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Case Report

Utilizing Liquid Biopsy for Treatment Management in Bone-Dominant Metastatic Breast Cancer: A Case Report

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Liquid biopsy · Bone metastasis · Bone-dominant · Breast cancer · *PIK3CA*

Abstract
Breast cancer is the most commonly diagnosed cancer and the second leading cause of cancer death among women in the United States. In clinical practice, the standard method to confirm metastatic disease and tailor treatment is to biopsy a metastatic site. Bone is the most common metastatic site, occurring in up to 70% of patients with advanced breast cancer. Standard-of-care management includes a needle biopsy with histopathological analysis to confirm tumor status and to evaluate for mutations. However, bone biopsies can be technically challenging and are oftentimes painful for patients. Given the challenges in acquisition and analysis of bone samples in metastatic breast cancer (mBC), a liquid biopsy is a less invasive alternative that can reveal clinically relevant alterations. Here, we report two cases of bone-dominant hormone-positive (HR+) mBC, in which circulating tumor DNA (ctDNA) was extracted from blood samples using two different next-generation sequencing (NGS) platforms to identify molecular targets for FDA approved treatment. In both patients, *PIK3CA* mutations were detected and subsequently started on alpelisib along with aromatase inhibitor or fulvestrant treatment. These cases demonstrate a feasible real-world clinical application to liquid biopsies.

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Introduction

Current goals for treatment of metastatic breast cancer (mBC) are to improve survival outcomes, prevent disease progression, and provide supportive care including symptom management. Even with treatment, the general prognosis of mBC is very poor with median overall survival being around 5 years. Standard of care therapy depends on the specific subtype of mBC. For instance, hormone-positive (HR+) mBC is treated with endocrine therapy followed by sequential single-agent chemotherapy; triple negative mBC is only treated with chemotherapy; HER2+ mBC treatment includes taxane plus trastuzumab and pertuzumab [1]. New insights and therapies are constantly emerging for the treatment of mBC according to specific molecular profiles of tumors. An example of targeted therapy is with the use of alpelisib for patients with HR+/HER2− mBC and a *PIK3CA* mutation.

Bone metastasis occurs in up to 70% of patients with advanced breast cancer [2]. In clinical practice, the standard way to confirm metastatic disease is through biopsy of a metastatic site. Obtaining a biopsy from a metastatic site can have limitations depending on the location such as bone. Bone biopsies can be technically challenging and may not yield enough specimens to be analyzed, as well as be painful and undesirable for patients [3, 4]. In addition, the decalcification step in a bone biopsy can lead to decreased DNA/RNA purity, poor DNA/RNA integrity, and loss of mutations [5]. Given the challenges in acquisition and analysis of bone samples in mBC, a liquid biopsy is a less invasive and less painful alternative that can reveal clinically relevant alterations.

Many studies have demonstrated that plasma derived circulating tumor DNA (ctDNA) can be utilized as an effective surrogate marker in mBC providing spatial and longitudinal information regarding tumor status and treatment response [6, 7]. In July 2020, the U.S. Food and Drug Administration (FDA) approved two comprehensive liquid biopsy tests for solid tumors: Guardant360 CDx and FoundationOne Liquid CDx [8]. The utilization of next-generation sequencing (NGS) technology can help identify tumor mutations and guide clinicians in determining clinical benefit from targeted therapy. Although tissue biopsies are still the conventional method for testing tumor mutational status, clinicians should first consider liquid biopsies in bone-dominant mBC (cancer metastasis exclusively to the bone) as it is more convenient and less invasive for patients, and then reflex to standard tissue biopsy if no actionable mutation is found. Here we report two cases of bone-dominant HR+ mBC, in which liquid biopsies were obtained and molecular targets were identified for FDA-approved treatment.

Case Presentations

The historical timeline of each patient's diagnosis with breast cancer, bone metastasis, and mutation with *PIK3CA* is shown in Figure 1. Patient 1 is a 42-year-old premenopausal woman with a history of high-risk HR+ BC (pT3N3-stage 3C), initial diagnosis at age 38 years, no germline genetic mutations detected who developed lower back pain while on adjuvant ovarian suppression and nonsteriodal aromatase inhibitor therapy. The patient has no significant smoking history, no family history of breast cancer, but has a maternal grandmother diagnosed with colon cancer at 34 years old. Staging scans showed multiple bone lesionswith no other sites of disease, and bone biopsy confirmed HR+ mBC. During her metastatic workup, we obtained a liquid biopsy for Guardant360® CDx liquid tumor biopsy testing (Guardant Health, Redwood City, CA, USA, http://www.guardanthealth.com/), and did not detect any ctDNA on April 30, 2020 (Fig. 2). She was started on standard treatment for mBC with continued ovarian suppression, steroidal aromatase inhibitor therapy, CDK4/6 inhibitor, and bisphosphonate therapy. During interval scans, she was noted to have additional bone lesions

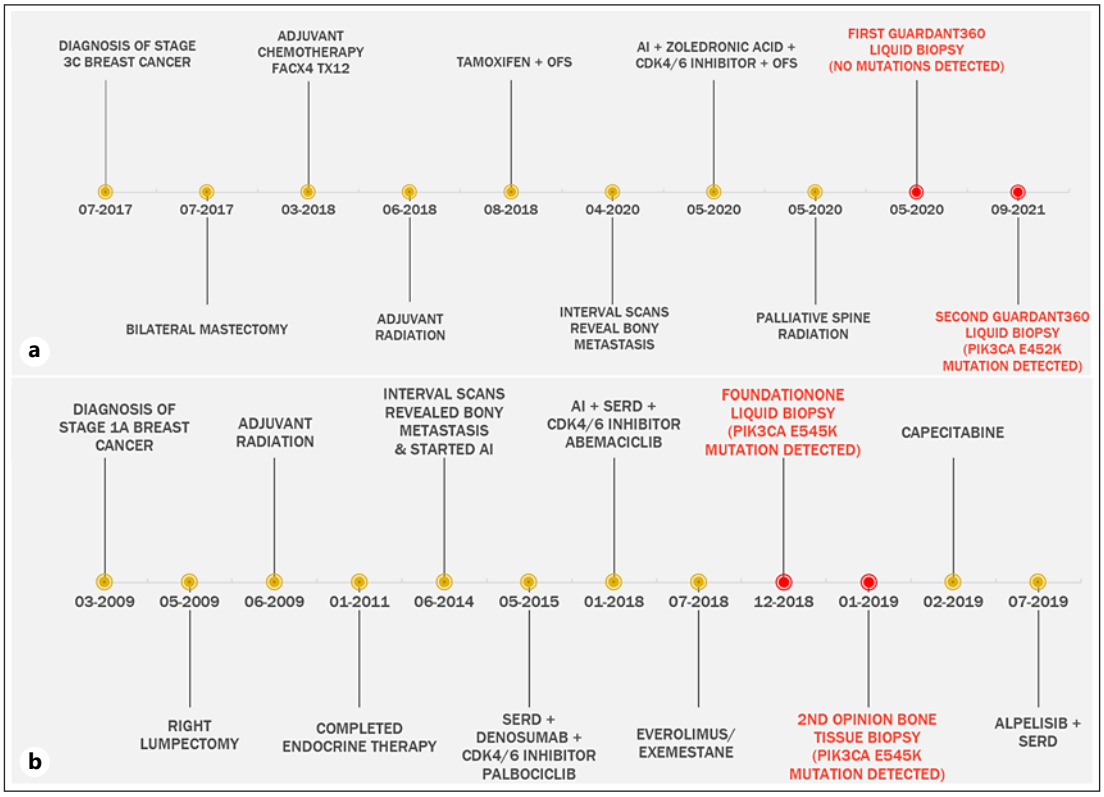

Fig. 1. a The timeline of Patient 1. The second Guardant360 liquid biopsy on September 2021 revealed a *PIK3CA* E452K-targetable mutation. **b** The timeline of Patient 2. The FoundationOne liquid biopsy on January 2019 and the following tissue biopsy on January 2019 both revealed a *PIK3CA* E545K mutation. FAC, doxorubicin, cyclophosphamide, and fluorouracil; T, paclitaxel; OFS, ovarian function suppression; AI, aromatase inhibitor; SERD, selective estrogen receptor degrader.

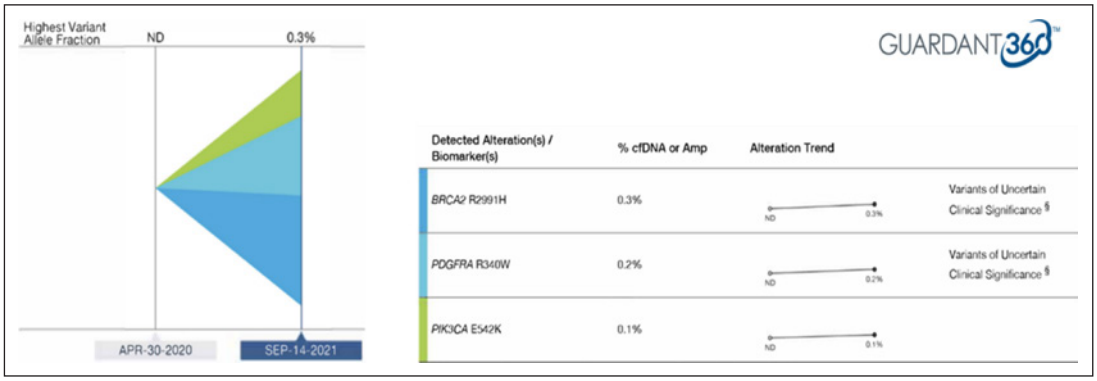

Fig. 2. Guardant360® CDx liquid biopsy testing performed on Patient 1. Each color represents a different genetic alteration (*BRCA2* R2991H, *PDGFRA* R340W, and *PIK3CA* E542K). At disease progression, a repeat liquid biopsy was performed. *PIK3CA*, *BRCA2*, and *PDGFRA* mutations were not identified in the initial testing (collection date: April 30, 2020) but identified in the repeat testing (collection date: September 14, 2021).

along with increasing tumor markers, indicating progression of disease. Another liquid biopsy was performed at that time with the same NGS platform to evaluate potential actionable molecular abnormalities, and a PIK3CA E542K mutation was found on September 14, 2021 (Fig. 2).

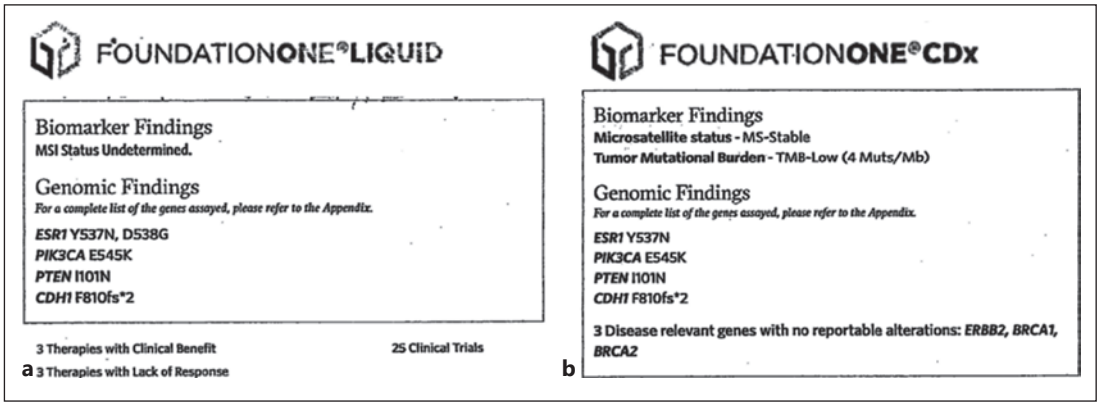

Fig. 3. a A FoundationOne®Liquid CDx testing that was collected via blood draw on Patient 2. *PTEN* I101N, *ESR1* Y537N and D538G, *CDH1* F810fs, and *PIK3CA* E545K mutations were found (Collection date December 19, 2018). **b** A FoundationOne®CDx testing from a tissue bone biopsy that was performed after academic center consultation. *PTEN* I101N, *ESR1* Y537N, *CDH1* F810fs, and *PIK3CA* E545K mutations, TMB-4Mut/Mb, and Microsatellite-stable were reported (collection date: January 21, 2019).

She subsequently was started on alpelisib with continued fulvestrant therapy and bisphosphonate treatment.

Patient 2 is a 77-year-old postmenopausal woman with a history of early-stage HR+ BC (pT1cN0-stage IA), initial diagnosis at age 65 years, who did not tolerate adjuvant endocrine therapy. The patient has no significant smoking history or family history of cancer. After 5 years, she had a new abnormality on breast imaging and staging scans showed multiple bone lesions. She then underwent palliative radiation and started on endocrine treatment and bisphosphonate therapy. After another 4 years and multiple therapies, she underwent FoundationOne®Liquid CDx NGS testing (Foundation Medicine, Cambridge, MA, USA, https://www.foundationmedicine.com/), which identified *PTEN* I101N, *ESR1* Y537N and D538G, *CDH1* F810fs, and *PIK3CA* E545K mutations (Fig. 3a). She was re-evaluated by a major academic institution and underwent a T10 biopsy that confirmed HR+ mBC. The bone tissue sample was also sent for NGS with FoundationOne® CDx (Foundation Medicine, Cambridge, MA, USA, https://www.foundationmedicine.com/), which again identified a *PTEN* I101N, *ESR1* Y537N, *CDH1* F810fs, *PIK3CA* E545K mutations, TMB-4Mut/Mb, and Microsatellite-stable (Fig. 3b). At that time, she was started on oral chemotherapy with capecitabine. After progression of disease and FDA approval in May 2019, she was started on alpelisib.

Discussion

In recent years, the availability of NGS through liquid biopsies has revolutionized the clinical management of patients with metastatic cancer. Additionally, blood-based sampling is noninvasive and accessible making it more favorable to tissue biopsies. *PIK3CA* is the most commonly mutated gene in hormone-positive (HR+) mBC (approximately 40%) and has been associated with resistance to endocrine therapy [9]. According to the National Comprehensive Cancer Network® (NCCN) guidelines, a tumor or liquid biopsy is indicated to identify *PIK3CA* mutations in patients with HR+/HER2– BC and recurrent unresectable (local or regional) or stage IV (M1) disease for treatment with alpelisib [10]. Several studies have demonstrated the feasibility of detecting mutations including *PIK3CA* and selection of targeted therapy using ctDNA in mBC [11, 12]. In addition to the detection of ctDNA, other tumor-derived

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material can be assessed with liquid biopsy to further understand the underlying disease processes in mBC. An extensive review done by Tellez-Gabriel et al. [13] demonstrated increasing research development in the detection of circulating tumor cells (CTCs) to assess tumor burden, CTC expression of RANK or HER2+ for treatment response, and extracellular vesicles containing proteins and RNA contents as potential biomarkers to monitor treatment resistance. Despite progress in blood-based technologies, there are still no standard guidelines for its use in breast cancer and is left for the clinician to determine when to best utilize liquid biopsy [14].

We have presented 2 cases of bone-dominant mBC, highlighting the use of liquid biopsy using two separate NGS platforms that were able to detect actionable *PIK3CA* mutations. This demonstrates a feasible real-world clinical application to liquid biopsies. Serial sampling in our first patient case showed the dynamic change found in tumor mutational status upon disease progression. In the second patient case, both liquid and tissue (T12, bone) biopsies provided similar mutational profiles. A study carried out by Lee et al. [15] demonstrated that bone-only mBC had a more favorable prognosis when compared to breast cancer with other sites of metastases. This supports the argument for obtaining liquid biopsies serially throughout the disease course in bone-dominant mBC to identify any changes in tumor mutational status so that appropriate treatments can be initiated promptly.

When the patients were interviewed, they both reported experiencing increased anxiety over the anticipation of pain that may occur during the bone biopsy procedures. They reported that the experience only became tolerable through anxiolytics preprocedure and conscious sedation during the procedure. This elicits the fact that these procedures can be quite painful, depending on the biopsy site and whether pharmacological intervention is provided as an option. Most importantly, both patients agreed that they would choose having a liquid biopsy over a bone biopsy.

Although tissue biopsy is standard for the detection of actionable mutations in mBC, liquid biopsy can be utilized in community practice especially at disease progression when tissue biopsies are difficult to obtain. By obtaining liquid biopsy, patients will experience less pain and have the convenience of scheduling a single blood draw. They will also undergo a procedure that is already familiar to patients. However in our practice, we would still advise the patients to have a tissue biopsy if no actionable mutation is detected on liquid biopsy.

Conclusion

In our clinical practice, we routinely obtain liquid biopsies at each disease progression to identify new mutations and potential treatments. This report provides an example of precision medicine in breast cancer oncology and how genomic characterization using liquid biopsies can help clinicians tailor treatment.

Acknowledgments

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Statement of Ethics

The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All procedures performed in studies involving human participants were in accordance with

the ethical standards of the institutional and/or national research committee(s) and with the Helsinki Declaration (as revised in 2013). Ethical approval is not required for this study in accordance with local or national guidelines. Written informed consent was obtained from both patients for publication of this case report and accompanying images. A copy of the written informed consent is available for review by the editorial office of this journal.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

Conceptualization: Jami Aya Fukui; data curation: Shirley Cheng and Edward Tri Nguyen; formal analysis: Shirley Cheng and Edward Tri Nguyen; funding acquisition: Jami Aya Fukui; investigation: Jami Aya Fukui, Shirley Cheng, and Edward Tri Nguyen; project administration: Jami Aya Fukui; resources: Jami Aya Fukui; software: Jami Aya Fukui, Shirley Cheng, and Edward Tri Nguyen; supervision: Jami Aya Fukui; validation: Jami Aya Fukui, Shirley Cheng, and Edward Tri Nguyen; visualization: Shirley Cheng and Edward Tri Nguyen; roles/writing – original draft: Shirley Cheng and Edward Tri Nguyen; writing – review and editing: Jami Aya Fukui, Shirley Cheng, and Edward Tri Nguyen.

Data Availability Statement

All data generated or analyzed during this study are included in this article. Further inquiries can be directed to the corresponding author.

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