

BRCA2 reversion mutation confers resistance to olaparib in breast cancer

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February 20, 2023

Abstract

A 34-year-old woman with breast cancer and the BRCA2: p.Gln3047Ter was treated with olaparib. After tumor progression, cancer genomic profiling testing revealed the BRCA2 p.Gln3047Ter and p.Gln3047Tyr, with 48.9% and 0.37% allele frequency, respectively. These findings shed light on reversion mutation as a resistance mechanism to olaparib in breast cancer.

Case report

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Data availability statement: Data sharing is not applicable to this article as no datasets were generated or analyzed during the current study.

Funding: none

Conflict of Interests

The authors declare that there are no conflicts of interest regarding the publication of this article.

Ethics Approval : This study was conducted ethically in accordance with the World Medical Association Declaration of Helsinki.

Patient consent: Written informed consent was obtained from the patient to publish this report in accordance with the journal's patient consent policy.

Key Clinical Message

Reversion mutation was identified as a mechanism underlying resistance to olaparib in breast cancer using cancer genomic profiling.

Abstract

A 34-year-old woman with breast cancer and the BRCA2: p.Gln3047Ter was treated with olaparib. After tumor progression, cancer genomic profiling testing revealed the BRCA2 p.Gln3047Ter and p.Gln3047Tyr, with 48.9% and 0.37% allele frequency, respectively. These findings shed light on reversion mutation as a resistance mechanism to olaparib in breast cancer.

Keywords

Breast neoplasms, BRCA, genomics, olaparib

Introduction

The protein-encoded *BRCA* is involved in homologous recombination repair and plays an important role in DNA double-strand break repair. PARP plays a vital role in DNA single-strand break repair.¹ Therefore, olaparib, a PARP inhibitor, is effective for tumors with a germline *BRCA* mutation owing to its synthetic lethality.² The OlympiAD trial demonstrated the efficacy of olaparib in patients with human epidermal growth factor receptor type2 (HER2)-negative metastatic breast cancer with a germline *BRCA* mutation.² The median progression-free survival was significantly longer in the olaparib group than in chemotherapy of the physician's choice group (7.0 vs. 4.2 months; $P < 0.001$).²

In recent years, several mechanisms of resistance to PARP inhibitors have been proposed,³ including protein alteration in the homologous recombination pathway, altered expression of a protein in the DNA replication fork protection pathway,⁴ epigenetic modifications, restoration of ADP-ribosylation, and pharmacological alterations.³ Importantly, reversion mutation has been recently reported as a mechanism of resistance to the PARP inhibitor olaparib.^{5,6}

Herein, we describe a case of a patient with breast cancer with a *BRCA2* pathogenic variant that was resistant to olaparib and was suspected to be a reversion mutation based on cancer genomic profiling.

Case presentation

A 34-year-old woman presented to our hospital with a mass in her right breast, which she noticed after giving birth to her second child. Breast cancer was diagnosed via core needle biopsy. The tumor was classified as invasive ductal carcinoma, estrogen receptor (ER) positive, progesterone receptor (PgR) positive, and HER2 positive. The timeline of the treatment course is shown in Figure 1. The patient received 8 courses of docetaxel–pertuzumab–trastuzumab therapy (75 mg/m² docetaxel on day 1, 420 mg/kg pertuzumab on day 1, and 6 mg/kg trastuzumab on day 1 every 3 weeks). Subsequently, right total mastectomy and axillary lymph node dissection were performed. The main pathological findings were a residual tumor diameter measuring 53 mm, a number of residual lymph node metastases found in four of 18 nodes, ER and PgR

positivity, HER2 negativity, and a histological response to preoperative therapy of grade 1a. Postoperatively, the patient received chemotherapy and endocrine therapy (pertuzumab–trastuzumab, tamoxifen, and a luteinizing hormone-releasing hormone agonist). Magnetic resonance imaging revealed liver metastases. After three courses of trastuzumab emtansine (3.6 mg/kg every 3 weeks), the metastatic lesions had progressed. Subsequently, adriamycin and cyclophosphamide (AC) therapy (60 mg/m² adriamycin on day 1 and 600 mg/m² cyclophosphamide on day 1 every 3 weeks) was administered. Considering cardiotoxicity, AC therapy was completed after 11 courses (total adriamycin dose: 495 mg/m²). Thereafter, lapatinib (1250 mg/kg daily) plus capecitabine (3600 mg/kg on days 1–14 every 3 weeks) therapy was administered. After 20 months, the metastatic tumors showed progression (Figure 2a). *BRCA* genetic testing was performed and a pathogenic variant was detected. The variant using hg19 as the reference genome was *BRCA2* : c.9139C>T (p.Gln3047Ter) (Figure 3). Therefore, olaparib (600 mg/kg daily) was administered. The tumor size reduced after olaparib administration (Figure 2b); however, progression of liver metastases was noted after 12 months of olaparib administration (Figure 2c). Repeated biopsy of the liver metastasis revealed an ER-positive, PgR-positive, and HER2-negative tumor. Abemaciclib (150 mg twice daily) plus fulvestrant (500 mg every 4 weeks) therapy was administered. After 8 months, metastatic lesions progressed. Trastuzumab deruxtecan (5.4 mg/kg every 3 weeks) was administered. After 4 months, metastatic lesions progressed; therefore, bevacizumab (10 mg/kg on days 1 and 15 every 4 weeks) plus paclitaxel therapy (90 mg/m² on days 1, 8, and 15 every 4 weeks) was administered. Cancer genomic profiling (FoundationOne[®] Liquid CDx) was performed during bevacizumab plus paclitaxel therapy. The sample at the time of surgery was >3 years old, and the amount of specimen at the time of liver biopsy was insufficient; therefore, liquid biopsy was performed. Figure 2d shows computed tomography images of liver metastasis when cancer genomic profiling testing was performed. The variants detected were *BRCA2* c.9139C>T (p.Gln3047Ter) and *BRCA2* c.9139-9141delinsTAC (p.Gln3047Tyr) mutation, with 48.9% and 0.37% allele frequency, respectively (Figure 3). After 9 months of bevacizumab plus paclitaxel therapy, metastatic lesions progressed; therefore, eribulin was administered (1.1 mg/m² on days 1 and 8 every 3 weeks). However, after 3 months, metastatic lesions progressed; thus, irinotecan (100 mg/m² on days 1, 8, and 15 every 5 weeks) was administered. After one course, the patient's general condition deteriorated, and the policy of best supportive care was adopted; 4 months later, the patient died.

Discussion

A reversion mutation is a well-discussed mechanism of resistance to PARP inhibitors. Waks et al. reported a reversion mutation as the most commonly observed mechanism of resistance to PARP inhibitors or platinum chemotherapy in eight patients with metastatic breast cancer with *BRCA1/2* mutations.⁴ Tobalina et al. reported the occurrence of reversion mutations in 26.0% (86/327) patients with *BRCA1* or *BRCA2* mutations, including 27 patients with breast cancer,⁷ with a higher percentage in *BRCA2* compared to *BRCA1* (30.7% vs. 22.0%).⁷

Pettitt et al. analyzed and reported 308 homologous recombination gene reversions associated with a PARP inhibitor or platinum resistance.⁸ In *BRCA1*, the reversion mutations occurred throughout the *BRCA1* coding sequence. However, in *BRCA2*, there were “hotspots” and “deserts.” The frequency of reversions 3' to coding sequence position 7617 (exon 16 onward) was significantly lower than the expected frequency based on the incidence dataset. However, numerous reversion mutations in the N-terminal c.750-775 regions have occurred.⁸ Therefore, our presented case might be relatively rare. However, as the authors' indicated, PARP inhibitors are routinely used in clinics and some reversion mutations will no longer be considered novel enough to be reported. Therefore, reversion mutations in this area may be underestimated.

Weigelt et al. reported the detection of reversion mutations using circulating cell-free DNA in breast and ovarian cancers.⁹ Tumor tissue was not synchronously collected; therefore, it was not possible to validate the presence of the putative reversion mutations in the tumor, which was a limitation in Weigelt et al.'s study. Similarly, reversion mutation was not confirmed using tissue biopsy in our case herein. Repeat tumor biopsy from liver metastases and liquid biopsy to confirm the amplification of newly detected secondary variants were planned. However, these were not performed due to the deterioration of the patient's condition and

the fact that no additional treatment would be administered.

In addition, there was a concern in our case. The occurrence of true reversions to wild-type was previously reported⁸; however, true reversions are challenging to identify using liquid biopsy alone. Thus, the prevalence of true reversions may be underestimated, and it is possible that our case also had a true reversion.

There are several reports on treatment after PARP inhibitor resistance. Reversions have been predicted to encode immunogenic neopeptides; thus, immunotherapies may also be an option for direct targeting of the revertant protein.⁸ As another strategy for acquired resistance to PARP inhibitors, a cyclin-dependent kinase 12 inhibitor¹⁰ or WEE1 kinase inhibitor¹¹ can be used. To prove the efficacy of these treatments, it is important to continue to investigate PARP inhibitor resistance cases.

Conclusion

We experienced a case in which the possibility of a reversion mutation was suggested as a mechanism of resistance to olaparib. The amount of research into these cases has so far been insufficient to elucidate the mechanism of resistance to olaparib; therefore, reporting each case is of paramount importance.

Authors' Contributions

SY: collected the clinical data, analyzed the data, wrote the manuscript, approved the final manuscript

KK, YF, SA, KN, MS. MO, AY, ST, MS, MT: collected the clinical data, revised the article, approved the final manuscript

CH: collected the clinical data, wrote the manuscript, approved the final manuscript

RT: collected the clinical data, approved the final manuscript

CK, IE: revised the article, approved the final manuscript

Acknowledgments

None

References

- Walsh CS. 2015. Two decades beyond BRCA1/2: Homologous recombination, hereditary cancer risk and a target for ovarian cancer therapy. *Gynecol Oncol* 137(2):343-350. doi: 10.1016/j.ygyno.2015.02.017.
- Robson M, Im SA, Senkus E, et al. Olaparib for metastatic breast cancer in patients with a germline BRCA mutation. *N Engl J Med* 377(6):523-533. doi: 10.1056/NEJMoa1706450.
- Li H, Liu ZY, Wu N, Chen YC, Cheng Q, Wang J. 2020. PARP inhibitor resistance: The underlying mechanisms and clinical implications. *Mol Cancer* 19(1):107. doi: 10.1186/s12943-020-01227-0.
- Waks AG, Cohen O, Kochupurakkal B, et al. 2020. Reversion and non-reversion mechanisms of resistance to PARP inhibitor or platinum chemotherapy in BRCA1/2-mutant metastatic breast cancer. *Ann Oncol* 31(5):590-598. doi: 10.1016/j.annonc.2020.02.008.
- Pan JN, Lei L, Ye WW, Wang XJ, Cao WM. 2021. BRCA1 reversion mutation confers resistance to Olaparib and camrelizumab in a patient with breast cancer liver metastasis. *J Breast Cancer* 24(5):474-480. doi: 10.4048/jbc.2021.24.e39.
- Gornstein EL, Sandefur S, Chung JH, et al. 2018. BRCA2 reversion mutation associated with acquired resistance to Olaparib in estrogen receptor-positive breast cancer detected by genomic profiling of tissue and liquid biopsy. *Clin Breast Cancer* 18(2):184-188. doi: 10.1016/j.clbc.2017.12.010.
- Tobalina L, Armenia J, Irving E, O'Connor MJ, Forment JV. 2021. A meta-analysis of reversion mutations in BRCA genes identifies signatures of DNA end-joining repair mechanisms driving therapy resistance. *Ann Oncol* 32(1):103-112. doi: 10.1016/j.annonc.2020.10.470.

8. Pettitt SJ, Frankum JR, Punta M, et al. 2020. Clinical BRCA1/2 reversion analysis identifies hotspot mutations and predicted neoantigens associated with therapy resistance. *Cancer Discov* 10(10):1475-1488. doi: 10.1158/2159-8290.CD-19-1485.
9. Weigelt B, Comino-Mendez I, de Bruijn I, et al. 2017. Diverse BRCA1 and BRCA2 reversion mutations in circulating cell-free DNA of therapy-resistant breast or ovarian cancer. *Clin Cancer Res* 23(21):6708-6720. doi: 10.1158/1078-0432.CCR-17-0544.
10. Johnson SF, Cruz C, Greifengberg AK, et al. CDK12 inhibition reverses de novo and acquired PARP inhibitor resistance in BRCA wild-type and mutated models of triple-negative breast cancer. *Cell Rep* 17(9):2367-2381. doi: 10.1016/j.celrep.2016.10.077.
11. Drean A, Williamson CT, Brough R, et al. 2017. Modeling therapy resistance in BRCA1/2-mutant cancers. *Mol Cancer Ther* 16(9):2022-2034. doi: 10.1158/1535-7163.MCT-17-0098.

Figure legends

Figure 1 Timeline of the treatment course

Abbreviations:

TP: trastuzumab and pertuzumab, T-DM1: trastuzumab emtansine, AC: adriamycin and cyclophosphamide, L+C: lapatinib and capecitabine, Abema+Flu: abemaciclib and fulvestrant, T-Dxd: trastuzumab deruxtecan, PTX+Bev: bevacizumab and paclitaxel, CPT-11: irinotecan, BSC: best supportive care, Bt+Ax: total mastectomy and axillary lymph node dissection

Figure 2 Liver metastasis on computed tomography

- a) Liver metastasis before olaparib administration (Arrows)
- b) Liver metastasis has shrunk (Arrows)
- c) Liver metastasis has progressed after 12 months of olaparib administration (Arrows)
- d) Liver metastasis (Arrows) at the time of cancer genomic profiling testing

Figure 3 Schema of the BRCA2 mutation

Middle row: Result of BRCA genetic testing. The following pathogenic variant in BRCA2 is detected: p.Gln3047Ter.

Lower row: Result of Cancer Genomic Profiling testing. The following new mutation is identified: p.Gln3047Tyr.

Abbreviations: CGP: cancer genomic profiling testing

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