Treatment Response Monitoring Using a Tumor-Informed Circulating Tumor DNA Test in an Advanced Triple-Negative Breast Cancer Patient: A Case Report

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Abstract
Triple-negative breast cancer (TNBC) is highly aggressive disease that is often refractory to surgery and multiple lines of therapy. Although the repertoire of FDA-approved treatments has expanded, there is an unmet need for biomarkers that can aid in appropriate selection and timing of therapy. We present a case of highly aggressive treatment-resistant TNBC that employed a comprehensive genomic profiling (CGP)-based assay to identify therapeutic targets, followed by longitudinal circulating tumor DNA (ctDNA) testing. For this, a tumor-naïve next-generation sequencing-based targeted panel was used to aid in therapy selection, along with longitudinal personalized and tumor-informed ctDNA testing to monitor tumor response to treatment. Longitudinal ctDNA testing using the tumor-informed assay detected post-surgical molecular residual disease, and rise in ctDNA levels during the surveillance period provided rationale for switching between four lines of therapy. Overall, the combined use of CGP assay with longitudinal ctDNA testing resulted in a potential prolonged survival in this highly aggressive case of TNBC.
Introduction

Triple-negative breast cancer (TNBC) is the most aggressive subtype of breast cancer and is associated with poor clinical outcome [1]. TNBC is characterized by a lack of expression of estrogen receptor/progesterone receptor and lack of amplification of human epidermal growth factor receptor 2 (HER2), thereby making hormone receptor and anti-HER2 targeted therapies ineffective [1]. Chemotherapy is currently the standard-of-care for metastatic (m) TNBC patients; however, it is associated with short initial responses and poor prognosis [1]. Treatment options for mTNBC are increasing with FDA-approved immune checkpoint blockade-based treatments (atezolizumab), poly ADP-ribose polymerase (PARP) inhibitors (i.e., olaparib), and more recently, the novel antibody-drug conjugate, sacituzumab govitecan [2,3].

Monitoring disease progression and response to treatment in TNBC currently poses a clinical challenge, due to the lack of a predictive biomarker. Toward this, assessment of circulating tumor DNA (ctDNA) in blood for detection of molecular residual disease (MRD) has emerged as a minimally invasive approach in both early and advanced stage disease [4–6] and has shown to predict treatment response as well as acquired resistance to therapy [6–9]. Here, we present a case study of a patient with advanced TNBC using a personalized tumor-informed ctDNA assay for MRD detection to monitor treatment response and aid in therapy adjustment.

Case Report

In April 2019, a 40-year-old female presented with a palpable mass in her left breast, which on biopsy was diagnosed as advanced TNBC. The patient was treated with neoadjuvant chemotherapy with weekly paclitaxel and carboplatin (80 mg/m²) followed by dose-dense doxorubicin (60 mg/m²) and cyclophosphamide (600 mg/m²) once every 2 weeks. A month after completion of chemotherapy, she underwent bilateral mastectomy, where her pathological staging showed ypT2pN0. The patient was monitored through periodic radiological imaging (computed tomography [CT] and positron emission tomography [PET]/CT scan).

Next-generation sequencing (NGS) was performed on the primary tumor biopsy using the commercially available comprehensive genomic profiling (CGP) assay, FoundationOne (Foundation Medicine, Cambridge, MA, USA) (Table 1), to determine targets for her treatment. For therapy selection, the patient was evaluated using a tumor-naïve NGS-based targeted panel (Guardant360). Concurrently, a personalized, tumor-informed multiplex-PCR (m-PCR)-NGS assay (Signatera™) was designed to longitudinally analyze ctDNA levels as a readout for response to treatment. In addition, the patient’s plasma cell-free DNA (cfDNA) levels were monitored, to better inform measured ctDNA levels and fluctuations over time. Since the patient aggressively progressed on different therapies, a biopsy of the metastatic lesion was evaluated for HER2 expression using immunohistochemistry and analyzed by NGS and RNA-Seq, using the CGP Caris Molecular Intelligence® (Caris, TX, USA) assay (Table 1).

Post-surgical assessment performed in December 2019 (2 weeks after surgery) with the tumor-informed assay revealed elevated ctDNA levels (0.41 mean tumor molecules per mL of plasma [MTM/mL]), indicating MRD-positivity. This prompted a CT scan, which showed a suspicious finding, that was eventually confirmed to be a liver metastasis (Fig. 1). Interestingly, at this time point, the tumor-naïve ctDNA-static panel failed to detect any residual disease (Fig. 3). Based on the findings of the tumor-informed ctDNA assay, the patient was initiated on first line of therapy with 28-day cycles of nab-paclitaxel (100 mg/m², administered on day 1, day 8, and day 15) and atezolizumab (840 mg/m², administered on day 1 and day 15).

During treatment, the patient’s ctDNA levels continued to rise, leading to 1,000 MTM/mL at ~160 days post-surgery, suggestive of progressive disease (PD), which was later confirmed.
by imaging. Here, ctDNA was able to identify nonresponsiveness to therapy, as early as ∼80 days prior to imaging of PD. At this time point, several targets of the tumor-naïve assay corroborated these findings, showing an increase in the mean VAF of NOTCH1, TP53, PTEN, and CCND2 mutations. However, significant elevations in the mean VAF of BRAF, BRCA2, AR, APC, RAF1, HRAS, ARID1A, MAPK3, EGFR, RET, and ALK were not appreciable at this time point (Fig. 3).

Given the patient’s resistance to the existing therapy, she was switched to the second line of therapy with 28-day cycles of sacituzumab govitecan (100 mg/kg, administered by IV once per day on days 1 and 8), following which her ctDNA levels transiently decreased, but stayed detectable (0.8 MTM/mL, Fig. 1). PET/CT scan performed at this time (∼200 days postsurgery) showed a partial response to treatment (Fig. 2), suggesting acquired resistance to

Table 1. CGP tests performed on tumor tissue

| Primary tumor | Metastatic tumor, liver |
|---------------|-------------------------|
| Panel         | Caris                   |
| Date          | May 2019                |
| MSI status    | Stable                  |
| TMB           | Low, 4 mut/Mb           |
| LOH score     | N/A                     |
| PDL1 status   | IC 5% (SP142 assay)     |
| Therapy       | None                    |
| Genetic alterations | PTEN R233*, TP53 L145Q, ARID1A D1850fs, MSH3 K383fs, amplifications: CCND2, IRS2, KDM5A, KDR, KIT, PDGRFA, MLLS duplication intron 7-exon 11 | TP53 L145Q, R273C, NOTCH1 L1678P, PTEN N323fs, JAK1 K860fs, ASXL1 G967del, SDHD W43* |

* indicates a stop codon (nonsense mutation).

Fig. 1. Patient longitudinal plot based on a personalized, tumor-informed, mPCR-NGS ctDNA assay. PET, positron emission tomography; PR, partial response; ddAC, dose-dense andriamycin and cyclophosphamide.
the antibody-drug conjugate. Again, ctDNA results were validated by imaging, with a lead time of ∼60 days in identifying therapy resistance. In August 2020, the patient was subsequently switched to combination immunotherapy with ipilimumab (1 mg/kg) and nivolumab (3 mg/kg) administered once every 21 days, given MSI high status detected on liquid CGP. However, her ctDNA levels continued to rise, indicating resistance to therapy. The patient’s last scan in November 2020 confirmed PD. At this time, her ctDNA levels reached 24,827 MTM/mL. Ultimately, due to continuous disease progression, the patient elected to discontinue further treatment and ultimately succumbed to her disease.

Interestingly, the plasma cfDNA levels in the patient also seemed to be influenced by changes in tumor progression and therapy. Toward the end of sacituzumab govitcan therapy (day 265) and ipilimumab/nivolumab combination immunotherapy (∼day 320), the patient’s cfDNA level spiked to ∼60 ng/mL and >160 ng/mL, respectively. However, this high total
cfDNA did not impact/confound ctDNA levels measured via tumor-informed assay, suggesting that measuring ctDNA concentration in MTM/mL is an accurate representation of tumor burden. For this case study, an informed consent to publish information and/or images from the patient’s guardian was obtained.

Discussion

TNBC patients, despite presenting similar clinical and pathological features, respond differently to standard chemotherapies due to disease heterogeneity and genomic instability [10]. Moreover, predictive and prognostic biomarkers that could guide change in treatment are poorly defined in this setting [11]. Currently, treatment efficacy is monitored by periodic radiological imaging, which lacks sensitivity and can lead to false positives for disease recurrence and progression [12, 13]. Therefore, new approaches to monitor treatment response are urgently needed to make timely treatment decisions, given the aggressiveness of mTNBC, and its susceptibility to treatment resistance. Previous studies have elucidated the role of dynamic changes in ctDNA from baseline in response to therapy to be predictive and prognostic across various tumor types, including breast cancer [6]. A recent systematic review and meta-analysis of 8 studies (n = 739) showed the association of elevated ctDNA levels with shorter disease-free survival in patients with both early and metastatic breast cancer [14].

Our study showed that longitudinal ctDNA analysis using a personalized tumor-informed m-PCR-NGS assay detected poor response to treatment and acquired resistance to the anti-PD-L1 monoclonal antibody chemotherapy combination and to the antibody-drug conjugate, as early as ∼60 and ∼80 days prior to imaging, respectively. These findings are consistent with previously published studies [4, 8]. In our case study, the patient never cleared ctDNA and showed persistent rise in levels as the disease aggressively progressed despite changing the line of therapy multiple times. This is consistent with another recent study, which showed early detection of ctDNA to be predictive of nonresponse to therapy and poor prognosis with an increase in ctDNA levels (from baseline) indicative of inferior overall survival (p < 0.005) [7].

Currently approved treatments for TNBC are associated with significant side effects. Early assessment of treatment efficacy can spare patients of unnecessary exposure to toxic agents. In this study, the lead time of ctDNA to imaging provided an opportunity to switch the patient to second and subsequently to third and fourth lines of therapy, allowing for prompt adjustment of ineffective therapies.

For identifying and selecting targets for treatment, we performed an NGS-based tumor-naive CGP assay (Guardant360). Among the variants tested, a wide VAF distribution was observed (Fig. 3), where some variants showed patterns that were concordant with the tumor-informed ctDNA levels (NOTCH1, TP53, PTEN, and CCND2), whereas other variants were not detectable throughout the patient course (“low VAF”: BRCA2, AR, APC, RAPF1, HRAS, ARID1A, MAPK3, EGFR, RET, and ALK). One gene from the static panel, BRAF, became positive toward the end of treatment course (∼day +275; Fig. 3). The observed variability in tumor-naive variant levels over time makes ctDNA results difficult to interpret, using the static panel. The tumor-naive panel measures actionable oncogenes, tumor suppressor, and caretaker genes that are highly associated with TNBC [10]. We speculate that diversity of VAFs fluctuations is due to the clonal heterogeneity and high mutation rate that are characteristic of TNBC, particularly in genes with functional consequences [10]. Additionally, some alterations could also be secondary to clonal hematopoiesis, which increases on treatment [15]. The tumor-informed approach circumvents this issue by targeting passenger mutations specific to an individual’s tumor that are unlikely to be impacted by clonal selection
and therapeutic challenge. Our case study demonstrates the accuracy of tumor-informed ctDNA testing in monitoring disease progression as all appreciable ctDNA changes were validated by standard imaging.

We further observed that changes in plasma cfDNA levels of the patient were influenced by changes in tumor progression as well as changes in therapy (Fig. 2a). Interestingly, a sudden spike in cfDNA levels (gray line in Fig. 1) at day 265 and 360 did not impact the ctDNA levels. This highlights the unique ability of a tumor-informed ctDNA assay to sensitively detect and track the ctDNA levels, despite changes in the total cfDNA.

Since our study is limited to a single-patient longitudinal study, this alone cannot establish it as a disease-monitoring tool. Thus, larger prospective studies are needed to establish the role of a tumor-informed ctDNA assay for treatment response monitoring. Given the features described, it is reasonable to test this assay’s utility in multiple treatment settings and as a surrogate measure for novel treatments in clinical trials in mTNBC.

**Statement of Ethics**

This study was conducted ethically, in accordance with the World Medical Association Declaration of Helsinki. Ethical approval is not required for this study in accordance with local or national guidelines. The patient was deceased prior to manuscript preparation. Written informed consent was obtained from the patient’s health care proxy for publication of the details of patient’s medical case and accompanying images.

**Conflict of Interest Statement**

Georges Azzi is a speaker for Natera, Inc., and Guardant Health Inc. Ruben Ruiz Vega reports no conflict of interest. Shifra Krinshpun, Antony Tin, Allyson Koyen Malashevich, Meenakshi Malhotra, Angel Rodriguez, and Alexey Aleshin are employees of Natera, Inc., with stock/options to own stock in the company. Minu Maninder and Paul Billings were employees of Natera with stock/option to own stock in the company.

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**Author Contributions**

Conceptualization: Georges Azzi, Shifra Krinshpun, Antony Tin, Ruben Ruiz Vega, Angel Rodriguez, Paul R. Billings, and Alexey Aleshin. Data acquisition/curation: Georges Azzi, Shifra Krinshpun, and Antony Tin. Data analysis/interpretation: Shifra Krinshpun, Antony Tin, Meenakshi Malhotra, Minu Maninder, and Allyson Koyen Malashevich. Writing – original draft: Minu Maninder, Meenakshi Malhotra, and Allyson Koyen Malashevich. Writing – reviewing and editing: Georges Azzi, Shifra Krinshpun, Antony Tin, Minu Maninder, Meenakshi Malhotra, Allyson Koyen Malashevich, Ruben Ruiz Vega, Angel Rodriguez, Paul R. Billings, and Alexey Aleshin.
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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