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Evaluating ZNF217 mRNA Expression Levels as a Predictor of Response to Endocrine Therapy in ER+ Breast Cancer

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ZNF217 is a candidate oncogene with a wide variety of deleterious functions in breast cancer. Here, we aimed at investigating in a pilot prospective study the association between ZNF217 mRNA expression levels and the clinical response to neoadjuvant endocrine therapy (ET) in postmenopausal ER-positive (ER+) breast cancer patients. Core surgical biopsy samples before treatment initiation and post-treatment were obtained from 68 patients, and Ki-67 values measured by immunohistochemistry (IHC) were used to identify responders (n = 59) and non-responders (n = 9) after 4 months of ET. We report for the first time that high ZNF217 mRNA expression level measured by RT-qPCR in the initial tumor samples (pre-treatment) is associated with poor response to neoadjuvant ET. Indeed, the clinical positive response rate in patients with low ZNF217 expression levels was significantly higher than that in those with high ZNF217 expression levels (P = 0.027). Additionally, a retrospective analysis evaluating ZNF217 expression levels in primary breast tumor of ER+/HER2−/LN0 breast cancer patients treated with adjuvant ET enabled the identification of poorer responders prone to earlier relapse (P = 0.013), while ZNF217 did not retain any prognostic value in the ER+/HER2−/LN0 breast cancer patients who did not receive any treatment. Altogether, these data suggest that ZNF217 expression might be predictive of clinical response to ET.

Keywords: breast cancer, ZNF217, endocrine therapy, clinical response, predictive biomarker

Abbreviations: ER+, ERα-positive; ERα, estrogen receptor alpha; ET, endocrine therapy; HR, hormone receptor; IHC, immunohistochemistry; LN0, no invaded lymph nodes; PR, progesterone receptor; RFS, relapse-free survival; RT-qPCR, real-time quantitative PCR.
INTRODUCTION

In recent years, studies investigating neoadjuvant therapies have emerged improving both patient management by providing a means of performing less extensive surgery and our understanding of tumor biology and response to treatment (for review, Charehbili et al., 2014). Neoadjuvant ET is administered to HR-positive postmenopausal patients, as recommended by the 15th St. Gallen International Breast Cancer Conference (Morigi, 2017). The main advantage of such a preoperative systemic ET is the prospect of downsizing and downstaging large tumors, thus facilitating breast-conserving surgical interventions. Despite the use of standard biomarkers, the heterogeneity of response to therapy still represents a challenge to clinicians in terms of selecting the most suitable neoadjuvant therapy. Thus, there is an urgent need to discover predictive biomarkers capable to identify patients who will respond to neoadjuvant ET.

We previously described that high expression levels of ZNF217, a candidate oncogene, are associated with poor prognosis, shorter RFS in breast cancer (Vendrell et al., 2012; Bellanger et al., 2017). A functional crosstalk exists between ZNF217 and ER signaling (Nguyen et al., 2014), representing a potential mechanism to escape ET. Most interestingly, high ZNF217 expression levels confer resistance to ET in ER+ breast cancer cell lines, and ZNF217 expression silencing is associated with reversion of such resistance (Nguyen et al., 2014). Furthermore, a decrease in Ki-67 levels during neoadjuvant ET (considered alone or as part of a Preoperative Endocrine Prognostic Index) was shown to predict response to ET (Dowsett et al., 2005, 2007; Ellis et al., 2011, 2017; Iwamoto et al., 2017). The aim of this pilot study is to investigate the predictive value of ZNF217 mRNA levels for response to neoadjuvant ET in patients with ER+ breast cancer.

MATERIALS AND METHODS

Study Design

This was a prospective neoadjuvant ET study on breast cancers expressing the estrogen receptor (ER+) and having a clinical size exceeding 2 cm (T2). This study has been approved by the local ethics committee (Institut du Cancer de Montpellier, France). Patients were informed that their data could be used for research; all the patients signed an informed consent form and the study was conducted in accordance with the Declaration of Helsinki principles. A total of 111 patients were treated for 4 months with neoadjuvant ET (letrozole 2.5 mg/day or tamoxifen 20 mg/day), before being subjected to resection surgery (see Supplementary Material). The response to treatment was evaluated by monitoring the evolution of a biological marker of proliferation (Ki-67) before (initial tumor) and after 4 months of ET. Investigation of ZNF217 mRNA expression levels was also conducted in the initial breast tumor and in the post-treatment tumor samples.

Sample Collection

Three micro-biopsies were collected per patient: one for histopathological diagnosis and the other two were frozen in liquid nitrogen until further use. These tissues were later used for RNA extraction and ZNF217 mRNA expression analysis, respecting post-therapeutic medical diagnostic requirements. Moreover, IHC examination was carried out to assess the statuses of ER, PR, HER2, and Ki-67. Ki-67 IHC values were measured pre- and post-treatment for each patient and used to discriminate between responders and non-responders (Dowsett et al., 2007). Patients displaying a ΔKi-67 (Ki-67 IHC value post-treatment – Ki-67 IHC value pre-treatment) ≤0 were designated to be responders, while patients with ΔKi-67 > 0 were non-responders.

RNA Extraction and Real-Time Quantitative PCR (RT-qPCR)

Total RNA was extracted from frozen biopsies using the RNeasy Mini Kit (Qiagen, Hilden, Germany). After checking RNA quality, 68 tumor samples were deemed suitable for expression analysis (59 responders and nine non-responders) (Supplementary Table 1). Reverse-transcription and RT-qPCR measurements were performed as described in the Supplementary Material. A P-value of <0.05 was considered to be statistically significant (StatgraphicsTM Software). ROC-AUC was investigated using the SPSSTM Software.

The Kaplan-Meier Plotter (KMP) Breast Cancer Cohort

The KMP cohort investigation resulted from a meta-analysis of gene-expression profiles from 2,978 primary breast cancer specimens who had not received any therapy before surgery and with known adjuvant therapy and clinical follow-up (Gyorffy and Schafer, 2009). The SPSS™ Software was used to assess the prognostic value of ZNF217 or Ki-67 mRNA expression (univariate analysis). Data were divided into two groups with either high or low expression values according to the median value. Candidate prognostic factors for RFS with a 0.1 significance level in univariate analysis were entered in a multivariate Cox model, and a backward selection procedure was used to determine independent prognostic markers.

RESULTS

ZNF217 mRNA expression levels were not correlated with Ki-67 values, neither in the initial breast tumor (pre-treatment) (r = −0.169, P = 0.17), nor in the post-treatment samples (r = −0.026, P = 0.83), nor with the ΔKi-67 values (r = −0.136, P = 0.26), thus ruling out that investigating ZNF217 expression levels was merely a surrogate markers of Ki-67 expression (Spearman test).

In responders (n = 59) and in non-responders (n = 9), ZNF217 expression was associated with response to neoadjuvant ET, since ZNF217 mRNA expression levels tended to be significantly higher (P = 0.05) in the initial breast tumor in patients who did not respond to neoadjuvant ET (median = 5.98) than those who did (median = 3.01) (Figure 1A).
Fisher’s exact test was used to investigate the association between the dichotomized clinical response measures and the ZNF217 molecular marker. The 68 patients were separated in two groups, based on the median ZNF217 expression value. The positive clinical response rate in the low ZNF217 expression level group was significantly higher ($P = 0.027$) than in the high ZNF217 expression level group, with high ZNF217 expression levels being associated with the absence of response to neoadjuvant ET (Supplementary Table 2).

The ZNF217 low and high expression level groups were comparable in terms of Ki-67 values in the initial tumor (pre-treatment) ($P = 0.20$, data not shown). Changes in Ki-67
for individual patients before and after neoadjuvant ET response are shown in Figure 1B. In the ZNF217 low expression levels group (Wilcoxon signed-rank test, \( P < 0.00004 \)), only one patient was ET-resistant (displaying increased Ki-67 level), while 33/34 patients were responders (Figure 1B). In the ZNF217 high expression level group (Wilcoxon signed-rank test, \( P < 0.0025 \)), eight patients displayed an increase in Ki-67 (ET-resistant patients), whereas 26 patients were responders (Figure 1B).

Although our study is exploratory with a limited sample size (\( n = 68 \)) and with limited relapse events (\( n = 9 \)), we assessed the predictive power of ZNF217 expression level for response to ET. The AUC was 0.701 (95% confidence interval: 0.563–0.838, \( P = 0.05 \)), which represents a good/moderate discriminatory accuracy for a model including few events (\( n = 9 \)) (Figure 1C). Based on the ROC curve, the discriminating sensitivity and specificity were 100 and 51%, respectively.

A number of studies have suggested that post-treatment biomarkers (such as Ki-67 and ER) could have a better prognostic value than pre-treatment biomarkers, and investigating biomarkers in post-treatment samples is thus of interest (Dowsett et al., 2007; Ellis et al., 2008; Chia et al., 2010). In post-treatment tumor samples, no differences in ZNF217 expression levels were observed between responders and non-responders (\( P = 0.4 \), Mann-Whitney test, data not shown), suggesting that assessing ZNF217 expression levels in the initial tumor before ET is the most informative.

To support our finding, we then hypothesized that if ZNF217 expression retains any predictive value for ET response in the neoadjuvant setting, then, the biomarker value of ZNF217 would be different between ER+ breast cancer patients treated with adjuvant ET only and patients who did not receive any treatment. We thus performed a retrospective analysis of gene-expression array data from 2,978 breast cancer patients (KMP cohort). In this cohort, we have previously demonstrated that high levels of ZNF217 mRNA expression levels were strongly and significantly associated with shorter RFS (\( P < 10^{-5} \), Nguyen et al., 2014). Strikingly, when considering the ER+/HER2−/LN0 patients, high ZNF217 mRNA levels were predictive of earlier relapse for patients treated with adjuvant ET only (\( n = 399 \), \( P = 0.018 \), univariate analysis), but not for non-treated patients (\( n = 639 \), \( P = 0.74 \), univariate analysis) (Figure 2). In ER+/HER2−/LN0 patients, ZNF217, and Ki-67 mRNA expression levels were not correlated (\( r = -0.07 \), \( P = 0.14 \), Spearman test). Since Ki-67 mRNA levels were almost significantly correlated with RFS in this cohort (\( P = 0.094 \), univariate analysis), the two factors were entered in a multivariate Cox model, and both persisted in the model showing that they are independent biomarkers (\( P < 0.1 \)). A signature associating ZNF217 and Ki-67 mRNA levels displayed a prognostic value with regards to RFS (\( P = 0.01 \), univariate analysis). Interestingly, this signature was identified as the best fit for predicting clinical outcome of ET-treated patients (likelihood = 741.46), compared to the models integrating ZNF217 mRNA levels (likelihood = 746.65, \( P = 0.023 \)) or Ki-67 mRNA levels (likelihood = 749.24, \( P = 0.005 \)) only. Our data support that ZNF217 is a predictive biomarker for response to ET, and that, in the ER+/HER2−/LN0 cohort, the signature including both ZNF217/Ki-67 mRNA levels had the best predictive value.

**DISCUSSION**

Short-term pre-operative trials with specific groups of patients have proven to be highly promising in identifying biomarkers predictive for the efficacy of targeted anti-cancer therapies (Marous et al., 2015). Early evidence of endocrine drug effectiveness can be obtained in the pre-operative (neoadjuvant) setting by profiling baseline and on-treatment biopsy samples using the window-of-opportunity. This predictive evidence acquired during short-term neoadjuvant therapy can help in identifying individual patients who will potentially benefit from long-term adjuvant treatment enabling personalized approaches. Short-term reduction in Ki-67 is predictive of clinical response to ET (Dowsett et al., 2005, 2007; Ellis et al., 2011, 2017; Iwamoto et al., 2017). However, controversy remains regarding the reproducibility of Ki-67 measurements and international efforts are ongoing to standardize and validate Ki-67 by IHC (Polley et al., 2015; Rimm et al., 2018). An additional obstacle derives from intra-tumor heterogeneity of Ki-67 (Focke et al., 2016). Altogether, there is an urgent need for further biomarkers that might increase the accuracy of prediction of response to ET.

In a previous study, proliferation-associated genes, including cyclins, mini chromosome maintenance genes and mitotic spindle-associated genes were shown to be predictive of response to ET after 2 weeks but not before treatment (Turnbull et al., 2015). A four-gene signature measuring two genes pre-treatment and two genes after 2 weeks of treatment was shown to predict response to neoadjuvant ET in ER+ patients (Turnbull et al., 2015). The genes that predicted response to ET included two pre-treatment genes associated with immune response and apoptosis and two genes measured after 2 weeks of treatment, which were associated with proliferation. Altogether, these data suggest that transcriptomic changes that develop during treatment are representative of the drug’s mechanism of action, suggesting that suppression of proliferation is the main driver of response.

In the present pilot study, the clinical sample size used is small and included only nine non-responders. Nevertheless, we found that ZNF217 expression levels are predictive of neoadjuvant ET response in ER+ breast cancer. ZNF217 expression levels are not associated with Ki-67 values, neither in the initial nor in the treated tumor, ruling out that ZNF217 could only be a surrogate marker of cell proliferation. Of utmost interest is that the predictive value of ZNF217 expression levels seems to reside in the initial tumor and is not the reflection of transcriptional changes following ET in the treated tumors. This is the first pilot prospective study conducted in the neoadjuvant setting to relate ZNF217 expression levels with treatment efficacy, thus suggesting that aside from its prognostic value in luminal breast cancers (Vendrell et al., 2012; Nguyen et al., 2014), ZNF217 expression may also be predictive of response to ET. In this exploratory study, it is difficult to estimate the accuracy of ZNF217 mRNA
levels for predicting response to ET, due to the low numbers of non-responders (9 out of 68). However, while obtained in a small cohort, our preliminary data are encouraging and need to be extended to a larger cohort for validation.

Interestingly, evaluating ZNF217 expression levels in the primary breast tumor of ER+/HER2−/LN0 breast cancer patients treated by adjuvant ET led to the identification of poorer responders prone to earlier relapse, while in ER+/HER2−/LN0 breast cancer patients who did not receive any treatment the association between ZNF217 expression and RFS was not significant. Previous studies reported multi-gene genomic assays predicting response to neoadjuvant ET (Turnbull et al., 2015; Iwata et al., 2018), and we speculate that these coupling with the ZNF217 biomarker might improve their predictive performance. Indeed, we herein demonstrated in the ER+/HER2−/LN0 cohort that combining ZNF217 and Ki-67 expression levels was more performant at predicting relapse under ET, than each of these biomarkers taken individually.

Altogether, these data support the idea that in the luminal breast cancer subclass, ZNF217 expression levels relate to ET response and provide a novel candidate biomarker. Finally, there are several ongoing trials investigating the combination of ET and other targeted therapies to prevent/reverse endocrine resistance. The PI3K/mTOR pathway, CDK4/6, HDAC, and immune checkpoints are the most promising and widely investigated targets (Rugo et al., 2016). Further studies are needed to delineate whether the ER+/ZNF217high breast cancer subpopulation might benefit from combining ET with another therapy.

REFERENCES

Bellanger, A., Donini, C. F., Vendrell, J. A., Lavaud, J., Machuca-Gayet, I., Ruel, M., et al. (2017). The critical role of the ZNF217 oncogene in promoting breast cancer metastasis to the bone. J. Pathol. 242, 73–89. doi: 10.1002/path.4882

Charehbili, A., Fontein, D. B., Kroep, J. R., Liebers, G. J., Mieog, J. S., Nortier, J. W., et al. (2014). Neoadjuvant hormonal therapy for endocrine sensitive breast cancer: a systematic review. Cancer Treat. Rev. 40, 86–92. doi: 10.1016/j.ctrv.2013.06.001

Chia, Y. H., Ellis, M. J., and Ma, C. X. (2010). Neoadjuvant endocrine therapy in primary breast cancer: indications and use as a research tool. Br. J. Cancer 103, 759–764. doi: 10.1038/sj.bjc.6605845

Dowsett, M., Smith, I. E., Ebbs, S. R., Dixon, J. M., Skene, A., AL’Hern, R., et al. (2007). Prognostic value of Ki67 expression after short-term presurgical endocrine therapy for primary breast cancer. J. Natl. Cancer Inst. 99, 167–170. doi: 10.1093/jnci/djk020

Dowsett, M., Smith, I. E., Ebbs, S. R., Dixon, J. M., Skene, A., Griffith, C., et al. (2005). Short-term changes in Ki-67 during neoadjuvant treatment of primary breast cancer with anastrozole or tamoxifen alone or combined correlate with recurrence-free survival. Clin. Cancer Res. 11, 9515–9586.

Ellis, M. J., Suman, V. J., Hoog, J., Goncalves, R., Sanati, S., Creighton, C. J., et al. (2017). Ki67 proliferation index as a tool for chemotherapy decisions during and after neoadjuvant aromatase inhibitor treatment of breast cancer: results from the american college of surgeons oncology group z1031 trial (Alliance). J. Clin. Oncol. 35, 1061–1069. doi: 10.1200/JCO.2016.69.4406

Ellis, M. J., Suman, V. J., Hoog, J., Lin, L., Snider, J., Prat, A., et al. (2011). Randomized phase II neoadjuvant comparison between letrozole, anastrozole, and exemestane for postmenopausal women with estrogen receptor-rich stage 2 to 3 breast cancer: clinical and biomarker outcomes and predictive value of the baseline PAM50-based intrinsic subtype—ACOSOG Z1031. J. Clin. Oncol. 29, 2342–2349. doi: 10.1200/JCO.2010.31.6950

Flocke, C. M., Decker, T., and van Diest, P. J. (2016). Intratumoral heterogeneity of Ki67 expression in early breast cancers exceeds variability between individual tumours. Histopathology 69, 849–861. doi: 10.1111/his.13007

Gyorffy, B., and Schafer, R. (2009). Meta-analysis of gene expression profiles related to relapse-free survival in 1,079 breast cancer patients. Breast Cancer Res. Treat. 118, 433–441. doi: 10.1007/s10549-008-0242-8

Iwamoto, T., Katagiri, T., Niikura, N., Miyoshi, Y., Kochi, M., Nogami, T., et al. (2017). Immunohistochemical Ki67 after short-term hormone therapy identifies low-risk breast cancers as reliably as genomic markers. Oncotarget 8, 26122–26128. doi: 10.18632/oncotarget.15385

Iwata, H., Masuda, N., Yamamoto, Y., Fujisawa, T., Toyama, T., Kashiwabara, M., et al. (2018). Validation of the 21-gene test as a predictor of clinical response to neoadjuvant hormonal therapy for ER+, HER2-negative breast cancer: the TransNEOS study. Breast Cancer Res. Treat. doi: 10.1007/s10549-018-4964-y [Epub ahead of print].

Marous, M., Bieche, I., Paolletti, X., Alt, M., Razak, A. R., Stathis, A., et al. (2015). Designs of preoperative biomarkers trials in oncology: a systematic review of the literature. Ann. Oncol. 26, 2419–2428. doi: 10.1093/annonc/mdv378

Morigi, C. (2017). Highlights from the 15th St Gallen International Breast Cancer Conference 15–18 March, 2017, Vienna: tailored treatments for patients with early breast cancer. Ercancermedicalscience 11:732. doi: 10.3332/ecancer.2017.732

Nguyen, N. T., Vendrell, J. A., Poulard, C., Gyorffy, B., Goddard-Leon, S., Bieche, I., et al. (2014). A functional interplay between ZNF217 and estrogen receptor

AUTHOR CONTRIBUTIONS

PR and TM designed and supervised the neoadjuvant clinical trial. JV, PV, and LG performed the experiments. JV, PV, and CD performed the RT-qPCR data analysis. MJ performed the clinical data analysis. JV and BG performed the retrospective in silico analysis. PR, TM, and PC conceived and supervised the study. JV, JS, TM, and PC wrote the manuscript.

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SUPPLEMENTARY MATERIAL

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alpha exists in luminal breast cancers. *Mol. Oncol.* 8, 1441–1457. doi: 10.1016/j.molonc.2014.05.013

Polley, M. Y., Leung, S. C., Gao, D., Mastropasqua, M. G., Zabaglo, L. A., Bartlett, J. M., et al. (2015). An international study to increase concordance in Ki67 scoring. *Mod. Pathol.* 28, 778–786. doi: 10.1038/modpathol.2015.38

Rimm, D. L., Leung, S. C. Y., McShane, L. M., Bai, Y., Bane, A. L., Bartlett, J. M. S., et al. (2018). An international multicenter study to evaluate reproducibility of automated scoring for assessment of Ki67 in breast cancer. *Mod. Pathol.* 32, 59–69. doi: 10.1038/s41379-018-0109-4

Rugo, H. S., Vidula, N., and Ma, C. (2016). Improving response to hormone therapy in breast cancer: new targets, new therapeutic options. *Am. Soc. Clin. Oncol. Educ. Book* 35, e40–e54. doi: 10.14694/EDBK_159198

Turnbull, A. K., Arthur, L. M., Renshaw, L., Larionov, A. A., Kay, C., Dunbier, A. K., et al. (2015). Accurate prediction and validation of response to endocrine therapy in breast cancer. *J. Clin. Oncol.* 33, 2270–2278. doi: 10.1200/JCO.2014.57.8963

Vendrell, J. A., Thollet, A., Nguyen, N. T., Ghayad, S. E., Vinot, S., Bieche, I., et al. (2012). ZNF217 is a marker of poor prognosis in breast cancer that drives epithelial-mesenchymal transition and invasion. *Cancer Res.* 72, 3593–3606. doi: 10.1158/0008-5472.CAN-11-3095

**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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