Gynecologic Oncology Reports 21 (2017) 57–60

Contents lists available at ScienceDirect

Gynecologic Oncology Reports

journal homepage: www.elsevier.com/locate/gynor

Case report

BRCA1 reversion mutation acquired after treatment identified by liquid biopsy

Paul Mayor[a,**], Laurie M. Gay[b], Shashikant Lele[a], Julia A. Elvin[b,*]

[a] Roswell Park Cancer Institute, Buffalo, NY, USA
[b] Foundation Medicine, Inc., Cambridge, MA, USA

ARTICLE INFO

Keywords:
Ovarian serous carcinoma
BRCA1
Olaparib
Comprehensive genomic profiling

1. Introduction

Patients with breast or ovarian cancers harboring *BRCA1/2* mutations have clinically benefitted from treatment with inhibitors of poly (ADP-ribose) polymerase (PARPi) or platinum compounds, but acquired resistance to these therapies can limit clinical benefit. A variety of genomic alterations can lead to loss of *BRCA1/2* activity, including nonsense or frameshift alterations and large scale genomic deletions or rearrangements. Previous studies have identified secondary mutations in both *BRCA1/2* that restore, at least partially, homologous repair capabilities and allow tumors to escape inhibition by PARPi or platinum-based therapies (Wang et al., 2016a; Wang et al., 2016b; Drost et al., 2016; Bouwman and Jonkers, 2014). Detecting these reversion alterations in *BRCA1/2* after disease progression following treatment with PARPi or platinum compounds can provide valuable information to be considered when modifying treatment plans. We report here the acquisition of a reversion mutation in *BRCA1* following treatment of ovarian serous carcinoma with both platinum therapy and the PARPi olaparib. This mutation was detected from circulating tumor DNA using a blood-based next-generation sequencing assay that did not require a tissue biopsy for analysis.

2. Methods

Comprehensive genomic profiling (FoundationOne®, Foundation Medicine, Inc., Cambridge, MA) of a pre-treatment sample obtained during surgical resection was performed as described previously (Frampton et al., 2013). The sequenced sample was from the omentum, and was one of several observed tumor sites including the falciform ligament, peritoneum, pelvic wall, bilateral ovary and fallopian tube surfaces, serosal aspect colon and rectum, small bowel and hemidiaphragm. In brief, DNA was extracted from 40 μm of formalin-fixed, paraffin-embedded sections, and CGP was performed on a hybrid capture-based library of 315 genes, plus 28 select introns frequently rearranged in cancer (Supplemental Fig. 1). Sequence data were analyzed for clinically relevant classes of genomic alterations, including base pair substitutions, insertions/deletions, copy number alterations, and rearrangements.

At time of progression following treatment with olaparib (400 mg BID), a circulating tumor DNA (ctDNA) genomic profiling assay (FoundationACT™, Foundation Medicine, Inc., Cambridge, MA) was performed to assess potential resistance mechanisms and inform future therapeutic strategy for the patient. The assay is validated in accordance with the Clinical Laboratory Improvement Amendment (CLIA) and was conducted as follows. Two 10 mL aliquots of peripheral whole blood were collected in cell-free DNA blood collection tubes. A double-spin protocol was used to isolate plasma, and 50 ng to 100 ng of ctDNA was extracted to create an adapted sequencing library before hybrid capture and sample-multiplexed sequencing on an Illumina HiSeq 2500 sequencer (Illumina, San Diego, CA). The assay evaluates 62 genes (Supplemental Fig. 1) to ×5000 unique coverage and can identify alterations at low allele frequencies (0.1% for substitutions, 1% for indels and rearrangements, and 20% for copy number amplifications). These 62 genes are a subset of those interrogated with the tissue-based assay. Mutant allele frequency (MAF) for the tissue-based assay represents the percentage of DNA obtained from the mutation-containing tumor on

* Corresponding author at: Foundation Medicine, Inc., 150 Second Street, Cambridge, MA 02141, USA.
** Corresponding author.
E-mail addresses: paul.mayor@roswellpark.org (P. Mayor), jelvin@foundationmedicine.com (J.A. Elvin).

http://dx.doi.org/10.1016/j.gore.2017.06.010
Received 27 February 2017; Received in revised form 14 June 2017; Accepted 17 June 2017
Available online 21 June 2017

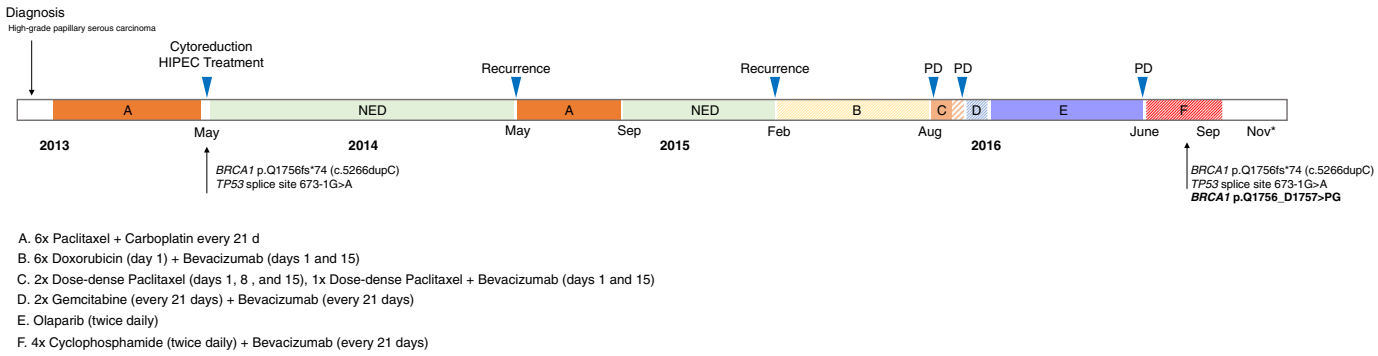

Fig. 1. Timeline of disease course from diagnosis onward. The patient received germline genetic testing in December 2015, which revealed the BRCA1 alteration c.5266dupC. Comprehensive genomic profiling was subsequently performed on the tumor resection sample obtained in May 2013 and on a liquid biopsy sample obtained in August 2016. The results of both CGP analyses revealed the germline *BRCA1* alteration, as expected. In addition, the ctDNA sequenced post-treatment (Aug 2016) revealed the acquisition of a *BRCA1* mutation that is expected to restore some level of BRCA1 protein functionality.

which a biopsy was performed. In the blood, MAF represents the percentage of ctDNA in the bloodstream that is harboring the mutation on a given day and time. For this reason, MAF for the tissue assay and ctDNA assays cannot be directly compared.

The listed price for each assay is $5800, although actual cost will vary depending on circumstances. Approval for this study, including a waiver of informed consent and a HIPAA waiver of authorization, was obtained from the Western Institutional Review Board (IRB# 20152817).

3. Results

The patient is a 58-year-old Caucasian female who was initially seen at our institution in December of 2012 after being diagnosed with peritoneal carcinomatosis based on computed tomography (CT) of her abdomen and pelvis (Fig. 1). A paracentesis was performed and the fluid sample was found to be positive for adenocarcinoma of uncertain origin. Given her uncertain primary disease site, she was initially managed by colorectal surgery and was taken to the operating room for a diagnostic laparoscopy with biopsy. This revealed a large volume of straw colored ascites, omental caking and large volume tumor implants along the anterior abdominal wall, both hemi-diaphragms, the sigmoid colon, and the peritoneal surfaces in the pelvis and upper abdomen. Pathology from her omentum and pleural effusions revealed high grade papillary serous carcinoma, likely of ovarian origin. Given her advanced stage disease, high tumor burden, and likely need for extensive resection to achieve an optimal tumor debulking status, she was initially treated with the neoadjuvant chemotherapy regimen of carboplatin AUC 6 plus paclitaxel 175 mg/m^2 every 21 days for 6 cycles, followed by interval cytoreduction and heated intraperitoneal chemotherapy (HIPEC) treatment with mitomycin C for 90 min. She completed this surgery in May of 2013. Both prior to and after her diagnosis of ovarian cancer, the patient did not have any other cancer diagnoses made.

She was without evidence of disease until May of 2014 when she underwent a surveillance CT scan of her chest, abdomen and pelvis which revealed a recurrence. Given her platinum sensitivity, she was started on carboplatin AUC 6 plus paclitaxel 175 mg/m^2 every 21 days and completed 6 cycles in September of 2014. She was without disease until February of 2015. She was started on doxorubicin 40 mg/m^2 (day 1) plus bevacizumab 10 mg/kg (days 1 and 15), completing 6 cycles in August 2015. She was noted to have disease progression at that time and was referred to the Gynecologic Oncology service for clinical trial consideration.

After review of her treatment course and current disease state by the gynecologic oncology service, she was referred to the Clinical Genetics service for germline genetic testing and started on dose dense paclitaxel 80 mg/m^2 (Days 1, 8, and 15). After 2 cycles, the patient was noted to have re-accumulation of large volume ascites requiring frequent paracentesis; therefore, bevacizumab 10 mg/kg (days 1 and 15) was added to her chemotherapy regimen. She completed a third cycle of dose dense paclitaxel with bevacizumab and CT imaging revealed progression of disease in December 2015. Her chemotherapy regimen was switched to gemcitabine 800 mg/m^2 plus bevacizumab 15 mg/m^2 every 21 days, and she completed two cycles. At this time, the patient met with the clinical genetics service and underwent germline genetic testing with the *OvaNext* panel through Ambry Genetics. This panel tests for deletion/duplication analysis of the following 23 genes: ATM, BARD1, BRCA1, BRCA2, BRIP1, CDH1, CHEK2, MLH1, MRE11A, MSH2, MSH6, MUTYH, NBN, NF1, PALB2, PMS2, PTEN, RAD50, RAD51C, RAD51D, SMARCA4, STK11, and TP53, as well as deletion/duplication analysis of the EPCAM gene. Her testing revealed a *BRCA1* gene mutation (c.5266dupC, p. Q1756fs*74) and the remainder of her germline results were negative. In January 2016, she had increased abdominal pain and CT revealed disease progression (Fig. 2). Given her *BRCA1* mutation, she started on olaparib 400 mg PO twice daily and completed approximately 6 months of therapy in June of 2016. While on olaparib the patient had a partial response to this therapy with reduction in size of her intraperitoneal tumor burden as evidenced on CT imaging done in May of 2016 (Fig. 2). She also had a plateau in her CA125 during her final three months of olaparib treatment: April, May and June (Fig. 3). Her olaparib was discontinued after 6 months of therapy due to intraperitoneal disease progression noted clinically by re-accumulation of ascites and CT imaging performed in June of 2016 showing progression. In May 2016, molecular genetic testing was completed on her omental tumor sample obtained in May of 2013 (FoundationOne). This testing revealed the previously identified germline *BRCA1* mutation (p.Q1756fs*74, c.5266dupC) as well as an inactivating *TP53* mutation (splice site 673-1G > A). This *BRCA1* alteration would prematurely truncate the protein and disrupt the BRCT domains of BRCA1, if expressed. Alternatively, protein levels could be reduced through nonsense mediated decay. The BRCT domains of BRCA1 are responsible for interactions with a variety of additional proteins and protein complexes.

In June of 2016, due to disease progression, she was started on cyclophosphamide 50 mg PO twice daily and bevacizumab 15 mg/kg once every 3 weeks. She remained on this therapy until the end of September. In late August of 2016, sequencing of the patient's ctDNA detected 3 mutations: the previously identified *TP53* splice site alteration and *BRCA1* p.Q1756fs*74, as well as *BRCA1* p.Q1756_D1757 > PG (c.5263_5272 > TCCCCAGGAC). The effect of the second *BRCA1* alteration, p.Q1756_D1757 > PG, appears to serve as a reversion alteration that restores the translational reading frame of the expressed BRCA1 protein (Fig. 4). This alteration would occur within a loop region of the BRCT domain, just past an alpha helical domain (see A), and would maintain a similar distance between folded elements of the