Gut Microbiota Signatures are Associated with Response in Triple Negative Breast Cancer Patients Treated with Standard Neoadjuvant Chemotherapy

Ariel A. Aptekmann[1], Leslie L. Montgomery[2], Iriana Colorado[1], Kira Goldgirsh[1], Enrique Corapi[1], Sara Hyman[2], Myesha Brito[2], Colleen McGuire[3], Ayaad Akand[3], Rajni L. Mehta[4], Rocco Carbone[4], Michelle Zupa[4], Marson Davidson[2], Claudine Isaacs[3], Candace B. Mainor[3], Deena Graham[5], Michael J. Goldfischer[2], Lajos Pusztai[4], Rena Feinman[1]

INTRODUCTION

The Challenge: Triple-Negative Breast Cancer (TNBC) is a heterogeneous and aggressive disease. A key challenge is to identify tumor-extrinsic biomarkers that can accurately predict refractory disease at an early stage.

The Question: Does the composition of patient's gut microbiota influence their response to standard NAC treatment?

Hypothesis: An unbalanced gut microbiome (dysbiosis) in TNBC patients is associated with a poor response to standard neoadjuvant chemotherapy.(NAC)

METHODS

1. Prospective Observational Study

Cohort: 49 newly diagnosed TNBC patients receiving standard neoadjuvant chemotherapy (NAC).

Data Collection: Stool samples were collected before, during and after NAC.

Goal: Identify differences in gut microbiota profiles between Responders (residual cancer burden-0, (RCB-0) and Non-Responders (RCB-I/II).

2. **Causality Study (E0771 TNBC Mouse Model)**

Fecal Microbiota Transplantation: Immunocompetent E0771 TNBC mice were treated with broad-spectrum antibiotics (Abx) for 7 days. Bacterial ablation was confirmed and immediately after Abx withdrawal, fecal homogenates from patients were gavaged into mice for 3 consecutive days. On the 3rd day of FMT, mice were injected with E0771 cells into the 4th mammary fat pad. FMT was repeated weekly.

Goal: Determine whether pre-NAC gut microbiota composition from RCB-0 and RCB-II patients is the cause or consequence of TNBC progression.

Table 1. Patient Characteristics

Age	(Yr)
Median	52
Youngest	27
Oldest	76
Pre-Tumor	
Stage	**N**
T1	9
T2	29
T3	9
T4	2
Race	**N**
White	29
Black	10
Other	11
Pre-Nodal	
Stage	**N**
N0	36
N1	11
N2	0
N3	2
Ethnicity	**N**
HL	11
NHL	38
NAC Regimen	**N**
Pembro	22
AC-T	14
AC-TC	5
Others	3

RESULTS

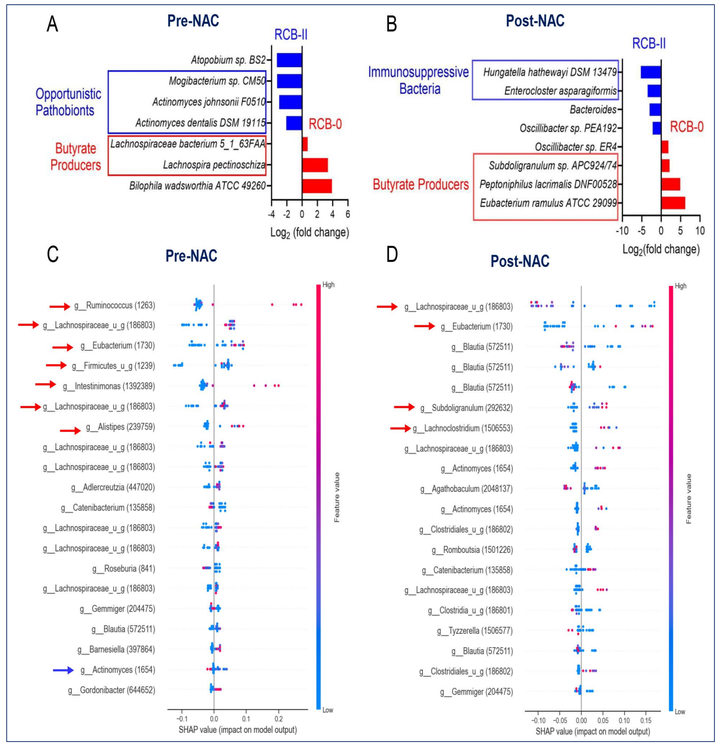


Figure 1. Gut Microbiota Signatures Predict Chemotherapy Response

Gut bacterial profiles differ significantly between patients who responded well (Responders, RCB-0) and those who did not (Non-Responders, RCB-II) before and after NAC.

Responders had more beneficial butyrate-producing bacteria, while Non-Responders were enriched with opportunistic pathobionts.

A and B, Bar plots show fold-changes between groups (Mann-Whitney adjusted p< 0.05). **C and D,** The importance ranking of each genus predicted by Random Forest Classifier and Shapley Additive exPlanations (SHAP), a post-hoc explainability algorithm analysis. Each dots X axis represents the SHAP value of a taxa for a patient and its color the relative abundance ranges from high (pink) to low (blue).Higher SHAP values suggests that the feature has a larger impact on the model predictions and illustrate the importance of these specific genera in predicting outcome.

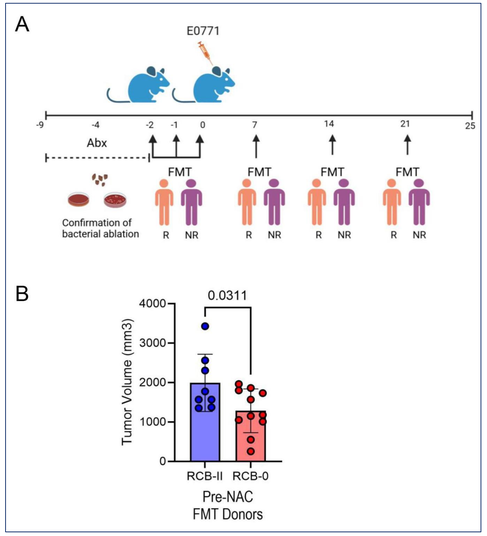


Figure 2. Non-Responder Microbiota Drives Tumor Growth in E0771 Mice.

A, The FMT study. **B,** FMT from human Non-Responders (RCB-II) caused significantly higher tumor volumes in the E0771 mouse model compared to FMT from Responders (RCB-0) (p=0.0311) Unpaired, Two-Tailed Lognormal Welch's t-test.

CONCLUSIONS

Predictive Biomarker: The gut microbiota profile at diagnosis is a strong independent predictor of response to TNBC neoadjuvant chemotherapy.

Causal Role: The gut microbiome actively influences TNBC progression and chemoresistance.

Therapeutic Target: These findings identify the gut microbiome as a promising new target for therapeutic intervention to improve TNBC patient outcomes.

This presentation is the intellectual property of the author/presenter. Contact them at Rena.Feinman@HMH-CDI.org for permission to reprint and/or distribute.

AFFILIATIONS

1. Center for Discovery and Innovation, Hackensack Meridian Health, Nutley NJ
2. Hackensack University Medical Center, Hackensack Meridian Health, Hackensack, NJ.
3. Georgetown Lombardi Comprehensive Cancer Center, Washington, DC.
4. Yale Cancer Center, Yale University School of Medicine, New Haven, CT.
5. John Theurer Cancer Center, Hackensack Meridian Health, Hackensack, NJ.

ACKNOWLEDGEMENTS

- Thank you to the patients and their families for participation in this sampling study
- Funding provided by BCRF and The Cannata Report/-HMH Foundation