J Breast Cancer. 2020 Apr;23(2):218-223
https://doi.org/10.4048/jbc.2020.23.e4
pISSN 1738-6756·eISSN 2092-9900

Journal of Breast Cancer JBC

Case Report

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ESR1 Mutation Detection and Dynamics in Meningeal Carcinomatosis in Breast Cancer

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OPEN ACCESS

Received: Sep 30, 2019
Accepted: Dec 3, 2019

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Conflict of Interest
François-Clément Bidard declares having submitted patents related to circulating tumor DNA detection. The other authors declare that they have no competing interests.

ABSTRACT

ESR1 mutation is frequently encountered in hormone receptor (HR)-positive, human epidermal growth factor receptor 2 (HER2)-negative metastatic breast cancer (MBC), especially after aromatase inhibitor (AI) therapy, as a mechanism of resistance to endocrine therapy. Circulating tumor DNA-based detection of *ESR1* mutation in plasma has been demonstrated as a prognostic and predictive factor for poor outcomes in subsequent AI therapy. In this case report, for the first time, we describe the detection of *ESR1* mutation (p.Tyr537Ser) only in the cerebrospinal fluid (CSF) and not in the plasma of a patient with isolated leptomeningeal progression who was treated with AI for HR-positive, HER2-negative MBC (bone metastasis only). Circulating tumor DNA levels also appeared to be correlated with clinical evolution. We suggest that in the presence of isolated leptomeningeal metastasis and when tamoxifen or AI has been prescribed for HR-positive MBC, CSF should be screened for *ESR1* mutations to potentially adjust systemic treatment.

Keywords: Breast neoplasms; Cerebrospinal fluid; Estrogen receptor alpha; Liquid biopsy; Meningeal carcinomatosis

INTRODUCTION

Leptomeningeal metastasis (LM) is the third most common metastatic complication of the central nervous system (CNS), which is defined by the seeding of the leptomeninges by malignant cells. The diagnosis is based on the cerebrospinal fluid (CSF) cytology with the detection of tumor cells or neuroimaging associated with suggestive clinical findings [1].

LM is reported in up to 5% of patients with solid tumors, most commonly breast cancer (approximately 5%), lung cancer, and melanoma, and its incidence has increased in recent years [1,2]. Despite considerable progress in the treatment of breast cancer (BC) and multimodal therapy, the prognosis of LM remains dismal with a median overall survival (OS) of about 4 months [1-3].

Author Contributions

Conceptualization: Cabel L; Data curation: Melaabi S; Writing - original draft: Carausu M; Writing - review & editing: Melaabi S, Pierga JY, Bidard FC, Cabel L.

The treatment for LM comprises active systemic therapy, intrathecal therapy, and radiotherapy or surgery when necessary. Due to lack of informative clinical trials, current recommendations are mostly based on expert opinion and consensus but with a low level of evidence, leaving a number of questions unresolved, such as the most reliable criteria for diagnosis and response to treatment, as well as the best indicators of time and type of treatment required for LM [2].

Circulating tumor DNA (ctDNA) is a fraction of cell-free DNA released by the tumor cells, which can be measured in bodily fluids, such as plasma, sputum, urine, or CSF, and correlates with the tumor burden; thus, it is emerging as a new, noninvasive method to monitor and characterize malignant diseases. Numerous ctDNA applications have been demonstrated and several others are still under investigation. It is an important method of diagnosis, genomic characterization, and identification of mechanisms of resistance for precision treatment, monitoring of response to therapy, and relapse prediction [4]. The application of this type of liquid biopsy is also being increasingly studied in BC, and the prognostic value of detection and monitoring of the variant allele frequency (VAF) of *ESR1* mutations (the gene encoding the main estrogen receptor) in plasma ctDNA has been demonstrated in hormone receptor (HR)-positive metastatic breast cancer (MBC) [5].

However, it has been shown that plasma ctDNA is not always a reliable marker of the genomic and quantitative analysis of primary or metastatic CNS tumors, as compared to the CSF ctDNA, which is a more sensitive biomarker for CNS lesions [6].

Here, we report the detection of an *ESR1* mutation only in the CSF ctDNA and not in the plasma ctDNA of a patient with isolated LM progression from MBC treated with the aromatase inhibitor (AI) anastrozole. VAF dynamics in CSF were correlated with clinical evolution.

CASE REPORT

A 37-year-old woman was diagnosed with locally advanced T2N1 (pN2a), grade SBR II (3+2+2), estrogen receptor (ER)-positive, progesterone receptor (PR)-positive, human epidermal growth factor receptor 2 (HER2)-negative invasive lobular carcinoma of the left breast, with 41% Ki-67 staining positivity. She was treated surgically and underwent tumorectomy and left axillary lymphadenectomy, completed by left mastectomy, followed by adjuvant chemotherapy with six cycles of doxorubicin/ cyclophosphamide, radiotherapy, and endocrine therapy. She received tamoxifen for 5 years and treatment was continued with letrozole during postmenopause for 3 years. Nine years after the end of the endocrine therapy, she presented with the first metastatic recurrence (bone); she was treated with metastatic first-line anastrozole endocrine and denosumab antiresorptive therapies. Due to the young age at diagnosis, the patient was referred to genetic counseling, which did not reveal any relevant family history; however, she was further recommended to undergo genetic analysis of *BRCA1*, *BRCA2*, and *PALB2*, which also did not demonstrate any deleterious constitutional mutations. Eighteen months later, she presented with facial dysesthesia, followed by confusion. The imaging assessment (based on computed tomography) was negative for CNS progression and showed stable extracranial disease, but a lumbar puncture confirmed the diagnosis of leptomeningeal metastasis (presence of malignant cells compatible with a breast origin and CSF protein levels increased to 13.6 g/L). Intrathecal chemotherapy with methotrexate (D1–D5, D1=D15) was initiated and systemic therapy with

AI was continued with initial clinical and laboratory improvement (CSF protein: 0.96 g/L). The patient received 3 months of intrathecal methotrexate injections prior to the onset of clinical symptoms of neurological deterioration, increasing levels of CSF proteins, and diffuse leptomeningeal disease and hydrocephalus by magnetic resonance imaging (MRI), suggestive of leptomeningeal metastasis resistant to intrathecal methotrexate chemotherapy. Intrathecal and systemic anticancer treatments were stopped and the patient was referred to palliative care.

Paired plasma and CSF samples were obtained at the initiation of intrathecal chemotherapy during the collection of routine samples for diagnosis owing to the isolated CNS progression. These samples were subsequently analyzed by digital droplet polymerase chain reaction (ddPCR), which revealed the presence of an *ESR1* mutation in exon 8 of the CSF ctDNA, with a concentration of 521 copies/ml and VAF of 28.6%. The mutation was detected based on a method previously described by Jeannot et al. [7] (**Figure 1**). These findings were confirmed by Sanger sequencing, which identified the c.1610A>C, p. (Tyr537Ser) mutation in exon 8. No *ESR1* mutations were detected in exon 5 or 8 by ddPCR (limit of detection: 0.1%) in the plasma. Interestingly, analysis of CSF ctDNA in a sample collected after one month of intrathecal chemotherapy, concomitant with the initial clinical improvement and decreased levels of CSF protein, showed a reduction in the previously detected *ESR1* mutation concentration to 80 copies/mL with a VAF of 4.6%.

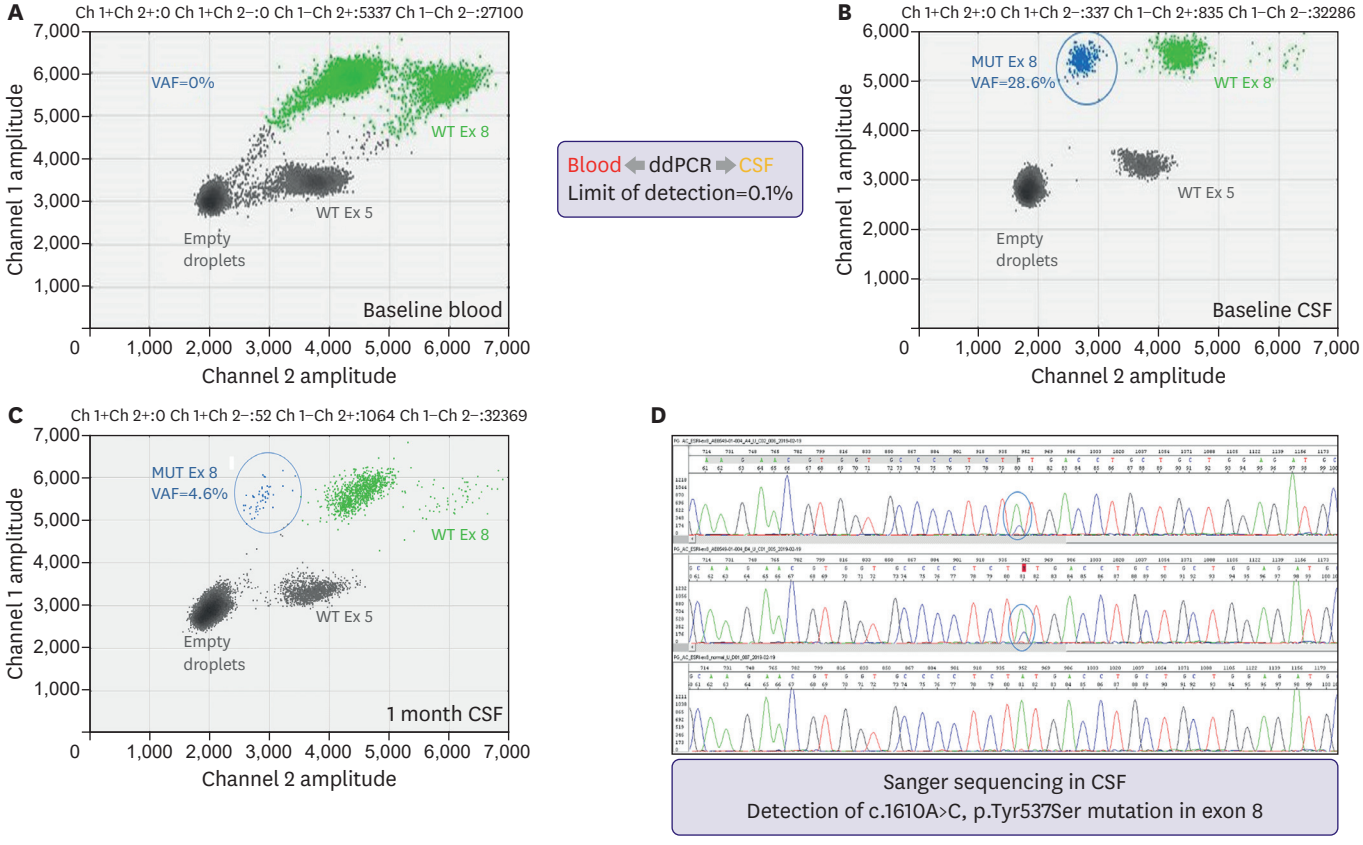

Figure 1. *ESR1* mutation detection in ctDNA. Graphical representation of ctDNA analysis in paired samples of plasma (A) and CSF, first sample, (B) second sample one month later, (C) by ddPCR. (D) Detection of *ESR1* mutation only in CSF and identification of c.1610A>C, p.Tyr537Ser mutation in exon 8 by Sanger sequencing. ctDNA = circulating tumor DNA; CSF = cerebrospinal fluid; ddPCR = digital droplet polymerase chain reaction; MUT Ex 8 = mutation in exon 8; WT Ex 8 = exon 8 wild-type; WT Ex 5 = exon 5 wild-type; VAF = variant allele frequency.

https://doi.org/10.4048/jbc.2020.23.e4

All procedures followed were in accordance with the ethical standards of the Helsinki Declaration of 1975 (in its most recently amended version). A patient consent could not be obtained because of the decease of the patient. All relating data have been anonymized and we present no identifiable material.

DISCUSSION

Due to anatomical considerations and diverged evolution that is frequently observed in CNS metastatic lesions [8], CSF is likely to be a more reliable source of ctDNA than plasma for liquid biopsy of CNS malignant lesions.

Several studies assessing the applicability of CSF ctDNA in CNS tumors have shown the detection of tumor mutations in CSF even in the presence of negative cytology [6,9]. These studies have also demonstrated the detection of actionable genomic alterations with a better concordance with CNS lesions (than in plasma), a correlation with CNS tumor burden, and detection of resistance mutations [6,9]. Importantly, it has been demonstrated that the blood-brain barrier may prevent ctDNA from entering the circulation [6]; therefore, the blood samples are likely to be uninformative for genomic characterization of CNS tumors.

Moreover, the discovery of target genetic alterations in CSF could have a major clinical impact [10,11]; for example, prescription of osimertinib for leptomeningeal metastatic non-small cell lung cancer (NSCLC) with epidermal growth factor receptor (EGFR)-T790M mutation detected only in CSF and not in blood, may confer a long-term benefit [10].

A few studies have even highlighted the utility of CSF ctDNA levels to monitor response to treatment of LM in NSCLC or melanoma, and these studies have reported modifications in VAF of genomic alterations according to response to treatment or relapse of disease [9]. Furthermore, in a patient with HER2-positive MBC with divergent intra- and extracranial responses to treatment, paired CSF and plasma ctDNA analysis mirrored the distinct courses of CNS and systemic disease [12].

In the present case, CSF ctDNA dynamics were correlated with the initial response to one month of intrathecal chemotherapy, with a significant decrease in *ESR1* mutation VAF (from 28.6% to 4.6%), concomitant with clinical and laboratory (decreased CSF protein levels) improvement. Regrettably, our ctDNA analysis was performed retrospectively and no sample was available after clinical deterioration. Although we used a sensitive method of detection (ddPCR), no *ESR1* mutation was detected in the plasma ctDNA, which could be explained by isolated CNS progression that was not associated with significant release of ctDNA into the blood, and acquisition of the *ESR1* mutation only in CNS.

Despite concerns regarding the intracranial diffusion of systemic therapies, current guidelines recommend that adapted systemic therapies may be considered for most patients with MBC and LM [2]. Systemic anastrozole therapy was maintained in our patient because of stable extracranial disease. Tamoxifen (selective estrogen receptor modulator; SERM) and letrozole (AI) have been shown to have a good distribution in the CNS [13]. Improved survival has also been reported with the continuation of endocrine therapy, including AI, tamoxifen or fulvestrant (selective estrogen receptor downregulator; SERD) for MBC in the presence of CNS metastases [13].

https://doi.org/10.4048/jbc.2020.23.e4

ESR1 mutations have been frequently found to be associated with HR-positive MBC, particularly after exposure to AI, suggesting that their occurrence may be a mechanism underlying secondary resistance to hormone deprivation therapy. These mutations have been shown to be prognostic factors for poor survival and predictive factors for poor outcomes in case of subsequent AI therapy [5].

Although some *ESR1* mutations maintain a certain degree of sensitivity to endocrine therapy agents other than AI, such as SERM or SERD, the Y537S mutation that was identified in the present case, appears to have the highest level of resistance to tamoxifen and fulvestrant [14].

Combination therapies with CDK4/6 inhibitors or PI3K-Akt-mTOR axis inhibitors appear to increase the efficacy of endocrine therapy in the presence of *ESR1* mutations [5], and the preclinical data suggest that palbociclib and abemaciclib (CDK4/6 inhibitors) could both achieve effective drug-free levels in the CNS, with better CNS diffusion of abemaciclib [15]. To the best of our knowledge, this is the first study to report the detection of an *ESR1* mutation in the CSF of a patient with isolated leptomeningeal progression, especially when this mutation was not detected in the plasma. We suggest that in the presence of isolated leptomeningeal metastasis and when tamoxifen or AI has been prescribed for HR-positive MBC, the presence of *ESR1* mutations should be assessed in CSF to possibly adjust systemic therapy.

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