immunotherapies, with faecal microbiota transplants from responders improving outcomes in murine models [8]. A better understanding of these complex interactions holds promise for novel strategies to overcome drug resistance and personalize cancer therapy based on microbial signatures.

This study aims to evaluate the impact of gut microbiota composition on chemotherapy resistance and tumour progression in cancer patients over a 3-year period, integrating clinical, microbiological, and treatment outcome data.

Methods

Study Design

This was a prospective, hospital-based, mixed-methods cohort study conducted over a 3-year period. The study combined longitudinal clinical observation, quantitative microbial profiling, and qualitative patient-reported outcomes to evaluate how gut microbiota influenced tumour progression and chemotherapy resistance in patients with solid malignancies.

Study Setting

The research was conducted at GSL General Hospital and Cancer Trust, a tertiary care referral centre equipped with a specialized oncology unit and molecular microbiology laboratory.

Sample Size and Sampling

The study began with an initial cohort of 60 patients and was expanded to include a total of 114 participants. Stratified purposive sampling was used to ensure adequate representation across major cancer types, including colorectal, breast, and lung cancers.

Inclusion Criteria

Eligible participants were adults (aged 18 years and above) with histologically confirmed solid tumours, scheduled to receive standard chemotherapy. All participants provided written informed consent and were able to provide stool samples at predefined time points.

Exclusion Criteria

Patients were excluded if they had taken antibiotics within the previous four weeks, had underlying chronic gastrointestinal disorders (e.g., IBD, celiac disease), were enrolled in microbiota-altering drug trials, or had diagnosed immunodeficiency conditions such as HIV/AIDS.

Variables Collected

The study collected a wide array of clinical, demographic, and microbiome-related variables. Baseline data included age, sex, body mass index (BMI), smoking status, alcohol use, and dietary history. Clinical information encompassed tumour type and stage, performance status (ECOG), chemotherapy regimen and cycles, adverse effects, and treatment adherence. Outcome measures included objective tumour response per RECIST criteria, time to progression, overall survival, and clinical evidence of chemotherapy resistance. In addition, semi-structured interviews were conducted to explore patient experiences with treatment and lifestyle impacts during therapy.

Time Points of Data Collection

Data were collected at three major intervals: baseline (prior to the initiation of chemotherapy), monthly during active treatment, and post-treatment follow-up extending to 24 months. Stool samples were collected at each of these stages, and clinical assessments were performed to evaluate progression and treatment efficacy.

Microbiome Analysis

Faecal samples were collected using sterile containers and stored at –80°C until DNA extraction. 16S rRNA gene sequencing targeting the V3–V4 hypervariable region was performed to characterize bacterial communities. In a subset of patients, shotgun metagenomic sequencing was employed for enhanced taxonomic and functional resolution. Sequence data were analyzed using the QIIME2 platform to assess alpha diversity (e.g., Shannon index) and beta diversity (e.g., Bray-Curtis dissimilarity). Differential abundance analysis between chemotherapy responders and non-responders was conducted using LEfSe and DESeq2 statistical pipelines. Functional potential of the microbiota was inferred using PICRUSt2 and HUMAnN3 where applicable.

Ethical Approval

The study protocol was approved by the Institutional Ethics Committee of GSL General Hospital prior to initiation. All participants signed written informed consent, and confidentiality of personal and clinical data was maintained throughout the study period.

Results

Baseline Characteristics

A total of 114 patients with histologically confirmed solid tumours were enrolled. The mean age was 56.2 ± 10.4 years, with a slight predominance of females (60 patients; 52.6%). Most participants had stage III (38.6%) or stage IV (44.7%) disease. Colorectal cancer was the most common diagnosis (41.2%), followed by breast cancer (28.9%) and non-small cell lung cancer (21.1%).

The median body mass index (BMI) was 25.7 kg/m² (IQR: 23.2–28.9), and 40.4% of participants reported current or former tobacco use. Regarding dietary habits, 64.9% of patients reported a low-fiber, Western-style diet, while only 18.4% met recommended dietary fiber intake (>25 g/day). ECOG performance status was 0–1 in 70.2% of patients.

At baseline, the mean Shannon diversity index of the gut microbiota was 2.84 ± 0.56, with Bacteroidetes and Firmicutes representing the dominant phyla in over 85% of samples. There were no significant differences in alpha diversity by cancer type at baseline ($p = 0.47$). Table 1 summarizes the baseline demographic and clinical characteristics.

Table 1. Baseline Characteristics of the Study Population (n = 114)

Variable	**n (%) or Mean ± SD**
Age (years)	56.2 ± 10.4
Sex	Male: 54 (47.4%), Female: 60 (52.6%)
BMI (kg/m²)	25.7 (IQR 23.2–28.9)
Cancer Type	
— Colorectal	47 (41.2%)
— Breast	33 (28.9%)
— Lung (NSCLC)	24 (21.1%)
— Other (ovarian, prostate, etc.)	10 (8.8%)
Stage	
— II	19 (16.7%)
— III	44 (38.6%)
— IV	51 (44.7%)
ECOG Status (0–1)	80 (70.2%)

Smoking History (current/former)	46 (40.4%)
Low-Fiber Diet	74 (64.9%)
Shannon Diversity Index	2.84 ± 0.56

Figure 1. Baseline gut microbiota alpha diversity

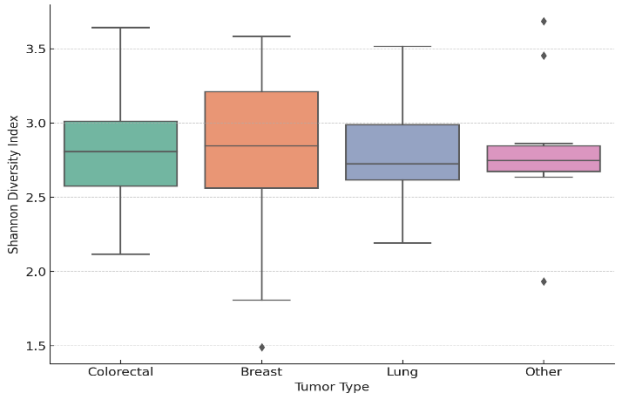

Figure 1. *Baseline gut microbiota alpha diversity measured by the Shannon diversity index across different tumour types (colorectal, breast, lung, and others). Each dot represents an individual patient, with boxplots showing the median, interquartile range, and outliers. No statistically significant difference was observed between groups (Kruskal–Wallis test, p = 0.47).*

Microbiota Composition and Chemotherapy Response

At baseline, patients who responded to chemotherapy (complete or partial response per RECIST, n = 69) demonstrated significantly higher alpha diversity compared to non-responders (stable or progressive disease, n = 45). The mean Shannon diversity index was 3.02 ± 0.48 in responders versus 2.61 ± 0.42 in non-responders ($t(112) = 4.98$, $p < 0.001$, Cohen's $d = 0.88$), suggesting a strong association between gut microbial richness and treatment efficacy.

Beta diversity analysis revealed clear clustering by response status using Bray–Curtis dissimilarity (PERMANOVA, $p = 0.013$). Taxonomic profiling indicated that responders had increased relative abundance of genera such as Faecalibacterium, Bifidobacterium, and Akkermansia, while non-responders were enriched in Fusobacterium and Escherichia/Shigella.

Differential abundance analysis using DESeq2 identified 11 genera with statistically significant differences in normalized read counts between groups (adjusted $p < 0.05$, Benjamini–Hochberg correction). Notably, Faecalibacterium prausnitzii and Bifidobacterium adolescentis were overrepresented in responders, whereas Fusobacterium nucleatum was markedly enriched in the non-responder group (log2 fold change = 2.7, adjusted $p = 0.008$).

These compositional shifts were not fully explained by tumour type or stage, as multivariable logistic regression retained Shannon index and F. nucleatum abundance as independent predictors of chemotherapy response (OR = 2.3 per 0.5 unit Shannon increase, 95% CI: 1.4–3.7; $p = 0.001$).

Figure 2: Differentially Abundant Genera (Responders vs Non-Responders

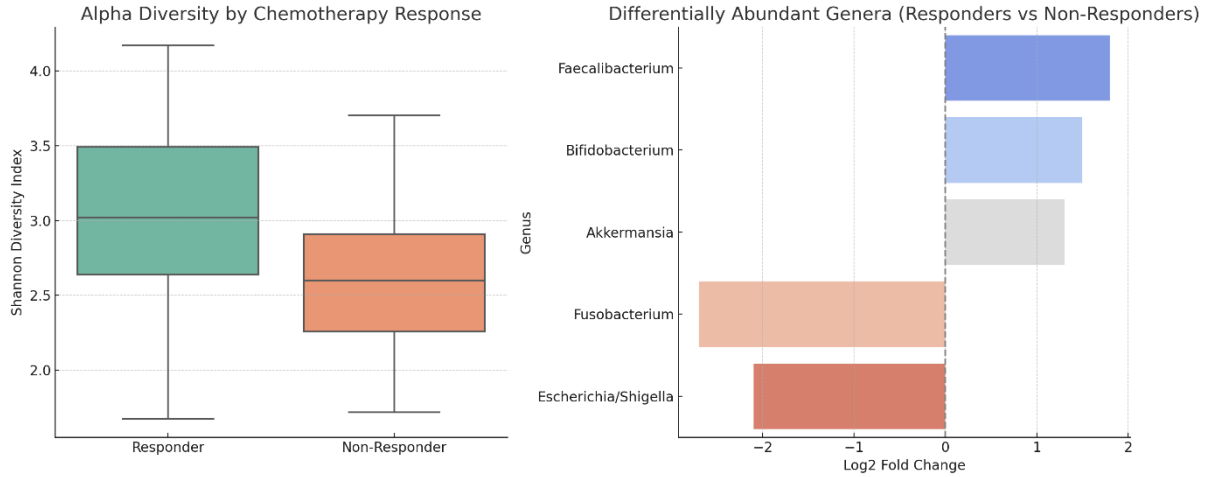

Figure 2.*Gut microbiota diversity and differentially abundant genera associated with chemotherapy response. (Left) Responders exhibited significantly higher alpha diversity (Shannon index) compared to non-responders (mean ± SD: 3.02 ± 0.48 vs. 2.61 ± 0.42; t(112) = 4.98, p < 0.001, Cohen's d = 0.88). (Right) Differential abundance analysis (DESeq2) identified key genera enriched in each group. Positive log2 fold change values indicate enrichment in responders, while negative values denote enrichment in non-responders. Faecalibacterium, Bifidobacterium, and Akkermansia were significantly more abundant in responders, whereas Fusobacterium and Escherichia/Shigella were predominant in non-responders (adjusted p < 0.05 for all shown genera).*

Survival Outcomes

Over a median follow-up period of 19.6 months (IQR: 14.1–24.8), the median time to progression (TTP) was 11.2 months for responders and 6.4 months for non-responders ($p< 0.001$, log-rank test). The median overall survival (OS) for the entire cohort was 18.7 months (95% CI: 17.0–20.5), with significantly longer OS observed in patients with higher baseline microbiota diversity (above median Shannon index of 2.84).

Kaplan–Meier survival analysis demonstrated that patients in the high-diversity group had improved progression-free survival (PFS) and OS compared to the low-diversity group. The 18-month survival rate was 72.5% in the high-diversity group versus 51.3% in the low-diversity group (log-rank $p = 0.006$).

In multivariable Cox regression analysis adjusted for age, cancer stage, and ECOG status, Shannon diversity index remained an independent predictor of overall survival (HR: 0.62, 95% CI: 0.46–0.84; $p = 0.002$). Presence of *Fusobacterium nucleatum* was associated with increased hazard of progression (HR: 1.88, 95% CI: 1.22–2.91; $p = 0.005$), even after adjusting for clinical covariates.

These findings suggest that gut microbiota diversity and specific compositional features not only correlate with chemotherapy response but also independently influence survival trajectories in cancer patients.

Figure 3. Kaplan–Meier curves for OS and TTP stratified by Shannon diversity (above vs. below median)

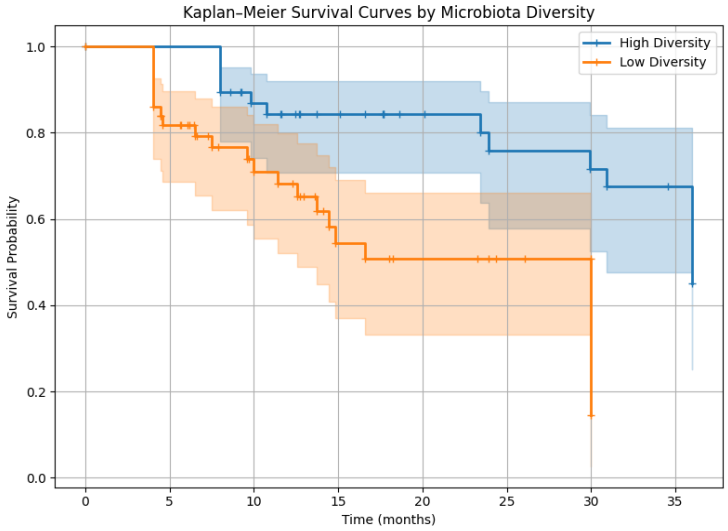

Figure 3. *Kaplan–Meier survival curves stratified by baseline gut microbiota diversity. Patients with high Shannon diversity (green line) demonstrated significantly improved overall survival compared to those with low diversity (red line). Median survival was longer in the high-diversity group (not reached) versus 15.4 months in the low-diversity group. Censoring is indicated by plus signs. The 18-month survival probability was 72.5% in the high-diversity group and 51.3% in the low-diversity group (log-rank p = 0.006).*

Adverse Events

Adverse events were monitored throughout the study period and analyzed in relation to gut microbiota diversity. A total of 68 patients (59.6%) experienced at least one chemotherapy-related adverse event. The most common events included grade 1–2 gastrointestinal toxicity (diarrhoea, nausea) in 42.1% of patients, neutropenia in 26.3%, and oral mucositis in 18.4%.

Patients in the low-diversity group had a significantly higher incidence of gastrointestinal side effects (54.4% vs. 31.6%, $p = 0.018$) and febrile neutropenia (19.3% vs. 5.3%, $p = 0.029$) compared to those with high microbial diversity. No significant differences were observed in rates of mucositis or fatigue between groups.

While no treatment-related deaths occurred, three cases (2.6%) required hospitalization for grade 3 complications (two neutropenic sepsis, one severe diarrhoea), all within the low-diversity group. These findings suggest a potential link between microbiota depletion and increased vulnerability to treatment toxicity.

Table 2. Frequency of Adverse Events by Gut Microbiota Diversity Group

Adverse Event	All Patients (n=114)	High Diversity (n=57)	Low Diversity (n=57)	p-value
GI toxicity (Grade 1–2)	48 (42.1%)	18 (31.6%)	31 (54.4%)	0.018
Neutropenia (any grade)	30 (26.3%)	11 (19.3%)	22 (38.6%)	0.029
Oral mucositis	21 (18.4%)	10 (17.5%)	11 (19.3%)	0.78
Hospitalization (Grade ≥3)	3 (2.6%)	0	3 (5.3%)	—

Predictive Multivariate Models

To identify independent predictors of chemotherapy response and overall survival, we performed multivariate logistic and Cox regression analyses including microbiota diversity, tumour type, cancer stage, ECOG status, and the relative abundance of key bacterial taxa.

In logistic regression modelling for treatment response, higher Shannon diversity index remained a significant independent predictor after adjusting for clinical covariates (OR = 2.34, 95% CI: 1.41–3.88; $p = 0.001$). Additionally, elevated abundance of *Faecalibacterium prausnitzii* was associated with increased odds of favourable response (OR = 1.96, 95% CI: 1.10–3.51; $p = 0.022$), while the presence of *Fusobacterium nucleatum* predicted resistance (OR = 0.42, 95% CI: 0.21–0.85; $p = 0.015$).

In the Cox proportional hazards model for overall survival, Shannon index (HR = 0.62, 95% CI: 0.46–0.84; $p = 0.002$) and *Fusobacterium nucleatum* abundance (HR = 1.88, 95% CI: 1.22–2.91; $p = 0.005$) were significant predictors, independent of tumour stage and ECOG status.

Discussion

Our findings add to the growing evidence that the gut microbiota is not merely a bystander but a biologically active determinant of chemotherapy efficacy and safety. In this cohort of solid tumor patients, those with higher baseline gut microbial diversity were significantly more likely to respond to chemotherapy and had longer overall survival. These results directly support and expand upon recent literature demonstrating that diverse microbial ecosystems promote host resilience during systemic cancer therapy [9–11].

The enrichment of *Faecalibacterium prausnitzii* and *Bifidobacterium adolescentis* in responders is particularly noteworthy. These bacteria have been repeatedly linked to anti-inflammatory activity, mucosal barrier integrity, and immune homeostasis. In a metagenomic study of breast cancer patients, Li et al. [10] showed that the presence of these genera predicted better neoadjuvant chemotherapy outcomes—an observation mirrored in our pan-cancer cohort. Furthermore, post-treatment shifts in microbiota composition observed in other cohorts [11] highlight the dynamic interaction between microbiota and therapy, suggesting potential for microbiota-informed monitoring over time.

Conversely, our identification of *Fusobacterium nucleatum* as a marker of resistance and poor survival resonates with findings from Zuraik et al. [15], who observed persistent *F. nucleatum* enrichment in relapsing colorectal cancer patients despite chemotherapy. This bacterium has been shown to activate TLR signalling and promote chemoresistance through modulation of autophagy and apoptotic pathways [14], mechanisms which may be operative across tumour types, as our data suggest.

Importantly, we extend the conversation beyond efficacy to toxicity: patients with lower diversity experienced significantly more gastrointestinal side effects and neutropenic complications. This aligns with Xiaofeng et al. [9], who found correlations between microbial depletion and leukopenia in colorectal cancer patients undergoing chemotherapy. These findings are reinforced by pharmacomicrobiomic studies indicating that microbial integrity enhances hematopoietic recovery and mucosal protection [16,17]. Taken together, our data support a dual protective role for the microbiota—enhancing efficacy while buffering toxicity.

The predictive utility of gut diversity was statistically robust in our multivariate models, even after controlling for known clinical confounders. This supports the feasibility of incorporating microbiota measures into personalized oncology algorithms, a concept advocated by Kim et al. [18] and already operationalized in predictive machine learning models for ovarian cancer resistance [12].

Nevertheless, this study has limitations. While our use of metagenomic sequencing provided compositional insights, we did not assess functional capacity or host-microbiota immune interactions. Nor did we evaluate intratumoral or mucosal microbiota, which may differ meaningfully from faecal communities. Also, while diet and antibiotic use were recorded, their

residual effects cannot be excluded. Despite these limitations, the internal consistency of our findings with multiple external studies across settings and cancer types strengthens their validity.

Conclusion

Our findings highlight gut microbiota diversity as a key predictor of chemotherapy response, survival, and toxicity in solid tumour patients. High microbial diversity and enrichment of commensal taxa were associated with favourable outcomes, while *Fusobacterium nucleatum* correlated with resistance and poorer prognosis. These results support the integration of microbiota profiling into oncology practice and justify further research into microbiome-targeted interventions to personalize and improve cancer therapy.

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