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case reports

Exquisitely Platinum-Sensitive Triple-Negative Breast Cancer, Time for *BRCA* Methylation Testing?

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JCO Precis Oncol 6:e2200309. © 2022 by American Society of Clinical Oncology

Introduction

Triple-negative breast cancer (TNBC) is an aggressive subtype of breast cancer associated with poor prognosis.[1-3] There has been significant progress in the treatment of metastatic TNBC (mTNBC) that was until recently treated primarily and nonselectively with chemotherapy. With evolving technologies, there are now established biomarkers identifying patients who are eligible for novel targeted therapies. Immunotherapy, for example, in combination with chemotherapy, is indicated in the 40% of programmed cell death-ligand 1 (PD-L1) expressing mTNBC, and poly (ADP-ribose) polymerase (PARP) inhibitors are indicated in the treatment of up to 5% of advanced breast cancers associated with germline pathogenic *BRCA* mutations.[4-8] The *BRCA* genes are archetypal DNA repair genes involved in stabilization of the replication fork and homologous recombination (HR).[9] Alterations of the *BRCA* genes result in phenotypic HR deficiency (HRD) characterized by heightened sensitivities to platinum salts and PARP inhibitors.[10] A significant proportion of TNBCs are identified on mutational signature testing to be HRD, and yet only a minority of TNBCs (10%-15%) have an identified pathogenic germline *BRCA* mutation.[11-13]

We report here two cases of exquisitely platinum-sensitive mTNBC, one associated with a germline *BRCA* mutation and the other with biallelic (homozygous) methylation of *BRCA1*, both detected on a proprietary HRD panel which analyses 18 genes involved in HRD in addition to methylation of *BRCA1* and *RAD51C* gene promoters. The oncoReveal *BRCA1* and *RAD51C* Methylation assay was codesigned and codeveloped by Pillar Biosciences (Natick, MA) and XING Genomic Services (XGS) and manufactured by Pillar Biosciences. It has been analytically and clinically validated by XGS as an ISO15189 NATA/RCPA accredited in-house in vitro diagnostic and is available as a commercial clinical test via XGS.[14] A manuscript detailing technical aspects and clinical utility of this assay is currently in preparation.

The patients provided informed consent for their cases to be published and for the Individualized Molecular Profiling for Allocation to Clinical Trials (IMPACT) Project which is approved by the institutional ethics committee (Singhealth CIRB 2019/2170) allowing the use of clinical and molecular profiling data for research purposes.

Case Report 1

A 45-year-old woman with a history of pT2N0M0 estrogen receptor low (1%) human epidermal growth factor receptor 2–negative breast cancer in 2012 was treated with left simple mastectomy (SM) and sentinel lymph node biopsy (SLN), adjuvant docetaxel/cyclophosphamide, followed by tamoxifen for 3 years. She developed a new TNBC, pT1bN0M0, in the contralateral right breast in 2015, treated with wide excision and SLN, declined adjuvant therapy. She presented 1 year later in 2016 with local recurrence of a 2-cm TNBC, treated with right SM, again declined adjuvant therapy. In January 2020, this patient presented with new onset vertigo. MRI of the brain revealed multiple brain metastases. Computed tomography imaging showed multiple necrotic mediastinal lymph nodes with collapse consolidation of the right middle lobe. After right occipital craniotomy and excision of the occipital tumor, she underwent whole-brain radiation therapy. Histology of the resected brain metastases was consistent with TNBC. She had a significant family history of breast cancer, and germline testing of blood identified a pathogenic *BRCA1* mutation (c.3858_3861delTGAG, p.Ser1286fs). Testing for PD-L1 expression was not performed at that time. She consented to systemic therapy on a clinical trial which evaluated a taxane chemotherapy in combination with a PD-L1 inhibitor regardless of PD-L1 status. After one cycle of study treatment, she progressed clinically with new-onset hemoptysis, hoarseness of voice and worsening dyspnea. She received salvage chemotherapy with nab-paclitaxel in combination with carboplatin with rapid relief of respiratory symptoms. Because of worsening myelosuppression, she was keen for a break after three cycles. She was switched to talazoparib to which she responded well to for approximately 7 months before worsening respiratory symptoms recurred. Treatment was switched to eribulin and subsequently capecitabine, but her disease remained progressive. Finally in June 2021, her systemic therapy was switched to gemcitabine in combination with cisplatin. She responded rapidly to

Author affiliations and support information (if applicable) appear at the end of this article.

Accepted on September 26, 2022 and published at ascopubs.org/journal/po on November 3, 2022: DOI https://doi.org/10.1200/PO.22.00309

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this regimen until March 2022 when she presented with seizures secondary to progressive brain metastases. Next-generation sequencing (NGS) of the resected brain metastases (in 2020) using the Foundation One panel showed the same *BRCA1* pathogenic variant (S128sfs*20) as was identified on germline testing in addition to MYC amplification, *PIK3R1* loss, *TP53* P153fs*28, tumor mutation burden of 6.3, microsatellite stable. Testing with the oncoReveal NGS-based HRD panel test identified the same *TP53* and *BRCA1* gene variant.

Case Report 2

A 48-year-old woman with a history of right pT2N0M0 TNBC in 2014 was treated with SM, SLN, followed by adjuvant doxorubicin, cyclophosphamide, and docetaxel. She had a local recurrence 2 years later in 2016 which was excised. She received postoperative chemotherapy with paclitaxel, carboplatin, and bevacizumab, followed by adjuvant chest wall radiotherapy. In February 2020, she presented with cough and dyspnea. Imaging demonstrated extensive disease in the pleura with accompanying pleural effusion. Pleural biopsy confirmed TNBC, and PD-L1 testing with SP 142 assay was negative. There was no family history of note, and germline genetic testing of blood on a multigene panel that tests 57 genes was negative for pathogenic variant(s). She was treated with eribulin for 4 months with response followed by rapid clinical progression with recurrence of dyspnea, cough, and reaccumulation of a large pleural effusion. She was switched to gemcitabine in combination with carboplatin in September 2020 with a complete response. As of May 2022, she remained on alternate weekly carboplatin (area under the curve 2) with well-controlled disease and minimal chemotherapy-related toxicity. NGS using the Foundation One panel showed *CDKN2A* loss, *CDKN2B* loss, *MTP* loss, *MYC* amplification, *NRAS* Q61R, *TP53* V173M, tumor mutation burden of 12.61, and microsatellite stable. Testing with the oncoReveal NGS-based HRD panel test identified the same *TP53* V173M mutation and *BRCA1* promoter methylation. The sequenced tumor specimen contained 90% tumor cells with a raw level of 70% methylation representing high somatic promoter methylation postulated to account for her exceptional and durable response to platinum chemotherapy.

Discussion

We present two phenotypically similar cases of exquisite and durable platinum sensitivity. One case was associated with a pathogenic germline alteration identified through standard clinical sequencing platforms and another was associated with biallelic (homozygous) *BRCA1* promoter methylation which was occult on routine clinical sequencing.

It has long been reported that *BRCA1* can undergo epigenetic inactivation through gain of DNA methylation at the dinucleotide clusters of cytosine nucleotide followed by guanine nucleotide (CpG island) located near the transcription start site.[15] *BRCA1* promoter methylation results in gene silencing reducing *BRCA1* protein levels conferring an HRD phenotype and similar degree of platinum sensitivity as *BRCA1* mutations.[15] The genetic and epigenetic phenotypes of early TNBC (n = 237) have previously been reported, identifying *BRCA1* hypermethylation to occur twice as frequently (24%) as the mutually exclusive *BRCA1*-inactivating variants where approximately three fourth are germline and one fourth are somatic variants.[16] Approximately 90% of the hypermethylated cases show concurrent loss of heterozygosity of *BRCA1*.[16]

Current predictors of HRD, primarily for research use, include gene-specific approaches such as sequencing of known HR genes, multiplex ligation-dependent probe amplification for copy number changes, promoter hypermethylation assays, transcriptional signatures, HRD index, genomic scars, functional assays, and mutational signatures on the basis of whole-genome sequencing.[11,17-20] For clinical use, HRD testing in the context of breast cancer is primarily through sequencing of germline DNA for disease-causing *BRCA* variants with approval of PARP inhibitors as adjuvant therapy and the treatment of advanced/metastatic human epidermal growth factor receptor 2–negative breast cancers that are associated with pathogenic germline *BRCA1/2* variants.[6-8] Qualitative *BRCA1* and *RAD51C* methylation assays using methylation-sensitive high-resolution melting have been available for clinical use, but the method is not commercially available as a kit hence patient samples must be outsourced. Moreover, these assays do not quantitate the level of methylation.

It has been proposed that the observation of platinum and PARP inhibitor sensitivity may be extended from *BRCA1/2*-mutated to *BRCA1*-methylated breast cancers. This was demonstrated in the breast cancer cell line (UACC3199) which harbors *BRCA1* CpG island hypermethylation associated with loss of *BRCA1* protein expression.[21] This cell line had the same degree of sensitivity to three tested PARP inhibitors as did the *BRCA1*-mutated cell line.[21] In the window of opportunity PETREMAC trial, responses to olaparib monotherapy were seen among patients not harboring HR mutations with enrichment for *BRCA1* promoter methylation (six of eight responders, overall response rate [ORR], 75%; 95% CI, 40.9 to 92.9 *v* 3 of 13 nonresponders, ORR, 23.1%; 95% CI, 8.2 to 50.3; $P = .03$).[22] Contrasting results were, however, noted in the randomized phase III TNT trial evaluating the use of carboplatin versus docetaxel in advanced/metastatic *BRCA 1/2*-mutated and TNBC.[23] A total of 376 patients were treated on this study, of whom 43 (11.4%) patients had germline *BRCA 1/2* mutation.[23] Of the 212 with known methylation status, 33 (15.6%) had *BRCA1* promoter methylation.[23] As expected, patients with deleterious *BRCA 1/2* germline mutations had significantly better response to carboplatin compared with docetaxel (ORR 68% *v* 33%; $P = .03$) and a median progression-free survival of 6.8 versus 4.4 months; $P = .002$.[23] However, patients with *BRCA1* methylation did not have

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better responses to carboplatin as compared with docetaxel (ORR 21.4% *v* 42.1%; *P* = .28).[23]

The differences noted might in part be explained by collection of archival tissue in the TNT trial and varying levels of *BRCA1* promoter methylation (5%-100%) which have been reported in breast and ovarian cancer samples. On the basis of results presented by several groups, it is clear that all alleles must be epigenetically silenced i.e., the level of methylation needs to be complete/high/biallelic/homozygous to establish HRD in the affected cell.[24-27] Clinical samples are mixtures of tumor cells and surrounding stromal or immune cells. Some estimation of tumor cell content in any given sample needs to be made in conjunction with methylation quantitation to determine if methylation is complete/biallelic/homozygous. The oncoReveal assay analysis pipeline includes this estimation, either from pathology assessment of tumor cell content and/or *TP53* variant allele frequency (if present), to adjust the quantitated methylation level.[27] On the basis of the distribution of adjusted methylation levels seen across a number of patient samples, the threshold used for complete/high/biallelic/homozygous similar to Menghi et al is 70%.[27] This was observed with case 2 wherein homozygous somatic promoter methylation of *BRCA1* was identified and therefore predicted sensitivity to platinum salts and/or PARP inhibitor therapy.

Before the development of the oncoReveal *BRCA1* and *RAD51C* methylation assay, NGS-based approaches have been attempted, but methylation quantitation has not been accurate enough for clinical use, presumably because of biases in sequencing reads (personal communication). Notably, the oncoReveal assay includes SLIMamp technology which demonstrably removes any sequencing-related bias, allows the assay to be sensitive even with highly degraded DNA, and requires < 10 ng of input DNA. Therefore, the methylation assay applied here can be adopted by any diagnostic laboratory with access to short read sequencing, thus becoming an in-house NGS laboratory-developed test. This assay can be run in combination with Pillar Biosciences' HRD NGS assay, on the same flowcell (Product Code HDA-HR-1003-96) or with other commercially available HRD assay kits to identify both genetic or epigenetic causes of HRD.

We would suggest testing for *BRCA1* promoter methylation at the time of diagnosis of mTNBC, in the first-line treatment setting, to aid in identifying the best choice of chemotherapeutic with or without programmed cell death protein-1/PD-L1 immune checkpoint inhibitor. The potential of maintenance therapy with a chemotherapy-free alternative such as a PARP inhibitor after clinical response is an attractive option to preserve quality of life and a strategy currently being evaluated in the DORA study (ClinicalTrials.gov identifier: NCT03167619) and KEYLYNK-009 (ClinicalTrials.gov identifier: NCT04191135).[28]

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SUPPORT

T.J.T. is supported by the NMRC research training fellowship. XING Genomic Services provided the oncoReveal assays used in this case report.

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Financial support: Daniel S. Tan
Administrative support: Tira J. Tan, J. Lynn Fink, Timothy K. Tay, Kevin M. Koo, Rebecca A. Dent
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Manuscript writing: All authors
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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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Honoraria: AstraZeneca, Roche/Genentech, Novartis, MSD Oncology
Consulting or Advisory Role: AstraZeneca, Lilly, Novartis, MSD Oncology, Everest Medicine, DKSH
Speakers' Bureau: Novartis
Research Funding: Bayer (Inst), Novartis (Inst), AstraZeneca (Inst), Odonate Therapeutics (Inst), Seattle Genetics (Inst), Roche/Genentech (Inst), Daiichi Sankyo/Astra Zeneca (Inst), Sanofi (Inst)
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Travel, Accommodations, Expenses: Roche, Pfizer, Amgen, Merck, AstraZeneca

No other potential conflicts of interest were reported.

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