npj Breast Cancer

https://doi.org/10.1038/s41523-026-00922-3

Article in Press

The landscape of the intestinal microbiome among patients with newly diagnosed invasive breast cancer and ductal carcinoma in situ (DCIS)

Received: 18 August 2025

Accepted: 16 February 2026

Cite this article as: Sammons, S.L., Kuntz, T.M., DiLullo, M. *et al.* The landscape of the intestinal microbiome among patients with newly diagnosed invasive breast cancer and ductal carcinoma in situ (DCIS). *npj Breast Cancer* (2026). https://doi.org/10.1038/s41523-026-00922-3

Sarah L. Sammons, Thomas M. Kuntz, Molly DiLullo, Xochitl C. Morgan, Alyssa Martin, Melissa E. Hughes, Tasnim Rahman, Romualdo Barroso-Sousa, Esther Ritah Ogayo, Julia Giordano, Sean Ryan, Adrienne G. Waks, Ilana Schlam, Jennifer Ligibel, Nancy U. Lin, Ana C. Garrido-Castro, Elizabeth A. Mittendorf & Sara M. Tolaney

Title: The landscape of the intestinal microbiome among patients with newly diagnosed invasive breast cancer and ductal carcinoma *in situ* (DCIS)

Authors: Sarah L. Sammons[1,2,3], Thomas M. Kuntz[4], Molly DiLullo[1,2], Xochitl C. Morgan[4], Alyssa Martin[1,2], Melissa E. Hughes[1,2 *], Tasnim Rahman[1,2], Romualdo Barroso-Sousa[5], Esther Ritah Ogayo[1,2,3], Julia Giordano[6], Sean Ryan[1 **], Adrienne G. Waks[1,2,3], Ilana Schlam[1,2], Jennifer Ligibel[1,2,3], Nancy U. Lin[1,2,3], Ana C. Garrido-Castro[1,2,3], Elizabeth A. Mittendorf[2,3,7**], Sara M. Tolaney[1,2,3]

Affiliations: [1]Medical Oncology, Dana-Farber Cancer Institute, Boston, MA ; [2]Breast Oncology Program, Dana-Farber Brigham Cancer Center, Boston, MA; [3]Harvard Medical School, Boston, MA, USA; [4]Microbiome Analysis Core, Harvard T.H. Chan School of Public Health, Boston, MA; [5]DASA Oncology, Brasilia Hospital, Brasília, Brazil; [6]Perelman School of Medicine, Philadelphia, PA, USA; [7]Division of Breast Surgery, Department of Surgery, Brigham and Women's Hospital, Boston MA, USA

*Current Affiliation: Yale Cancer Center, New Haven, CT, USA

**Current affiliation: Beth Israel Deaconess Medical Center, Boston, MA, USA

Running title: Intestinal microbiome in patients with invasive breast cancer and DCIS

Corresponding Author:

Dr. Sara M. Tolaney

Dana-Farber Cancer Institute

450 Brookline Avenue

Boston, MA, USA 02215

Email: Sara_Tolaney@DFCI.HARVARD.EDU

Phone: 617-632-3800

ORCID #: 0000-0002-5940-8671

X (formerly Twitter) account: @stolaney1

Word count: 2,245 **Methods section: 880**

Tables: 1 **Figures: 4** **Supplementary Figures: 5** **Supplementary Data: 7**

Abstract

The intestinal microbiome shapes immune responses and is associated with patient outcomes in cancer following immunotherapy. We evaluated differences between the intestinal microbiome profiles of patients with early-stage invasive breast cancer (BC) and ductal carcinoma *in situ* (DCIS) by subtype using whole genome metagenomic sequencing. There were no significant differences in microbiome composition between DCIS and invasive BC as measured by alpha diversity ($p = 0.20$, ANOVA) or beta diversity ($p = 0.52$, PERMANOVA). Within invasive BC, patients with hormone receptor-positive (HR+)/HER2+ BC differed significantly in beta diversity relative to other subtypes ($p < 0.05$), with differences in six species ($q < 0.25$). *Bacteroides ovatus* was significantly more abundant in patients with stage III BC vs. stage I ($p = 0.0003$). Functional pathway analysis using HUMAnN3 revealed stage-specific enrichment of amino acid biosynthesis and nucleotide-related pathways. Altogether, these findings highlight potential microbial signatures associated with BC subtype and stage.

Introduction

Worldwide, invasive breast cancer is the most common and deadliest form of cancer diagnosed in women.[1] Approximately 90% of patients with breast cancer are initially diagnosed with early-stage disease (stage I-III) [2] and are treated with surgery, radiation therapy, and often neoadjuvant and/or adjuvant systemic therapy.[3] Despite these treatments, some patients experience cancer recurrence. The risk of recurrence varies by breast cancer stage, subtype, and other clinical, pathologic, and biological factors.[4-7] Breast cancer is a heterogeneous disease, and the identification of additional biomarkers that distinguish patients may help to further stratify the risk of recurrence.

The intestinal microbiome comprises all the microorganisms that reside in the intestinal tract.[8] The composition of the intestinal microbiome is intricately linked to human health, including regulating the immune response[9-12], the development of cancer[13] , and the response to systemic anti-cancer therapies.[14-16] Moreover, the intestinal microbiome contains a large community of bacterial species that metabolize estrogens and thus may influence the risk of developing hormone receptor-positive (HR+) breast cancer.[13,17] Thus ,the intestinal microbiome represents a potentially modifiable biomarker that could shed light on the mechanisms of breast cancer pathogenesis and progression.

We previously characterized the intestinal microbiome in patients with metastatic breast cancer undergoing treatment with chemotherapy alone or in combination with immunotherapy.[18,19] However, the intestinal microbiome has remained underexplored in patients with early-stage breast cancer and those with ductal carcinoma *in situ* (DCIS). DCIS is a precancerous lesion that

consists of a localized proliferation of neoplastic cells within the ducts and lobules of the breast. DCIS has the potential to evolve into invasive breast cancer if the neoplastic cells invade through the ductal basement membrane into surrounding tissues.[20,21] Currently, there is a paucity of data analyzing microbiome differences between DCIS and invasive breast cancer, though small studies suggest no major differences in alpha and beta diversity.[22]

To gain a greater understanding in this area, we performed whole genome metagenomic sequencing on stool samples collected from a large cohort of patients with DCIS or early-stage invasive breast cancer. Our primary objectives were to compare the baseline intestinal microbiome between patients with DCIS and invasive early-stage breast cancer and investigate differences amongst subtypes within invasive breast cancer.

Results

Patient Population

Baseline stool samples collected prior to any systemic treatment or surgical resection from 278 patients were sequenced, including 36 patients with DCIS and 242 with invasive breast cancer (**Table 1**). The study population was enriched for patients with triple-negative breast cancer (TNBC) (36.3%) and for patients with stage I (32.01%) and stage II (38.85%) breast cancer. Most patients were White (83.45 %) and were older than or equal to age 50 (70.5%).

Comparison of the stool microbiomes of patients with DCIS and invasive breast cancer

In both invasive breast cancer and DCIS samples, the most abundant species were typical commensals of the healthy gut, such as *Faecalibacterium prausnitzii* and various *Alistipes, Bacteroides,* and *Ruminococcus* species (**Figure 1**). The baseline stool microbiome composition

of patients with DCIS and invasive breast cancer was comparable and not significantly different as measured by alpha diversity (p = 0.20) nor beta diversity (p = 0.52) (**Figure 2**), nor were any individual microbial taxa or pathways associated with DCIS or invasive breast cancer.

Association of age, body mass index (BMI), cancer stage and stool microbiome of invasive breast cancer patients

Within invasive breast cancer, patient age was not associated with alpha diversity (p = 0.160) but was associated with beta diversity (p = 0.014) and the abundance of 26 species and 133 MetaCyc pathways (q < 0.25) (**Supplementary Figure 1; Supplementary Data 1**). Baseline BMI was associated with the abundance of 6 species and 3 pathways (**Supplementary Figure 2; Supplementary Data 2**), but not alpha (p = 0.820) nor beta diversity (p = 0.101). Similarly, cancer stage was not associated with alpha (p > 0.05) nor beta diversity (p = 0.25). However, *Bacteroides ovatus* was significantly more abundant in patients with stage III (n = 32) versus stage I (n = 89) breast cancer (p = 0.0001, q = 0.08; **Figure 3**). *B. ovatus* abundance between stage I and II differed numerically but findings were not significant after correction for false discovery (p = 0.0193, q = 0.702).

To account for any potential confounders, we conducted multivariable analyses for microbial species (**Supplementary Figure 3; Supplementary Data 3**) and MetaCyc pathways (**Supplementary Figure 4; Supplementary Data 3**) using age, stage, BMI, and hormone receptor status as covariates. The multivariable models generally agreed with the univariate models, albeit with lower power, and thus lower sensitivity.

In total, 211 MetaCyc pathways were significantly associated with stage ($q < 0.25$) (**Supplementary Data 4**). Of these, 111 pathways were significant in both stage II and stage III, with changes more pronounced in stage III. Sixteen pathways were more abundant in later-stage cancer, and most were related to the biosynthesis of amino acids, including histidine, methionine, arginine, lysine, and threonine, as well as nucleotide-related pathways such as inosine biosynthesis **(Supplementary Data 5).**

The microbiome of HR+/HER2+ cancer was distinct from that of other subtypes.

There were no differences in alpha diversity between invasive breast cancer subtypes. However, patients with HR+/HER2+ had a unique intestinal microbiome profile based on pairwise testing, with significantly different beta diversity relative to all other breast cancer subtypes (**Figure 4**). This corresponded to eight species that were differentially abundant between HR+/HER2+ and any other subtype (**Supplementary Figure 5**). The only other subtype-associated difference in species abundance was *Blautia wexlerae SGB4837*, which was more abundant in HR-/HER2+ than HR+/HER2- ($p = 0.001$, $q = 0.201$). Hormone receptor status was also associated with sixteen MetaCyc pathways (**Supplementary Data 6**); alanine metabolism was the most affected process.

Discussion

The average human colon contains roughly 38 trillion bacterial cells, most of which belong to the Bacteroidetes and Firmucutes phyla.[23,24] Intestinal bacteria have a tremendous influence on human health and development. Their metabolic activity supplies the human body with essential nutrients, and interactions between intestinal bacteria and human immune cells are essential for

proper immune system development.[11,12] Intestinal dysbiosis (disruption of the gut microorganisms) has been linked to a variety of chronic illnesses, including diabetes, inflammatory bowel disease, and cancer.[11,12] In addition, the composition of the intestinal microbiome has been linked to the response to systemic anti-cancer therapy, particularly immune checkpoint inhibitors.[14-16]

To date, studies into the role of the intestinal microbiome in the development of breast cancer and response to therapy have produced conflicting results. One small pilot case-control study used 16S rRNA gene sequencing to investigate the intestinal microbiome in baseline fecal samples from 48 postmenopausal women with breast cancer and 48 postmenopausal women without breast cancer. This analysis revealed that the alpha diversity of the intestinal microbiome was significantly lower in patients with breast cancer compared to healthy controls ($p < 0.004$). Beta diversity also varied significantly between patients with breast cancer and healthy controls ($p = 0.01$). Compared to healthy controls, patients with breast cancer had higher levels of *Clostridiaceae*, *Faecalibacterium*, and *Ruminococcaceae*, and lower levels of *Dorea* and *Lachnospiraceae*.[25,26]

A different study used metagenomic shotgun sequencing to evaluate the intestinal microbiome in baseline fecal samples from 18 premenopausal patients with breast cancer, 25 premenopausal healthy controls, 44 postmenopausal breast cancer patients, and 46 postmenopausal healthy controls. Among premenopausal women, there was no significant difference in alpha diversity ($p = 0.777$) or beta diversity ($p = 0.056$) between breast cancer patients and healthy controls. In contrast, the alpha diversity ($p = 0.003$) and beta diversity ($p < 0.001$) were both significantly higher in postmenopausal breast cancer patients compared to postmenopausal healthy controls.[27]

In the present study, we explored the baseline intestinal microbiome in patients with DCIS (n = 36) and stage I-III invasive breast cancer (n = 242). When we compared the intestinal microbiomes between patients with DCIS and invasive breast cancer, we observed no significant differences in alpha or beta diversity. When we focused on invasive breast cancer, we found that patients with HR+/HER2+ breast cancer were distinguished from patients with all other subtypes in terms of beta diversity ($p = 0.005$). The reason for this difference remains unclear and should be validated in larger datasets.

We also observed differences by stage, with an enrichment of *Bacteroides ovatus* in patients with stage III vs. stage I breast cancer. *Bacteroides ovatus* has been implicated as a predictor of response[28,29] or resistance[30,31] to therapies in other tumor types. In a study by Li *et al*., stool samples were collected before the initiation of treatment and after the end of treatment from 31 patients with locally advanced or metastatic esophageal squamous cell carcinoma who received paclitaxel with cisplatin. The baseline abundance of *Bacteroides ovatus* was identified as a predictor of patients who achieved a partial response to therapy.[28] In another study, Heshiki *et al.* performed shotgun metagenomic sequencing on baseline and on-treatment stool samples from 26 patients with eight different cancer types (including seven patients with breast cancer) who were treated with a variety of systemic therapies. *Bacteroides ovatus* was one of 22 bacterial species that was enriched in responders. In a mouse lung cancer model, greater tumor control with erlotinib was achieved in mice that had been colonized with *Bacteroides ovatus* compared to mice that had not received the bacteria ($p = 0.032$).[29] In contrast, Peters *et al.* found that the baseline abundance of *Bacteroides ovatus* was associated with shorter progression-free survival

(PFS) among 27 patients with metastatic melanoma receiving immunotherapy. Metagenomic pathway analysis suggested that shorter PFS was associated with breakdown of L-rhamnose and biosynthesis of pyridoxal 5-phosphate, 6-hydroxymethyl-dihydropterin diphosphate, pantothenate, and coenzyme A by *Bacteroides ovatus*.[30] Teng *et al.* also identified *Bacteroides ovatus* as one of the species associated with nonresponse to neoadjuvant chemoradiotherapy among patients with locally advanced rectal cancer.[31] Future studies should investigate how the composition of the baseline intestinal microbiome impacts treatment response in early-stage breast cancer.

The present study adds to the growing body of knowledge regarding the composition of the intestinal microbiome in early-stage breast cancer. In a recent study, the intestinal microbiome was evaluated using 16S rRNA sequencing in 25 patients with early-stage TNBC who received neoadjuvant anthracycline and taxane-containing chemotherapy. Fecal samples were collected before initiation of therapy (t0), at one-week post-neoadjuvant chemotherapy (t1), and at eight weeks post-neoadjuvant chemotherapy (t2). At t0, patients who achieved a pathologic complete response rate (pCR) had significantly higher alpha diversity compared to patients with residual invasive disease ($p = 0.049$), though there was no significant difference in beta diversity between the two groups ($p = 0.965$). Beta diversity differed significantly based on BMI ($p = 0.039$) and menopausal status ($p = 0.035$).[32]

Furthermore, our HUMAnN3-based pathway analysis revealed that the intestinal microbiome undergoes stage-specific functional shifts, with more pronounced changes observed in stage III versus stage II breast cancer. The enrichment of amino acid biosynthesis pathways, including

histidine, methionine, arginine, lysine, and threonine, suggests that microbial communities in later-stage cancer may contribute to an increased supply of metabolites critical for tumor growth, protein synthesis, and cellular proliferation. The concurrent upregulation of nucleotide-related pathways, such as inosine biosynthesis, further indicates that the microbiome may influence nucleotide availability, potentially supporting rapid DNA replication and repair in tumor cells. We hypothesize that higher tumor burden/stage may later tumor-associated nutrient/metabolite utilization that could alter substrate availability and thereby shift the microbiome. We also acknowledge that stage may correlate with other exposures (clinical status, host factors, or peri-diagnostic factors) that could contribute to observed differences, and we clarify that mechanistic conclusions cannot be drawn from this observational dataset. This highlights the potential for microbiome-derived functional signatures to serve as biomarkers of disease stage and raises the possibility that targeting specific microbial metabolic pathways could complement conventional therapies or modulate tumor growth.

This study was limited by a relatively small sample size of patients with DCIS (n = 36), which limits the power of any comparison with the larger sample of patients with stage I-III breast cancer (n = 242). We also only sequenced baseline samples, which did not allow for investigations into changes in the composition of the intestinal microbiome during treatment, nor any influence of the baseline (or on-treatment) microbiome on treatment outcome. Moreover, the univariate analyses performed in this study were vulnerable to confounding. We attempted to address this by performing multivariate analyses, which generally agreed with the univariate analyses, but were less powered and thus less sensitive. Finally, the use of certain medications (such as antibiotics and proton pump inhibitors), prebiotics, and probiotics, as well as diet, can

impact the composition of the intestinal microbiome.[33-35] Data about the use of these agents were not included in this analysis. This limitation will also have to be considered in future analyses, in which we will assess changes in the intestinal microbiome before and during treatment, as well as response to cancer-directed therapies in this cohort. Given the observational design and potential for residual confounding, these findings should be considered hypothesis-generating and warrant confirmation in independent cohorts and future studies.

In summary, our study contributes to the growing body of evidence that the intestinal microbiome exhibits individualized and stage-specific features in breast cancer. While overall differences in microbiome composition are modest, functional shifts, particularly in amino acid and nucleotide metabolism, may play a role in tumor progression and represent potential targets for biomarker development or therapeutic modulation. Future studies leveraging longitudinal sampling, larger cohorts, and integration of functional and taxonomic data will be essential to fully understand the interplay between the intestinal microbiome, breast cancer biology, and treatment response.

Methods

Patient Population and Stool Collection

Baseline (pre-treatment) stool samples were collected from stage 0-III breast cancer patients enrolled in two prospective clinical trials (NCT04425018, DFCI protocol number 20-068, April 15, 2020 initial release date; and NCT02999477, DFCI protocol number 16-466, December 20, 2016 initial release date) and two biospecimen collection registries (DFCI protocol numbers 93-085 and 18-617). MARGOT (20-068, NCT04425018) an open-label, randomized phase II trial,

enrolled patients with stage II-III human epidermal growth factor receptor 2 (HER2)-positive breast cancer with a CD16A low-affinity genotype (FF or FV). Patients provided written consent and all trials were conducted in line with the Declaration of Helsinki. Participants were randomized 2:1 to receive neoadjuvant paclitaxel, pertuzumab, and margetuximab (Arm A) or paclitaxel, pertuzumab, and trastuzumab (Arm B).[36] Stool samples were collected at baseline, during cycle 2 of neoadjuvant therapy, and after completion of neoadjuvant therapy, prior to surgery. For the purposes of this study, only baseline stool samples were sequenced and analyzed. Western Institutional Review Board (Baylor)-FWA00000286, Albert Einstein College of Medicine IRB(Montefiore)-FWA00002558, Fred Hutchinson Cancer Research Center Institutional Review Office(UWash)-FWA00000334, U.T. M. D. Anderson Cancer Center Institutional Review Board- FWA00000363, MHRI-Georgetown University Oncology IRB-FWA00000504 DFCI protocol number 16-466 (NCT02999477) was a pilot study that enrolled patients with early-stage hormone receptor-positive (HR+)/HER2- breast cancer. Participants were randomized 1:1 to receive a two-week window of neoadjuvant nab-paclitaxel or pembrolizumab. Subsequently, all participants received neoadjuvant nab-paclitaxel in combination with pembrolizumab to complete neoadjuvant therapy. Stool samples were collected at baseline (prior to initiation of window neoadjuvant therapy), after one week of window monotherapy (nab-paclitazel or pembrolizumab), and after exposure to the combination of neoadjuvant nab-paclitaxel and pembrolizumab (week 4). For the purposes of this study, baseline stool samples were sequenced and analyzed. Project SHARE (93-085) is a multi-institutional biobanking repository protocol that enrolls patients with all stages and subtypes of invasive breast cancer, as well as DCIS. For the purposes of this study, baseline stool samples from patients with stage I-III invasive breast cancer and DCIS were sequenced and analyzed. DFCI

protocol number 18-617 is a longitudinal, multi-institutional registry of patients with newly diagnosed treatment-naive TNBC. Participants receive neoadjuvant systemic therapy at the discretion of their treating physician. Stool samples are collected at baseline, during neoadjuvant therapy, and after surgery using the DNA Genotek Omnigene-Gut Microbiome Collection Kit according to the manufacturer's instructions. Samples are processed according to manufacturer's instruction, aliquoted and stored at -80 °C until sequencing. For the purposes of this study, baseline stool samples were sequenced and analyzed from patients enrolled in Cohort B, including patients with stage I-III TNBC who were eligible for preoperative systemic therapy.

Whole Genome Sequencing

Stool samples were sequenced at the Alkek Center for Metagenomics and Microbiome Research at Baylor College of Medicine in Houston, TX, USA. Total genomic DNA was extracted using the Qiagen DNeasy PowerSoil Pro kit according to the manufacturer's instructions, automated on the QIAcube HT instrument. Libraries were constructed using the Illumina DNA Prep library kit. Samples were barcoded using kit-appropriate unique dual index adapter sets (UD indexes). Completed libraries were quality controlled using PicoGreen (Thermo), Qubit (Invitrogen), Fragment Analyzer (Agilent), and Tapestation (Agilent) to assess concentration and fragment size distribution. Whole Genome shotgun sequencing of pooled libraries was carried out via the Illumina NovaSeq platform, using the 2x150 bp paired-end protocol.

Bioinformatic Processing

Whole genome stool sequence data were processed using the bioBakery software pipeline, with default parameters. KneadData v0.12.0 was used for quality control of raw reads, including

trimming, adapter removal, and removal of human reads from the default hg37 and human contamination database. The average final read count was 66 million reads per sample, with a minimum of 19M. Taxonomic profiling was performed using MetaPhlAn v4.0.6[37] with the mpa_vOct22_CHOCOPhlAnSGB_202212 database, generating relative abundance data. MetaPhlAn 4 provides the per-sample abundance of species-level genome bins (SGBs), which are comparable to species but allow identification of taxa not currently annotated at the species level, as well as differentiation of taxonomic groups previously believed to be single species.[37] Pathway analysis was performed using HUMAnN3 to profile the functional potential of the intestinal microbiome and identify metabolic pathways associated with cancer stage.

Statistical Analyses

Data were analyzed using R 4.3.0. The vegan package was used for calculation of ecological diversity measurements and statistical tests. Species were filtered at a threshold of minimum 0.1% relative abundance in more than 10% of samples. Of the 2343 SGBs detected in the data, 223 met prevalence and abundance thresholds. Alpha and beta diversity were quantified as Shannon diversity and Bray-Curtis dissimilarity, respectively. Shannon diversity describes the complexity of a sample and is influenced by both richness (number of features) and evenness of distribution. Bray-Curtis dissimilarity measures compositional overlap between a pair of samples (0 = identical, 1 = nothing in common) and is influenced by the features and their abundances. Alpha diversity associations were tested by ANOVA and beta diversity by PERMANOVA (**Supplementary Data7**). Associations between clinical characteristics at the individual species level were tested by linear modeling using the MaAsLin2 framework[38]. Total sum scaling was used, and species were log transformed with a pseudocount of half the minimum non-zero value

for variance stabilization. The p-values from linear modeling were adjusted for false discovery using the Benjamini and Hochberg method via the p.adjust function in R.

Data Availability

Sequencing data for this project are deposited at the NCBI Sequencing Read Archive (SRA) under BioProject PRJNA1295573.

Code Availability

Not applicable.

Acknowledgements

This study was funded by Lori and Randy Benderson; by the Elaine and Eduardo Saverin Foundation; by a Massachusetts Life Sciences Center grant (Dana-Farber Cancer Institute project number 6395601) (to SMT); by Merck; by MacroGenics; and by a National Cancer Institute (NCI) Breast Cancer SPORE (P50CA168504 to DF/HCC).

The funders played no role in study design, data collection, analysis and interpretation of data, or the writing of this manuscript.

The authors wish to thank Timothy K. Erick, PhD, for medical writing support, Joanna Baginska, PhD for reviewing and providing feedback on the manuscript, and Valerie Hope Goldstein for medical editing and submission support.

The authors also wish to thank clinical research coordinators Eileen Wrabel, Jillian Alberti, Ashley Root, and Stefan Atanasov.

Author Contributions

Conceptualization: ST, EM, MH, RB, AW, IS, JL, AGC

Data curation: SS, TK, ML, XM, AM, MH, TR, ERO, JG, SR

Formal Analysis: XM, TK, SS, ST

Funding acquisition: ST

Investigation: All authors

Methodology: ST, XM, TK, SS

Supervision: ST, XM

Writing – original draft: SS

Writing – review & editing: All authors

Disclosures

SS reports serving as a consultant for and/or on the advisory boards of AstraZeneca, Daiichi Sankyo, Gilead Sciences, Lilly, Novartis, Pfizer, Relay Therapeutics, and Seagen; research funding from AstraZeneca, Alterome, BioNtech, Daiichi Sankyo, Iambic, Lilly, and Relay Therapeutics; and travel, accommodations, and expenses from AstraZeneca and Pfizer. **NUL** declares institutional research support from Genentech, Pfizer, Merck, Seattle Genetics, Zion Pharmaceuticals, Olema Pharmaceuticals, and AstraZeneca; consulting honoraria from Seattle Genetics, Daiichi-Sankyo, AstraZeneca, Olema Pharmaceuticals, Stemline/Menarini, Artera Inc., Eisai, and Shorla Oncology; royalties from UptoDate (book); and travel support from Olema, AstraZeneca, and DSI. **AGW** declares research funding to the institution from Gilead, Genentech, Macrogenics, and Merck; serving on the steering committee of AMBRX; and serving as a consultant and paid speaker for AstraZeneca.**AG-C** declares research funding to institution from Gilead Sciences, AstraZeneca, Daiichi- Sankyo, Merck, Zenith Epigenetics, Bristol-Myers Squibb, Novartis, Biovica, Foundation Medicine, 4D Path, Precede Biosciences, and Bicycle Therapeutics; scientific advisory board service/consulting for AstraZeneca, Daiichi-Sankyo,

Novartis, Pfizer, and Gilead Sciences; serving as a speaker for/honoraria from AstraZeneca, Daiichi-Sankyo, Gilead Sciences, and Roche/Genentech; and travel/other support from Roche/Genentech, Gilead Sciences, AstraZeneca, Daiichi Sankyo, Novartis, and Merck. **EAM** reports compensated service on scientific advisory boards for AstraZeneca, BioNTech, Merck and Moderna; uncompensated service on steering committees for Bristol Myers Squibb and Roche/Genentech; speakers honoraria and travel support from Merck Sharp & Dohme; and institutional research support from Roche/Genentech (via SU2C grant) and Gilead. EAM also reports research funding from Susan Komen for the Cure for which she serves as a Scientific Advisor, and uncompensated participation as a member of the American Society of Clinical Oncology Board of Directors. **SMT** reports consulting or advisory roles for Novartis, Pfizer/Seagen, Merck, Eli Lilly, AstraZeneca, Genentech/Roche, Eisai, Bristol Myers Squibb/Systimmune, Daiichi Sankyo, Gilead, Blueprint Medicines, Reveal Genomics, Sumitovant Biopharma, Artios Pharma, Menarini/Stemline, Aadi Bio, Bayer, Jazz Pharmaceuticals, Natera, Tango Therapeutics, eFFECTOR, Hengrui USA, Cullinan Oncology, Circle Pharma, Arvinas, BioNTech, Launch Therapeutics, Zuellig Pharma, Johnson&Johnson/Ambrx, Bicycle Therapeutics, BeiGene Therapeutics, Mersana, Summit Therapeutics, Avenzo Therapeutics, Aktis Oncology, Celcuity, Boehringer Ingelheim, Samsung Bioepis, Olema Pharmaceuticals, Tempus, and Boundless Bio; research funding from Genentech/Roche, Merck, Exelixis, Pfizer, Lilly, Novartis, Bristol Myers Squibb, AstraZeneca, NanoString Technologies, Gilead, Seagen, OncoPep, Daiichi Sankyo, Menarini/Stemline, Jazz Pharmaceuticals, and Olema Pharmaceuticals; and travel support from Lilly, Gilead, Jazz Pharmaceuticals, Pfizer, Arvinas, and Roche. The remaining authors declare no conflicts of interest.

Prior presentation:

This study was presented at the 2024 American Society of Clinical Oncology (ASCO) Annual Meeting held May 31-June 4, 2024 in Chicago, IL.

References

1 Bray, F. *et al.* Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* **74**, 229-263 (2024). https://doi.org/10.3322/caac.21834

2 Dawood, S., Broglio, K., Ensor, J., Hortobagyi, G. N. & Giordano, S. H. Survival differences among women with de novo stage IV and relapsed breast cancer. *Ann Oncol* **21**, 2169-2174 (2010). https://doi.org/10.1093/annonc/mdq220

3 Corti, C. *et al.* Systemic Therapy in Breast Cancer. *Am Soc Clin Oncol Educ Book* **44**, e432442 (2024). https://doi.org/10.1200/EDBK_432442

4 Lafourcade, A. *et al.* Factors associated with breast cancer recurrences or mortality and dynamic prediction of death using history of cancer recurrences: the French E3N cohort. *BMC Cancer* **18**, 171 (2018). https://doi.org/10.1186/s12885-018-4076-4

5 van Maaren, M. C. *et al.* Ten-year recurrence rates for breast cancer subtypes in the Netherlands: A large population-based study. *Int J Cancer* **144**, 263-272 (2019). https://doi.org/10.1002/ijc.31914

6 Leone, J. P. *et al.* Factors associated with late risks of breast cancer-specific mortality in the SEER registry. *Breast Cancer Res Treat* **189**, 203-212 (2021). https://doi.org/10.1007/s10549-021-06233-4

7 Pan, H. *et al.* 20-Year Risks of Breast-Cancer Recurrence after Stopping Endocrine Therapy at 5 Years. *N Engl J Med* **377**, 1836-1846 (2017). https://doi.org/10.1056/NEJMoa1701830

8 Ursell, L. K., Metcalf, J. L., Parfrey, L. W. & Knight, R. Defining the human microbiome. *Nutr Rev* **70 Suppl 1**, S38-44 (2012). https://doi.org/10.1111/j.1753-4887.2012.00493.x

9 Zheng, D., Liwinski, T. & Elinav, E. Interaction between microbiota and immunity in health and disease. *Cell Research* **30**, 492-506 (2020). https://doi.org/10.1038/s41422-020-0332-7

10 Belkaid, Y. & Hand, T. W. Role of the microbiota in immunity and inflammation. *Cell* **157**, 121-141 (2014). https://doi.org/10.1016/j.cell.2014.03.011

11 Shreiner, A. B., Kao, J. Y. & Young, V. B. The gut microbiome in health and in disease. *Curr Opin Gastroenterol* **31**, 69-75 (2015). https://doi.org/10.1097/MOG.0000000000000139

12 Durack, J. & Lynch, S. V. The gut microbiome: Relationships with disease and opportunities for therapy. *J Exp Med* **216**, 20-40 (2019). https://doi.org/10.1084/jem.20180448

13 Plottel, C. S. & Blaser, M. J. Microbiome and malignancy. *Cell Host Microbe* **10**, 324-335 (2011). https://doi.org/10.1016/j.chom.2011.10.003

14 Matson, V. *et al.* The commensal microbiome is associated with anti-PD-1 efficacy in metastatic melanoma patients. *Science* **359**, 104-108 (2018). https://doi.org/10.1126/science.aao3290

15 Routy, B. *et al.* Gut microbiome influences efficacy of PD-1-based immunotherapy against epithelial tumors. *Science* **359**, 91-97 (2018). https://doi.org/10.1126/science.aan3706

16 Gopalakrishnan, V. *et al.* Gut microbiome modulates response to anti-PD-1 immunotherapy in melanoma patients. *Science* **359**, 97-103 (2018). https://doi.org/10.1126/science.aan4236

17 Kwa, M., Plottel, C. S., Blaser, M. J. & Adams, S. The Intestinal Microbiome and Estrogen Receptor-Positive Female Breast Cancer. *J Natl Cancer Inst* **108** (2016). https://doi.org/10.1093/jnci/djw029

18 de Sousa, R. B. *et al.* Abstract P3-09-16: Fecal microbiome and association with outcomes among patients (pts) receiving eribulin (E) +/- pembrolizumab (P) for hormone receptor positive (HR+) metastatic breast cancer (MBC). *Cancer Research* **80**, P3-09-16-P03-09-16 (2020). https://doi.org/10.1158/1538-7445.SABCS19-P3-09-16

19 Barroso-Sousa, R. *et al.* Abstract PD11-05: PD11-05 Gut microbiome signatures correlate with overall survival among patients receiving eribulin with or without pembrolizumab for hormone receptor-positive metastatic breast cancer. *Cancer Research* **83**, PD11-05-PD11-05 (2023). https://doi.org/10.1158/1538-7445.SABCS22-PD11-05

20 Allred, D. C. Ductal carcinoma in situ: terminology, classification, and natural history. *J Natl Cancer Inst Monogr* **2010**, 134-138 (2010). https://doi.org/10.1093/jncimonographs/lgq035

21 van Seijen, M. *et al.* Ductal carcinoma in situ: to treat or not to treat, that is the question. *Br J Cancer* **121**, 285-292 (2019). https://doi.org/10.1038/s41416-019-0478-6

22 Yang, P., Wang, Z., Peng, Q., Lian, W. & Chen, D. Comparison of the Gut Microbiota in Patients with Benign and Malignant Breast Tumors: A Pilot Study. *Evolutionary Bioinformatics* **17**, 11769343211057573 (2021). https://doi.org/10.1177/11769343211057573

23 Sender, R., Fuchs, S. & Milo, R. Revised Estimates for the Number of Human and Bacteria Cells in the Body. *PLoS Biol* **14**, e1002533 (2016). https://doi.org/10.1371/journal.pbio.1002533

24 Bull, M. J. & Plummer, N. T. Part 1: The Human Gut Microbiome in Health and Disease. *Integr Med (Encinitas)* **13**, 17-22 (2014).

25 Goedert, J. J. *et al.* Investigation of the association between the fecal microbiota and breast cancer in postmenopausal women: a population-based case-control pilot study. *J Natl Cancer Inst* **107** (2015). https://doi.org/10.1093/jnci/djv147

26 Mikó, E. *et al.* Microbiome-Microbial Metabolome-Cancer Cell Interactions in Breast Cancer-Familiar, but Unexplored. *Cells* **8** (2019). https://doi.org/10.3390/cells8040293

27 Zhu, J. *et al.* Breast cancer in postmenopausal women is associated with an altered gut metagenome. *Microbiome* **6**, 136 (2018). https://doi.org/10.1186/s40168-018-0515-3

28 Li, N. *et al.* Impact of the gut microbiome on response and toxicity to chemotherapy in advanced esophageal cancer. *Heliyon* **10**, e32770 (2024). https://doi.org/10.1016/j.heliyon.2024.e32770

29 Heshiki, Y. *et al.* Predictable modulation of cancer treatment outcomes by the gut microbiota. *Microbiome* **8**, 28 (2020). https://doi.org/10.1186/s40168-020-00811-2

30 Peters, B. A. *et al.* Relating the gut metagenome and metatranscriptome to immunotherapy responses in melanoma patients. *Genome Med* **11**, 61 (2019). https://doi.org/10.1186/s13073-019-0672-4

31 Teng, H. *et al.* Gut microbiota-mediated nucleotide synthesis attenuates the response to neoadjuvant chemoradiotherapy in rectal cancer. *Cancer Cell* **41**, 124-138 e126 (2023). https://doi.org/10.1016/j.ccell.2022.11.013

32 Vernaci, G. *et al.* Characterization of Gut Microbiome Composition in Patients with Triple-Negative Breast Cancer Treated with Neoadjuvant Chemotherapy. *Oncologist* **28**, e703-e711 (2023). https://doi.org/10.1093/oncolo/oyad060

33 Vich Vila, A. *et al.* Impact of commonly used drugs on the composition and metabolic function of the gut microbiota. *Nat Commun* **11**, 362 (2020). https://doi.org/10.1038/s41467-019-14177-z

34 Conlon, M. A. & Bird, A. R. The impact of diet and lifestyle on gut microbiota and human health. *Nutrients* **7**, 17-44 (2014). https://doi.org/10.3390/nu7010017

35 Weersma, R. K., Zhernakova, A. & Fu, J. Interaction between drugs and the gut microbiome. *Gut* **69**, 1510-1519 (2020). https://doi.org/10.1136/gutjnl-2019-320204

36 Adrienne Waks, H. H., Paula Pohlmann, Mothaffar Rimawi, Natalie Sinclair, Rachel L. Yung, Jesus Anampa, Claudine Isaacs, Thomas O'Connor, Nadine Tung, Adam Brufsky, Lisa Carey, Rita Nanda, Nancy U. Lin, Antonio Wolff, Clare Strickland, Michelle DeMeo, Ashley Root, Tasnim Rahman, Abigail Recko, Molly DiLullo, Ashka Patel, Esther R. Ogayo, Philip & Poorvu, A. G., Erica Mayer, Tari King, Eric P. Winer, Elizabeth Mittendorf, Nabihah Tayob, Sara M. Tolaney, Ian E. Krop. in *San Antonio Breast Cancer Symposium.*

37 Pasolli, E. *et al.* Extensive Unexplored Human Microbiome Diversity Revealed by Over 150,000 Genomes from Metagenomes Spanning Age, Geography, and Lifestyle. *Cell* **176**, 649-662 e620 (2019). https://doi.org/10.1016/j.cell.2019.01.001

38 Mallick, H. *et al.* Multivariable association discovery in population-scale meta-omics studies. *PLoS Comput Biol* **17**, e1009442 (2021). https://doi.org/10.1371/journal.pcbi.1009442

Figure Legend

Figure 1. Landscape of baseline intestinal microbiota species relative abundance in patients with early-stage breast cancer (n = 242) and DCIS (n = 36). The per-relative abundance of the 20 most-abundant species in the data is shown.

Abbreviations: DCIS, ductal carcinoma *in situ*

Figure 2. The baseline stool microbiota of patients with DCIS (n = 36) and invasive breast cancer (n = 242) are similar and are not differentiated by beta diversity (p = 0.52, PERMANOVA).

Abbreviations: DCIS, ductal carcinoma *in situ*

Figure 3. *Bacteroides ovatus* abundance by breast cancer stage. Abundance was significantly higher with advanced stage in early-stage breast cancer when comparing stages I (n = 89) and III (n = 32) (p = 0.0001; q = 0.08). For all box plots, the boxes range from quartile 1 to quartile 3 (Q1 to Q3), with the median value indicated by the line within the box. The whiskers extend to the most extreme points within 1.5 × interquartile range (IQR) from the box edges. Individual values outside the whiskers are considered outliers.

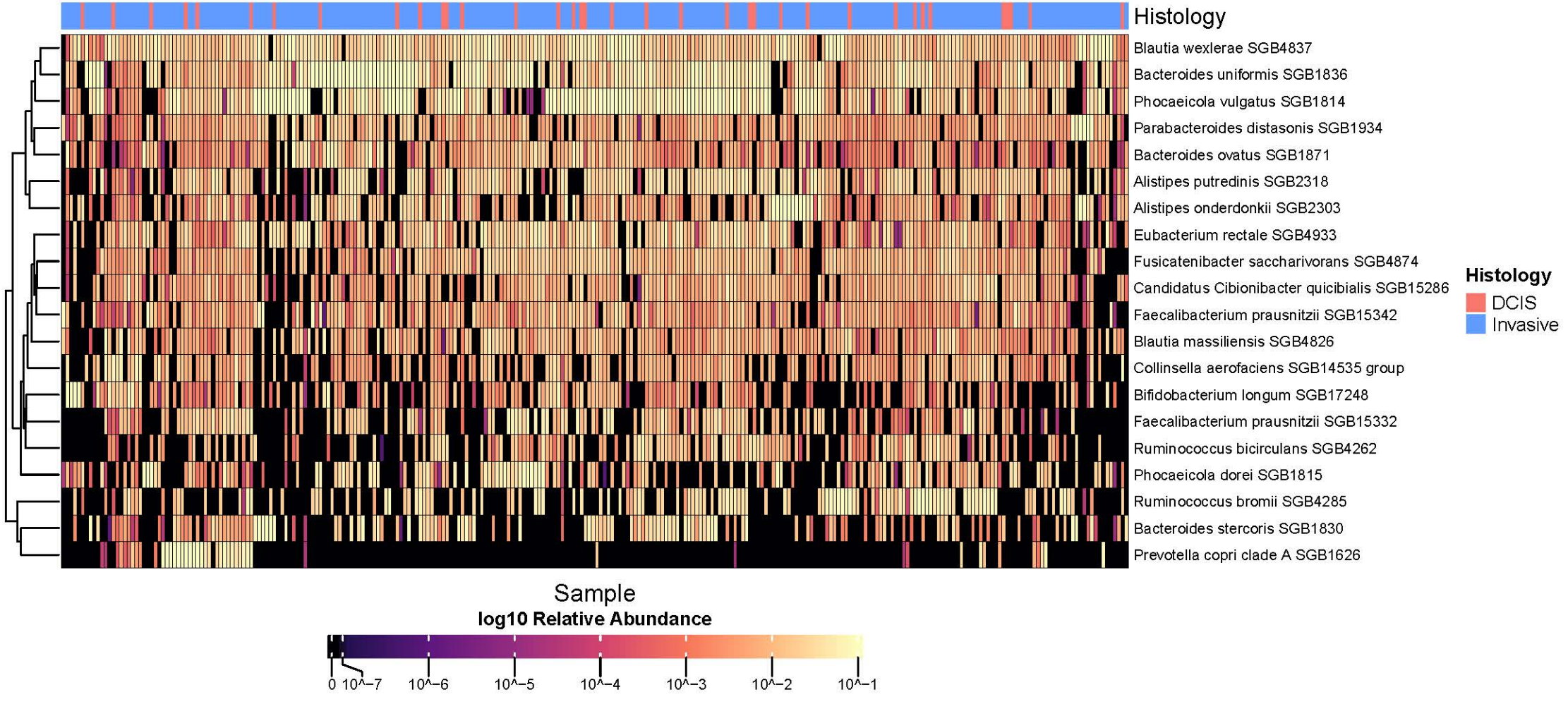
Figure 4. The baseline stool microbiome of HR+/HER2+ invasive breast cancer is distinct from other HR subtypes ($p < 0.05$, $q < 0.1$, PERMANOVA).

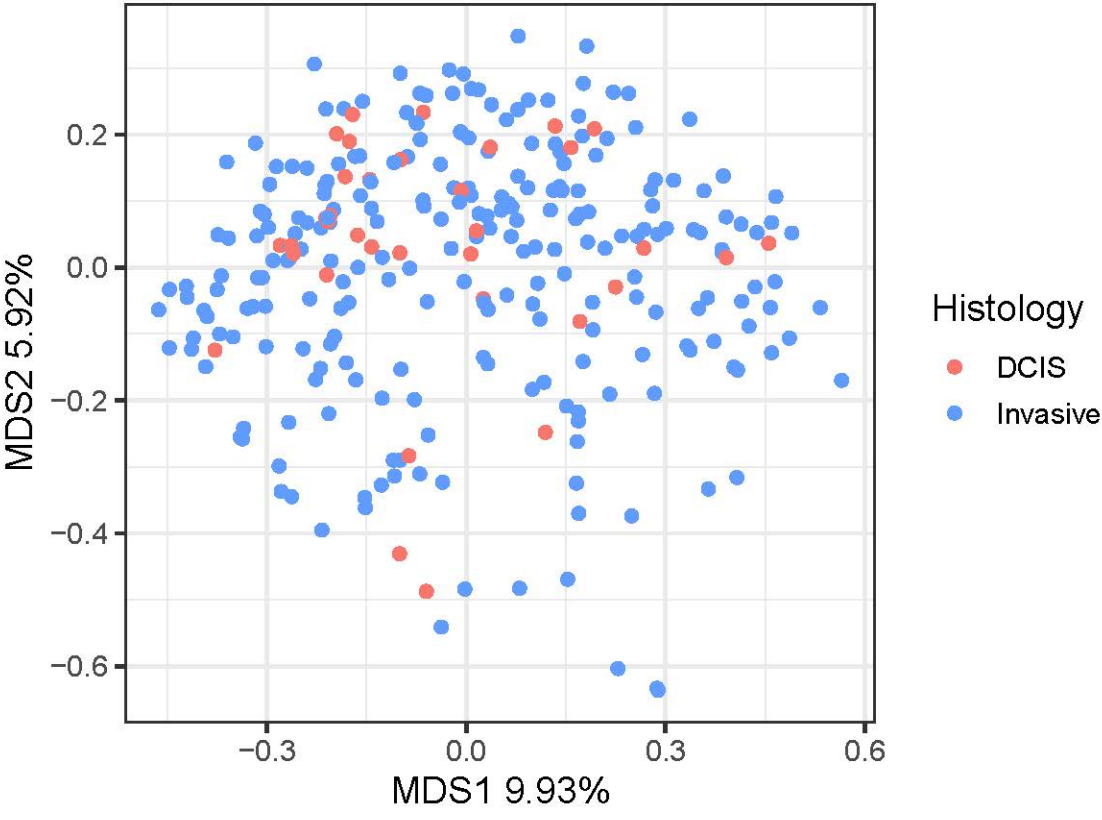
Abbreviations: HR, hormone receptor; HER, human epidermal growth factor receptor 2; TNBC, triple-negative breast cancer

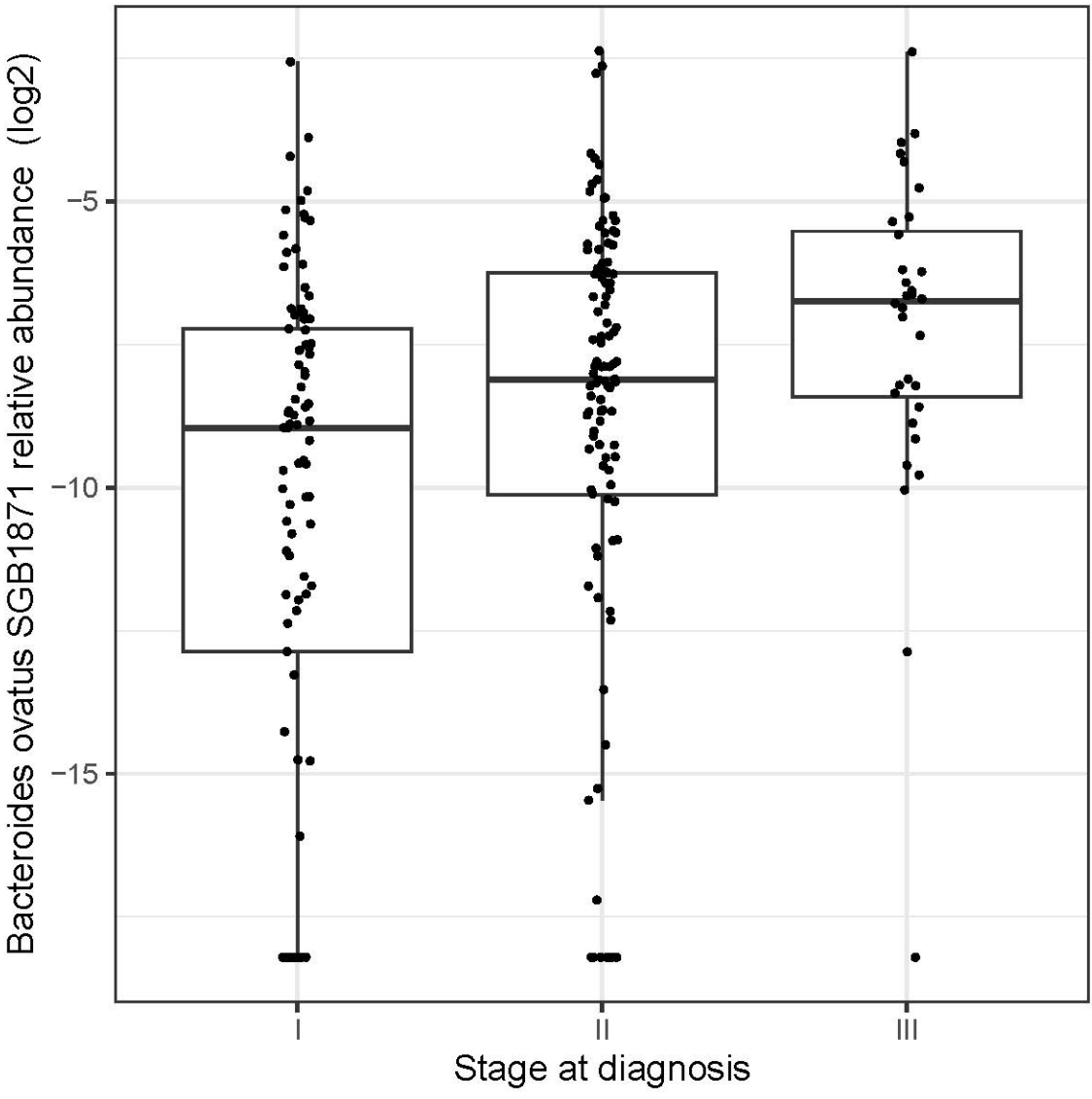
Table 1. Baseline characteristics of invasive breast cancer and DCIS patients with baseline stool microbiome analysis prior to neoadjuvant systemic therapy or surgery

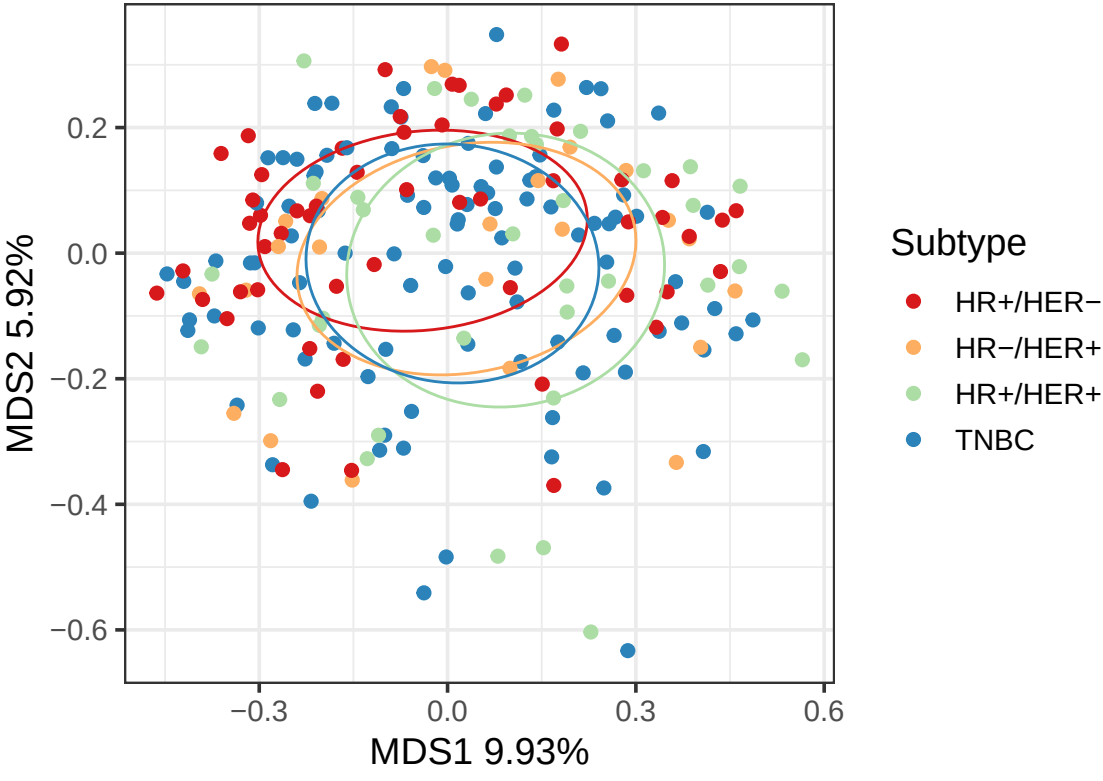
Clinical Characteristics (n=278)		
Age at Initial Diagnosis	**N**	**%**
≥18-<50	82	29.50
≥50	196	70.50
Race		
White	232	83.45
Black	18	6.47
Asian or Pacific Islander	9	3.24
Other	8	2.88
Unknown	11	3.96
Ethnicity	**N**	**%**
Hispanic or Latino	16	5.76
Non-Hispanic	252	90.65
Unknown	10	3.60
Gender	**N**	**%**
Female	278	100.00
Histology	**N**	**%**
DCIS	36	12.95
Invasive	242	87.05
Clinical Stage at Initial Diagnosis	**N**	**%**
Stage 0	48	17.27
Stage I	89	32.01
Stage II	108	38.85
Stage III	32	11.51
Unknown	1	0.36
Tumor Subtype at Initial Diagnosis	**N**	**%**
HR+/HER2 Negative	54	19.42
HR+/HER2 Positive	38	13.67
HR-/HER2 Positive	23	8.27
TNBC	101	36.33
Unknown	62	22.30

Abbreviations: DCIS, ductal carcinoma *in situ*; HR, hormone receptor; HER2, human epidermal growth factor receptor 2; TNBC, triple-negative breast cancer


Histology
Blautia wexlerae SGB4837
Bacteroides uniformis SGB1836
Phocaeicola vulgatus SGB1814
Parabacteroides distasonis SGB1934
Bacteroides ovatus SGB1871
Alistipes putredinis SGB2318
Alistipes onderdonkii SGB2303
Eubacterium rectale SGB4933
Fusicatenibacter saccharivorans SGB4874
Candidatus Cibionibacter quicibialis SGB15286
Faecalibacterium prausnitzii SGB15342
Blautia massiliensis SGB4826
Collinsella aerofaciens SGB14535 group
Bifidobacterium longum SGB17248
Faecalibacterium prausnitzii SGB15332
Ruminococcus bicirculans SGB4262
Phocaeicola dorei SGB1815
Ruminococcus bromii SGB4285
Bacteroides stercoris SGB1830
Prevotella copri clade A SGB1626
Histology
DCIS
Invasive
Sample
log10 Relative Abundance
0 10^−7 10^−6 10^−5 10^−4 10^−3 10^−2 10^−1


0.2
0.0
−0.2
−0.4
−0.6
MDS2 5.92%
−0.3
0.0
0.3
0.6
MDS1 9.93%
Histology
DCIS
Invasive




MDS2 5.92%
MDS1 9.93%
0.2
0.0
−0.2
−0.4
−0.6
−0.3
0.0
0.3
0.6
Subtype
HR+/HER−
HR−/HER+
HR+/HER+
TNBC