Strategies in Overcoming Homologous Recombination (HR) Proficiency and Poly (ADP-Ribose) Polymerase Inhibitor (PARPi) Resistance

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Conflicts of Interest statement: Dr. Rebecca Arend is on the advisory boards for Merck, GSK, Caris, Clovis, Astra Zeneca, Leap Therapeutics. The other contributing authors have no conflicts of interest.
Ovarian cancer is the second most common gynecologic malignancy in the United States (US) and the most common cause of gynecologic cancer-related death. The majority of ovarian cancers ultimately recur despite excellent response rates to upfront platinum and taxane-based chemotherapy. Maintenance therapy after frontline treatment has emerged in recent years as an effective tool for extending the platinum-free interval of these patients. Maintenance therapy with poly (ADP-ribose) polymerase inhibitors (PARPi) in particular has become part of standard of care in the upfront setting and in patients with platinum-sensitive disease. HR deficient (HRD) tumors have a nonfunctioning homologous recombination repair (HRR) pathway and respond well to PARPi, which takes advantage of synthetic lethality by concomitantly impairing DNA repair mechanisms. Conversely, patients with a functioning HRR pathway, i.e. HR proficient (HRP) tumors, can still elicit benefit from PARPi, but the efficacy is not as remarkable as what is seen in HRD tumors. PARPi are ineffective in some patients due to HR proficiency, which is either inherent to the tumor or potentially acquired as a method of therapeutic resistance. This review seeks to outline current strategies employed by clinicians and scientists to overcome PARPi resistance – either acquired or inherent to the tumor.
I. Introduction

Gynecologic malignancies are among the leading cause of cancer-related death in women in the United States (US) with ovarian cancer being the deadliest. The American Cancer Society (ACS) predicts that over 21,000 women will be diagnosed with ovarian cancer in 2020 in the United States (US), and approximately 14,000 women will die from their disease [1]. Historically, patients with HGSOC have been treated with a combination of surgical cytoreduction with platinum- and/or taxane-based chemotherapy. Despite promising initial responses to therapy that include complete responses (CRs), approximately 80% of women experience disease progression or recurrence [2]. For this reason, further research into superior treatment options for these patients is of necessary.

Poly (ADP-Ribose) Polymerase (PARP) proteins are involved in the repair of both single- and double-stranded DNA breaks (SSB and DSB) through several repair pathways, including mismatch repair (MMR), nonhomologous end joining (NHEJ), and nucleotide excision repair (NER) [3]. Notably, PARP proteins are integral to base excision repair in which a single misplaced base is exchanged with the correct base (Figure 1). In cells with a non-functional homologous recombination (HR) repair pathway, PARP-mediated base excision is utilized for cell survival. Inhibiting these pathways via PARP inhibition (PARPi) causes an accumulation of SSBs that eventually stall replication forks and prohibit replication, causing an accumulation of DSBs. The failed repair of DSBs due to HRD can result in insurmountable DNA damage that ultimately leads to cancer cell death. [4]. HRD can arise from mutations in the tumor suppressor genes BRCA1/2, and other genes involved in the HRR pathway, including ATM, CHEK2, BRIP1, RAD51C, and PALB2.

Once it was discovered that cells with mutations in BRCA1/2 had increased sensitivity to PARPi, [5], the concept of synthetic lethality quickly became the focus of many preclinical and clinical
studies. Synthetic lethality occurs when cells that are genetically predisposed to the inactivation of one pathway are targeted by the intentional inactivation of a second pathway, whereas the inactivation of either pathway alone would not be enough to kill the cell [6]. The SOLO1 trial (NCT01844986) led to the Food and Drug Administration (FDA) approval of a PARPi, olaparib, for upfront maintenance therapy in BRCA-mutated patients and demonstrated an unprecedented improvement in progression free survival (PFS) of 36 months compared to placebo [7]. Similarly, the SOLO2 trial evaluated the use of maintenance olaparib in recurrent, platinum sensitive, BRCA1/2-mutated ovarian cancers and demonstrated an improvement of PFS in the olaparib group. The recently published SOLO2 data showed a benefit of 13 months in OS, further proving the efficacy of maintenance PARPi in this patient population [8]. Additionally, niraparib maintenance therapy has now been approved in the frontline setting regardless of BRCA or HR status based on the PRIMA trial (NCT02655016), although the magnitude of benefit is not as great in the HR proficient (HRP) population [9]. Three PARPi’s (niraparib, olaparib, and rucaparib) are currently approved for use in the maintenance setting of treatment for platinum sensitive HGSOC.

II. Mechanisms of PARPi Resistance

Despite the revolutionary impact of PARPi on the HRD population, PARPi resistance (PIR) has been observed in many patients [10]. Among those mechanisms of resistance studied includes the upregulation of drug efflux pumps such as p-glycoprotein. This upregulation was seen after treatment of BRCA-1 deficient breast cancer cells in mice that developed PARPi resistance [11]. This mechanism of resistance through drug efflux is also a commonly described mechanism of resistance to platinum-based chemotherapy through a variety of transporters including CTR1, CTR2, ATP7A, and ATP7B [12].
The vast majority of PIR mechanisms revolve around the restoration of HRR in cells that are deficient. Previous studies have shown that the restoration of wild-type BRCA1/2 (wtBRCA) phenotype in patients whose tumors harbor BRCA1/2 mutations – either through secondary mutations or epigenetic modifications – can cause significant PIR [13, 14]. One study showed that nearly 50% of platinum-resistant ovarian carcinomas had a secondary mutation that restored the wt-BRCA gene and thus HR function [13]. Other HR gene mutations have been shown to be reversed by secondary mutations, including PALB2, RAD51C, and RAD51D. In fact, this mechanism of resistance via restoration of HR has been shown to play a role in the resistance of ovarian cancer cells to platinum-based chemotherapy as well [15]. Particular sensitivity of ovarian cancer cells with HRD to platinum-based chemotherapy has been described, as it allows for the accumulation of DNA DSBs that are rendered irreparable by the lack of HR functionality. Subsequently, the theoretical resistance to platinum-based therapy as a result of regaining HR function has been proven true as seen by BRCA reversion mutations in patients with platinum-resistant ovarian cancer [16]. This overlap in resistance allows for the further evasion of HGSOC from advanced therapy.

An additional mechanism of PIR that has been described is the rewiring of HRR and the DNA damage response by replication fork protection. HR proteins such as BRCA1/2 function to prevent replication fork stress and therefore protect genomic stability [17]. Resistant cells have been shown to bypass the loss of fork protection through mechanisms like the downregulation of MRE11 or loss of CDH4 [18]. Since restoring replication fork protection is independent of DNA repair, combination therapy targeting this mechanism could result in synergistic lethality with PARPi.

Downregulation of PARP1 proteins is another classically described mechanism of PIR. During PARPi treatment, the cytotoxicity that occurs as a result of PARP1-trapped DNA complexes can
lead to a depletion of PARP protein expression rather than cell death [19]. Moreover, a clinical case of PIR has been seen as a result of a \textit{PARP1} mutation [20]. The loss of \textit{PARP1} protein in cancer cells was only found in patients with either some or all of the functioning HR pathway. Thus, it can be conferred that patients with complete loss of HR do not show this mechanism of PARPi resistance.

According to the Cancer Genome Atlas (TCGA), approximately half of ovarian cancers are considered to be HRP [21]. Additionally, PIR occurs in over 50% of patients with ovarian cancer, which poses a question of how clinicians can overcome both acquired and \textit{de novo} HRP. These tumors have an overall worse response to platinum-based chemotherapy and dismal survival outcomes, as shown by a worse OS in \textit{wtBRCA} cases compared to \textit{BRCA}-mutated cases (median OS ~40 months vs ~60 months, respectively) [22]. Because of the unmet need to improve outcomes in HRP patients, many studies are actively trying to better understand ways to “switch” an HRP tumor to have an HRD phenotype. This review focuses on research regarding the utilization of various targeted agents (Tables 1 and 2) to sensitize HRP ovarian cancers to PARPi or re-sensitize HRD patients that have become resistant to PARPi.

III. ATR inhibitors

Historically, ataxia telangiectasia Rad3-related (ATR) is known to be a major regulator of a cell cycle checkpoint signaling pathway controlled by checkpoint kinase 1 (Chk1). This pathway functions during the G2/M phase of the cell cycle to recognize DNA irregularities and induce cell cycle arrest for DNA repair [23]. Beyond its role in the Chk1 pathway of DNA repair, ATR has also been implicated as a regulator of several proteins in the HRR pathway, including activation of \textit{BRCA1}, \textit{PALB2}, and \textit{RAD51}, which suggests the potential for mechanistic synergism between ATR inhibitors (ATRi) and PARPi [24]. In fact, the use of ATRi has been shown to sensitize both HRP and HRD ovarian cancer cells to the use of PARPi in preclinical models [25].
Several clinical trials have explored this combination treatment, including an ongoing phase I trial investigating the combination therapy of cisplatin, veliparib, and VX-970 (ATRi) in the treatment of refractory solid tumors (NCT02723864).

ATRi have also been found to impair replication fork protection, a known mechanism of PIR. They target this mechanism by inhibiting Rad51-loading onto stalled replication forks in HRD cells, subsequently subjecting the cell to synthetic lethality induced by PARPi [26]. The ATRi VE-821 has been shown to enhance the degradation of stalled replication forks using ex vivo DNA fiber analyses. Unfortunately, this effect was not seen in cells with wtBRCA proteins, showing that the use of ATRi may not extend to innately HRD tumors [27].

Previous studies have demonstrated a relationship between PARPi response and the expression of SLFN11, a gene involved in cell-lethal replication inhibition [28]. By prolonging cell cycle arrest and inducing replication fork damage, high SLFN11 expression can cause hypersensitivity to PARPi [29]. PIR was further demonstrated after the SLFN11 gene was inactivated in preclinical models [28]. These cells were no longer able to go through PARP trapping at prolonged cell arrest points and thereafter increased the reliance on ATR checkpoints to promote cell survival [28]. Using this data as a foundation, ATRi could be of particular use for PARPi sensitization in cancer cells with PIR.

IV. PI3K inhibitors

The phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K) pathway has been heavily investigated given its multiple roles in cancer progression. Aberrations in this pathway can contribute to both the initial development and the survival of ovarian cancer cells, providing an opportunity for targeted therapies. Despite this, there has been minimal clinical benefit seen when treating EOC patients with single-agent PI3K inhibitors (PI3Ki) [30]. In contrast, there is an
abundance of preclinical data supporting the synergism between PARPi and PI3Ki as PI3Ki can increase the antimetabolic effects of PARPi [31]. One study showed that PI3Ki caused the cessation of DNA synthesis as well as cytoskeletal functions, processes that are further exacerbated by PARPi. Utilizing mouse models of mammary epithelial cells, treatment with PARPi + PI3Ki resulted in a decrease in S-phase cell cycle progression due to impairment of nucleotide synthesis [32].

When considering the combinational use of PARPi and PI3Ki in the treatment of ovarian cancer, the crosstalk of the PI3K and HRR pathways must be taken into account. Beyond its mechanisms of cell cycle regulation and metabolic influence, PI3K helps control the repair of DSBs by acting as a sensor of genomic instability and detecting DSBs [33]. One preclinical study showed that PI3Ki resulted in the downregulation of BRCA1/2 and induced a HRD phenotype in the cell [34]. Additionally, a phase Ib study published in 2019 showed that combining the PI3Ki alpelisib with olaparib in recurrent HGSOC was safe; and potential efficacy was seen with an overall response rate of 33% [35]. Currently, there is a phase I clinical trial (NCT01623349) examining the oral PI3Ki’s, BKM120 and BYL719, in conjunction with olaparib in patients with HGSOC and triple-negative breast cancer (TNBC). Preliminary data from this trial has shown a 29% response rate in patients with wtBRCA1/2 tumors regardless of platinum sensitivity, recommending phase II studies to further examine this relationship [31]. The current data fully supports continued investigation of the clinical synergism between PI3Ki and PARPi.

V. Glutaminase inhibitors

The metabolic alterations in tumor cells have been thoroughly investigated, with particular focus on the adjustments that tumor cells make to enable their survival in hypoxic conditions. It has been demonstrated that cancer cells have a decreased ability for glucose to enter the tricarboxylic acid (TCA) cycle, which results in reliance on other carbon sources for the cell,
including glutamine [36]. This shift towards using glutamine is an integral part of the
development and progression of invasive and advanced ovarian cancer.

Previous data has shown that inhibition of glutaminase can cause an arrest in the growth of
HGSOC [37]. When exploring this mechanism as a potential cancer therapeutic target, one
must consider the role of phosphate-activated mitochondrial glutaminase (GLS1), a key enzyme
involved in glutamine metabolism that allows the conversion of glutamine to glutamate [38]. A
study investigating the effects of the GLS1 inhibitor compound 968 found that glutaminase
inhibition caused cessation of ovarian cancer cell proliferation and reduced the glutamine
metabolism for the sustainment of cancer cells [39]. This inhibitor also increased ROS
formation, cell apoptosis, and induced cell cycle arrest leading to tumor cell destruction. The use
of glutaminase inhibitors to sensitize ovarian cancer to PARPi is still being explored. Glutamine
is a necessary nitrogen source for nucleotide synthesis and subsequent DNA synthesis. Thus,
by inhibiting the metabolism of glutamine, the cell would experience a depletion of completed
nucleotides and would be unable to repair DNA effectively [40]. This buildup of DNA replication
stress can thereafter be exploited by PARPi in cancer cells. Okazaki et al. found synergism
between glutaminase inhibitors and PARPi in the treatment of renal cell carcinoma due to von
Hippel-Lindau (VHL) disease [41]. Currently, a phase I/II clinical trial is investigating the results
of treating metastatic solid tumors with a combination of the glutaminase inhibitor, CB-839, and
PARPi, talazoparib (NCT03875313). Additionally, an upcoming investigator-initiated clinical trial
at our institution will explore the efficacy of combining CB-839 with niraparib in the treatment of
platinum resistant, wtBRCA1/2 ovarian cancer (NCT03944902). This could provide insight as to
possible mechanism of overcoming HRP, although more research exploring the potential to
reverse this phenotype with glutaminase inhibitors is warranted.
VI. HDAC Inhibitors

HDAC Class 1 expression is prevalent in ovarian cancers and has been examined as a potential cause of resistance to platinum-based chemotherapy [42]. HDAC expression in HGSOC is associated with a poor prognosis and provides an opportunity for targeted pharmacotherapy. HDAC inhibitors (HDACi) have been shown to downregulate the transcription of wild-type HRR genes such as *RAD51*, leading to a further increase of irreparable DNA damage and providing an opportunity for combination treatment with PARPi [43].

The HDACi romidepsin has been shown to enhance the antitumor effects of cisplatin by further inflicting DNA damage [44]. In a study using the HDACi sodium butyrate, tumor radiosensitivity was enhanced due to the DNA damage caused by downregulation of HRR genes [43]. This theory was further explored by Konstantinopoulos *et al.*, who showed through preclinical microarray assays that vorinostat, also known as suberanilohydroxamic acid (SAHA), induced the downregulation of critical HRR genes *RAD51* and *BRCA* [45]. Currently, there is a phase I clinical trial underway looking at the combinational treatment of olaparib with vorinostat in the treatment of metastatic breast cancer (NCT03742245). Future work could explore this combination therapy in patients with wt*BRCA* or *BRCA*-mutated ovarian cancer resistant to PARPi therapy.

VII. Immune Checkpoint Inhibitors

The response of HGSOC to immune checkpoint modulators has been variable, usually seen only in patients with high microsatellite instability (MSI-H) [46]. For this reason, other agents such as PARPi have been explored to be used in conjunction with immunotherapy in the treatment of HGSOC. It has previously been demonstrated that HRD tumors are more sensitive to immune checkpoint blockade (ICB) therapies than HRP tumors [47]. In a preclinical study investigating the efficacy of treating ovarian cancer cells with a PARPi prior to the use of ICB
therapy, combining niraparib with full dose anti-PD-1 resulted in significant tumor growth inhibition compared to either drug used alone [48]. Although preclinical studies strongly suggest that the use of PARPi and ICB therapy is of clinical benefit in patients with HRD, the utility was not demonstrated in patients with HRP [47].

A single-arm phase I/II clinical trial (NCT02657889) investigating the clinical response of patients with HGSOC to both PARPi and ICB therapy using pembrolizumab demonstrated promising antitumor activity. In fact, even patients with a functioning HR pathway had higher antitumor activity than those treated with monotherapy niraparib or pembrolizumab [49]. These findings suggest the potential for clinical combination of ICB therapies and PARPi in the setting of HRP.

VIII. VEGFR and EGFR inhibitors

The vascular endothelial growth factor (VEGF) protein family consists of growth factors that promote increased vascularity and angiogenesis in response to hypoxic conditions. VEGF is induced by hypoxia inducible factors (HIFs) that are upregulated to allow tumors to thrive in a hypoxic environment, which plays a part in several diseases when over-expressed [50]. Various agents targeting VEGF have been clinically utilized in several malignancies with the intention of destroying the tumor vascular supply [51].

A phase II clinical trial investigated the combination of olaparib and cediranib, a potent inhibitor of VEGF receptors (VEGFR), in the treatment of platinum resistant ovarian cancers (NCT01116648). The combination treatment resulted in an increase in PFS compared treatment with olaparib alone [52]. Specifically, the added activity of cediranib increased tumor regression even in patients with wtBRCA1/2, suggesting that synergism induced by cediranib extends to HRP tumors [52]. Despite this, GY004 (NCT02446600), a recent phase III open-label clinical
trial that was designed to expand on NCT01116648, showed no significant improvement in the PFS of patients treated with the combination treatment compared to olaparib alone (regardless of HR status). This mechanism could be explained by data showing that VEGFR inhibition in ovarian cancer cells is associated with decreased expression of wtBRCA1/2 [52].

Furthermore, the epidermal growth factor receptor (EGFR/ERBB/HER) has been shown to be associated with accelerated tumor growth when overexpressed. The use of the EGFR inhibitor neratinib has been shown in preclinical studies to act in synergy with niraparib to accentuate ovarian cell death via exploitation of synthetic lethality to heighten levels of DNA damage [53]. This is being further explored in an ongoing Phase I/ Ib clinical trial utilizing the combination therapy in the treatment of platinum-resistant ovarian cancer (NCT04502602). Overall, the use of EGFR inhibitor therapy in combination with PARPi should be explored further to understand its use in the treatment of HR positive ovarian cancers and whether it can overcome that barrier of treatment.

IX. WEE1 inhibitors

Wee-1-like kinase (WEE1) is highly upregulated in certain cancers, including HGSOC, as well as glioblastoma, osteosarcoma, melanoma, and breast and vulvar carcinoma [54]. By targeting the G2-M checkpoint via WEE1 inhibition (WEE1i), tumor cells with dysfunctional G1-S checkpoints are selectively targeted and ultimately undergo cell death due to irreparable DNA damage [55].

In recent years, WEE1 has been linked to the HRR pathway via its interaction with DNA repair mechanisms, making it an interesting target to study in combination with PARPi. A study using the WEE1i AZD1775 in combination with gemcitabine-radiation in pancreatic cancer cells
showed that upon treatment with AZD1775 and chemoradiation, only HRP cells were sensitive
to chemoradiation [56].

One proposed mechanism for WEE1’s role in HRR is through nucleotide exhaustion and PARP
trapping. A previous study investigating the combination AZD1775 with olaparib found that only
KRAS-mutant non-small cell lung cancer (NSCLC) were more sensitive to radiotherapy than
cells treated with either agent alone [57]. Additionally, through experiments using nucleotide
depletion, it was observed that PARP1 trapping must be present for WEE1i to sensitize tumors
to radiotherapy. Although PARPi alone can radio-sensitize a tumor regardless of PARP1
trapping activity, the current theory for the synergy of WEE1i with PARPi is that the two agents
impair DNA replication at multiple time points [57]. More experiments must be done to further
characterize how WEE1i impacts the HRR pathway.

**X. BET inhibitors**

Similar to WEE1i, BET inhibitors (BETi) have been increasingly linked to the HRR pathway by
synergizing with PARPi via induction of DNA damage [58]. The BET family of proteins includes
BRD2, BRD3, BRD4, and BRDT, each with a conserved N-terminal bromodomain. One study
has shown that **BRD4** is a necessary factor for survival of ovarian cancer cell lines such as
OVCAR8 [59]. Additionally, it was observed that HGSOC tumors with **BRD4** amplifications may
derive the most clinical benefit from BETi [60]. This finding is particularly interesting as the
**BRD4** overexpressing subtype often do not harbor **BRCA1/2** mutations, rendering these
cancers limited in their treatment options.

**BRD4** expression has also been recognized as a component of PIR. In a study investigating
aldehyde dehydrogenase (ALDH) expression in ovarian cancer cell lines, cells with acquired
resistance to olaparib demonstrated increased activity in ALDH via elevated expression of
This increase in ALDH1A1 expression stems from BRD4 over-expression, which can be induced by exposure to olaparib. BETi has been shown to suppress the expression of both WEE1 and TOPBP, which sensitized wtBRCA1/2 cells to a PARPi. Additionally, BETi treatment was able to re-sensitize mBRCA2 cells with acquired olaparib resistance to PARPi [58]. Inhibition of BRD4 has also been shown to result in inhibition of ALDH1A1 expression, which assisted in overcoming PIR in HGSOC cells [61].

The BRD4 inhibitor INCB054329 caused decreased activity in both BRCA1 and RAD51 as well as HR reporter activity in an HRP ovarian cancer cell line, supporting its ability to induce an HRD phenotype [62]. Additionally, cell lines treated with the combination of INCB054329 and olaparib showed increased tumor cytotoxicity compared to cells treated with either agent alone [62]. A similar study demonstrated that the three BETi: JQ1, I-BET762, and OTX015 were able to sensitize HRP cells to PARPi [63]. Cumulatively, these findings suggest that the combination of BET and PARP inhibition warrants further investigation in HRP tumors.

XI. BCL2 Inhibitors

B-cell lymphoma 2 (BCL2) is a group of proteins that are key cell death regulators, making it a potential key player to target cancer cells [64]. The expression of BCL2 genes have been shown to be associated with increased chemotherapy resistance, specifically taxane and platinum-based therapies [65].

Synergy has been observed between rucaparib and the BCL2 inhibitor navitoclax – the combination therapy induced more apoptosis than navitoclax monotherapy [64]. Preferential apoptotic activity was seen in cells with mutations in HRR genes, indicating that cells with a dysfunctional HRR system may rely more heavily on the anti-apoptotic BCL group of proteins. Furthermore, Stover et.al. demonstrated the effectiveness of BCL2 inhibition in sensitizing
HGSOC cells to PARPi [65]. Lastly, the use of combination therapy with navitoclax and talazoparib in the treatment of three wt-BRCA HGSOC cell lines showed a greater cytotoxic effect than seen with monotherapy, suggesting a potential clinical benefit in HRP tumors [66].

Currently, an ongoing phase II clinical trial (NCT02591095) is seeking to determine survival of patients with platinum-resistant/refractory ovarian cancer while treated with single agent use of the BCL2 inhibitor ABT263. Further clinical trials are warranted investigating the clinical use of combinational therapy with BCL2 inhibitors and PARPi in the treatment of ovarian cancer.

XII. CDK1 inhibitors

Cyclin dependent kinase 1 (CDK1) is a key cell cycle regulator whose function is essential for cell proliferation [67]. It forms a complex with cyclins to regulate G1/S phase gene transcription, ultimately promoting cell cycle progression through the phosphorylation of cell cycle regulators. It has previously been demonstrated that CDK1 modulates the BRCA1 protein, and a preclinical study demonstrated the cessation of S phase checkpoint activation due to short hairpin RNA (shRNA)-mediated CDK1 depletion in TNBC [68]. This inhibition causes a decrease in the phosphorylation of cell cycle regulator, BRCA1, resulting in the inhibition of BRCA1-mediated foci formation at sites of DNA damage [68]. By disrupting BRCA1 function, it was theorized that CDK1 could inhibit not only cell cycle checkpoints, but other critical DNA damage repair pathways as well.

This theory was later investigated in a study which found that decreased CDK1-mediated BRCA1 phosphorylation was able to inhibit the HRR pathway [69]. Furthermore, CDK1 inhibition (CDK1i) resulted in an 80% decrease in RAD51 foci formation in an in vitro lung adenocarcinoma model. RAD51 function plays a critical role in the HRR pathway, and its decreased expression is associated with BRCA1-deficient cells [69]. This data demonstrated...
that CDK1i could inflict similar damage to both wtBRCA1/2 and mBRCA1/2 cells, and therefore leaving the cells susceptible to PARPi. As such, combining a PARPi with a CDK1i, such as dinaciclib, could induce synthetic lethality within HRP tumors.

An ongoing phase I trial combining dinaciclib and veriparib in the treatment of advanced solid tumors (NCT01434316) is investigating both wtBRCA and BRCA-mutated tumors to determine their sensitivity to PARPi with and without a CDK1i. More preclinical and clinical data are needed to determine the effect on PARPi sensitization using dinaciclib in HGSOC patients with and without functioning HRR.

XIII. Hsp90 inhibitors

Mutated BRCA proteins can cause defective protein folding, leading to an unstable secondary structure that ultimately subjects the protein to degradation. Heat shock protein 90 (Hsp90) is a chaperone protein responsible for the stabilization of proteins against this very mechanism. The stabilization of mBRCA proteins via Hsp90 can result in enhanced RAD51 loading onto stalled replication forks that bypass the HRR pathway [70]. The interaction of Hsp90 with BRCA proteins as a mechanism of PIR has been a recent focus of investigation in cancer therapeutics.

Beyond the potential for Hsp90 inhibitors (Hsp90i) to reverse acquired PIR in HRD tumors, preclinical studies have shown that this could be applied even to HRP cells. In a study exposing HRP breast cancer cells to the HSP90i 17-AAG, treatment resulted in decreased activity of the HRR pathway, ATM serine/threonine kinase, and Fanconi Anemia DNA repair pathways [71]. Treatment with 17-AAG also down-regulated wtBRCA1 and RAD51 levels, thus inducing an HRD state in initially HRP cancer cells. Most remarkably, the exposure of 17-AAG to HRP EOC cells sensitized the cells to olaparib and platinum-based chemotherapy, as measured by DNA damage using a γH2AX assay measurement [71]. This sensitization was not seen in EOC cells...
CONCLUSION

Targeting the HRR pathway in ovarian cancer through PARPi has been a paradigm-shifting treatment strategy over the past decade. However, approximately half of patients have a limited response to PARPi due to HRP. Therefore, there has been a focus of research in the last decade as to whether innate or acquired HRP can be overcome in order to induce PARPi sensitivity. Various agents have shown promise in this area either by working synergistically with PARPi or by targeting the HRR pathway through an alternate strategy, both of which have potential clinical benefit in patients with otherwise limited options. The pharmacotherapies mentioned in this review are currently under preclinical or clinical investigation for use in combinational treatment with PARPi to improve response rates and survival outcomes. A variety of clinical trials across the country have the goal of maximizing clinical benefit from PARPi and helping to overcome or prevent therapeutic resistance. Genetic and molecular data are rapidly being integrated into cancer care. These analyses have immense potential to improve patient outcomes by providing more targeted treatments that can be used in combination with PARPi. As the scientific and clinical communities continue to better understand the underlying genetics of the disease and how to manipulate mechanisms that repair DNA damage, we are optimistic that these approaches will ultimately improve the survival of women with ovarian cancer.

REFERENCES


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Table 1. Summary of pre-clinical studies utilizing PARPi combinational therapy

<table>
<thead>
<tr>
<th>Drug Mechanism</th>
<th>First Author, Year</th>
<th>Drugs Used</th>
<th>Model</th>
<th>Summary of Results</th>
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<tbody>
<tr>
<td><strong>ATR Inhibitors</strong></td>
<td>Yazinski et.al., 2017</td>
<td>Olaparib + VE-821, AZ-20</td>
<td>mBRCA1 cells, mBRCA2 PDX</td>
<td>Disruption of resistance mechanisms and overcoming of resistance to PARPi Increased survival of PARPi-resistant cells</td>
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<tr>
<td></td>
<td>Kim et. al., 2020</td>
<td>Olaparib + AZD6738</td>
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<tr>
<td><strong>PI3K Inhibitors</strong></td>
<td>Wang et.al., 2016</td>
<td>Olaparib + BKM120</td>
<td>mBRCA2 OVCA433; mBRCA1 OVCA8; mBRCA1/2 OVCA8</td>
<td>Attenuation of DNA repair impairment compared to PARPi treatment alone; decreased growth</td>
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<tr>
<td></td>
<td>Juvekar et al., 2012</td>
<td>Olaparib + BKM120</td>
<td>mBRCA1 HCC1937</td>
<td>Delay in tumor growth</td>
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<tr>
<td></td>
<td>Ibrahim et al., 2012</td>
<td>Olaparib + BKM120</td>
<td>MDA-MB-468, MDA-MB-231, HCC70, HCC1143, BT20</td>
<td>Reduction in tumor growth and downregulation of BRCA expression</td>
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<tr>
<td><strong>Glutaminase Inhibitors</strong></td>
<td>Emberly et al., 2018</td>
<td>Niraparib/Talazoparib + CB-839</td>
<td>TNBC, CRC, non-small cell lung carcinoma, ovarian and prostate cancer cells in vivo VHL-deficient/VHL-replete</td>
<td>Enhanced anti-tumor activity</td>
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<tr>
<td></td>
<td>Okazaki et al., 2017</td>
<td>Olaparib + GLS1 inhibitors</td>
<td>UMRC2, UMRC3, RCC4, UOK102</td>
<td>Suppression of tumor cell growth</td>
</tr>
<tr>
<td><strong>BET Inhibitors</strong></td>
<td>Karakashev et al., 2017</td>
<td>Olaparib + JQ1</td>
<td>wtBRCA OVCA3</td>
<td>Synergistic increase in DNA damage and apoptosis</td>
</tr>
<tr>
<td></td>
<td>Wilson et al., 2018</td>
<td>Olaparib/Rucaparib + JQ1/ INB054329/INCB057643</td>
<td>wtBRCA1 OVCA3, OVCA4, SKOV3</td>
<td>Reduced HR activity and sensitized cell to PARPi, DNA damage, and cell death</td>
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<tr>
<td></td>
<td>Yang et al., 2017</td>
<td>Olaparib + JQ1/I-BET762/OTX015</td>
<td>wtBRCA OVCA10</td>
<td>Impaired transcription of HR genes, sensitized tumors to PARPi</td>
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<td><strong>VEGFR Inhibitors</strong></td>
<td>Kaplan et al., 2019</td>
<td>Olaparib + Cediranib</td>
<td>IGROV1</td>
<td>Down-regulation of BRCA gene expression</td>
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<tr>
<td><strong>WEE1 Inhibitors</strong></td>
<td>Fang et al., 2020</td>
<td>Talazoparib + Adavosertib</td>
<td>OVCAR8</td>
<td>Marked tumor regression with combination therapy</td>
</tr>
<tr>
<td></td>
<td>Parsels et al., 2018</td>
<td>Olaparib + AZD1775</td>
<td>Calu-6 and H23 NSCLC</td>
<td>Enhanced radiosensitization seen with combinational therapy</td>
</tr>
<tr>
<td></td>
<td>Ha et al., 2020</td>
<td>Olaparib + AZD1775</td>
<td>MDA-MB-157, MDA-MB-231, MDA-MB-468, HCC1143, BT-549, Hs 578 T</td>
<td>Induced apoptotic cell death with combinational therapy</td>
</tr>
<tr>
<td><strong>CDK1 Inhibitors</strong></td>
<td>Johnson et al., 2011</td>
<td>Rucaparib + AG024322</td>
<td>MDA-MB-436</td>
<td>Sensitization of cells to PARPi in vitro</td>
</tr>
<tr>
<td></td>
<td>Xia et al., 2013</td>
<td>Olaparib + RO3306</td>
<td>MDA-MB-231, HCC1937, SKBR-3, MCF-7</td>
<td>Decrease in cell growth with combinational therapy</td>
</tr>
<tr>
<td><strong>Hsp90 Inhibitors</strong></td>
<td>Choi et al., 2014</td>
<td>Olaparib + 17-AAG</td>
<td>HR proficient: Hs578T, MCF-7, MDA-MB-157, T47D, MDA-MB-231, MDA-MB-436, HCC1937, UACC3199</td>
<td>Sensitization of HR proficient cell lines to PARPi</td>
</tr>
<tr>
<td></td>
<td>Gabbasov et al., 2019</td>
<td>Talazoparib + Ganetespib</td>
<td>OVAR3, OC-1, OC-6, MCF-7</td>
<td>Synergistic decrease in OC cell viability</td>
</tr>
<tr>
<td></td>
<td>Jiang et al., 2017</td>
<td>ABT-888 + Ganetespib</td>
<td>MCF-7</td>
<td>Synergistic inhibition of tumor growth</td>
</tr>
</tbody>
</table>
Table 2: Summary of completed or currently active clinical trials utilizing combination PARPi therapy in ovarian cancer patients

<table>
<thead>
<tr>
<th>Drug Mechanism</th>
<th>Study Phase</th>
<th>NCT ID #</th>
<th>Drugs Used</th>
<th>Study Status</th>
<th>Results Available</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ATR inhibitors</strong></td>
<td>Ib</td>
<td>NCT04267939</td>
<td>Niraparib + BAY 1895344</td>
<td>Recruiting</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>I</td>
<td>NCT04149145</td>
<td>Niraparib + M4344</td>
<td>Not yet recruiting</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>NCT04065269</td>
<td>Olaparib + AZD6738</td>
<td>Recruiting</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>NCT03462342</td>
<td>Olaparib + AZD6738</td>
<td>Recruiting</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>Ib</td>
<td>NCT02264678</td>
<td>Olaparib + Ceralasertib</td>
<td>Recruiting</td>
<td>No</td>
</tr>
<tr>
<td><strong>PI3K Inhibitors</strong></td>
<td>I</td>
<td>NCT01623349</td>
<td>Olaparib + BKM120 or BYL719</td>
<td>Active, not recruiting</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>I</td>
<td>NCT04586335</td>
<td>Olaparib + CYH33</td>
<td>Recruiting</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>I</td>
<td>NCT03586661</td>
<td>Niraparib + Copanlisib</td>
<td>Recruiting</td>
<td>No</td>
</tr>
<tr>
<td><strong>Glutaminase Inhibitors</strong></td>
<td>I</td>
<td>NCT03944902</td>
<td>Niraparib + CB-839</td>
<td>Not yet recruiting</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>NCT02340611</td>
<td>Olaparib + Cediranib</td>
<td>Completed</td>
<td>Yes</td>
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<tr>
<td></td>
<td>I/II</td>
<td>NCT01116648</td>
<td>Olaparib + Cediranib</td>
<td>Active, not recruiting</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>III</td>
<td>NCT02446600</td>
<td>Olaparib +/- Cediranib vs non-</td>
<td>Active, not recruiting</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>III</td>
<td>NCT03278717</td>
<td>Olaparib +/- Cediranib</td>
<td>Recruiting</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>NCT03326193</td>
<td>Niraparib + Bevacizumab</td>
<td>Active, not recruiting</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>NCT02889900</td>
<td>Olaparib + Cediranib</td>
<td>Active, not recruiting</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>NCT03314740</td>
<td>Olaparib + Cediranib vs Paclitaxel</td>
<td>Active, not recruiting</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>I</td>
<td>NCT02855697</td>
<td>Olaparib +/- Cediranib</td>
<td>Active, not recruiting</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>NCT03117933</td>
<td>Olaparib +/- Cediranib vs Paclitaxel</td>
<td>Active, not recruiting</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>I</td>
<td>NCT02345265</td>
<td>Olaparib + Cediranib</td>
<td>Active, not recruiting</td>
<td>No</td>
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<tr>
<td></td>
<td>I</td>
<td>NCT00989651</td>
<td>Carboplatin + Paclitaxel +</td>
<td>Active, not recruiting</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>NCT04566952</td>
<td>Olaparib + Anlotinib</td>
<td>Recruiting</td>
<td>No</td>
</tr>
<tr>
<td><strong>VEGFR Inhibitors</strong></td>
<td>II</td>
<td>NCT03579316</td>
<td>Adavosertib +/- Olaparib</td>
<td>Recruiting</td>
<td>No</td>
</tr>
<tr>
<td><strong>WEE1 Inhibitors</strong></td>
<td>II</td>
<td>NCT03579316</td>
<td>Adavosertib +/- Olaparib</td>
<td>Recruiting</td>
<td>No</td>
</tr>
<tr>
<td><strong>CDK1 Inhibitors</strong></td>
<td>I</td>
<td>NCT01434316</td>
<td>Veliparib + Dinaciclib</td>
<td>Recruiting</td>
<td>No</td>
</tr>
<tr>
<td><strong>Hsp90 Inhibitors</strong></td>
<td>II</td>
<td>NCT03783949</td>
<td>Niraparib + Ganetespib</td>
<td>Active, not recruiting</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>I</td>
<td>NCT02898207</td>
<td>Olaparib + Onalespib</td>
<td>Active, not recruiting</td>
<td>No</td>
</tr>
</tbody>
</table>
Figure 1: Overview of the cellular mechanisms that may be targeted to overcome Homologous Recombination Proficiency.
Molecular Cancer Therapeutics

Strategies in Overcoming Homologous Recombination (HR) Proficiency and Poly (ADP-Ribose) Polymerase Inhibitor (PARPi) Resistance

Nidhi Goel, McKenzie E. Foxall, Carly Bess Scalise, et al.

Mol Cancer Ther Published OnlineFirst June 25, 2021.

Updated version
Access the most recent version of this article at:
doi:10.1158/1535-7163.MCT-20-0992

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