

EVALUATING EVIDENCE FOR THE USE OF PARP INHIBITORS IN CANCERS
WITHOUT BRCA1 OR BRCA2 MUTATIONS

By

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Abstract

PARP inhibitors are a class of drugs FDA approved to treat cancers with *BRCA1* and *BRCA2* mutations. This review focuses on the use of PARP inhibitors in cancers without *BRCA1* and *BRCA2* mutations and tries to determine how PARP inhibitors can be used to target cancers with DNA repair defects beyond *BRCA1* and *BRCA2* mutations. There is evidence that *SLFN11* may be a biomarker for sensitivity to PARP inhibitors in small cell lung cancer and Ewing sarcoma. There is also evidence that enzalutamide can downregulate genes in homologous recombination pathway to sensitize prostate cancer to PARP inhibitors. However, this evidence has mostly been shown preclinically and the clinical data is not sufficient for treatment of any of these cancer types without *BRCA1* or *BRCA2* mutations.

Introduction

BRCA1 and *BRCA2* are genes that encode proteins in the homologous recombination (HR) DNA repair pathway. *BRCA1* and *BRCA2* proteins are required for the recruitment of the DNA repair machinery to the site of double stranded DNA breaks. If there is a loss of function mutation in both copies of *BRCA1* or *BRCA2*, double strand DNA breaks can no longer be repaired through homologous recombination. When homologous recombination is lost, non-homologous end joining (NHEJ) and microhomology-mediated end joining (MMEJ) are still able to repair the double strand DNA breaks. However, these repair pathways are much more error prone than HR.¹

Poly [ADP-ribose] polymerase 1 (PARP1) is involved in NHEJ and MMEJ. PARP inhibitors are a class of drugs that inhibit the PARP1 enzyme (Table 1). This inactivates

the alternative pathways for DNA repair and prevents the repair of double strand breaks. Because there is significant damage to the DNA, the cell can no longer survive. This process is referred to as the synthetic lethality mechanism of PARP inhibitors. Cancer cells that are deficient in proteins in the HR pathway are able to be killed by inactivating the other DNA repair pathways.¹

Table 1. Summary of the different PARP inhibitors available

PARP Inhibitor	FDA approval
Olaparib	Ovarian cancer with a <i>BRCA1</i> and <i>BRCA2</i> mutations after three or more lines of chemotherapy
Rucaparib	Maintenance treatment for ovarian cancer with <i>BRCA1</i> and <i>BRCA2</i> mutation that responds to chemotherapy Prostate cancer with <i>BRCA1</i> and <i>BRCA2</i> mutation alongside androgen receptor-directed therapy and chemotherapy
Talazoparib	HER2 negative breast cancer with <i>BRCA1</i> and <i>BRCA2</i> mutation that is locally advanced or metastatic
Veliparib	Not FDA approved
Niraparib	Maintenance treatment for ovarian cancer with <i>BRCA1</i> and <i>BRCA2</i> mutation that responds to chemotherapy

Currently, PARP inhibitors are only used in the treatment of cancers with BRCA mutations. Table 1 shows the different PARP inhibitors available and which cancers they are FDA approved to treat. There are PARP inhibitors that are approved for use in ovarian, breast, and prostate cancer with *BRCA1* and *BRCA2* mutations. This review focuses on the use of PARP inhibitors in cancers without *BRCA1* and *BRCA2* mutations and tries to determine how PARP inhibitors can be used to target cancers with DNA repair defects beyond *BRCA1* and *BRCA2* mutations.

Methods

I used the University of Arizona library and PubMed to find papers. I started by searching PARP inhibitors and found a review paper that discussed the use of PARP inhibitors in cancers that are not BRCA positive. Then, I searched for PARP inhibitors and the specific cancer type I was looking at. My search terms were Small Cell Lung Cancer PARP Inhibitor, Prostate Cancer PARP inhibitors, Ewing Sarcoma PARP inhibitors, SLFN11, and Leukemia PARP inhibitors. I excluded papers that only discussed PARP inhibitors as a treatment for BRCA mutations. After finding papers from the library or PubMed, I searched for ongoing clinical trials using clinicaltrials.gov.

Small Cell Lung Cancer

Small cell lung cancer (SCLC) is an aggressive form of lung cancer that is fast growing and metastasizes rapidly. It is responsive to chemotherapy and radiation, but often patients relapse and become resistant to treatment. Non-small cell lung cancer (NSCLC) has been highly responsive to therapies targeting *EGFR* mutations or *EML4-ALK* fusions. However, there are significant differences in the pathways involved in the development of different lung cancer types, so the treatments for NSCLC are not relevant to SCLC. Currently, the genetic changes in SCLC, like loss of Rb and c-Myc activation, do not have successful targeted therapies.² Researchers are trying to better characterize the disrupted signaling pathways of SCLC and find targeted therapies that could treat SCLC.

Small cell lung cancer does not seem like it would be a good candidate for treatment with PARP inhibitors. PARP inhibitors are used to treat cancers with *BRCA1*

and *BRCA2* mutations and small cell lung cancer is not caused by these mutations. However, there are other genes in the homologous recombination pathway that could be biomarkers for PARP inhibition besides *BRCA1* and *BRCA2*.

A potential marker for sensitivity to PARP inhibition is *Schlafen 11 (SLFN11)*. The *Schlafen* family of genes play roles in cell cycle arrest, differentiation, and cancer cell invasion. *Schlafen 11* is a gene in this family that is found only in humans. Its function is not completely characterized, but it plays a role in blocking the replication of retroviruses in infected cells and in sensitizing malignant cells to DNA damaging agents.³ It has been shown that knocking down *SLFN11* can cause resistance to chemotherapy in cultured cells.⁴

Lok, et. al. found that *Schlafen 11 (SLFN11)* expression correlates with sensitivity to PARP inhibition for small cell lung cancer in vitro.² They used the Genomics of Drug Sensitivity in Cancer database to find genes that correlated with sensitivity to olaparib, veliparib, and rucaparib. The Genomics of Drug Sensitivity in Cancer database contains information about the response of around 700 cancer cell lines to 138 anticancer drugs that has been compiled from over 75,000 studies.⁵ Then they tested the effect of the PARP inhibitor, talazoparib, on cell lines that had different gene expression for various genes. Talazoparib was chosen because it had shown clinical activity in a phase I clinical trial in patients with small cell lung cancer. They found that cell lines that highly expressed *SLFN11* were more sensitive to talazoparib. Also, when *SLFN11* was knocked down the SCLC cells were more viable when treated with veliparib. The results were similar in a patient derived xenograft (PDX) mouse model. They used seven different small cell lung cancer cell lines from patients that they characterized for

SLFN11 expression for the PDX model. The mice were injected with one of the seven cell lines and observed tumor growth with and without talazoparib. The mice were immunocompromised to prevent rejection of the cells. SLFN11 expression still correlated with a better response to talazoparib. Finally, they showed that small cell lung cancer generally has high *SLFN11* expression. These results show that high *SLFN11* expression could be a marker for increased sensitivity to PARP inhibitors in small cell lung cancer.

Byers, et. al. found that small cell lung cancer is sensitive to PARP inhibition in combination with DNA damaging chemotherapy.⁶ First, they found that PARP is expressed higher in small cell lung cancer than non-small cell lung cancer (NSCLC). They looked at the combination of the PARP inhibitors, olaparib and rucaparib, with cisplatin, an alkylating agent⁷, and etoposide, a topoisomerase II inhibitor⁸. They found that PARP inhibitors can decrease poly ADP ribose (PAR) expression in vitro, which suggests that SCLC could be sensitive to PARP inhibition.

Byers, et. al. used a RAD51 assay to see if there was a defect in the homologous recombination (HR) pathway in small cell lung cancer.⁶ RAD51 is a protein involved in the HR pathway. RAD51 is in a complex with BRCA2, which gets recruited by BRCA1 in HR and assists in the repair of the double strand breaks.⁹ The RAD51 assay uses RAD51 recruitment as a marker for homologous recombination.¹⁰ The cells are irradiated causing double strand breaks. If HR is functioning properly, RAD51 should increase initially and eventually decrease once the DNA is repaired. In SCLC, RAD51 was recruited but stayed at high levels indicating a problem with the homologous recombination pathway. This shows that PARP inhibitors could potentially cause

synthetic lethality in SCLC, because the HR pathway is not completely functional. This study provides a basis for the use of PARP inhibitors in small cell lung cancer, but there are issues with some of the data and the evidence is not strong.

There is one phase II clinical trial for the use of veliparib in combination with the chemotherapy drug temozolomide for small cell lung cancer. Temozolomide is an alkylating agent that creates cross linkages in the DNA preventing cellular replication.¹¹ All of the patients had been treated with chemotherapy prior to the study and had become resistant to platinum based chemotherapy. Pietanza, et. al. found that in general veliparib did not increase progression-free survival and overall survival.¹² However, patients that were positive for *SLFN11* responded better to the PARP inhibitor and had an increase in progression-free survival. This provides more evidence for *SLFN11* expression being a marker for sensitivity to PARP inhibition.

Ewing Sarcoma

Ewing sarcoma is a rare cancer of the bone and soft tissue. It primarily affects children and young adults. It metastasizes to many other tissue types and usually has a low response rate to treatment, with 25% of patients with localized tumors and 75% of patients with metastasis who do not respond to treatment. There is a common genetic change in Ewing sarcoma. Approximately 85% of patients with Ewing sarcoma have a chromosome translocation, involving the *EWSR1* and *FLI1* genes, that encodes a chimeric transcription factor called EWS-FLI1.⁴

Tang et al. found that *SLFN11* is a transcriptional target of EWS-FLI1, suggesting that Ewing sarcoma would respond to PARP inhibitors.⁴ They used chromatin

immunoprecipitation (chIP) sequencing to see the interaction between EWS-FLI1 and the *SLFN11* promoter. They found that it binds to the promoter and increases expression in an Ewing sarcoma cell line with the EWS-FLI1 translocation and a kidney epithelial cell line as a control. Then, they showed that mRNA and protein levels for *SLFN11* were increased when EWS-FLI1 was present. Next, they used clinical samples to show that *SLFN11* expression is positively correlated with EWS-FLI1 expression and that there is a longer tumor free survival in patients with high *SLFN11* expression. Then, they used siRNA to knock out *SLFN11* expression. When *SLFN11* was knocked down, cells had a much higher percent viability and were more viable than the nontargeting siRNA control in the presence of niraparib. In cells that highly express *EWS-FLI1*, knocking down *SLFN11* causes a higher viability. This suggests that high expression of *SLFN11* could make cells more susceptible to PARP inhibition. The interaction between *SLFN11* and *EWS-FLI1* suggests that PARP inhibitors could be used to treat Ewing Sarcoma.

Tang et al. also identified other genes from breast and prostate cancer that are correlated with high *SLFN11* expression. They found that *ETS1* is positively correlated with *SLFN11* expression and with further testing found that knocking down *ETS1* also lowers mRNA levels for *SLFN11*. *ETS1* encodes a transcription factor that activates genes involved in stem cell development, cell senescence, cell death, and tumorigenesis.¹³ *ETS1* could potentially be another marker for sensitivity to PARP inhibition.

There has only been one clinical trial testing PARP inhibitors as a treatment for Ewing Sarcoma and there are no ongoing clinical trials at the moment. Choy et al. ran a

phase II clinical trial of olaparib in patients with refractory Ewing Sarcoma as secondary treatment after chemotherapy.¹⁴ The trial had 12 adult participants between the ages of 18 and 70. Ewing Sarcoma is usually a pediatric cancer, but it can occur in adults. Four of the twelve participants had stable disease. The rest of the patients had progressive disease and one patient had new metastatic lesions. The median progression free survival was 5.7 weeks. All twelve of the patients experienced toxicities from the treatment. Four out of twelve patients experienced grade 3 toxicities and one patient had a life-threatening toxicity. However, the researchers did not attribute two of the grade 3 toxicities and the life-threatening toxicity to olaparib. The toxicities and low progression free survival indicate that PARP inhibitors are not a good treatment option for Ewing Sarcoma despite the genetic markers indicating that it could be effective.

Prostate Cancer

Prostate cancer is a common cancer in men. It is treatable in the early stages, but once it metastasizes it is difficult to treat.¹⁵ One common treatment is androgen deprivation therapy, because androgens can cause growth and proliferation of prostate cancer. However, prostate cancer often becomes resistant to androgen deprivation therapy. Prostate cancer that is resistant to androgen deprivation therapy is called castration resistant prostate cancer.

Rucaparib is a PARP inhibitor that is FDA approved to treat castration resistant prostate cancer with *BRCA1* or *BRCA2* mutations. Researchers are looking at the potential to use PARP inhibitors to treat cancers with other defects in other genes that encode proteins in the homologous recombination pathway.

Li et al. found that it might be possible to use androgen receptor inhibitors to knock down homologous recombination, so that prostate cancer could be targeted by PARP inhibitors.¹⁵ They looked at how inhibiting the androgen receptor in prostate cancer can induce homologous recombination deficiencies and cause synthetic lethality with PARP inhibition. They started by looking at two data sets from other researchers that identified HR genes that are upregulated in castration-resistant prostate cancer. They looked in more depth at the ten genes that are expressed the most. These genes include *CHEK1*, *BRCA1*, *EXO1*, *BLM*, *RMI1*, *RAD54L*, *RAD51*, *LIG1*, *XRCC3*, and *RMI2*. Then, they treated three different cell lines with enzalutamide, an androgen receptor inhibitor, and olaparib. The VCaP cell line was androgen-receptor positive and androgen-dependent. The LNCaP cell line was also androgen-receptor positive, but less dependent on androgens due to a mutation leading to altered hormone specificity for the receptor. The CWR22Rv1 cell line has mutant androgen receptors that make it androgen independent. The VCaP and LNCaP cell lines had lower relative mRNA levels of the upregulated HR genes when exposed to enzalutamide compared to DMSO control. For one of the cell lines, the combination of enzalutamide and olaparib caused further downregulation of the genes. For the AR-positive and androgen independent cell line, olaparib downregulated the HR genes, but less than the other cell lines. They referred to the downregulation of the genes involved in HR as inducing “BRCAness” because they respond similarly to cells with *BRCA1* and *BRCA2* inactivating mutations. This suggests that enzalutamide and olaparib combined could make prostate cancer cells susceptible to PARP inhibition by downregulating genes involved in homologous recombination.

Li et al. did further testing to show that it was possible to induce “BRCAness” and treat prostate cancer with PARP inhibitors.¹⁵ They started by using siRNA to knock down *BRCA1*, *RAD51*, *RAD54L*, and *RMI2* and treating the cells with olaparib. They found that there is a higher percentage of sub-G1 cells when the cells with knocked down HR genes are treated with olaparib indicating that more cells are dying. Sub-G1 cells are cells that are in the late stage of apoptosis and have fragmented DNA. This shows that knocking down genes involved in HR can make cells more susceptible to olaparib, which will cause them to undergo apoptosis. They used γ H2AX and RAD51 foci as markers for DNA damage and homologous recombination. γ H2AX is histone variant that is formed as an early cellular response to DNA double-strand breaks. A higher percentage of γ H2AX foci to RAD51 foci indicates that there are double-strand breaks in the DNA that are not being repaired by homologous recombination.¹⁶ They found that in the presence of both enzalutamide and olaparib and olaparib alone there is a much higher percentage of cells with γ H2AX foci than RAD51 foci. In cells treated with just enzalutamide or the DMSO control there was a low percentage of both foci. This shows that DNA repair is suppressed when the cells are treated with olaparib and the combination of olaparib and enzalutamide. These results show that there is a potential for combination of enzalutamide and olaparib to inhibit homologous recombination, which would make prostate cancer cells susceptible to PARP inhibition.

Li et al. further tested the combination treatment of enzalutamide and olaparib mice. They injected mice with three different prostate cancer cell lines.¹⁵ There was a subcutaneous patient-derived xenograft model (PDX model) and two cell lines that were injected orthotopically. The mice were castrated and immunodeficient to avoid rejection

of the cells. They then treated the mice with a control, enzalutamide alone, olaparib alone, a combination of enzalutamide and olaparib, or enzalutamide and olaparib that was started before the cells were implanted. The tumor volume of the PDX model was significantly lower in all of the treatment groups compared to the control, but the combination of enzalutamide and olaparib that was given before the cells were implanted had the lowest tumor volume. This is not relevant to the treatment of prostate cancer, because we wouldn't be able to treat patients before they had prostate cancer. Enzalutamide alone and the combination treatment had similar tumor volumes and were the next lowest of the treatment groups. They also looked at the expression of *BRCA1* and Ki67, a marker for cell proliferation¹⁷, in the PDX models after treatment. The enzalutamide and olaparib combination treatment had lower levels of both BRCA1 and Ki67. Lower expression of these proteins shows that homologous recombination and cell proliferation are both downregulated by the treatment. The results of the mouse model are promising and show that there is potential for the combination of enzalutamide and olaparib to treat prostate cancer in humans by knocking down homologous recombination and causing synthetic lethality.

Discussion

There is some evidence for new biomarkers for sensitivity and different mechanisms of PARP inhibitors in cancers without mutations in *BRCA1* or *BRCA2*. High SLFN11 expression correlates with PARP inhibitor sensitivity in small cell lung cancer and Ewing sarcoma in preclinical studies. However, only small cell lung cancer has clinical data showing increased efficacy of PARP inhibitors with high *SLFN11*

expression. SLFN11 could be used as a biomarker for PARP inhibitor sensitivity in small cell lung cancer with more testing. There is also preclinical evidence for the use of PARP inhibitors in castration resistant prostate cancer without *BRCA1* or *BRCA2* mutations. It may be possible to induce “BRCAness” using androgen receptor inhibitors to sensitize prostate cancer to PARP inhibitors. However, there is no clinical data on this treatment.

Realistically, there is not enough evidence clinically for the use of PARP inhibitors to treat cancers without *BRCA 1* or *BRCA 2* mutations at this point. There are potential mechanisms and biomarkers for efficacy, but without good clinical data it is not a possible treatment option. It is also unlikely that there will be many more clinical trials soon, because the treatment is not tolerated well. There are many adverse effects associated with all PARP inhibitors. Niraparib, Olaparib, and Rucaparib are all associated with anemia, gastrointestinal issues, and fatigue. Rucaparib use has also resulted in elevated creatinine in patients, which could indicate renal toxicities. These toxicities are similar to toxicities from chemotherapy and other targeted cancer therapies, but they are still harmful to patients and should be considered. PARP inhibitors have similar adverse effects in the cancer types they are approved for currently, but in those cases the effectiveness of the treatment outweighs the adverse effects. The treatment is not very effective in clinical trials for cancers without *BRCA1* and *BRCA2* mutations. The one trial of PARP inhibitors for Ewing sarcoma only had a median progression-free survival of 5.7 weeks. This is not a promising result, and the researchers didn't continue testing the drug due to the poor results. Overall, the clinical

data for the use of PARP inhibitors in cancers without *BRCA1* or *BRCA 2* mutations is not promising.

Despite the poor clinical data, the preclinical studies finding new biomarkers and mechanisms for PARP inhibitors are still important. It is worth testing new potential new uses of an existing drug to see if there are other contexts that it can be used in.

Targeted therapies, including PARP inhibitors, have provided more treatment options for patients and have improved patient outcomes. Continuing to test new targeted therapies and new uses for existing targeted therapy is important for finding new ways to treat cancer.

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