# Gut microbiome composition predicts pathologic complete response in patients with early-stage HER2-positive breast cancer receiving neoadjuvant HER2-targeted therapy +/- immunotherapy with pembrolizumab

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## Introduction

- The gut microbiome influences anti-tumor immunity and has been shown to affect immune checkpoint inhibitors (ICI) response across a range of solid tumors.[1]
- The randomized, multicenter, open-label phase II neoHIP trial (NCT03747120) investigated the addition of ICI to neoadjuvant HER2-targeted therapy in patients with early-stage HER2-positive (HER2+) breast cancer and demonstrated encouraging synergistic activity with dual HER2-blockade plus ICI.[2]
- The study included three arms, with the following pathologic complete response (pCR; as defined by ypT0/TisypN0) rates:
  - Arm A paclitaxel (T), trastuzumab (H), and pertuzumab (P); THP: 48.3%
  - Arm B THP-pembrolizumab (K): 67.2%
  - Arm C TH-K: 25%
- The aim of this study was to explore the association between gut microbiome composition and clinical outcomes in patients with early-stage HER2+ breast cancer as part of the phase II neoHIP trial.

## Methods and Materials

- Fecal samples were optionally collected at baseline (cycle 1 day 1), cycle 3 day 1 (prior to treatment), at surgery (+/- 3 days), and at 30 and 60 days (+/- 1 week) after surgery.
- 99 fecal samples (Arm A: n=32; Arm B: n=48; Arm C: n=19) from 27 patients were included in the analysis.
- Samples were analyzed using deep shotgun metagenomics sequencing. Taxonomic profiling was conducted using MetaPhlAn4. Functional profiling was performed using HUMAnN3.
- Pre-operative fecal samples were analyzed according to pCR and onset of diarrhea during the neoadjuvant period.
- Comparisons were adjusted to control for false discovery rates (q-value).

## Results

**Table 1.** Patient and clinical characteristics of patients with fecal samples collected.

| Characteristic | Arm A: THP (n = 9; 32 samples) | Arm B: THP-K (n = 12; 48 samples) | Arm C: TH-K (n = 6; 19 samples) |
|---|---|---|---|
| **Median age, yr (range)** | 52 (36-69) | 58 (42-65) | 52 (32-63) |
| **Race, n (%)** | | | |
| White | 8 (88.9%) | 10 (83.3%) | 5 (83.3%) |
| Asian | 0 | 1 (8.3%) | 1 (16.7%) |
| Black | 1 (11.1%) | 0 | 0 |
| American Indian | 0 | 0 | 0 |
| Other/unknown | 0 | 1 (8.3%) | 0 |
| **Ethnicity, n (%)** | | | |
| Non-Hispanic | 8 (88.9%) | 11 (91.7%) | 5 (83.3%) |
| Hispanic or Latino | 1 (11.1%) | 1 (8.3%) | 1 (16.7%) |
| **Clinical T stage, n (%)** | | | |
| T1 | 1 (11.1%) | 1 (8.3%) | 2 (33.3%) |
| T2 | 7 (77.8%) | 10 (83.3%) | 4 (66.7%) |
| T3 | 1 (11.1%) | 1 (8.3%) | 0 |
| T4 | 0 | 0 | 0 |
| **Nodal status, n (%)** | | | |
| Positive | 7 (77.8%) | 9 (75%) | 4 (66.7%) |
| Negative | 2 (22.2%) | 3 (25%) | 2 (33.3%) |
| **Hormone receptor status, n (%)** | | | |
| Positive | 5 (55.6%) | 7 (58.3%) | 4 (66.7%) |
| Negative | 4 (44.4%) | 5 (41.7%) | 2 (33.3%) |
| **pCR (ypT0/TisypN0), n (%)** | 8 (88.9%) | 9 (75%) | 2 (33.3%) |

**Figure 1.** Beta diversity using Jaccard and Bray Curtis analyses demonstrates that bacterial diversity was significantly different in patients who achieved pCR vs. non-pCR across all timepoints and treatment arms (all p=0.001; q=0.001).

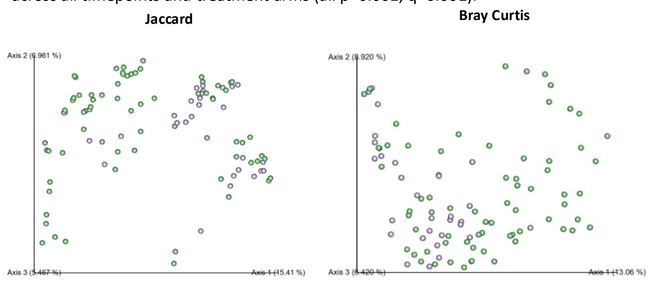



**Figure 2.** ANCOM-BC analysis identified multiple species in differential abundance among patients who achieved pCR versus non-pCR across the 3 arms.

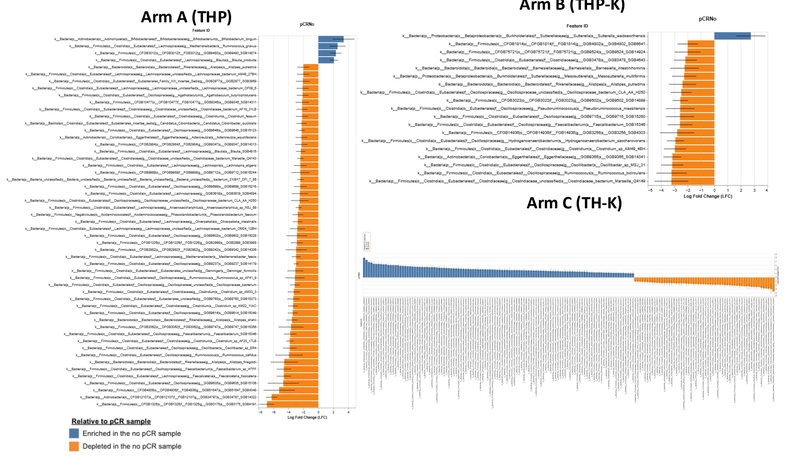



**Figure 3.** ANCOM-BC analysis identified several functional pathways in differential abundance among patients who achieved pCR versus non-pCR across the 3 arms (Arm B pictured).

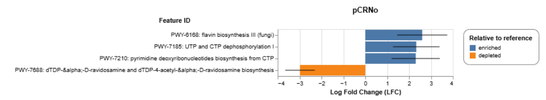
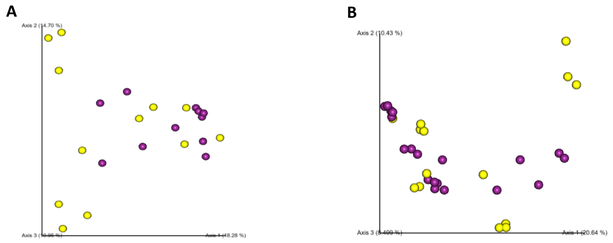



**Figure 4.** In patients with diarrhea vs. without, beta diversity was significantly different in the A) THP arm by Bray Curtis analysis and B) THP-K arm by Jaccard analysis (all p<0.05; q<0.05)




## Discussion

- Gut microbiome composition and bacterial diversity were significantly different according to pCR status across the 3 treatment arms, with multiple species found to have differential abundances between groups.
- In patients who achieved pCR in the THP-K and TH-K arms, notable bacterial genera that were in greater abundance include Faecalibacterium, Bifidobacterium, Ruminococcus, and Oscillibacter. These genera have previously been associated with ICI efficacy.[1]
  - These bacteria produce short chain fatty acid (SCFA), which maintain gut barrier integrity and promote CD8+ T cell differentiation.[3]
- In patients who achieved pCR in the THP-K arm, the dTDP-α-D-ravidosamine biosynthesis pathway was enriched. This pathway produces glycosylated metabolites that may modify immune interactions.[4, 5]
- In the THP and THP-K arms, patients who developed treatment-related diarrhea had significantly different gut microbiome diversity compared to those who did not develop diarrhea, which suggests that diversity of the gut microbiome influences gastrointestinal toxicity.

## Conclusions

- This is the first study to demonstrate that the pre-operative gut microbiome influences response to ICI in patients with early-stage HER2+ breast cancer.
- Patients who achieved pCR had significantly different microbiome profiles compared to those with residual disease, with greater abundance of several bacterial species previously associated with ICI efficacy.
- This study highlights the role of the gut microbiome in influencing response to ICI in patients with breast cancer treated in the curative intent setting.

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