MICROBIOTA DISRUPTION WITH ORAL ANTIBIOTICS HAS A DETRIMENTAL EFFECT ON HER2-POSITIVE BREAST CANCER THERAPY EFFICACY

Romina E. Araya[1,*], Lily J. Parker[1], Payal Mitra[1], Nofar Erlichman[1], Pavani Chalasani[2], Rong Li[1]

[1] Department of Biochemistry and Molecular Medicine, School of Medicine and Health Sciences, George Washington University.
[2] Hematology-Oncology Division, George Washington Cancer Center.
*contact: **romina.araya@gwu.edu**

THE GEORGE WASHINGTON UNIVERSITY
WASHINGTON, DC

PS3-12-02
SAN ANTONIO BREAST CANCER SYMPOSIUM®
UT Health San Antonio Mays Cancer Center | AACR American Association for Cancer Research

Copies of this poster obtained through Quick Response (QR) Code are for personal use only and may notbe reproduced without permission from SABCS® and the author

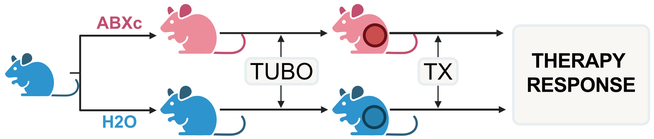
BACKGROUND

Cancer patients are particularly susceptible to infections. Approximately 20-60% of patients may receive antibiotics at some point during their therapy, including perioperative care and periods of neutropenia. Retrospective studies suggest that antibiotics negatively impact outcomes in various cancers. A pilot study focused on breast cancer (BC) indicated that HER2+ BC patients experience greater reductions in survival following antibiotic exposure than HER2-negative cases. While this effect is thought to arise from disruptions in the host microbiota, the underlying mechanisms remain poorly defined.

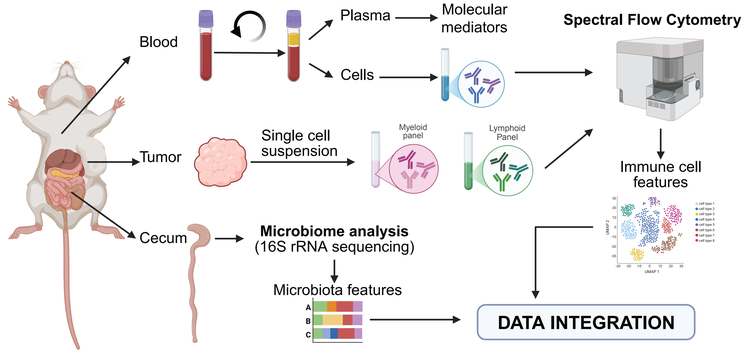
We have previously demonstrated that microbiota signaling modulates innate immune cells in different tumor types, with consequences for the efficacy of chemotherapy and immunotherapy. However, HER2+ BC patients receive targeted therapies such as anti-HER2 antibodies with small-molecule tyrosine kinase inhibitors in case of therapy resistance. Therefore, the impact of microbiota specifically on HER2 therapies requires further study.

EXPERIMENTAL DESIGN AND METHODOLOGY

Analysis of antibiotic effect on therapy outcome



Analysis of antibiotic and therapy impact on microbiota and immune system



RESULTS

1. Oral antibiotics have a detrimental effect on targeted therapies against HER2+ breast cancer

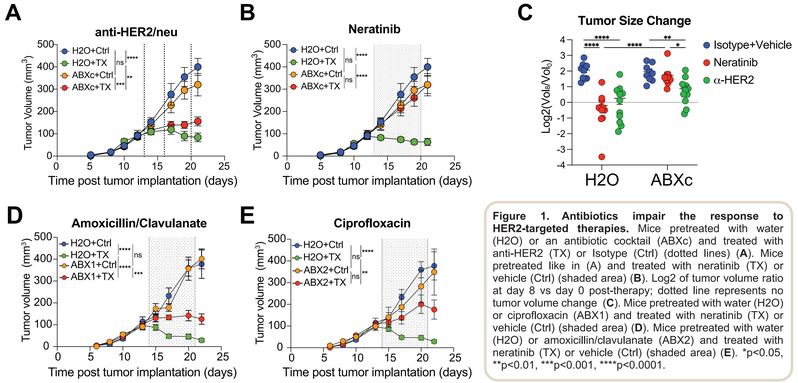


Figure 1. Antibiotics impair the response to HER2-targeted therapies. Mice pretreated with water (H2O) or an antibiotic cocktail (ABXc) and treated with anti-HER2 (TX) or Isotype (Ctrl) (dotted lines) (**A**). Mice pretreated like in (A) and treated with neratinib (TX) or vehicle (Ctrl) (shaded area) (**B**). Log2 of tumor volume ratio at day 8 vs day 0 post-therapy; dotted line represents no tumor volume change (**C**). Mice pretreated with water (H2O) or ciprofloxacin (ABX1) and treated with neratinib (TX) or vehicle (Ctrl) (shaded area) (**D**). Mice pretreated with water (H2O) or amoxicillin/clavulanate (ABX2) and treated with neratinib (TX) or vehicle (Ctrl) (shaded area) (**E**). *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

2. HER2 targeted therapies and antibiotics change the microbiota composition, but antibiotics reduce microbial diversity

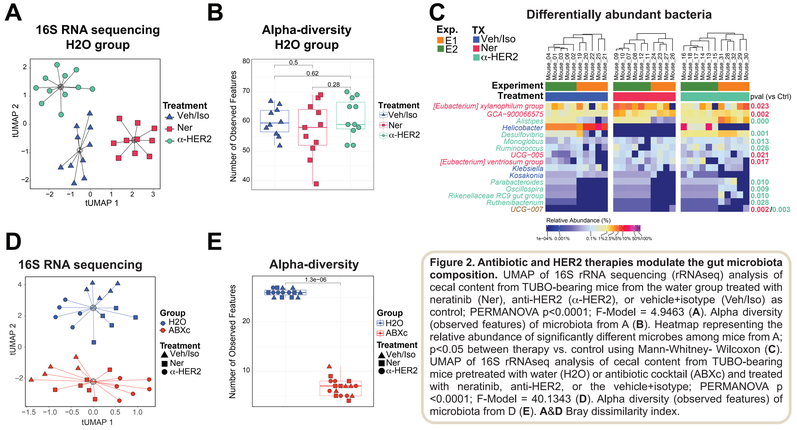


Figure 2. Antibiotic and HER2 therapies modulate the gut microbiota composition. UMAP of 16S rRNA sequencing (rRNAseq) analysis of cecal content from TUBO-bearing mice from the water group treated with neratinib (Ner), anti-HER2 (α-HER2), or vehicle+isotype (Veh/Iso) as control; PERMANOVA p<0.0001; F-Model = 4.9463 (**A**). Alpha diversity (observed features) of microbiota from A (**B**). Heatmap representing the relative abundance of significantly different microbes among mice from A; p<0.05 between therapy vs. control using Mann-Whitney- Wilcoxon (**C**). UMAP of 16S rRNAseq analysis of cecal content from TUBO-bearing mice pretreated with water (H2O) or antibiotic cocktail (ABXc) and treated with neratinib, anti-HER2, or the vehicle+isotype; PERMANOVA p <0.0001; F-Model = 40.1343 (**D**). Alpha diversity (observed features) of microbiota from D (**E**). **A&D** Bray dissimilarity index.

3. Microbiota disruption with oral antibiotics halts the tumor immune microenviroment remodeling induced by HER2 targeted therapies

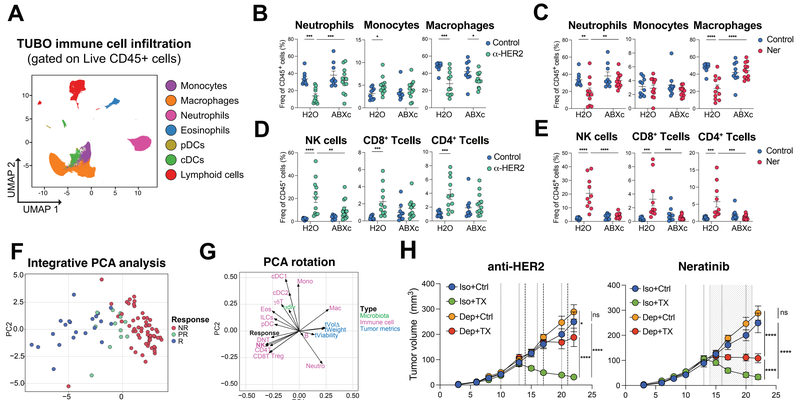
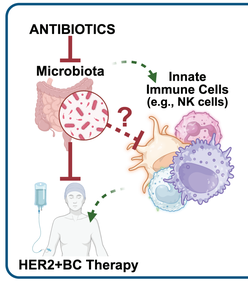


Figure 3. Oral antibiotics impair the tumor immune microenvironment in response to HER2 therapies. UMAP representing TUBO tumor-infiltrating immune cells from mice pretreated with oral antibiotic cocktail (ABXc) or regular drinking water (H2O) and treated with vehicle+isotype (Control), neratinib (Ner), or anti-HER2 (α-HER2) (**A**). Frequency of myeloid populations on total leukocytes in anti-HER2 vs control mice (**B**). Frequency of myeloid populations on total leukocytes in neratinib vs control mice (**C**). Frequency of lymphoid populations on total leukocytes in anti-HER2 vs control mice (**D**). Frequency of lymphoid populations on total leukocytes in neratinib vs control mice (**E**). PCA analysis integrating immune cell populations, microbiome, and tumor features; response was defined as tumor volume ratio<1 is responder (R), tumor volume ratio>1 & <1.5 is partial responder (PR), and tumor volume ratio>1.5 is non-responder (NR) (**F**). PCA rotation parameter from F (**G**). NK cell depletion post-tumor inoculation with anti-Asialo-GM1 (Dep) or IgG2 isotype control (Iso) for mice receiving anti-HER2 (TX, left panel) or neratinib (TX, right panel) or vehicle+isotype (Ctrl) (**H**). *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

CONCLUSIONS AND FUTURE PERSPECTIVES



Our results demonstrate that antibiotic-induced disruption of the gut microbiota in preclinical models **recapitulates their detrimental effect on HER2+ breast cancer therapies** observed in the clinic.

Tumor **NK cell** recruitment is increased in response to both HER2-targeted therapies and **needed for complete efficacy.**

Antibiotics impede beneficial immune cell recruitment, including NK cells, and **sustain pro-tumor innate immune cells**.

Further studies will address the specific microbiota and innate immune pathways impacted by antibiotics to find **strategies to mitigate their detrimental effects.**