MOLECULAR ONCOLOGY XXX (2015) 1–8

available at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/molonc

Gene expression profiling of sequential metastatic biopsies for biomarker discovery in breast cancer

Theodoros Foukakis[a,*], *John Lövrot*[a], *Patricia Sandqvist*[b], *Hanjing Xie*[a], *Linda S. Lindström*[a,c,d], *Carla Giorgetti*[e], *Hans Jacobsson*[b], *Elham Hedayati*[a], *Jonas Bergh*[a]

[a]*Department of Oncology-Pathology, Cancer Center Karolinska, Karolinska Institutet and University Hospital, Stockholm, Sweden*
[b]*Department of Radiology and Nuclear Medicine, Karolinska Institutet and University Hospital, Stockholm, Sweden*
[c]*Department of Biosciences and Nutrition, Karolinska Institutet, Stockholm, Sweden*
[d]*Department of Surgery, University of California at San Francisco, USA*
[e]*Pfizer Oncology, Milan, Italy*

ARTICLE INFO

Article history:
Received 10 November 2014
Received in revised form
10 March 2015
Accepted 23 March 2015
Available online ■

Keywords:
Breast cancer
Relapse biopsy
Positron emission tomography
Hypoxia
Sunitinib

ABSTRACT

The feasibility of longitudinal metastatic biopsies for gene expression profiling in breast cancer is unexplored. Dynamic changes in gene expression can potentially predict efficacy of targeted cancer drugs.

Patients enrolled in a phase III trial of metastatic breast cancer with docetaxel monotherapy versus combination of docetaxel + sunitinib were offered to participate in a translational substudy comprising longitudinal fine needle aspiration biopsies and Positron Emission Tomography imaging before (T1) and two weeks after start of treatment (T2). Aspirated tumor material was used for microarray analysis, and treatment-induced changes (T2 versus T1) in gene expression and standardized uptake values (SUV) were investigated and correlated to clinical outcome measures.

Gene expression profiling yielded high-quality data at both time points in 14/18 patients. Unsupervised clustering revealed specific patterns of changes caused by monotherapy vs. combination therapy ($p = 0.021$, Fisher's exact test). A therapy-induced reduction of known proliferation and hypoxia metagene scores was prominent in the combination arm. Changes in a previously reported hypoxia metagene score were strongly correlated to the objective responses seen by conventional radiology assessments after 6 weeks in the combination arm, Spearman's $\rho = 1$ ($p = 0.017$) but not in monotherapy, $\rho = -0.029$ ($p = 1$). Similarly, the Predictor Analysis of Microarrays 50 (PAM50) proliferation metagene correlated to tumor changes merely in the combination arm at 6 and 12 weeks ($\rho = 0.900$, $p = 0.083$ and $\rho = 1$, $p = 0.017$ respectively). Reductions in mean SUV were a reliable early predictor of objective response in monotherapy, $\rho = 0.833$ ($p = 0.008$), but not in the combination arm $\rho = -0.029$ ($p = 1$).

Gene expression profiling of longitudinal metastatic aspiration biopsies was feasible, demonstrated biological validity and provided predictive information.

* *Corresponding author*. Department of Oncology, Radiumhemmet, Karolinska University Hospital, 17176, Stockholm, Sweden. Tel.: +46 851779953; fax: +46 851779524.
E-mail address: Theodoros.Foukakis@ki.se (T. Foukakis).

http://dx.doi.org/10.1016/j.molonc.2015.03.011
1574-7891/

1. Introduction

In early breast cancer, gene expression has emerged as a method for classifying into biologically and clinically relevant subtypes, defining prognosis and selecting patients for treatment (Prat et al., 2012). In metastatic breast cancer (MBC) the data on gene expression and its impact on treatment effect or survival are scarce. Classical prognostic and predictive biomarkers that are determined on primary tumor are used. Accumulating evidence suggests that this strategy is suboptimal as the metastatic disease is often different from the primary, owing to clonal evolution, tumor heterogeneity and effect of adjuvant treatments (Foukakis et al., 2012).

This limitation has been recognized, and both retrospective (Lindstrom et al., 2012) and prospective (Amir et al., 2012; Thompson et al., 2010) studies have demonstrated that determining diagnosis and estrogen receptor and human epidermal growth factor 2 (HER2) on metastatic biopsies have an impact on treatment selection and in some cases on survival (Botteri et al., 2012). Several efforts have focused on identifying surrogate biomarkers in peripheral blood, not limited to proteins by classical biochemical assays, but also circulating tumor cells (Bidard et al., 2013) and more recently circulating tumor DNA (Dawson et al., 2013). All these are however in early stage of development and their clinical validity and utility remain to be proven. Similarly, evolving imaging methods using ^{18}F-2-fluoro-2-deoxyglucose positron emission tomography/computed tomography (FDG-PET/CT) appear promising for therapy monitoring and prediction but are to date not utilized clinically (Cachin et al., 2006; De Giorgi et al., 2009; Dose Schwarz et al., 2005).

Sunitinib is a multi-targeted tyrosine kinase inhibitor with antiangiogenic and tumor inhibitory effects both in vivo and in vitro (Abrams et al., 2003; O'Farrell et al., 2003). Targets of sunitinib include vascular endothelial growth factor receptors, platelet-derived growth factor receptors and stem cell factor receptor. In MBC, single agent sunitinib demonstrated antitumoral effect with overall response rate of 11% in a phase II trial (Burstein et al., 2008). In a subsequent phase III trial the addition of sunitinib to standard first line treatment with docetaxel was assessed (Bergh et al., 2012). The objective response (OR) rate was higher with the combination than with monotherapy (55% vs. 42%). However, this did not translate into improvements in median progression-free survival (PFS, 8.6 vs. 8.3 months) or overall survival (24.8 vs. 25.5 months). The patients included in the trial at Karolinska Hospital were also offered to participate in a translational substudy utilizing sequential metastatic fine needle aspiration biopsies (FNAB) and FDG-PET/CT. Here, we report the results of the substudy, as a proof of concept for this strategy aiming at understanding drug action and identifying potential biomarkers.

2. Materials and methods

2.1. The docetaxel ± sunitinib phase III clinical trial

The randomized phase III trial of docetaxel ± sunitinib in MBC has been reported in detail elsewhere (see Supplementary Methods for the full study protocol) (Bergh et al., 2012). Briefly, it was a prospective, multicenter, randomized, open-label, phase III study, comparing docetaxel (75 mg/m^2 intravenously every 3 weeks (q3w)) combined with sunitinib (37.5 mg orally once daily from days 2–15 q3w) versus docetaxel alone (100 mg/m^2 q3w), as first line therapy in patients with HER2 negative MBC. Patients were stratified based on the number of metastatic sites, estrogen receptor status and disease-free interval after prior adjuvant treatment. Measurable disease by Response Evaluation Criteria in Solid Tumors (RECIST) version 1.0 or bone-only disease was allowed. The primary endpoint was PFS, defined as the time from the date of randomization to the first evidence of progression or death from any cause. Conventional radiological tumor assessments were performed at baseline and at 6 weeks intervals (based on RECIST) and were evaluated by the investigators and retrospectively by an independent central review laboratory blinded to treatment assignment (RadPharm, Princeton, NJ). OR were confirmed at least 6 weeks after having been initially documented.

2.2. The exploratory substudy at Karolinska

All patients included in the phase III trial at Karolinska University Hospital were offered to participate in this exploratory substudy (see Supplementary Methods for the main study protocol including the substudy amendment). The main objective of the study was to determine whether gene expression profiling is feasible using sequential, intra-patient FNAB. Secondary objectives were to identify potential biomarkers of early response by gene expression and/or FDG-PET/CT and to explore drug action in vivo by changes in gene expression.

Patients were subjected to baseline FDG-PET/CT assessment, followed by Ultrasound/CT-guided FNAB of one tumor lesion prior to start of treatment (time point T1). The aspirated material allowed the morphological confirmation of the lesion and RNA isolation. FDG-PET/CT and FNAB of the same lesion were repeated at Day 14 ± 1 (time point T2). Patients were treated and evaluated according to the main study protocol. The randomization algorithm of the phase III trial was not stratified by site and was not affected by the participation in the substudy.

The study was approved by the ethics committee of the Karolinska Institutet. A separate written informed consent for the substudy was obtained from all patients.

2.3. Functional imaging with 18F-FDG-PET/CT

The methodology, technical details and equipment used for the 18F-FDG-PET/CT are detailed in the Supplementary Methods. Tumor assessments were performed at T1 and T2, using the same radiopharmaceutical and activity at both times. The radioactive uptake in the selected regions of interest and of the same tumor areas was calculated and evaluated by the investigators who were blinded to study arm. Three-dimensional isocontour-ROI's were used to assess standardized uptake values (SUV) of the lesion targeted for FNAB. PET response was stratified by the metabolic response criteria

Please cite this article in press as: Foukakis, T., et al., Gene expression profiling of sequential metastatic biopsies for biomarker discovery in breast cancer, Molecular Oncology (2015), http://dx.doi.org/10.1016/j.molonc.2015.03.011