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CCR Translations

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ESR1 Mutations in Breast Cancer: Proof-of-Concept Challenges Clinical Action

Guowei Gu[1] and Suzanne A.W. Fuqua[1,2]

Wang and colleagues demonstrate that digital droplet PCR (ddPCR) identified *ESR1* mutations in 7% of primary breast cancers. *ESR1* mutations were also readily detected in metastatic tissues and circulating tumor DNA in the blood. These results suggest that ddPCR may be amendable for monitoring tumor burden, and to predict relapse. *Clin Cancer Res; 22(5); 1034–6. ©2015 AACR.*

See related article by Wang et al., p. 1130

In this issue of *Clinical Cancer Research*, using an ultrasensitive digital droplet PCR (ddPCR) technique, Wang and colleagues demonstrate that some of the *ESR1* mutations reported in metastatic tumors are indeed present in primary breast tumors (1). In the past 2 years using next-generation sequencing, researchers have confirmed that the ligand-binding domain (LBD) of the estrogen receptor (*ESR1* gene) is frequently mutated in approximately 20% of metastatic breast cancers, and these somatic mutations can arise in estrogen receptor (ER)-positive cancer metastases after progression on endocrine therapy (2, 3). In their study, Y537S, Y537C, and D538G *ESR1* mutations were found at low allele frequencies in 7% (3/43) of primary tumors. Previously, it was generally accepted that *ESR1* mutations were either very low (<1%) or undetectable in primary breast cancer. However, Takeshita and colleagues, also using ddPCR, reported an *ESR1* mutation rate of 2.5% (7/270) in primary tumors (4). The differences in mutation frequency between these two studies probably reflect differences in selected cut-off values; however, these studies highlight that *ESR1* mutations might be present in primary breast tumors at levels below detection using next-generation sequencing. Whether *ESR1* mutation status in primary tumors is associated with outcomes of endocrine therapy is an important clinical issue, but neither study was powered to address this critical question.

Recently, circulating blood biomarkers such as circulating tumors cells (CTC) and cell-free plasma tumor DNA (cfDNA) have been considered as alternative sources of tumor material, especially as sampling of metastatic biopsies is often not practical, or biopsies do not yield sufficient material for analysis. High-depth targeted massively parallel sequencing (MPS) analysis of cfDNA has revealed the genomic complexity and extensive intratumor genetic heterogeneity of breast tumors (5), thus single-tissue biopsies may not represent an ideal source for global monitoring of metastatic disease course. Emerging data demonstrate that *ESR1* mutations and mutational profiles can often vary between metastatic sites within a patient (6). Wang and colleagues (1) also conducted longitudinal monitoring of *ESR1* mutation status in the cfDNA of 4 patients, and found that *ESR1* mutation allele frequencies changed during treatment, and they conclude that ddPCR assays could potentially monitor tumor burden in treated patients. These anecdotal data complement what has been shown using MPS of cfDNA where response to treatment was associated with reductions in the level of potential driver mutations (7). Importantly, mutation tracking of serial patient cfDNA samples after neoadjuvant chemotherapy identified early breast cancer patients at high risk of relapse, whereas mutations in baseline cfDNA prior to treatment was not statistically associated with disease-free survival (8). Collectively, these data suggest that evolving mutations in residual or micrometastatic disease before relapse may be useful to follow treatment response or to identify new therapeutic targets in metastatic disease.

The results to date also suggest that *ESR1* mutations are frequently acquired during progression of hormone resistance, especially in the context of estrogen deprivation therapy with aromatase inhibitors. Unfortunately, there are no clinical data to fully defend this conclusion. Serial blood sampling in the study by Wang and colleagues revealed three polyclonal *ESR1* mutations in 1 patient, with enrichment of Y537C and D538 mutations, but loss of a Y537S mutation in a subsequent sample after treatment with an aromatase inhibitor, the mTOR inhibitor everolimus, and chemotherapy (1). In addition, Wang and colleagues found that *ESR1* mutations could present in cfDNA but not in biopsied metastatic tissues, suggesting that cfDNA might be a better "snapshot" of tumor heterogeneity and evolution of multiple metastatic sites during treatment. Chu and colleagues have recently reported a prospective sampling from metastatic breast cancer patients where no *ESR1* mutations were detected in metastatic tissues, but ddPCR was able to detect them in half of the patients (9). Interestingly, in the Takeshita study 2 patients acquired *ESR1* mutations in their metastatic lesions without intervening endocrine therapy, thus mutations can arise without endocrine selection (4). Although it has been suggested that the hormone-independent activity of these LBD-mutant receptors could confer a selective advantage during estrogen deprivation therapy, it is also possible they confer a selective growth advantage or an enabling driver metastatic function in the absence of treatment

[1]Lester and Sue Smith Breast Center, Baylor College of Medicine, Houston, Texas. [2]Dan L Duncan Cancer Center, Baylor College of Medicine, Houston, Texas.

Corresponding Author: Suzanne A.W. Fuqua, Lester and Sue Smith Breast Center, Baylor College of Medicine, One Baylor Plaza, MS 600, Houston, TX 77030. Phone: 713-798-1671; Fax: 713-798-1673; E-mail: sfuqua@bcm.edu

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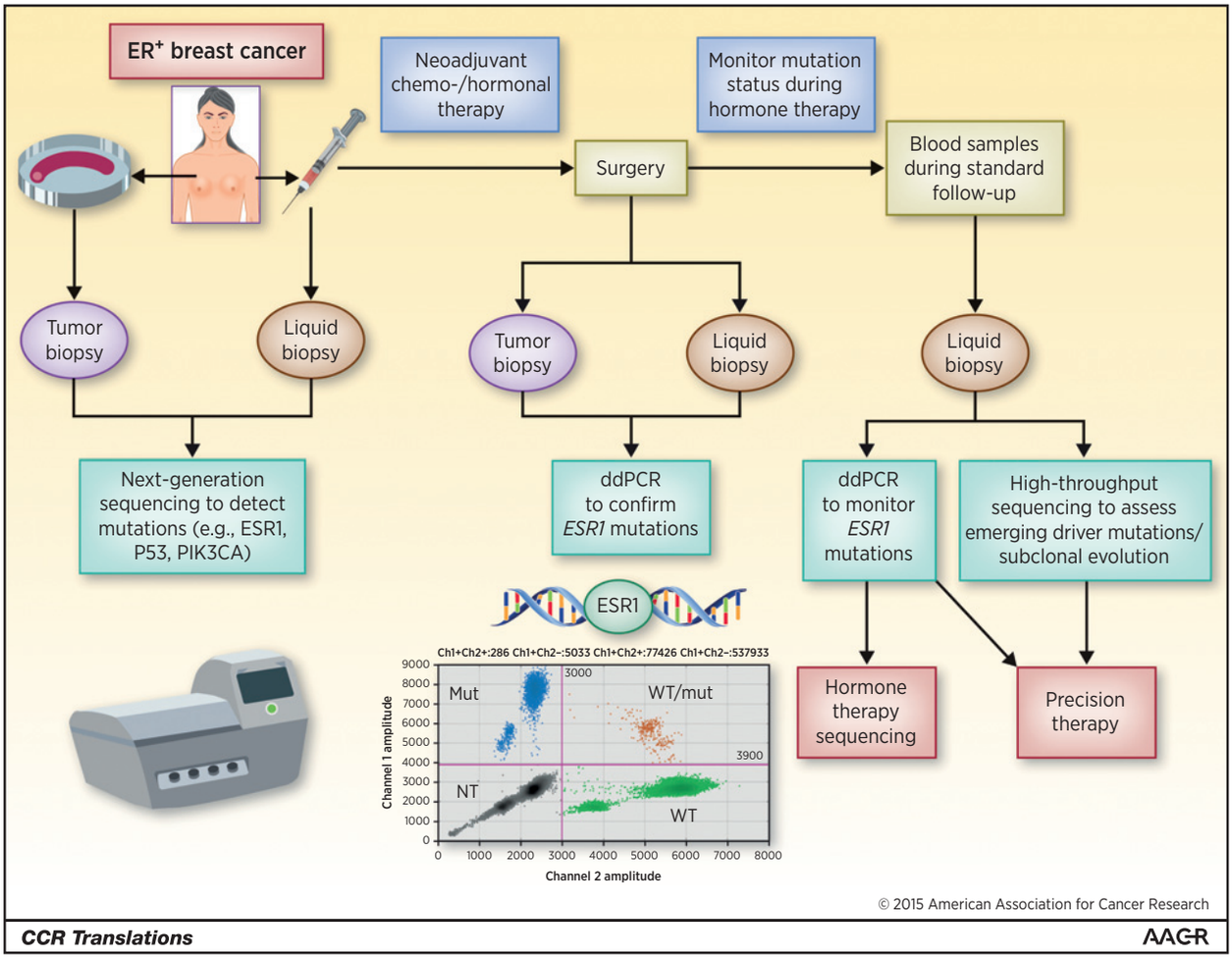

Figure 1.
Upon diagnosis of breast cancer, both tumor biopsies and blood samples can be collected from ER-positive breast cancer patients. Baseline biopsies could be analyzed using next-generation sequencing to detect relevant mutations in breast cancer such as ESR1, PIK3CA, p53, etc. Patients can receive chemotherapy or hormonal therapy before surgery. After neoadjuvant therapies, residual tumor biopsies and liquid biopsies can be collected and analyzed using ddPCR to confirm preexisting mutations and compare mutation frequency with matched baseline patient samples. After surgery, mutation status is monitored by collecting patient liquid biopsies. *ESR1* mutations can be assessed by ddPCR and other emerging driver mutations or subclonal evolution evaluated using high-throughput captured sequencing. If *ESR1* mutation allele frequencies increase in the plasma DNA, hormone therapy can be tailored to emerging mutations. This approach allows precision therapy for patients. Mut, mutated; NT, negative; WT, wild type.

(10). An enrichment in allele frequencies of the LBD *ESR1* mutations in metastatic disease compared with primary breast tumors also supports an important role in metastatic tumor dissemination (7, 11).

A remaining important clinical question is whether the *ESR1* mutations are actionable? Preclinical data are supportive that they can be targeted clinically. The LBD *ESR1* mutations confer partial resistance to both tamoxifen and the ER degrader fulvestrant, therefore effective treatment will probably require better antiestrogens (2, 3). The study by Chu and colleagues reported a noteworthy patient with bone-only ER-positive metastatic disease who developed an *ESR1* mutation while on the aromatase inhibitor letrozole, but who is clinically without evidence of progression on fulvestrant (9). This demonstrates that *ESR1* mutations do not necessarily exclude a response to fulvestrant. *ESR1* mutations occur along with wild-type receptor in tumors, thus responses to hormonal agents can be achieved via blockade of the normal receptor. Two new antiestrogens with mixed selective ER modulator/degrader activity (bazedoxifene and pipdendoxifene) were shown to be effective in an acquired tamoxifen-resistant model expressing wild-type ESR1, and bazedoxifen treatment alone significantly inhibited growth of a human patient–derived mouse xenograft model expressing the Y537S *ESR1* mutation (12). Whether inhibition of other survival/growth pathways, such as cyclins CDK4/6 or mTOR, in combination with agents with improved mixed antiestrogen activities will provide additional benefit or synergy in ESR1 mutant tumors remains to be determined. In an innovative study using *ex vivo* culture of CTCs, Yu and colleagues demonstrates that targeting heat shock protein 90, an ER chaperone, was effective in treating the Y537S *ESR1* mutation in combination with an antiestrogen and fulvestrant (11). An emerging picture is that targeted therapy for *ESR1* mutations may

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be best determined by a complete understanding of the genomic complexity and molecular portrait of each patient. Individual treatment response for ESR1 patients should be determined in a cell type–specific way.

Women with ER-positive breast cancer fear relapse during and after adjuvant therapy. We currently have no systematic genomic follow-up during this time period, and a potential treatment paradigm is shown in Fig. 1. At the time of diagnosis, tumor tissue and baseline liquid biopsies could be used for mutation detection using sensitive next-generation sequencing or MPS. Following neoadjuvant therapy, ddPCR could be employed to confirm *ESR1* mutations. ddPCR could also be used to monitor mutation status during hormone therapy via blood sampling during standard follow-up visits. These liquid biopsies could also be used to monitor emerging driver mutations and subclonal evolution during therapy which would affect hormone therapy sequencing and enable precision therapy on an individual basis. The study by Wang and colleagues (1) provides strong support for a call to action clinically for prospective investigation into the role of *ESR1* mutations in hormone resistance and metastatic progression of breast cancer.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: S.A.W. Fuqua
Development of methodology: S.A.W. Fuqua
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): S.A.W. Fuqua
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): S.A.W. Fuqua
Writing, review, and/or revision of the manuscript: G. Gu, S.A.W. Fuqua
Study supervision: S.A.W. Fuqua
Other (figure preparation): G. Gu

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