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BACTERIAL PRETA STATUS AND CAPECITABINE TREATMENT RESPONSE ACROSS MOUSE POPULATIONS

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Abstract:

This analysis examines whether microbiome related differences in chemotherapy response remain consistent across experiments, addressing the transferability of preclinical cancer evidence. A secondary descriptive analysis uses 552 longitudinal measurements from 73 mice bearing colorectal tumour xenografts across two experiments through day 17. Comparisons assess means, medians, dispersion and treatment contrasts within bacterial preTA knockout and over expression conditions. Capecitabine corresponds to lower mean tumour growth than vehicle in every condition. In the main experiment, treatment differences reach 335.51 percentage points under knockout and 41.72 under over expression. In the comparison experiment, corresponding differences are 81.02 and 100.90 percentage points, reversing their relative ordering. Temporal comparisons likewise show experiment dependent patterns. These findings distinguish recurring treatment associated growth reduction from inconsistent modification by bacterial gene status. The analysis refines pharmacomicrobiomic interpretation by separating observed tumour response from attribution to a metabolic mechanism. For preclinical research, the evidence supports evaluating experimental consistency and individual trajectories before prioritising microbial targets for translational development.

Key Words: Pharmacomicrobiomics; Capecitabine; Colorectal Cancer; Tumour Growth; Descriptive Longitudinal Analysis.

1. Introduction:

A microbial contribution to chemotherapy response becomes useful for treatment research only when its relationship with tumour control can be distinguished from variation between experimental conditions. This problem matters for colorectal cancer, which ranked third in global cancer incidence and second in cancer mortality in the GLOBOCAN estimates for 2020, as reported by Sung et al., 2021. Understanding variation in treatment response therefore requires attention to factors that influence drug activity alongside tumour characteristics. The gut microbiome is one such factor, but evidence of microbial drug metabolism does not automatically establish a consistent difference in tumour response across experimental settings. Pharmacomicrobiomics explains treatment outcomes through interactions among drugs, microbial functions and host processes. Lam et al., 2019, describe how bacterial transformation of therapeutic compounds can influence drug disposition, efficacy and toxicity. Functional variation matters because bacteria differ in the genes and enzymes capable of modifying particular substrates. Zimmermann et al., 2019, demonstrate this specificity through systematic screening of drug metabolism across human gut bacterial strains. These findings establish a basis for examining bacterial gene conditions rather than treating microbial presence as a uniform exposure. They also imply that a microbial contribution must be interpreted in relation to the drug and experimental conditions under examination.

Capecitabine provides a relevant setting for examining this conditional relationship. Javdan et al., 2020, identify microbial transformations of capecitabine and related fluoropyrimidines, showing that microbial activity can alter compounds within this therapeutic class. Such transformations establish biological plausibility, but their consequences depend on the reaction and the compound involved. The explanatory distinction is between microbial capacity to transform a drug and the resulting pattern of tumour control. A tumour outcome can reveal a treatment contrast without identifying the metabolic contribution responsible for that contrast. The closest experimental evidence comes from Spanogiannopoulos et al., 2022, who demonstrate that the bacterial preTA operon enables conversion of fluorouracil to an inactive metabolite and can interfere with capecitabine activity in mice. Their investigation combines bacterial genetics, biochemical measurements, pharmacokinetic experiments and tumour xenografts. It therefore establishes the mechanistic foundation and supplies the experimental observations used here. The present analysis is a secondary descriptive comparison of selected tumour experiments from that investigation. Its contribution concerns the consistency of the observed tumour response contrasts across those experiments rather than discovery of the preTA mechanism.

The analytical question separates two relationships that need not recur together. Capecitabine may correspond to lower tumour growth within both bacterial conditions, while the difference between its treatment contrasts under knockout and over expression may vary between experiments. Bacterial preTA status thus

conditions the treatment comparison rather than serving only as an additional group characteristic. Observation day adds a temporal dimension because an endpoint contrast may emerge gradually, fluctuate or narrow during follow up. This conditional logic is consistent with Haiser et al., 2013, who demonstrate that microbial drug inactivation depends on bacterial functional capacity and environmental conditions. It motivates examining the ordering of treatment contrasts across experiments rather than assuming that a functional mechanism produces an invariant outcome pattern. This study aims to describe how capecitabine associated tumour growth differences vary across bacterial preTA conditions and observation days in two published mouse experiments. The research question is whether lower tumour growth under capecitabine recurs within each bacterial condition and whether the relative treatment contrast under knockout versus over expression retains the same direction across experiments. The analysis uses 552 measurements from 73 female athymic nude mice bearing HCT116 colorectal tumour xenografts. The main experiment contributes 41 mice observed on eight occasions through day 17, while the comparison experiment contributes 32 mice observed on seven occasions over the same selected window. The descriptive approach compares means, medians, dispersion, shrinkage counts and treatment contrasts separately by experiment.

Tumour response is measured as net percentage change from each animal's baseline. This measure distinguishes slower growth from shrinkage and permits proportional comparisons among animals with different starting volumes. It does not eliminate differences in biological starting conditions. Reporting baseline distributions alongside relative growth therefore preserves information relevant to interpretation. Retaining the experimental schedules also avoids creating observations through interpolation. The design enables a transparent comparison of observed trajectories while keeping the individual mouse distinct from its repeated measurements. The findings show lower mean tumour growth with capecitabine within every represented bacterial condition. However, the larger treatment contrast under knockout appears in the main experiment and does not recur in the comparison experiment. The contribution is the separation of a recurring descriptive treatment pattern from an experiment dependent bacterial contrast. This distinction qualifies how the selected observations support a general account of microbiome related treatment response. Its relevance extends to preclinical research concerned with the transportability of experimental findings, while the evidence remains confined to the represented mouse experiments and does not establish a clinical treatment rule.

2. Theory, Conceptual Framework and Hypothesis Development:

This analysis adopts a pharmacomicrobiomic framework in which chemotherapy response emerges from interactions between the administered drug, bacterial metabolic capacity and the host. Microbial activity can alter the compounds available for absorption and subsequent action against tumour cells. Spanogiannopoulos et al., 2016, establish the conceptual basis for treating microbial metabolism as part of drug disposition. This perspective makes bacterial function relevant to treatment response rather than treating microbiome variation as a background characteristic. The framework links three observed treatment dimensions to tumour growth response: bacterial preTA status, capecitabine administration and elapsed treatment time. These dimensions represent distinct experimental conditions rather than indicators of a latent index. The consolidated data contain 552 measurements from 73 mice across two experiments. Tumour growth response is measured as percentage change from baseline, with higher values indicating poorer tumour control. The conceptual relationship is conditional: the association between capecitabine administration and tumour growth may differ between bacterial gene conditions and across observation days.

Bacterial preTA status distinguishes knockout from over expression conditions. The theoretical expectation is that altering bacterial metabolic capacity changes the extent to which bacteria interfere with chemotherapy activity. Zimmermann et al., 2019, demonstrate that bacterial drug metabolism depends on specific microbial genes and varies across bacterial strains. Their findings support a functional approach in which gene status provides a more precise experimental contrast than bacterial presence alone. For the present comparison, over expression is expected to weaken tumour control under capecitabine relative to knockout. This expectation concerns the difference in treatment response between gene conditions, rather than an unconditional association between gene status and tumour growth. Vehicle groups provide the corresponding comparison without capecitabine. The relevant test therefore examines whether the capecitabine versus vehicle difference changes between knockout and over expression conditions.

H1: The association between capecitabine administration and lower tumour growth is weaker under bacterial preTA over expression than under knockout.

Capecitabine administration distinguishes chemotherapy exposure from vehicle treatment. Its expected association with tumour growth depends on whether the administered compound produces sufficient antitumour activity within each bacterial condition. Javdan et al., 2020, identify microbial transformations of capecitabine and related fluoropyrimidines, demonstrating that microbial metabolism can alter compounds within this therapeutic class. These findings justify examining treatment response within bacterial gene conditions rather than assuming a uniform response across all mice. Microbial transformation does not imply a uniformly beneficial or harmful treatment effect. Wallace et al., 2010, show that bacterial enzymatic activity can reactivate an anticancer drug metabolite and increase intestinal toxicity, while selective enzyme inhibition reduces that

toxicity in mice. The direction of a microbial contribution therefore depends on the substrate and reaction involved. For the available tumour measurements, the narrower expectation is that capecitabine is associated with lower tumour growth within the knockout condition. This comparison separates the treatment association from the interaction specified in H1.

H2: Within the bacterial preTA knockout condition, capecitabine administration is associated with lower tumour growth than vehicle treatment at day 17, conditional on baseline tumour volume.

Elapsed treatment time captures the development of tumour response within each experimental condition. It is an observation dimension, not a direct measure of cumulative drug exposure. Repeated measurements distinguish an endpoint difference from a difference in response trajectory. Lam et al., 2019, describe drug outcomes as products of interacting microbial and host processes, supporting the expectation that microbial effects need not appear as a constant separation between treatment groups. The framework supports complementary endpoint and longitudinal comparisons. The endpoint specification relates day 17 tumour volume to capecitabine administration, bacterial preTA status and their interaction, while adjusting for baseline tumour volume. The longitudinal specification examines treatment, gene status and observation day jointly, accounting for repeated measurements within mice. Both specifications assess the same dependent construct, tumour growth response. We analyse the experiments separately and use the second experiment to assess whether the treatment contrast and interaction pattern recur. Hypotheses express theoretical expectations; the comparisons allow supporting, opposing or inconclusive evidence.

3. Data:

This analysis documents the provenance, selection and measurement of the experimental observations used to examine microbiome related variation in capecitabine response.The consolidated workbook preserves source identifiers, separates reported measurements from calculated outcomes and supports reproducible descriptive comparisons.

3.1 Data Sources, Population, and Sample Construction:

The numerical data originate from the supplementary workbooks accompanying Host and gut bacteria share metabolic pathways for anti-cancer drug metabolism, available through the publisher and the repository record. Source Data Figure 4 supplies baseline tumour volumes, repeated relative tumour measurements and endpoint volumes for the main experiment. Source Data Extended Data Figure 5 supplies baseline volumes and repeated relative measurements for the comparison experiment. Worksheet identifiers provide the extraction locations: Fig4b, Fig4c and Fig4d for the main experiment, and EDFig5h and EDFig5i for the comparison experiment. The extraction date and direct download routes are retained in the workbook metadata. The files do not contain a numbered release designation. Publisher copyright applies, and public accessibility does not establish an unrestricted redistribution licence. The empirical setting comprises female athymic nude mice bearing HCT116 colorectal tumour xenografts in experiments conducted at the University of California, San Francisco. The source protocol reports allocation based on body weight and baseline tumour size, with tumour measurements collected without knowledge of treatment assignments. Bacterial conditions distinguish engineered Escherichia coli with preTA knockout from preTA over expression. Each condition includes capecitabine and vehicle groups. These observations represent a laboratory sampling frame rather than a geographical sample of patients.

The target of inference is tumour response within the represented experimental conditions. The accessible frame contains 109 mice across three tumour experiments. The consolidated workbook selects 41 mice from the main experiment and 32 from the comparison experiment. The earlier experiment containing 36 mice is outside the workbook selection. This selection was made during secondary dataset construction and was not established through a registered protocol. The longitudinal unit is a mouse observed on a specified experimental day. The main experiment contains measurements on days 0, 3, 6, 8, 10, 13, 15 and 17. The comparison experiment contains measurements on days 0, 3, 6, 10, 13, 15 and 17. Each experiment is balanced over its selected schedule. The combined dataset has unequal schedules because day 8 occurs only in the main experiment. Tumour volumes are measurements at observation times rather than cumulative flows or period averages.

Table 1: Sample construction

Stage	Mice	Recorded Measurements Through Day 17	Inclusion or Exclusion Rule
Accessible experimental frame	109	837	Three experiments represented in the source workbooks
Earlier experiment excluded	36	285	Experiment outside the consolidated workbook selection
Main experiment retained	41	328	Eight recorded measurements per mouse
Comparison experiment	32	224	Seven recorded measurements per

Stage	Mice	Recorded Measurements Through Day 17	Inclusion or Exclusion Rule
retained			mouse
Consolidated longitudinal sample	73	552	Unique experiment, mouse and day combinations
Endpoint descriptive sample	73	73	One day 17 observation per mouse
Loss during integration and quality checks	0	0	All selected records retained

The accessible frame contains 840 scheduled measurement positions, of which three are unavailable in the excluded experiment. All 552 selected measurements are present. Counts distinguish mice from repeated observations because repeated measurements do not create additional independent animals, consistent with Lazic et al., 2018.

3.2 Data Integration, Cleaning, and Quality Assurance:

Integration uses experiment and the original mouse identifier as the animal key. Adding experimental day produces the longitudinal key. Baseline and endpoint records join within experiment and mouse, while treatment and bacterial status remain constant within each animal. Mouse identifiers are preserved rather than treated as globally unique across experiments. The selected longitudinal data contain 552 unique keys and no duplicate keys. All selected mice match to baseline measurements and day 17 outcomes. Integration produces no unmatched selected animals or unexpected increases in observations. Multiple records for the same mouse represent measurements on different days rather than duplicate observations.

Quality checks confirm positive baseline and endpoint volumes, a reported relative volume of 100 at baseline and complete treatment, bacterial status and day fields. Within the main experiment, endpoint volume divided by baseline volume reproduces the reported day 17 percentage of baseline without discrepancy at the retained numerical precision. Within the comparison experiment, reconstructed endpoint volumes reproduce the source relative measurements to numerical rounding precision. This second check establishes arithmetic consistency rather than independent measurement agreement. Missingness is zero across the selected analytical fields for every retained mouse and day. No imputation, interpolation, replacement with zero or external matching is applied. Baseline net tumourchange equals zero because it is defined relative to the same baseline measurement. This zero is a substantive reference value rather than a missing value replacement.

No observations are removed through outlier thresholds, trimming or winsorisation. Extreme tumour responses remain in the sample and are represented through medians and ranges alongside means and standard deviations. The checks identify no nonpositive volume denominators or inconsistent baseline scaling. Maintaining the distinction between repeated measurements and independent replication follows the concern identified by Lazic, 2010. The dataset retains experimental days without converting them to equal intervals or calendar dates. The two measurement schedules remain separate. No frequency conversion or interpolation is used to manufacture a common schedule. The workbook represents a secondary extract of published observations rather than a contemporaneous information archive.

3.3 Variable Measurement, Construction, and Validation:

Bacterial preTA status, capecitabine administration and experimental day operationalise the three observed dimensions of the framework. They are not combined into a composite index. Gene status identifies an experimental bacterial condition rather than measured enzyme abundance or activity. This distinction accords with the functional separation between microbial gene content and drug metabolism established by Zimmermann et al., 2019. The source trajectory tables label their measurements as percentage change, but baseline values equal 100. The supplied numbers therefore represent tumour volume as a percentage of baseline. Equation 1 converts this measure to net percentage change.

$$G_{it} = R_{it} - 100$$

Here, G_{it} denotes net percentage tumour change for mouse i on day t, and R_{it} denotes the reported percentage of baseline volume. A value of zero indicates unchanged volume, while positive values indicate growth. The workbook also calculates the natural logarithm of relative tumour volume using Equation 2.

$$L_{it} = \ln \frac{R_{it}}{100}$$

Here, L_{it} denotes logarithmic relative tumour volume. All selected relative volumes are positive, so this transformation requires no replacement constants. Net percentage change and logarithmic relative volume express the same outcome on different scales. Main experiment endpoint volumes are reported directly. Comparison experiment endpoint volumes are reconstructed using Equation 3.

$$\widehat{V}_{i17} = V_{i0} \frac{R_{i17}}{100}$$

Here, $\hat{V}_{i17}$ denotes reconstructed day 17 volume, V_{i0}denotes reported baseline volume and R_{i17}denotes reported day 17 percentage of baseline. Reconstructed volumes inherit the precision of the reported baseline values.

Table 2: Variable dictionary

Variable or Indicator	Analytical Role	Definition and Construction	Source and Identifier	Unit and Frequency	Transformation and Direction	Missing Value Rule	Coverage and Limitation
Net tumour change	Outcome	Reported percentage of baseline minus 100	Fig4c and EDFig5i	Percentage at each recorded day	Higher values indicate greater growth	None required	552 observations
Logarithmic relative volume	Alternative outcome scale	Natural logarithm of reported percentage divided by 100	Derived from trajectory worksheets	Log ratio at each recorded day	Higher values indicate greater relative volume	Positive inputs required and verified	552 observations
Bacterial preTA status	Experimental condition	Knockout or over expression	Source colonisation and group fields	Category, constant within mouse	Over expression code equals 1; knockout equals 0	None required	73 mice; gene condition rather than enzyme activity
Capecitabine administration	Experimental condition	Capecitabine or vehicle	Source treatment and group fields	Category, constant within mouse	Capecitabine code equals 1; vehicle equals 0	None required	73 mice; binary treatment indicator
Experimental day	Temporal dimension	Elapsed day relative to treatment initiation	Source trajectory headers	Days at irregular intervals	Larger values indicate later observation	No interpolation	Eight main and seven comparison occasions
Baseline tumour volume	Baseline characteristic	Reported volume before treatment	Fig4b and EDFig5h	Cubic millimetres, once per mouse	Level retained	None required	73 positive values
Day 17 tumour volume	Endpoint characteristic	Reported in the main experiment; reconstructed in the comparison experiment	Fig4d, EDFig5h and EDFig5i	Cubic millimetres, once per mouse	Higher values indicate greater tumour burden	None required	41 reported and 32 reconstructed values
Experiment identifier	Stratification key	Main or comparison experiment	Source workbook and experiment labels	Category	No numerical interpretation	None required	Distinguishes experimental protocols

The measurements capture tumour response without directly measuring the metabolic pathway responsible for it. Javdan et al., 2020, demonstrate why microbial drug transformation requires dedicated functional measurements. Accordingly, the tumour variables are not interpreted as direct measures of capecitabine metabolism.

Table 3: Descriptive statistics for baseline and endpoint measures

Experiment and Measure	Valid Mice	Mean	Standard Deviation	Median	Minimum	Maximum
Main baseline volume, cubic millimetres	41	99.40	12.23	99.24	78.65	119.42
Main day 17 volume, cubic millimetres	41	334.36	198.63	313.45	50.34	838.24
Main day 17 net change, percentage	41	233.74	189.17	209.56	−48.22	773.87
Comparison baseline volume, cubic millimetres	32	126.51	70.39	106.25	40.70	334.60
Comparison reconstructed day 17 volume, cubic millimetres	32	322.74	259.13	247.08	52.88	1052.45
Comparison day 17 net change, percentage	32	139.23	111.90	119.09	−31.49	369.42

3.4 Analytical Framework, Identification, and Reproducibility:

The implemented analysis is descriptive. It reports sample composition, arithmetic consistency, outcome distributions and treatment group summaries separately by experiment. No regression, significance test, confidence interval or causal effect estimate has been computed for this secondary analysis. Statistical significance is therefore not assigned to the observed differences, consistent with the distinction between numerical evidence and inferential conclusions emphasised by Wasserstein and Lazar, 2016. The main experiment contains ten mice in each group except over expression with vehicle, which contains eleven. The comparison experiment contains eight mice in each of its four groups. Table 4 reports day 17 net tumour change without treating the repeated measurements as additional independent animals.

Table 4: Day 17 net tumour change by experimental condition

Experiment	Bacterial Condition	Administration	Mice	Mean Percentage Change	Standard Deviation	Median Percentage Change
Main	Knockout	Capecitabine	10	38.60	64.71	45.65
Main	Knockout	Vehicle	10	374.11	211.00	299.22
Main	Over expression	Capecitabine	10	237.97	112.23	247.20
Main	Over expression	Vehicle	11	279.68	166.86	359.44
Comparison	Knockout	Capecitabine	8	69.17	38.16	61.63
Comparison	Knockout	Vehicle	8	150.19	94.29	175.28
Comparison	Over expression	Capecitabine	8	118.33	116.87	109.66
Comparison	Over expression	Vehicle	8	219.23	133.75	230.38

The experiments remain separate because their baseline distributions, enrolment procedures and antibiotic preparation differ. The comparison cohort also continues beyond the selected observation window in the source protocol. Consequently, it provides an additional experimental comparison rather than automatic external validation. The workbook preserves source worksheet locations, mouse identifiers, observation days and transparent transformation formulas. Reported and reconstructed volumes remain distinguishable. These features align the analytical measures with their source definitions while limiting interpretation to the selected experimental animals and observed tumour responses.

4. Methods:

4.1 Research Design and Analytical Scope:

This analysis uses a secondary descriptive design to examine whether capecitabine associated tumour growth contrasts recur across bacterial preTA conditions and experimental settings. The analytical targets are the observed treatment difference within each bacterial condition and the difference between those treatment contrasts. These quantities describe the selected experimental samples without estimating a population causal effect. The mouse is the biological unit, while the longitudinal record represents a mouse observed on a specified experimental day. Treatment comparisons distinguish capecitabine from vehicle within knockout and over expression conditions. Repeated measurements describe trajectories within animals rather than additional independent experimental units. This distinction follows Lazic et al., 2018, who emphasise that sample size must reflect the relationship between experimental units and interventions.

The analytical sample contains 73 mice and 552 measurements through day 17. The main experiment contributes 41 mice and 328 measurements, while the comparison experiment contributes 32 mice and 224 measurements. Sample eligibility, exclusions and source provenance are documented in the sample construction table and variable dictionary in Section 3. Both experiments contain complete observations over their selected schedules, although the schedules differ because day 8 occurs only in the main experiment. The experiments are analysed separately because their baseline distributions and experimental procedures differ. This preserves the distinction between variation within an experiment and variation between experiments. The comparison experiment serves as an additional assessment of descriptive consistency rather than an independent clinical validation sample. Relative growth accounts arithmetically for starting volume but does not establish equivalence in biological starting conditions.

4.2 Outcome Scaling and Descriptive Contrast Specification:

Net percentage tumour change is the principal outcome. Equation 1 defines its construction from the reported percentage of baseline volume. Equation 2 defines logarithmic relative volume as an alternative scale retained in the workbook. Equation 3 reconstructs endpoint volume for the comparison experiment. The reported treatment comparisons use net percentage change without fitting a regression to either outcome scale. At each observation day, the treatment contrast within an experiment and bacterial condition is calculated using Equation 4.

$$\Delta_{egt} = \overline{G}_{egt,CAP} - \overline{G}_{egt,Vehicle}$$

Here, Δ_{egt} denotes the observed treatment contrast in experiment e, bacterial condition g and day t. The quantities $\overline{G}_{egt,CAP}$ and $\overline{G}_{egt,Vehicle}$ denote mean net percentage tumour change among mice receiving capecitabine and vehicle, respectively. Each mouse contributes one observation to its group mean on a given day. A negative contrast indicates lower mean growth with capecitabine. The contrast is expressed in percentage points. The difference between treatment contrasts under over expression and knockout is calculated using Equation 5.

$$D_{et} = \Delta_{e,OE,t} - \Delta_{e,KO,t}$$

Here, D_{et} denotes the descriptive contrast between bacterial conditions within experiment e one day t. The terms OE and KO identify over expression and knockout. A positive value indicates a more favourable capecitabine versus vehicle contrast under knockout; a negative value indicates a more favourable contrast

under over expression. Equation 5 compares observed group means and is not an estimated causal interaction coefficient. Temporal comparisons retain the observed day categories. The cross-experiment presentation uses shared observation days without interpolating additional measurements. Baseline net change equals zero by construction, so baseline equality on this scale is not interpreted as evidence of comparable treatment groups. Endpoint comparisons use day 17 because both selected experiments contain observations for every retained mouse at that point.

4.3 Analytical Procedure and Numerical Implementation:

The procedure begins with the cleaned observational records and linked baseline measurements. Experiment and original mouse identifier define the animal key, while experimental day completes the longitudinal key. Treatment labels and bacterial conditions are harmonised into consistent categories without changing their source meaning. Reported percentages of baseline are converted to net percentage change before aggregation. The analysis then groups observations by experiment, bacterial condition, treatment and day. Arithmetic means describe group trajectories. Endpoint distributions are summarised using valid counts, means, sample standard deviations, medians, minima and maxima. Sample standard deviations use the number of mice minus one as the denominator.

Tumour shrinkage is counted when net percentage change at day 17 is below zero. This separates regression below baseline from slower positive growth. The treatment contrasts in Equation 4 are calculated from unrounded group means. Equation 5 is then applied to those contrasts. Numerical results are rounded only for presentation. All selected mice receive equal weight within their experimental groups. The experiments are not pooled into a common treatment estimate. The unequal group size in the main experiment is retained rather than corrected through resampling or weighting. Means and medians are compared to assess whether the descriptive ordering depends on the choice of central tendency. Calculations use Python 3.12.14 with the standard library modules json, statistics and collections. The statistics module supplies arithmetic means, medians and sample standard deviations. The procedure is deterministic and uses no random sampling, optimisation or random seed. Spreadsheet formulas retain the documented outcome transformations, while numerical summaries are calculated from the extracted source values.

4.4 Verification, Sensitivity and Interpretive Boundaries:

Verification checks assess key uniqueness, record matching, variable completeness, admissible numerical values and consistency between reported and calculated measures. The selected records contain no duplicate experiment, mouse and day combinations. Every retained mouse matches to its baseline and endpoint information. Baseline and endpoint volumes are positive, and reported relative volume equals 100 at baseline.

For the main experiment, dividing reported endpoint volume by baseline volume and multiplying by 100 reproduces the source endpoint percentage. For the comparison experiment, reconstructed endpoint volume reproduces the source relative measurement to numerical rounding precision. This latter check verifies calculation consistency rather than agreement between independent measurements. No selected analytical values require deletion or imputation. No interpolation, trimming, winsorisation or replacement of missing values with zero is applied. Valid extreme responses remain in the summaries. Reporting dispersion and median values alongside means preserves information that a single group average would conceal.

The implemented sensitivity assessment compares mean and median endpoint ordering and examines treatment contrasts across shared observation days. It also evaluates whether the direction of the contrast between bacterial conditions recurs across experiments. These comparisons assess descriptive consistency without establishing robustness to confounding or alternative causal specifications. The analysis does not treat repeated records as independent replications, addressing the pseudoreplication concern discussed by Lazic, 2010. The secondary selection and comparisons were not preregistered. The workbook preserves source worksheet identifiers, observation locations and transformation formulas, allowing the reported quantities to be traced to their inputs. The method contributes a transparent comparison of experimental response patterns through established descriptive tools, without claiming a new estimator or identifying design.

5. Findings:

This analysis examines tumour growth across bacterial preTA conditions, capecitabine administration and observation days. The findings distinguish treatment contrasts within each experiment from differences between experiments. Interpretation rests on calculated descriptive evidence without claiming statistical or mechanistic validation. The analytical sample contains 73 mice and 552 repeated measurements through day 17. The main experiment contributes 41 mice and 328 measurements across eight occasions. The comparison experiment contributes 32 mice and 224 measurements across seven occasions. No selected observations contain missing analytical values or require imputation. Repeated measurements remain observations within animals rather than additional independent experimental units, consistent with the distinction established by Lazic et al., 2018.

Tumour growth represents net percentage change from each animal's baseline. Zero indicates unchanged volume, positive values indicate growth and negative values indicate shrinkage. Baseline volumes are more concentrated in the main experiment, with a mean of 99.40 cubic millimetres and a standard deviation

of 12.23. The comparison experiment has a mean baseline volume of 126.51 cubic millimetres and a standard deviation of 70.39. These differences support reporting the experiments separately.

Table 1: Baseline characteristics and day 17 tumour growth

Experiment	Bacterial Condition	Administration	Mice	Mean Baseline Volume, Cubic Millimetres	Mean Tumour Change, Percentage	Standard Deviation	Median Tumour Change, Percentage	Mice With Tumour Shrinkage
Main	Knockout	Capecitabine	10	98.49	38.60	64.71	45.65	4
Main	Knockout	Vehicle	10	99.87	374.11	211.00	299.22	0
Main	Over expression	Capecitabine	10	97.87	237.97	112.23	247.20	0
Main	Over expression	Vehicle	11	101.19	279.68	166.86	359.44	0
Comparison	Knockout	Capecitabine	8	127.23	69.17	38.16	61.63	0
Comparison	Knockout	Vehicle	8	92.79	150.19	94.29	175.28	0
Comparison	Over expression	Capecitabine	8	116.83	118.33	116.87	109.66	1
Comparison	Over expression	Vehicle	8	169.21	219.23	133.75	230.38	1

In the main experiment, capecitabine under bacterial preTA knockout corresponds to substantially lower tumour growth than vehicle treatment. Mean net growth reaches 38.60 percent with capecitabine and 374.11 percent with vehicle, a difference of 335.51 percentage points. Median growth retains the same ordering, reaching 45.65 percent and 299.22 percent, respectively. Four of ten capecitabine treated knockout mice exhibit tumour shrinkage, compared with none receiving vehicle. Agreement across means, medians and shrinkage counts indicates that the contrast extends beyond a difference in group averages. The corresponding treatment contrast is smaller under preTA over expression. Mean growth reaches 237.97 percent with capecitabine and 279.68 percent with vehicle, a difference of 41.72 percentage points. Median growth is also lower with capecitabine, although no over expression mouse receiving capecitabine exhibits shrinkage. The difference between the two mean treatment contrasts is 293.79 percentage points. Within the main experiment, the descriptive pattern is therefore consistent with stronger tumour control under capecitabine when preTA is knocked out.

This pattern accords with evidence that specific bacterial genes can influence drug metabolism. Zimmermann et al., 2019, demonstrate variation in drug transformation across bacterial strains and identify microbial genes responsible for selected metabolic reactions. However, the present tumour measurements do not directly quantify drug transformation. The observed separation therefore describes treatment response within bacterial conditions rather than an independently measured metabolic mechanism. The comparison experiment produces a different conditional pattern. Within knockout mice, mean growth is 69.17 percent with capecitabine and 150.19 percent with vehicle, a difference of 81.02 percentage points. Within over expression mice, the corresponding values are 118.33 percent and 219.23 percent, a difference of 100.90 percentage points. Capecitabine corresponds to lower mean growth in both bacterial conditions, but its descriptive contrast is larger under over expression. The contrast between bacterial conditions is −19.88 percentage points, reversing the direction observed in the main experiment.

Median comparisons retain lower tumour growth with capecitabine in both bacterial conditions. Median growth reaches 61.63 percent with capecitabine versus 175.28 percent with vehicle under knockout, and 109.66 percent versus 230.38 percent under over expression. Nevertheless, shrinkage occurs in only one over expression mouse receiving capecitabine and one receiving vehicle. Reduced growth therefore does not imply tumour regression. The experiments converge on lower average tumour growth with capecitabine within each bacterial condition. They do not converge on a larger treatment contrast under knockout. Javdan et al., 2020, demonstrate that microbial transformations of capecitabine and related fluoropyrimidines depend on the compound and metabolic reaction involved. Their evidence supports examining specific functional relationships while avoiding an assumption that every microbial contribution produces the same treatment outcome.

Table 2: Capecitabine versus vehicle differences in mean net tumour growth

Experiment	Observation Day	Knockout Difference, Percentage Points	Over Expression Difference, Percentage Points	Over Expression Contrast Minus Knockout Contrast
Main	3	−45.89	−11.75	34.14
Main	6	−89.40	−8.79	80.60
Main	10	−171.72	−43.76	127.96
Main	13	−222.77	−50.10	172.67
Main	15	−256.24	−82.41	173.83

Experiment	Observation Day	Knockout Difference, Percentage Points	Over Expression Difference, Percentage Points	Over Expression Contrast Minus Knockout Contrast
Main	17	−335.51	−41.72	293.79
Comparison	3	−2.29	−23.12	−20.82
Comparison	6	−56.27	−44.40	11.86
Comparison	10	−50.79	−51.03	−0.23
Comparison	13	−66.29	−63.47	2.82
Comparison	15	−79.47	−94.52	−15.05
Comparison	17	−81.02	−100.90	−19.88

Source: Calculations from the consolidated experimental dataset. Treatment differences equal the capecitabine group mean minus the vehicle group mean within the same experiment, bacterial condition and day. Negative values indicate lower growth with capecitabine. The table includes shared observation days.

The trajectories show how the endpoint contrasts develop. In the main experiment, the knockout treatment difference expands from −45.89 percentage points on day 3 to −335.51 on day 17. Under over expression, the contrast remains smaller across the shared observation days and changes nonmonotonically. It reaches −82.41 percentage points on day 15 before narrowing to −41.72 on day 17. The endpoint separation therefore reflects differences in growth trajectories rather than a constant distance between treatment groups. The comparison experiment does not reproduce this persistent ordering. Its contrast between bacterial conditions changes sign across observation days. Treatment differences are nearly equal on day 10, while the over expression contrast becomes larger on days 15 and 17. The temporal evidence indicates that capecitabine related growth differences vary across days and bacterial conditions, with the ordering dependent on the experiment. This interpretation is consistent with the broader pharmacomicrobiomic framework described by Lam et al., 2019, in which treatment outcomes arise through interactions among microbial function, drug properties and host conditions.

Individual variation qualifies the interpretation of group averages. In the main experiment, day 17 growth ranges from −48.22 to 773.87 percent. The comparison experiment ranges from −31.49 to 369.42 percent. Differences between means and medians, together with the reported standard deviations, show that several groups contain heterogeneous responses. The endpoint distributions therefore support retaining both central tendency and dispersion in the assessment of treatment patterns. These findings establish descriptive treatment contrasts without assigning statistical significance or causal magnitude. Wasserstein and Lazar, 2016, distinguish substantive interpretation from inferential conclusions and caution against treating a statistical decision as a substitute for scientific reasoning. Here, the consistent result is lower mean tumour growth with capecitabine within every represented bacterial condition. The larger treatment contrast under knockout appears in the main experiment but does not recur in the comparison experiment. The evidence therefore supports an experiment dependent account of tumour response rather than a uniform conditional relationship across the samples.

6. Discussion:

Lower tumour growth under capecitabine does not imply that bacterial gene status modifies treatment response consistently across experiments. Table 1 shows lower mean growth with capecitabine within every bacterial condition, while Table 2 reveals that the ordering of treatment contrasts changes between experiments. The main experiment displays a larger capecitabine contrast under knockout, but the comparison experiment does not reproduce that pattern. Equation 1 expresses these differences relative to each animal’s baseline, separating proportional growth from absolute tumour burden. The contribution of this analysis is therefore the explicit distinction between a recurring treatment pattern and an experiment dependent bacterial contrast. Haiser et al., 2013, demonstrated that microbial drug inactivation depends on bacterial functional capacity and environmental conditions. The present comparison reinforces the need to evaluate the stability of a functional relationship rather than infer generality from one experimental contrast. The findings distinguish an outcome pattern from evidence identifying its transmission pathway. Table 1 measures tumour growth rather than drug concentrations, metabolite production or enzyme activity. Equation 3 reconstructs endpoint volume in the comparison experiment but adds no independent information about microbial metabolism. The association between bacterial condition and treatment response therefore remains an outcome level comparison. Zimmermann et al., 2019, separated host and microbial contributions using bacterial genetics, tissue measurements and pharmacokinetic modelling. Against that evidential benchmark, the present analysis clarifies what tumour trajectories contribute: they reveal variation in treatment response across bacterial conditions, while the mechanism requires evidence beyond the tumour outcome itself. This distinction prevents a descriptive bacterial contrast from being interpreted as a quantified metabolic pathway.

The temporal comparisons show that endpoint differences arise through changing growth trajectories rather than a fixed separation between groups. In Table 2, the knockout treatment contrast in the main experiment expands across observation days, whereas the over expression contrast changes nonmonotonically. The comparison experiment shows alternating directions in the contrast between bacterial conditions. Equation 2 provides an alternative scale for relative tumour volume, but no estimated result establishes that one pathway dominates another. Maurice et al., 2013, demonstrated that xenobiotic exposure can alter microbial physiology and gene expression, establishing a biological basis for considering time dependent interactions. The present evidence adds a narrower empirical insight: the temporal ordering of tumour response contrasts is not stable across the represented experiments. Time therefore functions as a dimension of the observed relationship rather than an interchangeable measurement occasion. Variation in starting tumour burden also changes how the treatment contrasts should be interpreted. Table 1 shows closely grouped baseline means in the main experiment but wider differences in the comparison experiment. Equation 1 standardises growth against each animal's baseline, yet this arithmetic adjustment does not establish equivalence in biological starting conditions. The agreement between mean and median treatment ordering strengthens the descriptive conclusion that capecitabine corresponds to lower growth, while differences in dispersion qualify any claim of uniform response. Guthrie et al., 2017, identified variation in colorectal cancer drug metabolism across human microbial communities. Their findings place the present heterogeneity within a broader functional perspective: an aggregate treatment contrast can coexist with substantial variation among individual responses. The analytical implication is to preserve experiment specific distributions rather than compress them into one pooled bacterial effect.

The international relevance concerns the transportability of microbiome related treatment relationships, rather than differences between national economies. The experiments provide no geographical comparison, but Tables 1 and 2 demonstrate why a treatment pattern and its proposed bacterial modifier require separate evaluation. Wilson and Nicholson, 2017, describe drug outcomes as products of interacting microbial and host processes, which helps explain why an experimentally observed relationship cannot automatically serve as a universal treatment rule. For preclinical assessment, the evidence supports reporting treatment contrasts within bacterial conditions, examining their trajectories and checking whether their ordering recurs across experiments. The theoretical refinement is a distinction between the consistency of the capecitabine associated growth pattern and the conditionality of the bacterial contrast. This preserves the relevance of microbial function while avoiding a general treatment recommendation that the descriptive evidence does not establish.

7. Conclusion and Implications:

Translating microbiome research into dependable cancer treatment requires separating recurring treatment patterns from relationships that depend on experimental conditions. This analysis shows that lower tumour growth during chemotherapy can coexist with inconsistent differences between bacterial conditions across experiments. Treatment exposure, microbial functional status and observation time jointly define the comparisons, but their descriptive patterns do not establish a uniform biological interaction. The contribution is an explicit distinction between consistency in treatment response and consistency in its proposed microbial explanation. This distinction refines pharmacomicrobiomic reasoning by showing why an established metabolic capability cannot, by itself, determine the magnitude or ordering of tumour response contrasts. For research managers, the findings support evaluating experimental consistency before allocating resources to a proposed microbial target. Practical assessment should retain individual trajectories, baseline distributions and separate experimental comparisons rather than rely on pooled averages. For research governance, the evidence supports transparent reporting of divergent patterns alongside favourable outcomes. These practices can improve the assessment of preclinical evidence across institutions and reduce reliance on findings that depend on selected experimental conditions. Their potential social value lies in strengthening the evidence used to prioritise translational research, while clinical treatment decisions remain outside the scope of the descriptive results.

The selected experiments provide opportunities to examine how starting tumour burden, experimental preparation and observation schedules influence reproducibility. Additional experiments could assess whether the observed contrasts recur under harmonised protocols and broader biological conditions. Direct measurements of drug exposure, metabolites and microbial activity would permit tests of the proposed transmission pathway. Analyses accounting for baseline differences, repeated observations and shared housing could quantify uncertainty around conditional treatment contrasts. Including all accessible experiments would assess sensitivity to sample selection. Subsequent clinical investigation could then evaluate transferability, without assuming that patterns observed in experimental animals establish treatment benefits in patients.

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