ESMO Research Research Fellowship

(January 2021 – December 2022)

Andri Papakonstantinou

FINAL REPORT

Host Institute: **Vall D'Hebron Institute of Oncology (VHIO), Barcelona, Spain**
Mentor: **Mafalda Oliveira**

Project title: **Impact of microbiota on breast cancer prognosis and treatment efficacy**

Home Institute: **Karolinska Institute, Stockholm, Sweden**

Introduction

The human body hosts a wide range of damaging and beneficial bacteria in balance, described as symbiosis or eubiosis. Sometimes this equilibrium is disrupted, leading to dysbiosis, which can have detrimental consequences on the health of the individual, leading to various pathological conditions, including neoplasia [1], [2]. Polymorphic microbiomes in the gut have been recently recognized as emerging mechanisms that enable cancer hallmark capabilities and there is an increasing interest on the significance of intratumoral microbiomes in cancer pathogenesis and natural history [3].

The breast has traditionally been considered a sterile tissue. However, the evolution of deep-sequencing technologies has allowed to identify and characterize the microbial composition of both normal and malignant breast tissue [2, 4-6]. The breast microbiome originates mainly from bacterial migration from the skin through the areola, although more distant migration from the gut may also occur [7]. It has also been suggested that gut bacteria may be transferred to the breast through an entero-mammary pathway, characterized by translocation of intestinal bacterial to secondary lymph nodes by immune cells, and thereafter transition to the breast through the blood or lymphatic circulation [8,9].

To describe the microbiome, three characteristics are usually used: (1) Alpha (α) diversity, which refers to the diversity within a community of microorganisms; (2) Beta (β) diversity, which refers to differences between communities; and (3) Taxonomic composition (measured in Operational Taxonomic Units, OTUs), which reports on the abundance (absolute or relative) of specific community members.

Other α-diversity parameters that are used to describe the microbiome diversity within a community are richness (number of species in a niche) and evenness (size of each of the species within a niche). The taxonomic composition based on OTUs, characterizes the microorganisms at various taxonomic levels down to species or strain level, depending on the sequencing method applied. The 16S rRNA gene is commonly used to identify the microbiome within a habitat, as it has two unique characteristics: on one hand, it has a well conserved region with a slow rate of evolution; on the other hand, it has nine regions that vary between microorganisms and usually-two or three of these regions (V1-V3 or V3-V5) are used for their distinction [10]. High-throughput 16S sequencing and metagenomic sequencing are the most commonly used methods for microbiome sequencing.

Despite methodological heterogeneity and challenges in the interpretation of the limited data available, Prevotella and Micrococcus species in normal breast tissue, and Lactobacillus and Fusobacterium have been described in normal and breast tumors respectively [2, 5, 6, 11]. Moreover, it is suggested that the different breast cancer subtypes are characterised by distinct microbiome composition, although the data is scarce for robust conclusions.

A growing amount of data suggest that a strong association exists between gut microbiome composition, tumor microenvironment and the host immune response. This interplay may impact on efficacy and toxicity of immune checkpoint inhibitors (ICI), ultimately impacting patients' survival [12-16]. However, there is currently no available data for breast cancer but given the emerging indications of ICI in breast cancer, the field is highly relevant.

In conclusion, there is an imperative need for the detection of novel biomarkers in breast cancer and predictors of therapy benefit and/or toxicity. The microbiome directly influences drug metabolism and can promote or compromise efficacy and toxicity while conversely cancer therapies directly affect microbiota leading to a spiral that can be either vicious or beneficial. The field of microbiome in breast cancer is relatively novel and data has just begun to emerge. It is a field that undeniably deserves further review for the identification of potential preventive, diagnostic, therapeutic or predictive strategies.

Rationale and Aim

Rationale

The tripartite relationship of Host – Gut/Breast Microbiome – Breast cancer is an important, yet largely unknown, aspect in breast cancer. The significance of microbiota in breast cancer prognosis and the influence of host microbial traits in treatment response is not fully understood. Deeper understanding and characterization of the interplay between Host/Breast cancer/Microbes (both in the normal and neoplastic breast and the gut) and its impact in diagnosis, prognosis, and response to treatment can fill a critical knowledge gap and improve breast cancer management and survival. Based on available data from other tumours, we posit that gut and breast microbiota composition could affect various aspects of breast cancer natural history and response to treatment.

Aim

The proposed research project had two major and an exploratory aim:

a. To investigate the characteristics and properties of breast microbiota and potential prognostic significance in breast cancer outcomes.

b. To investigate whether microbe enrichment (or lack thereof) can interact with response to therapy

c. To investigate the correlation of the relative abundance of breast microbiota with genomic alterations in breast cancer (exploratory aim).

Experimental design

A multi-step workflow including both retrospective and prospective data collection from different cohorts is planned, aiming to characterize the microbiome landscape in breast cancer, its prognostic significance, and its potential predictive value in patients treated with immune checkpoint inhibitors (ICI).

A. To investigate the characteristics and properties of breast microbiota and potential prognostic significance in breast cancer outcomes (retrospective study).
Given the worse prognosis in patients with triple negative breast cancer (TNBC) and the increasing indications for treatment with ICI we decided to focus on this subgroup. A marker-based approach using the 16S ribosomal RNA subunit gene (rRNA16S) was used (sequencing the V3-V4 variable regions to study bacterial diversity and to describe and quantify microbial alpha and beta diversity and study taxonomic profiles from phylum to species levels. The Illumina Miseq sequencing 300×2 approach was used and both positive and negative controls were used for quality control. The microbiome composition of patients with TNBC and a subgroup analysis per stage is performed. Bioinformatics and statistical analysis are on-going. The planned analysis also includes correlation of microbiome composition with probability of pathological complete response (pCR) and Residual Cancer Burden (RCB) as well as survival outcomes such as event-free survival and overall survival. In addition, microbiome composition in the tissue samples will be correlated to tumour infiltrating cells (TILs) and PD-L1 expression.

B. To investigate whether microbe enrichment (or lack thereof) can interact with response to immunotherapy (prospective study); the BRIOME study

This project is IRB approved (PR(AG)165/2021) and internal circuits have been established and implemented. Women eligible for treatment with an ICI and meet the following criteria are enrolled: i. Patients with TNBC with or without ICI treatment ii. No contraindication to undergo tumour biopsies iv. ICI may be received either as single agent or in combination with chemotherapy or other agents.
We collect saliva, stools, tumour (optional) and blood samples prior to ICI therapy, after the first cycle and at the end of treatment with ICI (patients with early breast cancer) or at progression (patients with metastatic disease). Questionnaires on oral hygiene, lifestyle and diet be collected at baseline and at the end of treatment. A 16S rRNA analysis of the microbiota is planned unless other method appears to be more appropriate. Predefined bioinformatics and statistical analyses will be employed for the analysis of the results and correlation to the predefined outcomes. Enrolment is actively ongoing.

C. To investigate the correlation of the relative abundance of breast microbiota with genomic alterations in primary breast cancer (exploratory study).
Given the novelty of the breast cancer microbiota field, little is known on the correlation between microbial composition and genetic alterations in the tumour. Therefore, we plan an exploratory analysis of 10% of the total sample size in the retrospective study to assess this issue. A panel with 450 cancer-related genes (VHIO-300) will be utilised to perform targeted gene sequencing of the primary tumours. DNA from fresh frozen primary tumours of the selected patients using QIAmp DNA Mini kit (Qiagen Inc., USA) will be extracted and sequenced using a VHIO-300 panel for targeted sequencing. The relative abundance of microbiome will be correlated with the results from targeted gene sequencing.

Results, Conclusions and Future Perspectives

A. Preliminary results from the retrospective study

Two different biobanks (one with surgery specimens and one with diagnostic biopsies) with more than 2000 samples have been investigated. Patients with primary TNBC and no previous history of malignancy were selected. We identified 115 TNBC patient samples: i) n=87 diagnostic biopsies, of which n=48 had thereafter received neoadjuvant therapy (NACT) and n=39 were operated up-front and ii) 28 unpaired surgery samples of which n=14 were from patients that had previously undergone NACT and n=14 from patients operated upfront. The latter, to control for different type of samples (diagnostic biopsy vs surgery). According to preliminary results, the different types of sampling (surgery vs biopsy) can impact the microbiome composition observed, a novel and significant observation for future studies and data interpretation. Further analysis of microbiome composition and its correlation with probability of pathological complete response (pCR), as well as survival outcomes is ongoing. An abstract is being prepared to be submitted to ESMO Breast 2023 and thereafter, a manuscript will be prepared.

B. Status of the Prospective Study (BRIOME)

During the first year of the Fellowship the study protocol and relevant operation procedures were prepared, as well as ethics approval. The study began enrolment in February 2022 and until December 2022, n=18 patients with TNBC were included (n=16 with NACT and ICI). The study was awarded a grant from the Spanish Society for Medical Oncology (SEOM), the SEOM-FECMA/Maset Grant, that will allow for the sample analysis for the first 10 patients, with the exploratory aim to correlated tumor molecular characteristics and microbiome composition in TNBC.

The fellow and the mentor continue collaboration on the projects and a trans-institutional agreement between the host institution (VHIO) and home institution (KI) is in process, for the continuous collaboration on the work initiated during the fellowship and strengthening the collaboration and exchange between the two institutions.

***List of Publications and Presentations Resulting from the Translational Research Project "*Impact of microbiota on breast cancer prognosis and treatment efficacy"**

Publications:

Papakonstantinou A, Nuciforo P, Borrell M, Zamora E, Pimentel I, Saura C, Oliveira M. *The conundrum of breast cancer and microbiome - A comprehensive review of the current evidence.* Cancer treatment reviews 2022 111; 102470-

Presentations

1. May 2022, Presentation at the clinic educational sessions titled: **"Breast Cancer and Microbiome"**

2. May 2022, Presentation at a Preceptorship in Triple Negative Breast Cancer, organized by VHIO. Lecture title: **"TILs and tumor associated microbiome"**

3. October 2022, Presentation at a Preceptorship in Breast Cancer, organized by VHIO. Lecture title: **"ctDNA & TILs as prognostic and predictive biomarkers of response to neoadjuvant therapy"**

List of Publications and Presentations resulting from other projects during the fellowship period (if applicable)

Publications:

1. **Papakonstantinou A,** Gonzalez NS, Pimentel I, Suñol A, Zamora E, Ortiz C, Espinosa-Bravo M, Peg V, Vivancos A, Saura C, Villacampa G, Oliveira M. *Prognostic value of ctDNA detection in patients with early breast cancer undergoing neoadjuvant therapy: A systematic review and meta-analysis.* Cancer treatment reviews 2022 104; 102362-

2. Garcia-Alvarez A, **Papakonstantinou A,** Oliveira M. *Brain Metastases in HER2-Positive Breast Cancer: Current and Novel Treatment Strategies*. CANCERS 2021 13;12

Two Poster Presentations at ASCO 2022:

1. **Papakonstantinou A***, Gonzalez-Medina A*, Matito J*, Ligero M, Ruiz-Pace F, Suñol A, Rivero J, Fasani R, Cruellas M, Peg V, Borrell M, Pimentel I, Escriva De Romani Munoz S, Balmana Gelpi J, Nuciforo P, Dienstmann R, Saura C, Perez-Lopez R, Oliveira M*, Vivancos A*. *Shedding of ctDNA, radiomics assessment of tumor disease volume (TDV), and concordance of mutations (mut) in synchronous liquid and tumor biopsies in metastatic breast cancer (MBC)*. J Clin Oncol 40, 2022 (suppl 16; abstr 1086).

   The poster was awared with the GRASP Advocate Choice Award and was also presented and discussed at a special GRASP event.

   Also, the findings of the poster were presented at an educational session of Pfizer: https://www.pfizerplay.se/video/onkologi/andri_papakonstantinou_om_j%c3%a4mf%c3%b6relse_av_mutation_i_flytande_och_v%c3%a4vnadsbiopsier

2. Gonzalez-Medina A*, **Papakonstantinou A***, Matito J*, Ruiz-Pace F, Bellet M, Suñol A, Arumí M, Zamora E, Ortiz C, Sanz L, Gómez Pardo P, Gómez-Rey M, Fasani R, Morales C, Peg V, Nuciforo P, Dienstmann R, Saura C, Vivancos A*, Oliveira M*. *Utility of liquid biopsy for identifying emerging mutations (mut) and novel treatment options in luminal metastatic breast cancer (LMBC)*. J Clin Oncol 40, 2022 (suppl 16; abstr 1061).

Other:

1. Article, in Swedish, reporting from ESMO Breast 2022, published in the journal of the Swedish Society for Medical Oncology: https://etidning.onkologi.org/shared/article/esmo-breast-2022-starkt-program-for-unga-onkologer/PIAKGK0Q

2. Interview, in Swedish, for Scandinavian oncology journal on the publication of the ctDNA meta-analysis: https://onkologisktidskrift.se/sjukdomar/10-brostcancer/364-preoperativt-ctdna-indikerar-okad-risk-for-aterfall-vid-brostcancer.html

Selection of Courses and Workshops Attended During the Fellowship

1. ASCO-SEOM Leadership Seminar for Oncologists, March 2022, Madrid, Spain
2. SOLTI ADCsessions in oncology 2022, March 2022, Madrid, Spain
3. Excel course organized by VHIO
4. Course of teaching in higher education, distance course, autumn 2022
5. The fellow also attended the ESMO Leaders Generation Programme Class 2021-2022 that coincided with the Fellowship Period.
6. Attended the virtual meetings of ESMO Breast 2021, ASCO 2021 and ESMO 2021 and in person ESMO Breast 2022, ASCO 2022 and ESMO 2022.

Acknowledgements

I would like to acknowledge Dr Josep Tabernero director of the Vall D´Hebron Institute of Oncology (VHIO), Dr. Cristina Saura, Head of the Breast Cancer Unit of VHIO, Dr Paolo Nuciforo and the group of Molecular Oncology at VHIO for their support in the projects, Anna Suñol, research nurse at the Breast Cancer Unit, Neus Bayo and Berta Colldeforns from the Project Management Group, Guillermo Villacampa and Laia Joval from the Oncology Data Science Group and all the colleagues of the breast cancer unit.

Personal Statement (not mandatory)

Beginning an ESMO Translational Research Fellowship in the middle of a pandemic was a challenge but it has rewarded me with a lifetime experience. The Fellowship provided me the opportunity to further improve my research skills, to find a new niche and to advance my career as a physician scientist. Visiting a centre of excellence, as Vall D´Hebron Institute of Oncology, gave me lessons and experiences that I will cherish and that I share with my home institution. I made friends for life and created strong research collaborations for the future. I wholeheartedly recommend the ESMO Fellowship Programme to all young oncologists around the globe.

References

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SIGNATURES

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Andri Papakonstantinou	10-01-2023

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