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The PARP inhibitor: the promises and challenges of targeted breast cancer therapy

Breast cancer is a complex heterogenic disease. It presents many different tumor phenotypes, and the progression of each tumor differs by the individual. Advances in breast cancer chemotherapy treatment over the past decade have improved survivorship through the application of biological research. With the end goal of selectively killing cancerous tumor cells in the body, novel targeted treatments have provided better survival with less damage to the body. As more biochemical research about cancer biology becomes unveiled, translational studies can deliver this information to the clinic and provide even better outcomes for patients.

In reviewing literature regarding targeted breast cancer therapies, there are multiple approaches to breast cancer chemotherapy treatment. One approach has the breast tumor biopsied and the cells are identified by hormone receptor and grading of tumor. The treatment is then designed around the termination of that specific type of tumor. For example, the use of monoclonal antibodies that modulate targets expressed at the cell surface of tumor cells including EGFR and HER2 (Sliwkowski et al.) has shown to perform well in the clinic, and these hormone positive breast cancers are generally less likely to relapse after treatment. For more aggressive breast cancers such as BRCA1/2 mutated cancer and triple negative breast cancer, prognosis is not as favorable and more targeted therapies need to be investigated.

To further understand the differences in subtypes of breast cancer, research has unveiled underlying genetic associations with different tumors. Additionally, there are polymorphic

variants associated with breast cancer susceptibility and pharmacologic response (Lymberis et al.). These genetic variants can be researched to unveil targeted therapies. For example, identifying BRCA1/2 functional loss in BRCA1/2 mutated tumor cells sparked research into the ability to selectively target these tumor cells by their dependency on base excision repair. Poly ADP-ribose polymerase (PARP) is an enzyme involved in base excision DNA repair in BRCA1/2 mutated cells, and thus this protein has been targeted for drug development. This case study of PARP inhibiting drugs highlights one novel approach to targeting breast cancer tumors using synthetic lethality. By understanding the PARP inhibition drug mechanism, its effectiveness in the clinic, and its challenges, the overall significance of this treatment can be summarized and evaluated for breast cancer patients moving forward.

PARP inhibition drug mechanism

Poly ADP-ribose polymerase (PARP) is an enzyme found to be involved in DNA repair. It is currently being targeted by inhibiting drugs that can then selectively kill tumor cells expressing PARP. Structurally, PARP-1 has 6 domains: Zn1, Zn2, Zn3, AD, WGR, and CAT (catalytic domain) (Langelier et al.). It exists as an extended monomer in solution in the absence of DNA and compacts on binding to damaged DNA. After binding to damaged DNA, PARP activates Poly-ADP ribose (PAR) synthesis. PAR then covalently attaches to target proteins that mediate gene transcription, DNA damage repair, and cell death signaling. Mutating the PARP-1 protein at key domain interfaces shows that certain domains alter DNA dependent activity (Langelier et al.). X-ray crystallography of PARP bound to DNA doubled stranded breaks reveal that Zn1, Zn3, and WGR-CAT are essential for PARP-1 activity. PARP-1 contacts the CAT domain through a network of interdomain communication that acts on the HD (helical subdomain of CAT). PARP-1 domains contact the ribose-phosphate backbone of DNA and

recruit the break of DNA through hydrophobic interactions with the exposed nucleotide bases. Specifically, Zn1 binds to DNA using two conserved regions termed backbone group and base-stacking loop. Zn3 binds to DNA adjacent to Zn1 using its N-terminal alpha helical region to span the minor groove. Overall, these DNA dependent regulatory domains work to distort the HD (helical subdomain of CAT) hydrophobic core to initiate binding to breaks in DNA and NAD+, an abundant coenzyme that enables PAR synthesis. This catalytic component of PARP is fundamental for design of PARP-1 inhibitors.

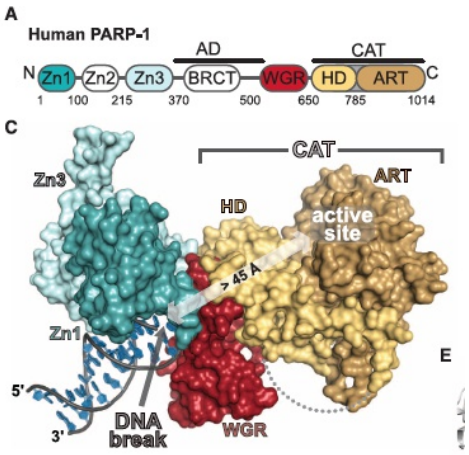

Figure 1. Structure of PARP-1 (Langelier et al.).

Because of the chemistry of PARP-1 and its affinity for binding to single stranded breaks in DNA, it was concluded that PARP-1 enzyme is involved in DNA repair. It signals DNA damage by its ability to recognize and rapidly bind to DNA single strand breaks (Lee et al.). It has been found to mediate base excision repair (or BER) by recruiting other proteins such as XRCC1, DNA ligase III, and DNA polymerase B. This DNA-bound activated PARP-1 recruits these proteins by using NAD+ as a substrate to polyADPribosylate nuclear target proteins and the site of DNA damage. This polyADPribosylation signals the need for both DNA single break and double break repair. Thus, by inhibiting PARP-1 activity, DNA repair does not occur, which leads to probable cell death for a given tumor cell.

This strategy has been seen to work particularly well in cells with a loss of BRCA1/2 function. Since PARP-1 is the rate-limiting enzyme in BER (Farmer et al.), it is a quality target for developing an inhibiting drug and potentially treating BRCA mutated cancer patients. The combination of BRCA function loss, dependency on BER repair, and PARP inhibition results in synthetic lethality. Synthetic lethality occurs because the HR deficient cells lose the ability to repair with either HR or BER. Because PARP inhibitors induce the loss the BER capacity, they also enhance the ability of DNA damaging cytotoxic agents. Many combination therapies are being explored for patients with BRCA1/2 mutations and associated cancers.

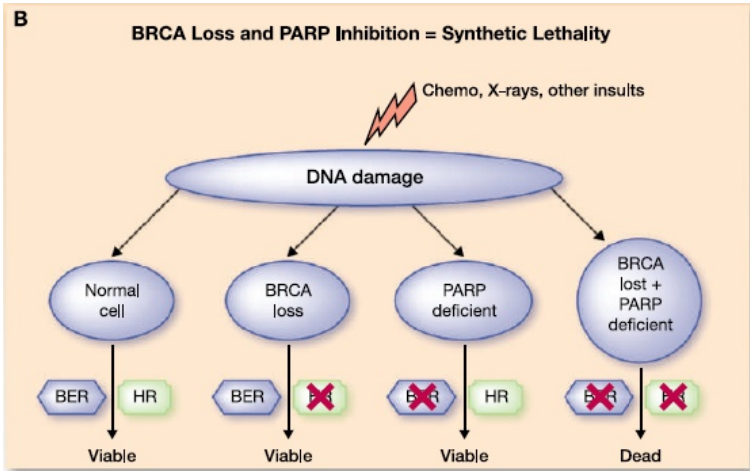

Figure 2. Synthetic lethality (Ford et al.)

Current PARP inhibitors involved in clinical trials

Several inhibitors were moved to clinical trials treating breast and ovarian cancer patients. There are potential lead drug molecules such as molecules that would disrupt the DNA binding affinity to PARP-1 by altering its binding site or that would compete for the active site by introducing a substrate similar to NAD+. Most PARP inhibitor chemistry that has been investigated includes NAD+ mimetics, and there are primarily six agents under study that alter effectiveness of PARP (See Figure 3, Lee et al.).

Iniparib is a unique inhibitor that targets the PARP zinc binding domains. When moved to the clinic in a Phase II trial, Iniparib was used to treat sporadic triple-negative advanced breast cancer. It had an impressive median overall survival for a metastatic cancer trial with patients surviving 9.2 months with treatment over 5.7 months (Ford et al.). This decision to move to clinical trials was based on testing different cancer cell lines in vitro with iniparib and tracking PARP activity. However, controversy over whether this PARP inhibitor was effective came to the attention of researchers when iniparib was launched further into later phase clinical trials and failed in a Phase III trial (Ledford). One study then again compared the effects of Iniparib to other NAD-like compounds in vitro. Iniparib, which is hypothesized to oxidize the zinc finger of PARP rather than compete competitively like NAD+-like compounds, did not show significant activity against PARP in functional enzyme assays whereas the use of NAD+-like inhibiting compounds showed single-digit nanomolar IC50 values for PARP (Liu et al.). This preclinical data conflicts with the previous data that confirmed the potency of iniparib for clinical use. The timeline of this drug development shows the importance of well-validated published preclinical data to secure the efficacy of a drug before moving to the clinic. Other inhibitors being researched, however, show robust preclinical data and as a result have been moved to more phase III clinical trials.

For example, Olaparib, a NAD-like resembling drug, is currently being investigated in a phase III clinical trial supported by Myriad genetics and AstraZeneca (Myriad Genetics). Previous preclinical trials and phase I and II clinical trials have shown significance and valid results for this drug (Fong et al.). Olaparib has also been researched in combination with other chemo toxins. For example, one study that looked at the effect of carboplatin and paclitaxel with the addition of Olaparib in patients with metastatic triple negative breast cancer showed

improvement in tumor reduction results (Ford et al.). Olaparib will continue to be investigated in phase III trials to determine its impact for the clinic.

One of the newest PARP inhibitors under investigation is BMN 673. As the strongest competitive inhibitor of PARP seen to date (Shen et al.), this drug is currently being tested for pharmakinetic properties in vivo and has been moved to phase I clinical trials to detect safety and efficacy. Because of its ability to inhibit PARP at lower concentrations (IC_{50} = 0.57nmol/L compared to Olaparib at IC_{50} = 1.94 nmol/L), BMN 673 shows promise as a more targeted PARP inhibitor and the potential for more tolerable dosage for patients.

Table1. Active PARP is under development

PARPi	Treatment	Cancer types	Phase
Olaparib (AstraZeneca)	-Monotherapy -Combinations with cytotoxic chemotherapy -Combinations with targeted agents -Combinations with RT	$BRCA1/2^{MUT+}$ associated BrCa/OvCa, BRCA-like tumors, Advanced hematologic malignancies and solid tumors, Maintenance study following remission in platinum sensitive OvCa (pending)	I/II/III
Veliparib (Abbott)	-Monotherapy -Combinations with cytotoxic chemotherapy -Combinations with targeted agents -Combinations with RT	$BRCA1/2^{MUT+}$ associated BrCa/OvCa, BRCA-like tumors, Advanced hematologic malignancies and solid tumors	I/II
BMN 673 (BioMarin)	- Monotherapy	Advanced hematologic malignancies and solid tumors	I
Rucaparib (Clovis)	-Monotherapy -Combinations (carboplatin)	Advanced solid tumors, Recurrent OvCa, $BRCA1/2^{MUT+}$ associated BrCa/OvCa	I/II
CEP-9722 (Cephalon)	-Monotherapy -Combinations with cytotoxic chemotherapy	Advanced solid tumors	I
Niraparib (MK-4827) (TesaroBio)	-Monotherapy -Combinations (temazolomide)	Advanced hematologic malignancies and solid tumors, $BRCA1/2^{MUT+}$ associated and HER2 negative BrCa, Maintenance study following remission in platinum sensitive OvCa (pending)	I/III

*OvCa, ovarian cancer; BrCa, breast cancer; RT, radiation therapy.

Figure 3. Current PARP inhibitors and clinical trials (as of April 2013) (Lee et al.).

Challenges to PARP inhibitors

While the synthetic lethality model and select promising inhibitors provide a targeted approach to attack tumor cells, there are challenges in translating PARP inhibitors to the clinic.

These challenges range from the detailed mechanism of the drug to the anti-tumor effects in a given patient. More importantly, identifying a targeted population that is sensitive to this PARP inhibitor treatment is central to the efficacy of these PARP inhibitors.

First, the biological mechanism of PARP needs to be further investigated. Recently, a study published potential mechanism of resistance because it has been noted that not all BRCA1/2 cell lines respond to PARP inhibition (Fong et al). Resistance mechanisms challenge the directness of synthetic lethality for tumor cells. These potential resistance mechanisms include: the loss of PARP1 expression, the up regulation of PgP transporter and thus the loss of PARP1 from cells, and restoration of homologous recombination that was initially not functional due to BRCA1/2 truncation or mutation (Lord et al., see Figure 4).

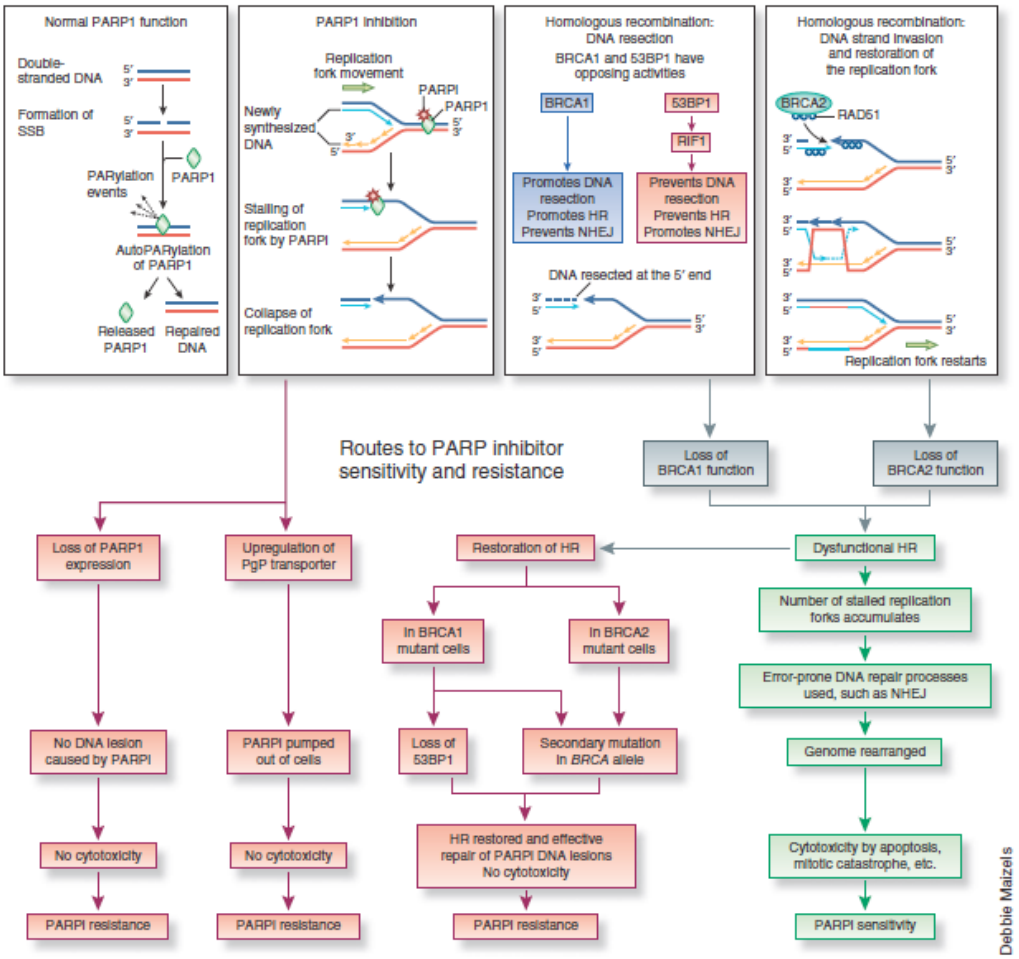

Figure 4. Mechanisms of PARP inhibition resistance (Lord et al.)

This resistance highlights the complexity of cancer polymorphisms and the trends associated with mutation in these DNA repair genes. While mutations can be detected, their phenotypic expression, in this case HR deficiency, depends on unique factors of the individual and thus treatment populations can be difficult to isolate. Restoration of HR is one particular example of how a given BRCA1/2 mutated patient could be resistant to PARP inhibition treatment. Therefore, rather than using the mutation status of a patient to validate PARP inhibition treatment, the “BRCAness” and biomarkers of the deficiency of HR need to be determined.

Summa et al. evaluate the potential of biomarkers in "BRCAness: a deeper insight in to basal-like breast tumors" (Summa et al.). This article highlights the commonalities between BRCA-related tumors and basal-like breast cancers. In order to find biomarkers for "BRCAness" tumors, there needs to be a consensus on what tumors have similar identities. Again, even within a subtype such as basal-like and BRCA1/2 mutated, the heterogeneity of breast cancer presents a challenge for defining populations.

Molecular subtypes	Receptor status	Enriched gene cluster
Luminal A	ER+/PR+/HER2+	**Adherens junction** *Apoptosis* *Insulin signaling pathway* *Renin-angiotensin system* *Androgen receptor signaling* **Calcium signaling pathway** *Estrogen receptor signaling* **Wnt signaling pathway**
Luminal B	ER+/PR+/HER2+	**Focal adhesion** *Cell motility* *Cell cycle* **p53 signaling pathway** *DNA replication and repair* **Insulin signaling pathway** *Estrogen receptor signaling* **B-cell receptor signaling**
HER2-enriched	ER-/PR?/HER2+	*TGF-β signaling* **EGFR pathway** *PTEN cell cycle arrest and apoptosis* **Cell cycle** **DNA replication and repair**
Basal-like	ER-/PR-/HER2-	*Cell cycle* **p53 signaling pathway** *DNA replication and repair* **Androgen and estrogen metabolism** **Fatty acid metabolism** **Insulin signaling pathway** **Estrogen receptor signaling** **PTEN cell cycle arrest and apoptosis**
Normal-like	ER−/PR+/HER2+	*Focal adhesion* *Cell motility* **Cell cycle** *p53 signaling pathway* **DNA replication and repair** *Estrogen receptor signaling* *mTOR signaling pathway* *TGF-β signaling*

Figure 5. Breast cancer molecular subtypes (Summa et al.)

In Summa et al., researchers classified different molecular subtypes of breast cancer by their hormonal status and associated molecular pathways (see Figure 5). Through DNA microarray, researchers subdivided breast cancer tumors into six major definitions: luminal A, luminal B, HER2-enriched, basal-like, normal-like, and claudin-low. Luminal A is positive for ER and PgR. Basal-like (BLBC) tumors have many shared features with BRCA-associated tumors (Foulkes, WD et al.) including impairment of double-strand break repair through HR leading to genomic instability. This loss of HR activity could be a viable biomarker for both of these tumor types. In addition, the basal-like tumors have over expression of basal markers (cytokeratines 5 and 8) and enrichment of genes involved in proliferation, DNA damage, and cell cycle checkpoint. Within basal-like tumors, triple negative breast cancers still lack clear markers and distinguishing between the different triple negative breast cancers also presents a challenge. However, studies investigating PARP inhibition within this subtype of breast cancer have shown increased sensitivity to this treatment.

For example, Alli et al. developed assays to research the sensitivity to ODD (oxidative DNA damage) and IQD PARP inhibitor in different breast cancer subtypes. They noted decreased BER activity in basal-like and BRCA1-mutated or deficient breast cancer cell lines using a novel assay for in vivo BER. By showing that triple-negative breast cancer cells are deficient in BER of ODD, this study suggests the use of PARP inhibitors in triple-negative breast cancers has potential for treatment. They then compared the sensitivity of PARP inhibition in different cell lines. First, BRCA mutated cells were compared to BRCA wild type cells; they found a 2.7-fold increase in sensitivity to IQD PARP inhibitor when comparing loss of BRCA1 to BRCA1 wild-type cells. Then, they looked at basal-like cell lines compared to luminal breast cancer cells. Basal-like cells were 6.7-fold more sensitive to IQD PARP inhibition compared with the luminal

breast cancer cell lines (see Figure 6, Alli et al.). However, it is important to note that there were differences in PARP sensitivity seen between the basal-like cell lines. While overall PARP sensitivity is increased in basal-like cells, the range of sensitivity highlights the need for more research on the factors that make these basal-like cells more sensitivity to PARP. Again, this finding relates the heterogeneity of breast cancer even within a subtype.

Table 2. IQD sensitivity in breast cancer cell lines

Cell line	IQD IC_{50} (μmol/L)	*P*
Luminal		
MCF7	450 ± 58	
BT474	550 ± 71	
T47D	325 ± 177	
Basal-like		0.0007*
BT549	95 ± 95	
HCC38	100 ± 0	
HCC1143	20 ± 0	
HCC1806	39 ± 49	

NOTE: Breast cell lines of the luminal or basal-like subtype as indicated were analyzed for IQD sensitivity by MTT assay. The average IC_{50} values determined from the dose-response curve of at least two independent experiments are shown as an average ± SE.
*$P < 0.01$.

Figure 6. PARP inhibitor sensitivity in luminal and basal-like breast cancer subtypes (Alli et al.)

Finding similarities between tumor types could unlock potential biomarkers and clearer definition for treatment subtypes. For BLBCs and BRCA-1 associated tumors, the IHC signature unveils lack of ER, HER2, and overexpression of cytokeratin (CK 5/6) and EGFR. As Sana et al. shows, BLBC have BRCA1 expression and enzymes involved in BER pathway in MCF-7 and T47D cell lines. This BRCA overexpression leads to two-fold increase in mRNA levels of OGG1 and NTHL1 of REF1/APE1, which is involved in processing of abasic sites and of the scaffold protein XRCC1. Also, 11-13% of BLBCs show BRCA-1 promoter methylation and ID4 transcriptional repressor of BRCA1. Also supporting this similar methylation pattern, Jacot et al. showed the analysis of PARP-1 activity, BRCA1 promoter methylation, and 53BP1 expression in

tumors without known BRCA1 mutation. The expression levels of BRCA in cancer tumors other than BRCA mutated tumors suggests that mutation status may not be the best way to distinguish breast cancer subtype or narrow PARP inhibition treatment.

Summa et al. suggests several potential markers that have potential for future studies on biomarkers that determine PARP inhibitor sensitive tumors. For example, more TP53 mutations were found in 90% of the BRCA1-mutated tumors and 95% of BLBCs than the luminal subtype (Holstedge). In addition, the expression of RAD51 is elevated in both subtypes (Lehmann). Actual PARP expression can also be investigated; only 7% of BRCA1 associated breast cancers do not exhibit nuclear expression of PARP1. Other biomarker targets include APE1 (a BER enzyme that is alternate to PARP) and MGMT (an enzyme of single-step mechanism of repair). IHC studies show that low expression of MGMT is associated with lack of HR and poor prognosis. Overall, there are number of expression markers that could be researched in tumor tissues from both BLBC and BRCA-1 mutated breast cancers to find if there exists a strong commonality between potential targeted cells.

Given the heterogeneity and nature of aggressive breast cancers, defined biomarkers relating the responsiveness to PARP inhibitors are the next step in developing targeted treatments. For example, a quantitative measure of HR deficiency could be used to determine the efficacy of a PARP inhibitor on a given tumor subtype. For now, however, the effectiveness of PARP inhibitors against various tumors and already defined subtypes such as triple negative breast cancer can be researched in clinical trials (see figure 7, Summa et al.). While confirmed mutation carriers are more sensitive to this treatment and it is difficult to fully differentiate between patient populations, preclinical data suggests that other patients with basal-like tumors could benefit from the treatment as well.

Table 2. Clinical trials (completed and ongoing) investigating PARPi in TNBCs

Title	Clinicaltrial.gov id	Phase	Intervention	Status	Primary outcome measures
A phase III, multicenter study of gemcitabine/carboplatin, with or without BSI-201, in patients with ER-, PR-, Her2-negative metastatic breast cancer	NCT00938652	III	Gemcitabine/carboplatin plus BSI-201	Completed	PFS
A phase II trial of standard chemotherapy, with or without BSI-201, in patients with triple-negative metastatic breast cancer	NCT00540358	II	Gemcitabine/carboplatin plus BSI-201	Completed	Clinical rate benefit
PARP inhibition for triple-negative breast cancer with BRCA1/2 mutations	NCT01074970	II	Cisplatin plus rucaparib following preoperative chemotherapy	Recruiting	2-year DFS
AZD2281 plus carboplatin to treat breast and ovarian cancer	NCT01445418	I	AZD2281 plus carboplatin	Recruiting	Safety and toxic effect
Phase I of BKM120/Olaparib for triple-negative breast cancer or high-grade serous ovarian cancer	NCT01623349	I	BMK120 plus Olaparib	Not yet open	To determine MDT
A phase II study of standard chemotherapy plus BSI-201 in the neoadjuvant treatment of triple-negative breast cancer	NCT00813956	II	Gemcitabine plus carboplatin plus BSI-201	Recruiting	Pathologic complete response
Study of SAR240550 (BSI-201) in combination with Gemcitabine/carboplatin in patients with metastatic triple-negative breast cancer	NCT01045304	II	Gemcitabine/carboplatin plus iniparib	Ongoing but not recruiting	ORR as CR and PR
ABT-888 with cyclophosphamide in refractory BRCA-positive ovarian, primary peritoneal or ovarian high-grade serous carcinoma, fallopian tube cancer, triple-negative breast cancer and low-grade non-Hodgkin's lymphoma	NCT01306032	II	ABT-888 plus cyclophosphamide	Ongoing but not recruiting	CR + PR
Phase II study of AZD2281 in patients with known BRCA mutation status or recurrent high-grade ovarian cancer or patients with known BRCA mutation status/triple-negative breast cancer	NCT00679783	II	AZD2281	Ongoing but not recruiting	ORR
Study to assess the safety and tolerability of a PARP inhibitor in combination with carboplatin and/or paclitaxel	NCT00516724	I	AZD2281 plus carboplatin and/or paclitaxel	Ongoing but not recruiting	MTD

Data from www.clinicaltrials.gov (update 26 July 2012).
PFS, progression-free survival; DFS, disease-free survival; MDT, maximum dose tolerated; ORR, objective response rate; CR, complete response; PR, partial response.

Figure 7. PARP inhibitor clinical trials in triple negative breast cancer patients. (Summa et al.)

Future directions for PARP inhibitors

Finding molecular markers for "BRCAness" is imperative to validate and improve the clinical use of PARP inhibition. Patients with confirmed BRCA1/2 mutations as well as sporadic breast cancers could be treated in a more targeted approach if these biomarkers are better understood. Furthermore, the patient population could be expanded for clinical trials. These biomarkers could also help researchers predict the course of a given cancer and ultimately lead to preventative strategies for breast cancer. As Vinayak and Ford reveal, these PARP inhibitors have the potential for chemoprevention therapy (Vinayak et al.). Currently, when a woman receives a BRCA genetic test in the clinic and finds out she has a high-risk mutation, her options are limited to watchful waiting or prophylactic surgery. Given the promise of PARP inhibitors, chemoprevention therapies could prove to be especially useful for high-risk patients with known BRCA1/2 mutations.

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