ESMO Translation Research Fellowship  
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“Circulating tumor DNA in metastatic breast cancer”

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Final Report

Host Institute: Institution Vall d’Hebron Institute of Oncology, Vall d’Hebron University Hospital  
Mentors: Josep Tabernero/Joan Seoane  
Project title: “Circulating tumor DNA in metastatic breast cancer”  
Home Institute: Santa Casa de Misericórdia de Belo Horizonte, Faculdade de Ciências Médicas de Minas Gerais, Belo Horizonte, MG, Brazil

Rationale and Aim

Introduction
Traditionally, the study of many cancer biomarkers, including mutation status, has been based in archival tumor-tissue. The study of such biomarkers in the original tumor tissue biopsy depends on the availability and quality of the specimen and may not be representative of the disease due to intra-tumor heterogeneity [1, 2].

The development of novel techniques for the evaluation of blood-borne biomarkers (i.e. liquid biopsies) in cancer such as circulating tumor cells (CTCs) and circulating cell-free tumor DNA (ctDNA) have changed the translational arena. Circulating biomarkers might constitute representative readouts of both primary tumour and metastatic deposits, and provide ways to expedite the discovery and validation of clinically useful predictive biomarkers [3]. In fact, blood represents a potential source of circulating tumor material, which may complement or replace the available tissue and has shown to be less invasive and repeatable. Circulating blood biomarkers hold promise to be non-invasive real-time surrogates for tumor tissue-based biomarkers.

Circulating tumor DNA & massively parallel sequencing
ctDNA in plasma or serum has been widely investigated as potential non-invasive surrogates for tumor tissue biopsies [4-6]. The detection, quantification, and molecular characterization of plasma ctDNA have introduced new means for investigating the metastatic process and the mechanisms of therapeutic resistance, and for monitoring the emergence of treatment-resistant clones [3].

Massively parallel sequencing strategies of ctDNA seem to be feasible [5, 6]. Toward this end, more accurate information may be derived from the analyses of genomic alterations derived from ctDNA. Screening ctDNA for driver cancer genomic alterations may be more comprehensive and informative than single biopsies, because spatially geographic distinct clones derived from the same patient seem to be all found mixed
together in blood. Consequently, massively parallel sequencing of ctDNA may open the way to better understand the pathways that drive cancer metastasis and to personalize cancer therapy.

**1st part**

Initially, we were working with the Sequenom Mass Array (OncoCarta Panel platform version 1.0), which utilizes pre-designed and pre-validated mass spectrometric single nucleotide polymorphism genotyping technology for the parallel analysis of 238 simple and complex mutations across 19 common oncogenes. Our aim was:

- To assess the feasibility of multiplex mutational detection with the OncoCarta Panel platform in plasma ctDNA and to match the somatic mutation profiling of both plasma ctDNA and patients’ tumor tissue biopsies.
- To investigate if the measurement of plasma ctDNA quantitative and qualitative alterations have prognostic value in breast cancer metastatic patients.

**Design**

Observational study in which metastatic breast cancer patients with known specific mutations in archival tumor tissue biopsies were screened prospectively in 3 time points at the start of a new therapy, either standard or experimental for plasma ctDNA alterations (quantification and somatic mutation profiling) (Figure 1).

**Figure 1.** Timeline of ctDNA collection and therapy administration

Results

In the training set, we analyzed samples from metastatic breast, colon and lung patients. We detected through the Sequenom Mass Array (OncoCarta Panel platform version 1.0) in plasma and matched mutation profiling of both plasma ctDNA and tumor tissue biopsies (Table 1).

<table>
<thead>
<tr>
<th>Tissue mutation</th>
<th>Sample type</th>
<th>Mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td>PIK3CA H1047R</td>
<td>Plasma</td>
<td>No mutation detected</td>
</tr>
<tr>
<td>PIK3CA E545K</td>
<td>Plasma</td>
<td>No mutation detected</td>
</tr>
<tr>
<td>PIK3CA E542K</td>
<td>Plasma</td>
<td>PIK3CA E542K</td>
</tr>
<tr>
<td>KRAS</td>
<td>Plasma</td>
<td>No mutation detected</td>
</tr>
<tr>
<td>KRAS G12V</td>
<td>Plasma</td>
<td>KRAS G12V</td>
</tr>
<tr>
<td>KRAS G13D</td>
<td>Plasma</td>
<td>KRAS G13D</td>
</tr>
<tr>
<td>EGFR</td>
<td>Plasma</td>
<td>No mutation detected</td>
</tr>
<tr>
<td>BRAFK601E</td>
<td>Plasma</td>
<td>BRAF K601E</td>
</tr>
<tr>
<td>KRAS Q61L</td>
<td>Plasma</td>
<td>KRAS Q61L</td>
</tr>
<tr>
<td>KRAS Q61L</td>
<td>Plasma</td>
<td>KRAS Q61L</td>
</tr>
</tbody>
</table>

In the prospective setting, we collected blood from metastatic breast cancer patients at 3 time points and...
alterations of the phosphatidylinositol-3-kinase (PI3K) pathway in metastatic breast cancer.

Plasma ctDNA results and their corresponding mutation in tumor-tissue, and the treatments were receiving at the time of blood draw (Presented in part at the European Society for Medical ESMO, Vienna, Austria, September 2012).

Indeed, we and others [5, 6] have seen that it is possible to monitor the course of therapy in metastatic through the assessment of the somatic mutation detected in plasma ctDNA (Figure 2). The frequency of mutant alleles detected in selected patients are in accordance with radiologic and biochemical (e.g. CA15.3 for breast cancer) and seem more sensitive indicators of disease status.

Table 2 shows plasma ctDNA results and their corresponding mutation in tumor-tissue, and the treatments that patients were receiving at the time of blood draw (Presented in part at the European Society for Medical Oncology (ESMO), Vienna, Austria, September 2012).

Indeed, [5, 6] have seen that it is possible to monitor the course of therapy in metastatic through the assessment of the somatic mutation detected in plasma ctDNA (Figure 2). The frequency of mutant alleles detected in selected patients are in accordance with radiologic and biochemical (e.g. CA15.3 for breast cancer) and seem more sensitive indicators of disease status.

Table 2. Plasma ctDNA results for metastatic breast cancer patients with PI3K-pathway alterations in tumor-

<table>
<thead>
<tr>
<th>Tissue PI3K-pathway alteration</th>
<th>Line of therapy</th>
<th>Administered Therapy</th>
<th>Best response</th>
<th>1st plasma sample (before starting a new line of therapy)</th>
<th>2nd plasma sample (after 1 month)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PI3K H1047R</td>
<td>6</td>
<td>AKT inhibitor</td>
<td>SD</td>
<td>H1047R</td>
<td>WT</td>
</tr>
<tr>
<td>PI3K E545K</td>
<td>4</td>
<td>PI3K alpha inhibitor</td>
<td>SD</td>
<td>N/A</td>
<td>WT</td>
</tr>
<tr>
<td>PI3K E542K</td>
<td>6</td>
<td>Etoposide</td>
<td>early PD</td>
<td>E542K</td>
<td>N/A</td>
</tr>
<tr>
<td>PI3K H1047R</td>
<td>4</td>
<td>Vinorelbine + trastuzumab</td>
<td>SD</td>
<td>H1047R</td>
<td>WT</td>
</tr>
<tr>
<td>PI3K E545K</td>
<td>2</td>
<td>Capecitabine +/- sorafenib</td>
<td>SD</td>
<td>E545K</td>
<td>WT</td>
</tr>
<tr>
<td>PI3K H1047R</td>
<td>4</td>
<td>Letrozol+PI3K/mTOR inhibitor</td>
<td>SD</td>
<td>WT</td>
<td>WT</td>
</tr>
<tr>
<td>PI3K H1047L</td>
<td>2</td>
<td>Tamoxifen</td>
<td>SD</td>
<td>WT</td>
<td>WT</td>
</tr>
<tr>
<td>PI3K E545K*</td>
<td>4</td>
<td>Paclitaxel + anthracycline</td>
<td>SD</td>
<td>WT</td>
<td>N/A</td>
</tr>
<tr>
<td>PI3K E545K</td>
<td>5</td>
<td>AKT inhibitor</td>
<td>early PD</td>
<td>H1047R</td>
<td>N/A</td>
</tr>
<tr>
<td>AKT1 E17K</td>
<td>2</td>
<td>Capecitabine</td>
<td>SD</td>
<td>WT</td>
<td>N/A</td>
</tr>
<tr>
<td>AKT1 E17K</td>
<td>4</td>
<td>AKT inhibitor</td>
<td>SD</td>
<td>AKT1 E17K</td>
<td>AKT1 E17K</td>
</tr>
<tr>
<td>PI3K H1047R</td>
<td>5</td>
<td>PI3K inhibitor</td>
<td>SD</td>
<td>H1047R</td>
<td>WT</td>
</tr>
<tr>
<td>PI3K H1047R</td>
<td>3</td>
<td>Afatinib + vinorelbiz</td>
<td>SD</td>
<td>WT</td>
<td>WT</td>
</tr>
<tr>
<td>PI3K G1049R</td>
<td>4</td>
<td>Capecitabine+ lapanitib</td>
<td>PR</td>
<td>WT</td>
<td>WT</td>
</tr>
<tr>
<td>PI3K H1047R</td>
<td>5</td>
<td>Vinorelbine + trastuzumab</td>
<td>PR</td>
<td>H1047R</td>
<td>H1047R</td>
</tr>
<tr>
<td>PI3K E110K</td>
<td>4</td>
<td>Paclitaxel + LCL161</td>
<td>PR</td>
<td>WT</td>
<td>WT</td>
</tr>
</tbody>
</table>

Legend: N/A, not available; PR, partial response; SD, stable disease; PD, progressive disease; WT, wild-type. * Per Oncocarta: PIK3CA E545K. ** Primary tumor: ER+/HER2-, M1: HER+.
In the second part of the project, we worked with massively parallel sequencing in tumor tissue and plasma-derived ctDNA. The use of massively parallel sequencing through “liquid biopsies” should provide means to uncover potential actionable targets and mechanisms of therapeutic resistance. This may provide ways to better select and monitor patients for specific targeted therapies.

Our aim was:
- To perform massively parallel sequencing in tumor tissue and ctDNA to decode genomic aberrations of metastatic or advanced breast cancer patients throughout patient treatment.
- To determine whether the collection of mutations detected in plasma ctDNA are representative of those obtained from the analysis of the tumor tissue, and if ctDNA can be used as a surrogate for tumor tissue.

Results of the feasibility study in collaboration with the Memorial Sloan-Kettering Cancer Center, New York, NY will be presented as a Poster Discussion Session at San Antonio Breast Cancer meeting (SABCS), San Antonio, Texas, December 2013.

Title: “Longitudinal Massively Parallel Sequencing Analysis of Circulating Cell-Free Tumor DNA”.

Figure 2. Assessment of ctDNA in the plasma for 3 selected patients with metastatic breast cancer. The graphs depict tumor dynamics as per concentration of total DNA [DNA] and the frequency of mutant alleles [% mutant alleles]. Both parameters are in accordance with radiologic and biochemical responses.
Future perspectives

Our goal now is to perform massively parallel sequencing of ctDNA in specific populations of cancer patients in order to discover predictive and prognostic molecular aberrations. In parallel with the analysis of the ctDNA of cancer patients, we aim to perform pre-clinical work with circulating biomarkers and patient-derived xenograft models. In fact, patient-derived xenograft models and the study of “liquid biopsies” may reproduce the molecular characteristics of the patients, powerfully modeling human cancer for biomarker discovery and potentially guiding personalized therapeutic decisions [7][8].

List of Publications Resulting from the Grant

Conference presentations:


**Selection of Courses & Workshops Attended During the Fellowship**

<table>
<thead>
<tr>
<th>Year</th>
<th>Event</th>
</tr>
</thead>
<tbody>
<tr>
<td>2011 &amp; 2012</td>
<td>ASCO Annual Meeting, American Society of Clinical Oncology, Chicago, USA.</td>
</tr>
<tr>
<td>2012</td>
<td>ESMO Congress, Vienna, AU.</td>
</tr>
</tbody>
</table>

**Awards and prizes**

- 2010 - 12º ECCO - AACR - EORTC - ESMO Workshop on Methods in Clinical Cancer Research, Flims, Switzerland. Special grant of Ontario Institute of Cancer Research.
- 2012 - Susan G. Komen for the Cure® travel grant. IMPAKT Breast Cancer Conference-Brussels, Belgium.
- 2013 - AACR Scholar-In-Training Award, San Antonio Breast Cancer symposium, San Antonio, TX, US.

**Other research projects:**

- Vall d’ Hebron Hospital, Breast Cancer Center and Drug Development Programme. Co-investigator of phase I, II and III trials (Ongoing).
- A Phase Ib, Open-label, Dose-Escalation Study Of The Safety And Pharmacology Of GDC-0068 In Combination With Either Docetaxel Or Fluoropyrimidine Plus Oxaliplatin In Patients With Advanced Solid Tumors. Co-Investigator (Ongoing).
- A Phase I Dose Escalation, Multi-Center, Open-label Study of HSP990 Administered Orally in Adult Patients With Advanced Solid Malignancies. Co-Investigator (Completed).
- A Two-Part, Adaptive, Randomized trial of Ridaforolimus in Combination with Dalotuzumab Compared to Exemestane or Compared to Ridaforolimus or Dalotuzumab Monotherapy in Estrogen Receptor Positive Breast Cancer Patients. Co-Investigator (Ongoing).
- A Randomized, Phase II, Multicenter, Double-blind, Placebo-controlled Study Evaluating the Safety and Efficacy of Metmab And/Or Bevacizumab in Combination With Paclitaxel In Patients With Metastatic, Triple Negative Breast Cancer. Co-Investigator (Ongoing).
- A Randomized Study Evaluating the Efficacy and Safety of Continued Or Re-induced Bevacizumab in Combination with Chemotherapy for Patients With Local Recurrent or Metastatic Breast Cancer After First Line Chemotherapy and Bevacizumab Treatment. Co-Investigator (Ongoing).
- A multicentric case-control study to determine the effect of pregnancy on breast cancer outcome in women with history of breast cancer. Co-Investigator (Completed) (Collaborative work with Dr. Hatem Azim, 2010 ESMO Translational Fellowship recipient).
Acknowledgments

I am extremely grateful for the opportunity that ESMO and Amgen gave me with the Translational Research Fellowship.

I would like to acknowledge all people that have contributed directly and indirectly to this project and have contributed to my academic background as a scientific physician.

To all my colleagues, friends and collaborators from Vall d’ Hebron Institute of Oncology/ Vall d’ Hebron University Hospital.

A special acknowledgement:
To Jose Baselga who gave me opportunity to work at the Vall d’ Hebron University Hospital.
To Javier Cortes who has mentored me at the breast cancer outpatient clinic and clinical trials.
To our collaborators, Johann De Bono (Royal Marsden Hospital, UK), and Jorge S Reis-Filho/Britta Weigelt (Memorial Sloan-Kettering Cancer Center, New York, NY).

And to my mentors Prof. Joan Seoane, for giving me a unique opportunity at his laboratory and for always guiding me and Dr. Josep Tabernero for his wonderful support.

Personal statement

I am a Brazilian medical oncologist and translational/ clinical investigator at the Translational Research Programme/Breast Cancer Center of Vall d’Hebron University Hospital, Barcelona. I have been conducting translational research projects and cutting edge clinical trials since 2009, after my residency in Medical Oncology.

From 2010 to 2012, I was involved with the European Society for medical Oncology (ESMO) Translational Research Fellowship, in which I started investigating the biology and molecular profiling of plasma circulating free DNA and circulating tumor cells in the metastatic breast cancer setting. With my enthusiasm and perseverance, and with the support of my mentors, I pioneered the programme of circulating biomarkers at Vall d’Hebron Hospital. Given its initial success, this programme now includes other cancer types in addition to breast cancer.

Based on the recent publications on massively parallel sequencing analysis of human cancers and my experience with the use of this technology for the analysis of primary breast cancers and metastasis, I have realized that intra-tumor genetic heterogeneity is one of the major challenges we currently face. In fact, I believe that understanding the impact of intra-tumor genetic heterogeneity on the biology and clinical behavior of breast cancers is absolutely essential for the realization of the potentials of precision medicine.

Given my involvement in the circulating biomarkers program at Vall d’Hebron University Hospital, one of the questions that fascinate me is whether massively parallel sequencing analysis of tumor DNA from plasma samples would constitute an alternative to sequencing of DNA extracted from biopsies, in particular for patients with metastatic disease, given that different metastatic deposits from the same patient have been shown to have distinct constellations of genetic aberrations and that biopsies of metastatic deposits are not uncommonly too risky to be performed. My vision is that ‘liquid biopsies’ may constitute an excellent means for the discovery of biomarkers, disease monitoring and tailoring the therapy of patients.
References


Photograph: Leticia De Mattos-Arruda