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PARP inhibitors to treat ovarian cancer: Mechanisms of action and resistance

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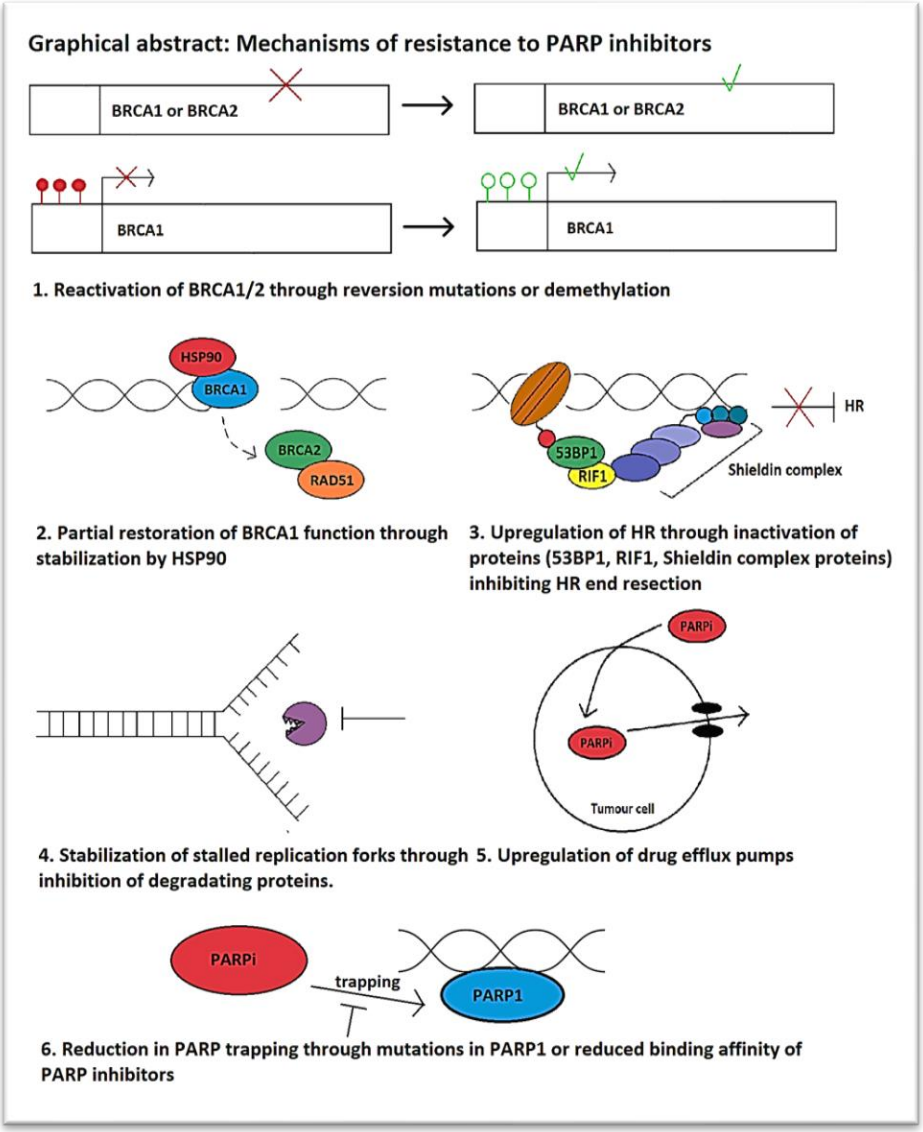
Abstract

Ovarian cancer is a rare type of cancer, but it causes relatively many deaths with a 5-year survival rate of less than 50%. This high death rate is thought to be the result of multiple factors, including the heterogeneity of the disease and the often-observed resistance against current treatment. Recently, a new type of treatment has been introduced, showing specifically promising response rates in tumours harbouring *BRCA1* or *BRCA2* mutations. These new drugs are called PARP inhibitors and their main function is the inhibition of poly(ADP-ribose) polymerase (PARP), a protein involved in several DNA repair mechanisms. PARP inhibition in cells deficient in homologous recombination leads to cell death by the mechanism of synthetic lethality. However, resistance to treatment with PARP inhibitors has been observed quite rapidly after their introduction in the clinic. Here, the mechanisms behind PARP inhibitors are discussed together with the mechanisms of resistance. Understanding these mechanisms could help to design improved PARP inhibitors or create new treatment combinations to reduce resistance. All in all, PARP inhibitors show promising results that could even be improved through adjustments or new treatment combinations.



Introduction to the thesis

The ovaries are two small female organs in the peritoneal cavity that are an important part of the reproductive system. Every month, several follicles start to develop in the ovaries, only to release one that might become fertilized. This process of ovulation is accompanied by changes in hormone levels and injury to the ovarian epithelium, subsequently repaired by proliferation. Different theories have been suggested to explain how these changes could contribute to the development of epithelial ovarian cancer (OC) (Cramer et al. 1983; Fathalla 1971). However, the exact pathway and the factors involved in the tumorigenesis of ovarian cancer remain to be uncovered.

One consistent risk factor that has been implied in ovarian cancer is a mutation in one of the *BRCA* genes. Both BRCA1 and BRCA2 perform functions in DNA repair and are found to be mutated in 5-15% of ovarian cancers (Ramus and Gayther 2009). While there are many subtypes of ovarian cancer, the majority of *BRCA1/2*-related cancers are of one specific type: high-grade serous ovarian cancers (HGSOC). This subtype is characterized by high mortality rates (Bowtell et al. 2015). The standard treatment for all ovarian cancer types has been the same for several years, consisting of surgery together with chemotherapy. However, the mortality rate of ovarian cancer remained high with a five-year survival of less than 50% (Malvezzi et al. 2016). Nevertheless, the search for more effective drugs continued and in 2014, a new type of treatment has been approved by the Food and Drug administration (FDA) called poly(ADP-ribose) polymerase (PARP) inhibitors. This category of drugs was demonstrated to be effective specifically in tumours with *BRCA1/2* mutations. Loss of function of BRCA1/2 proteins together with inhibition of PARP proteins results in the death of cancer cells (Bryant et al. 2005; Farmer et al. 2005). Since 2014, multiple PARP inhibitors have been introduced in the clinic and their results are promising.

However, almost immediately after the introduction of this new type of medication, the first cases of resistance to PARP inhibitors were observed (Montoni et al. 2013). This event has led to the question that will be discussed in this thesis: What is the mechanism behind PARP inhibitors and how do ovarian cancer cells develop resistance against this treatment? To answer this question, the thesis has been divided into 5 chapters. The first chapter will introduce ovarian cancer by discussing the prevalence, risk factors, different subtypes and the possible origin of OC. The second chapter explains the functions of the BRCA-proteins and how their loss of function might contribute to tumour development. The third chapter looks at the current diagnosis and treatment of OCs. In the fourth chapter, PARP inhibitors are introduced by looking at their mechanism of action and the different clinical types. And finally, the mechanisms behind PARP inhibitor resistance and solutions to this problem are discussed. Understanding the mechanisms behind PARP inhibitor resistance is important to optimise outcomes. It gives direction to future research on solutions to overcome resistance, which ultimately leads to prolonged survival of ovarian cancer patients.

The epidemiology of ovarian cancer

Worldwide incidence

According to data from a report on worldwide cancer numbers by the International Agency for Research on Cancer, there were 295,414 new cases of ovarian cancer in 2018 and 184,799 deaths due to ovarian cancer (Bray et al. 2018). Based on these numbers, OC is seventeenth on the list of most common female cancers and therefore a relatively rare disease. The lifetime risk of developing ovarian cancer is approximately 1,2% (Howlader et al. 2017). The size of the risk, however, can be influenced by different factors.

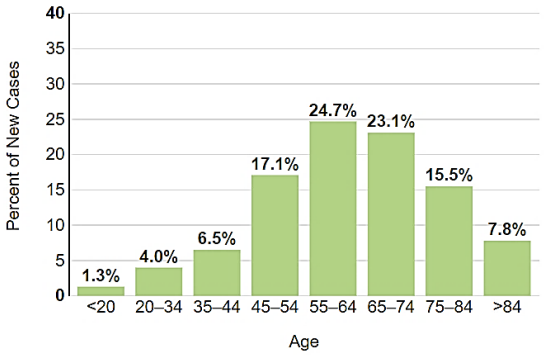


Figure 1: The distribution of new ovarian cancer cases per age category. Source: Based on the numbers of the report by Howlader et al, 2017.

First of all, the risk of acquiring ovarian cancer increases with age. The average age at which women get diagnosed with ovarian cancer is around 63 years (See figure 1). Secondly, the ethnic background of women influences the risk of developing OC. Caucasian white women have a higher incidence rate compared to other ethnicities, but a lower mortality rate than for example African-American women (Barnholtz-Sloan et al. 2002). However, the cause of this variety remains quite unknown (Wu et al. 2015). The presence of risk factors could contribute to the risk, but other studies contradict this theory (Grulich et al. 1992). Besides the influence of age and race on OC risk, other individual factors attribute to the chance of acquiring ovarian cancer.

Risk and protective factors for ovarian cancer

Indeed, several individual factors affect the chance of developing OC. First of all, reproduction-related aspects can contribute to an increased or decreased risk. Theories suggest that the number of ovulations contributes to the risk, as every ovulation is accompanied by hormonal changes and the breakdown and build-up of the ovarian epithelium. The incessant ovulation theory by Fathalla (1971) states that the proliferation needed to repair the epithelium might lead to DNA damage, which in turn could result in ovarian cancer. Several studies show that the number of ovulations indeed correlates with OC risk. Having your first menarche at early age or late onset of the menopause can increase the risk of having ovarian cancer (Parazzini et al. 1989; Wu et al. 1988). Furthermore, nulliparity enhances the risk, possibly through the increase in ovulations (Joly et al. 1974). Reversely, this indicates that pregnancy has a protective effect, which indeed has been shown by multiple studies (Chiaffarino et al. 2001; Cramer et al. 1983). In addition, breast feeding has also been identified as a protective factor for ovarian cancer (Luan et al. 2013).

Secondly, the gonadotropin theory suggests a role of gonadotropins in the pathogenesis of ovarian cancer and therefore a role for gonadotropin stimulating hormones such as oestrogen (Cramer et al. 1983). Oestrogens are a component of hormonal replacement therapy (HRT) for (post)menopausal women. HRT has been shown to increase ovarian cancer risk (Parazzini et al. 1994). On the other hand, use of oral contraceptives has been linked to a lower risk of developing ovarian cancer, up to a decrease of 20% (Cibula et al. 2010). This protection is possibly due to the effect of the progestin component. Progestins affect the apoptotic pathway in the target cells, which is important to prevent tumour growth (Rodriguez et al. 2002).

Thirdly, lifestyle factors could contribute to the risk. Obesity appears to be positively correlated with ovarian cancer (Calle et al. 2003; Fairfield et al. 2002). A clear link between any macro- or micronutrient and risk of ovarian cancer, on the other hand, has not yet been established. Outcomes of studies attempting to demonstrate significant effects have been highly debated (Hunn and Rodriguez 2012). Also, research on the influence of exercise on ovarian cancer risk is inconclusive.

Finally, the most consistent risk factor for developing ovarian cancer is a hereditary predisposition. Women who have family members with ovarian cancer are at higher risk of developing ovarian cancer themselves (Schildkraut, Risch, and Thompson 1989). Part of this hereditary origin of ovarian cancer is derived from the hereditary-non-polyposis-colorectal-cancer syndrome, also known as Lynch syndrome (Aarnio et al. 1999). This syndrome is characterized by mutations in DNA mismatch repair genes. Women with Lynch syndrome have a 12% chance of acquiring ovarian cancer, compared to the general chance of 1,2%. However, the majority of hereditary ovarian cancers originates from mutations in homologous recombination repair genes, most of them harbouring *BRCA1* or *BRCA2* mutations. The lifetime risk of ovarian cancer for women with *BRCA1* or *BRCA2* mutations is 39% and 11% respectively (Antoniou et al. 2003). This hereditary predisposition is highly associated with one specific type of ovarian cancer: high-grade serous ovarian cancer.

Histological subtypes of ovarian cancer

Ovarian cancer is very heterogeneous in nature. Many different subtypes have been classified by origin and histological and genetic characteristics. There have been three main types of cells identified from which ovarian cancer arises, namely, epithelial cells, germ cells and sex cord-stromal cells. Epithelial-derived tumours are the most abundant, they make up about 90% of all cases and are again divided into different groups: serous, mucinous, endometrioid, clear cell, Brenner, seromucinous and undifferentiated tumours (Carcangiu et al. 2014). The group of serous tumours accounts for approximately 50% off all epithelial tumours and has been separated into low- and high-grade serous carcinomas (Howlader et al. 2017). Low-grade carcinomas are often characterized by mutations in *BRAF* and *KRAS*, two protein-encoding genes involved in regulating growth signals (Hsu et al. 2004; Singer et al. 2003). HGSOCs, on the other hand, are highly associated with *TP53* mutations (Ahmed et al. 2010). The wild-type variant of this gene encodes for a protein called p53, which plays a regulatory role in numerous cell processes such as DNA repair, apoptosis and the cell cycle. Mutations in TP53 are involved in many cancers, but seem to have an exceptionally high share in HGSOCs, with a prevalence of almost a 100% (Ahmed et al. 2010; McAlpine et al. 2012; SALANI et al. 2008). Furthermore, several other genes are suggested to be involved in the development of high-grade tumours, amongst others *BRCA1* and *BRCA2* (Bonome et al. 2005). Of all the ovarian cancer cases with *BRCA1/2* mutations, more than half are HGSOCs (Risch et al. 2006).

The cellular origin of high-grade serous ovarian cancers

Even though the classification is rather clear, the origin of HGSOCs has recently been debated. Several researches have shown that the precursor cell of these tumours might be derived from the epithelial cells of the distal ends of the fallopian tube instead of the ovarian epithelium (Kim et al. 2012; Lee et al. 2007; Paik et al. 2012). However, it remains unclear to what extent the fallopian tube is responsible for the development of ovarian cancers. Besides this investigation of the cellular origin of HGSOCs, the molecular pathway by which these

cancers develop has also been examined. As mentioned in the previous part, *TP53* aberrations are present in almost all HGSOCs. The combination of different studies has led to the suggestion that the mutations in the *TP53* gene might be one of the earliest steps in the evolution from healthy cell to tumorigenic cell (Bowtell, 2010). Together with loss of BRCA1/2 function, *TP53* mutations cause genomic instability, especially copy number alterations (Ciriello et al. 2013). A pathway for the tumorigenesis of HGSOCs has been suggested by Bowtell (2010) and has been shown in figure 2. After the loss of both p53 and BRCA1/2, the instability of the chromosomes causes copy number alterations that will result in many different subclones within the tumour, leading to a highly heterogeneous tumour. Several loci have been identified as being amplified in HGSOCs, for example CCNE1 and Notch3 (Nakayama et al. 2007). These amplified genes are often involved in regulatory processes that are frequently disrupted in cancers. The exact pathway of tumorigenesis in high-grade serous ovarian cancers is not yet completely known, but the role of BRCA1/2 appears to be certain.

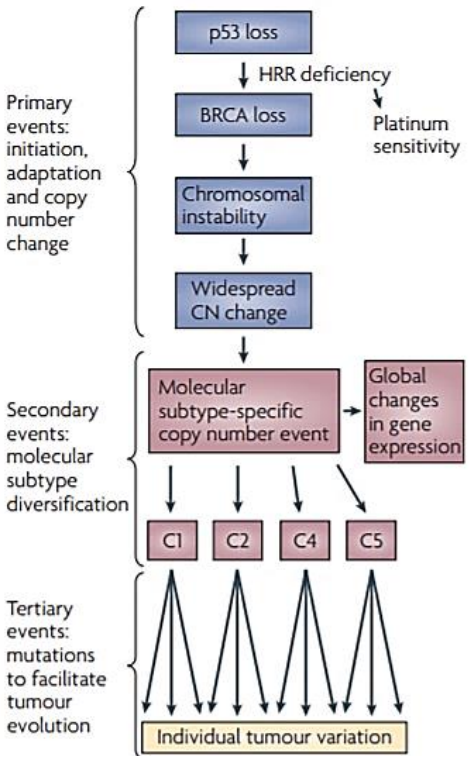


Figure 2: An overview of a proposed pathway for tumorigenesis in high-grade serous ovarian cancers. The loss of both p53 and BRCA results in genomic instability, which in turn leads to copy number alterations. Different strains develop and individual mutations lead to high variation within the tumour. Source: Bowtell, 2010.

BRCA1 and BRCA2: normal function and involvement in tumorigenesis

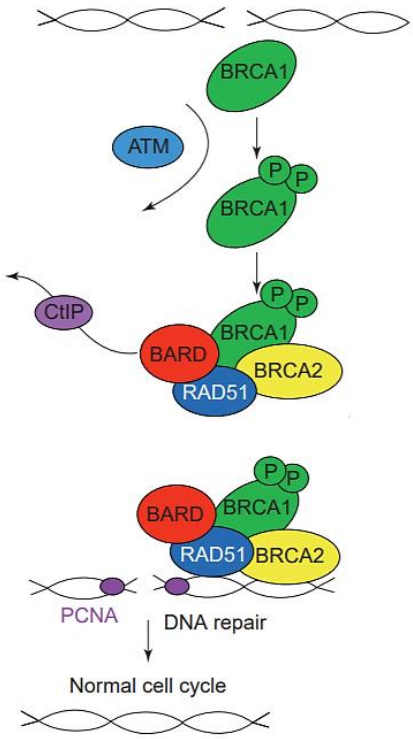


Figure 3: A schematic overview of the role of BRCA1 and BRCA2 in homologous repair. A complex containing BRCA1, BRCA2, BARD1 and RAD51 is recruited to the double-strand DNA break to initiate repair. Source: Welcsh, Owens, and King 2000.

BRCA1/2 normal function

BRCA1 and BRCA2 are two relatively large proteins that are, as mentioned above, frequently mutated in ovarian cancers. They belong to the group of tumour-suppressor genes, as their loss is associated with tumorigenesis (Hilton et al. 2002). One process in which both proteins are involved is DNA repair, specifically the repair of DNA double-strand breaks (DSBs). DSBs can be caused by multiple factors, for example exogenous agents such as ionizing radiation or endogenous agents such as reactive oxygen species. DSBs can be repaired by an error-prone process called non-homologous end joining (NHEJ), which simply ligates the loose ends of the DSB. In contrast to NHEJ, homologous recombination (HR) is an error-free mechanism to repair these breaks. BRCA1 and BRCA2 are involved in homologous recombination repair, thereby carrying out a caretaker function. BRCA1 interacts with BARD1 to form an E3 ubiquitin ligase complex (Mallery, Vandenberg, and Hiom 2002). This complex mediates the monoubiquitylation of the H2A histone, one of the first actions after DNA damage, possibly involved in the recruitment of other repair proteins (Celeste et al. 2003; Nakamura et al. 2004). BRCA2, on the other hand, interacts with RAD51 (Chen et al. 1998; Wong et al. 1997). BRCA2 has been shown to regulate the activity of RAD51, which is the ultimate recombinase enzyme in the repair of DSBs trough HR (Davies et al. 2001). Research has shown that these four proteins (BRCA1/BRCA2/BARD1/RAD51) form a complex that is recruited to the site of DNA damage to repair the DSB (See figure 3) (Welcsh, Owens, and King 2000).

Besides their function in DNA repair, both BRCA1 and BRCA2 are also involved in the stabilization of stalled replication forks to prevent their degradation and subsequent DNA damage (Schlacher, Wu, and Jasin 2012). Furthermore, BRCA1 appears to regulate the checkpoint arrest in the S-phase and G2/M-phase, a function also important to maintain chromosomal stability (Xu, Kim, and Kastan 2001). To sum up, both BRCA1 and BRCA2 have important functions in DNA repair and the prevention of DNA damage.

The role of BRCA1 and BRCA2 in tumorigenesis

BRCA1 and *BRCA2* germline mutations predispose women to several types of cancer, including ovarian cancer. Women with germline mutations only have one effective copy of the *BRCA1* or *BRCA2* gene. According to the Knudson's two-hit hypothesis, complete loss of function only occurs after the intact copy has also been inactivated (Knudson 1971). These women are therefore at higher risk to develop cancer, because one inactivation already leads to loss of function. Even though the inactivation of *BRCA1/2* is linked to cancer development, the pathway by which they initiate tumorigenesis is not yet completely uncovered. Research

in mice with germline Brca1 mutations has shown that homologous repair declines fivefold (Moynahan et al. 1999). BRCA2 mutations are also associated with reduced HR, but to a lesser extent (Moynahan, Pierce, and Jasin 2001). In addition, certain BRCA2 mutations demonstrate an increase in DNA repair through single-strand annealing (Tutt et al. 2001). This is a third type of DSB repair that is error-prone and an increase would therefore lead to more deviations in the DNA. In healthy cells, deficiencies in DNA repair would activate apoptosis or cell cycle checkpoints to prevent the persistence of DNA damage (See figure 4). However, in *BRCA1* mutated cells, a defect has been identified in the S-phase and G2/M-phase checkpoint (Xu et al. 2001). This means that in *BRCA1* mutated cells the cell cycle continues despite the unrepaired DNA damage. In case the DNA damage would lead to mutations in proliferation- or apoptosis-related genes, growth would be further stimulated in spite of accumulating DNA damage. This pathway could lead to hypermutated cells that start tumorigenesis.

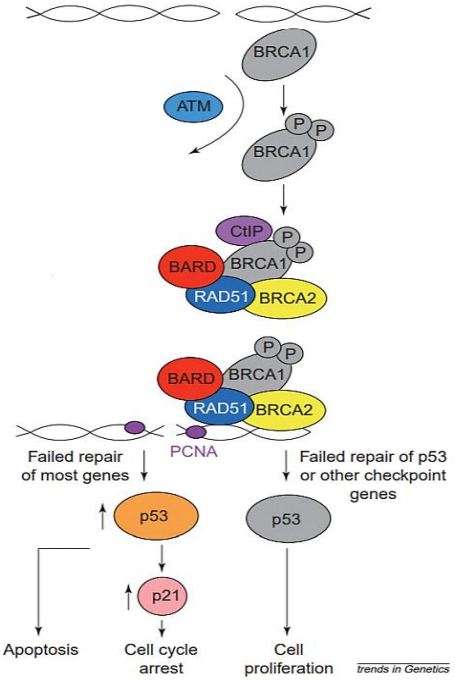


Figure 4: A schematic overview of the consequences of BRCA1 loss. Failed repair of most genes leads to the activation of checkpoint genes to induce apoptosis or cell cycle arrest. However, failed repair of checkpoint genes can lead to unwanted cell proliferation which could result in tumour formation. Source: Welsh et al, 2000.

Diagnosis and treatment of ovarian cancer

Current diagnosis of ovarian cancer

As mentioned in the introduction, the 5-year survival rate for ovarian cancer patients remains below 50%. This percentage depends, amongst others, on the stage of the cancer at the time of diagnosis. Unfortunately, the majority of ovarian cancers is discovered at a later stage, associated with a lower survival rate (Jemal et al. 2009). This seems to be the product of two factors: non-specific symptoms and ineffective screening methods. Ovarian cancer symptoms are usually only present at a later stage and are not specific for ovarian cancer, often leading to late diagnosis. Furthermore, the two known methods for screening appear to be rather ineffective. The two tests that are currently used are cancer antigen 125 (CA-125) blood tests and transvaginal ultrasounds (TVUS). However, both methods appear to have no effect on mortality (Buys et al. 2011). After diagnosis treatment is started, but outcomes are again suboptimal.

Current treatment of ovarian cancer

At the moment, the treatment of ovarian cancer consists mainly of surgery and/or chemotherapy. In most cases, system treatment is done first to remove (the largest part of) the tumour (neoadjuvant treatment). The following surgery is called cytoreductive or debulking surgery, often including abdominal hysterectomy, bilateral salpingo-oophorectomy and omentectomy (Baker 2001). After surgery, chemotherapy is prescribed, generally consisting of paclitaxel and a platin-based agent, such as cisplatin or carboplatin. Treatment with both surgery and chemotherapy has a response rate of up to 60%, but the majority of patients experiences a relapse (Schmid and Oehler 2014). Resistance to chemotherapy is a common event seen in ovarian cancer patients. Although treatment has evolved over the years to optimize outcomes, they are only slightly increased. Therefore, new targeted treatment options have been introduced, including: PARP inhibitors.

PARP protein function and mechanism behind PARP inhibitors

Functions of PARP proteins in DNA repair

The family of poly(ADP-ribose) polymerases consists of 18 members with PARP1 being the most abundant (Amé, Spenlehauer, and de Murcia 2004). After the occurrence of certain kinds of DNA damage, such as single- and double-strand breaks, PARP1 binds to the site of damage and attaches poly(ADP-ribose) (PAR) to itself and other proteins (Kim et al., 2005). This PARylation is done through the cleavage of NAD+ and leads to the recruitment of several proteins that are involved in DNA repair, making PARP1 a key player in the DNA damage response (See figure 5). Research has shown that PARP1 is involved in many different repair mechanisms, including single-strand break repair, base excision repair, nucleotide excision repair and double-strand break repair (Chaudhuri and Nussenzweig 2017).

Firstly, PARP1 has a role in single-strand break (SSB) repair. It recognizes these breaks and binds to the site of the SSBs. There it starts with the PARylation of itself and other proteins to recruit DNA repair proteins. The subsequent repair of the SSB can be done by either base excision repair (BER) or nucleotide excision repair (NER). In BER, PARP1 recruits one of the most important proteins called XRCC1, a scaffold protein for the other SSBR involved proteins (El-Khamisy et al. 2003). PARP1 also enacts a function in NER. It recruits proteins involved in NER, such as xeroderma pigmentosum group C-complementing protein (XPC). Furthermore, DNA damage binding protein 1 (DDB1) enhances PARP1 activity, leading to chromatin decondensation, which could stimulate NER (Luijsterburg et al. 2012).

Secondly, PARP proteins are involved in the repair of DSBs through the assistance in both NHEJ and HR. PARP1 senses the DSBs and recruits several repair proteins. One of the most important proteins in HR, BRCA1, is mobilized by PARP1 to the site of DNA damage (Li and Yu 2013). However, other mechanisms, independent of PARP1, can also recruit BRCA1. Furthermore, PARP1 has several functions in non-homologous end-joining both by recruitment of other proteins and PARylation of NHEJ proteins. Besides all these functions in DNA repair, PARP1 is also involved in stalled replication fork stability. It inhibits the function of a protein called RECQ1 to prevent fork collapse and the subsequent formation of DSBs (Chaudhuri et al. 2012) All in all, PARP1 has an important function in maintaining genomic stability.

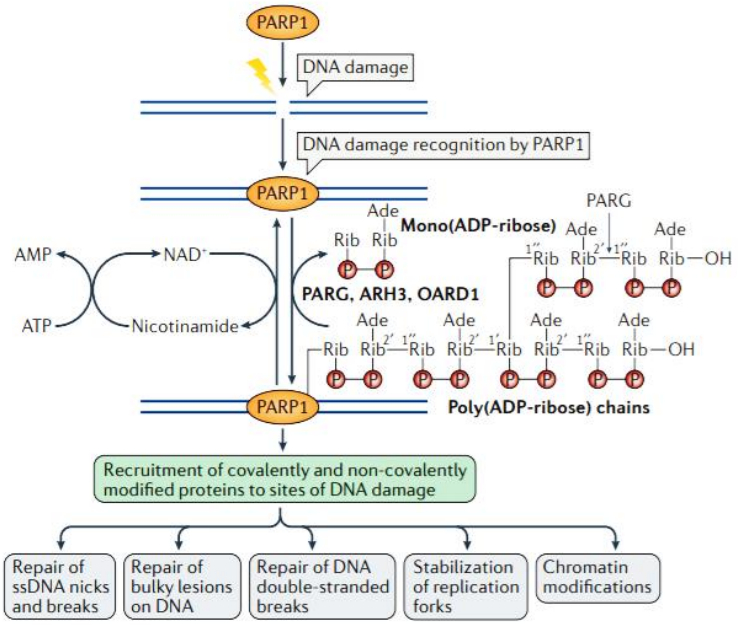


Figure 5: A schematic overview of the function of PARP1 in DNA repair. PARP1 binds to the site of DNA damage, where it starts to add Poly(ADP)ribose chains to itself and other proteins. This process results in the recruitment of DNA repair genes of many different repair mechanisms. Source: Chaudhuri and Nussenzweig, 2017.

The mechanism behind PARP inhibitors

In cancer treatment, the function of PARP1 in DNA repair is inhibited to create what is called synthetic lethality (Bryant et al. 2005; Farmer et al. 2005). Synthetic lethality is the concept of

two genes that are not lethal when loss of function occurs in either of them, but simultaneous loss of function in one cell results in cell death. This is the case with PARP and BRCA1/2. Cells that are already BRCA1 or BRCA2 deficient are sensitive to loss of PARP1 function. PARP inhibitors are thought to accomplish the loss of PARP1 function in two main ways.

The first effect of PARP inhibitors that may lead to synthetic lethality is the restriction of BER. If SSBs are not repaired, they can turn into DSBs during duplication. However, repair of DSBs is compromised in BRCA-deficient cells since the BRCA proteins are essential to HR. BER inhibition together with compromised HR could lead to cell death (See figure 6) (Ashworth 2008).

The second effect is accomplished by the entrapment of PARP1 on the DNA. Usually, the poly(ADP)ribosylation by the PARP1 itself facilitates the later dissociation of PARP1 from the DNA. If the activity of PARP is inhibited, the PARylation will not take place and PARP will be trapped on the DNA (Murai et al. 2012). This PARP-trapping affects the role of PARP1 in the stabilization of replication forks, resulting in increased collapse and subsequent DSBs.

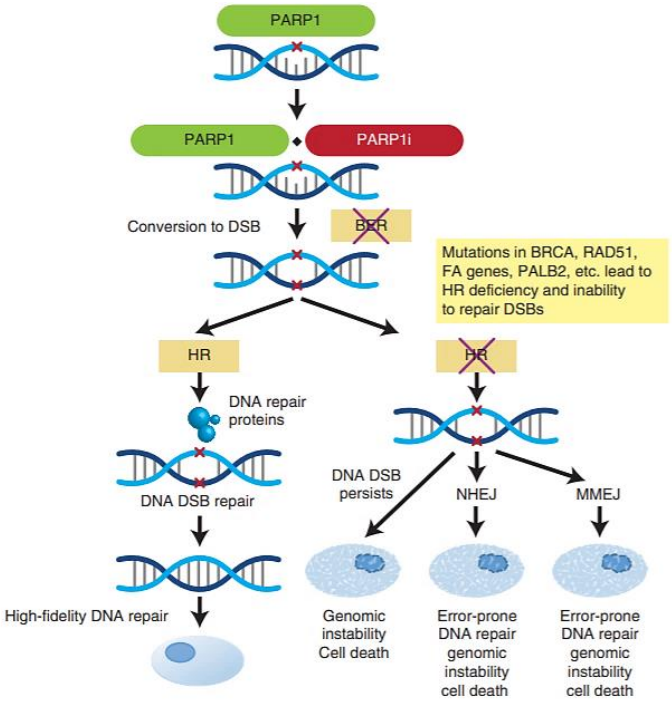


Figure 6: A schematic overview of the function of PARP inhibitors. PARP inhibitors restrict the function of PARP1 leading to a decrease in base excision repair. The unrepaired single-strand breaks can become double-strand breaks during DNA replication. In healthy cells, these DSB's are repaired through homologous repair, but in BRCA1/2 deficient cells HR is compromised. Therefore DSB's in these cells are either not repaired or by error-prone mechanisms, leading to genomic instability and ultimately cell death. Source: Konecny and Kristeleit, 2016.

In addition to BER-inhibition and PARP-trapping, other mechanisms for PARP inhibitors have been suggested, such as the reduction in BRCA1 recruitment or enhanced NHEJ. Interestingly, recent research has demonstrated a quite different function of PARP inhibitors. Ding et al. (2018) shows that PARP inhibitors affect the innate and adaptive immune response to tumours. The amount of T-cells was increased upon treatment with Olaparib, a PARP inhibitor, resulting in enhanced production of immune-stimulating cytokines. Furthermore, they illustrated that PARP inhibition activates the immune system through recognition of factors derived from BRCA-deficient cells. However, the first two mechanisms, BER restrication and PARP-trapping, are thought to contribute most to PARP inhibitor function and the effectiveness of different PARP inhibitors seems to be dependent on which of the two main functions they perform more.

The effectiveness of clinical PARP inhibitors

Clinical PARP inhibitors differ in their ability to inhibit PARP proteins. Some inhibitors appear to be more able to inhibit the enzymatic activity of PARP through binding at the active site, while others are better equipped to trap PARP by adhering to the PARP-chromatin complex (Konecny and Kristeleit 2016). To this moment, three clinical PARP inhibitors have been accepted in the treatment of ovarian cancer and five are investigated in clinical trials. The first PARP inhibitor that was approved by the FDA is Olaparib. This approval was based on the

positive outcomes of several clinical trials (Audeh et al. 2010; Fong et al. 2009; Gelmon et al. 2011). They show that the response to Olaparib in patients with *BRCA1/2* mutations might be as high as 41%. Treatment with PARP inhibitors increases the progression free survival and total survival compared to placebo treated patients. Furthermore, they demonstrate that *BRCA1/2* wildtype patients also respond to Olaparib, although to a lesser extent. The side-effects of Olaparib, such as nausea, vomiting and reduced appetite, are usually mild and less compared to standard chemotherapy. In addition, a trial by Kaufman et al. (2015) demonstrates that Olaparib might also be effective in *BRCA1/2* mutated breast, pancreas or prostate tumours.

Rucaparib was the next approved PARP inhibitor in 2016 and has also shown promising results. Coleman et al. (2016) examined the response rate of *BRCA1/2* mutation carriers to Rucaparib and found it to be approximately 80%. Furthermore, the progression free survival (PFS) with rucaparib was investigated in a later phase of the same clinical trial (Coleman et al. 2017). The PFS of treated patients significantly increased compared to the control group in both patients with *BRCA1/2* mutations and patients with other HR-deficiencies. In addition, the side-effects of Rucaparib are also mild, the majority of symptoms are grade 1/2.

The most recently approved PARP inhibitor is Niraparib. This drug has outcomes similar to those of Rucaparib, with the duration of PFS being even longer (Mirza et al. 2016).

In addition to these three PARP inhibitors approved for use in OC treatment, two other clinical PARP inhibitors are being investigated: Talazoparib and Veliparib. Talazoparib has been shown to have promising effects in *BRCA*-mutated breast cancers (Litton et al. 2018). Veliparib, on the other hand, shows mixed results.

As mentioned in the beginning, all clinical PARP inhibitors differ in their ability to either inhibit enzymatic activity or to trap PARP proteins. Two studies by Murai et al. (2012, 2014) have investigated the different abilities of the five afore mentioned PARP inhibitors. They argue that the competency of PARP inhibitors to trap PARP contributes most to their effectiveness. Olaparib and Rucaparib appear to have similar ability to trap PARP1/2. Veliparib, on the other hand, shows a much weaker PARP-trapping capacity. Niraparib has better trapping capacity than Olaparib and Rucaparib but Talazoparib seems to have the greatest potency to trap PARP proteins (See figure 7).

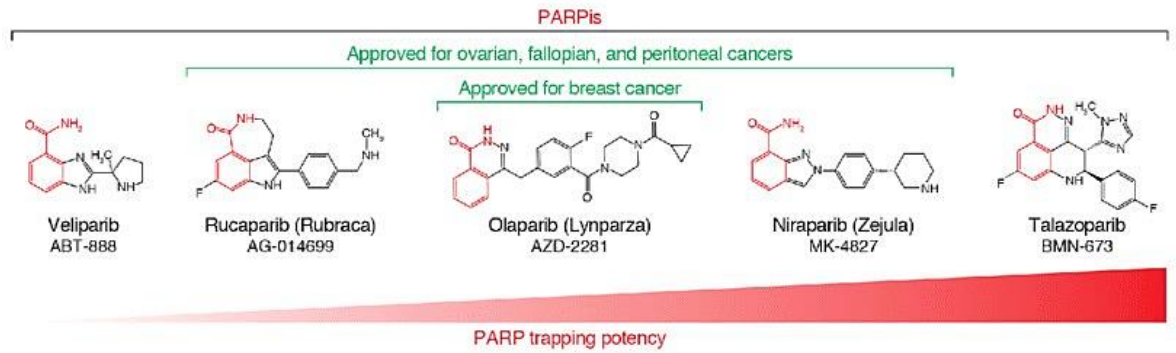


Figure 7: The 5 clinical PARP inhibitors in order of trapping capacity with Talazoparib being the most effective at PARP trapping. Source: Thomas, Murai, and Pommier (2018)

Clinical challenges of PARP inhibitors

Although the outcomes of the above-mentioned clinical trials and chemical studies are promising, there are still some challenges regarding PARP inhibitor treatment. First of all, the majority of adverse effects (AE) from PARP inhibitors are mild, but some patients experience more severe side effects (Pujade-Lauraine et al. 2017). The most frequently observed severe

AE of Olaparib are anaemia, abdominal pain and intestinal obstruction. In some rare cases, patients experienced Acute Myeloid Leukaemia, but this might have been the consequence of treatment prior to the PARP inhibitors. Nevertheless, the adverse effects should be closely monitored. The dosage could be personalised to keep AE to a minimum.

Secondly, it has been challenging to decide when to prescribe PARP inhibitors and to whom. At first the FDA approved PARP inhibitors mainly for maintenance therapy in patients with *BRCA*-mutated advanced ovarian cancer after 3 or more lines of chemotherapy (AstraZeneca pharmaceuticals LP, 2019). However, they are now also prescribed as first-line maintenance treatment to patients without *BRCA*-mutations. More research is necessary to determine the optimal timing of treatment with PARP inhibitors. In addition, it has been challenging to select the right patients to receive the treatment. Clinical trials have shown that response rates are the highest in *BRCA1/2* mutation carriers, but other patients also appear to respond to treatment. Current research intends to find a pattern that resembles BRCA1/2 deficiency (BRCAness) to predict the effectiveness of treatment with PARP inhibitors. Multiple studies investigate the loss of heterozygosity (LOH) in the tumour to predict treatment response. For example, Abkevich et al. (2012) shows that usage of a homologous recombination deficiency (HRD) score could help select patients. This score represents the number of regions with loss of heterozygosity caused by HRD. Birkbak et al. (2012) suggests another method, namely, determining the amount of allelic imbalances at the telomeres, the ends of the DNA. They showed that accumulation of these imbalances is correlated with low *BRCA1* expression in non *BRCA*-mutated ovarian cancers. Another method was proposed by Popova et al. (2012), in which they determined a cut-off point for the amount of large-scale state transitions (breaks in adjacent chromosomal regions of at least 10 Mb) to determine *BRCA1*-inactivation. However, more research is needed to decide on the best model or algorithm to determine which patients will benefit most from treatment.

The third challenge in PARP inhibitor treatment is resistance. Resistance often occurs when drugs are given repetitively, as is the case with PARP inhibitors. Identifying the mechanisms underlying resistance might help to avoid resistance and increase treatment effectiveness. The next chapter will discuss PARP inhibitor resistance in more detail.

Resistance to PARP inhibitors

Possible mechanisms behind PARP inhibitor resistance

Even though PARP inhibitors showed promising response rates, many patients become resistant to the treatment. Several studies have already investigated the mechanisms behind this resistance and multiple possibilities were discovered.

First of all, resistance can be caused by the restoration of homologous recombination in tumour cells. The pressure from PARP inhibitors on the tumour cells provides a selection advantage for cells that are HR-proficient. Research has shown that secondary mutations in HR-involved genes take place, leading to restored reading frames and therefore functional HR (Kondrashova et al. 2017; Weigelt et al. 2017). These mutations are called reversion mutations and take place in affected genes, such as *BRCA1*, *BRCA2*. Furthermore, reactivation of *BRCA1* can occur through demethylation of the promotor region (Kondrashova et al. 2018). The promotor of *BRCA1* is often hypermethylated in ovarian cancers, which leads to silencing of the gene (Esteller et al. 2000). Demethylation of the promotor region restores functional HR. In addition, BRCA1 can be stabilised by HSP90 to restore part of its function in HR (Johnson et al. 2013). Besides the reactivation of HR-involved genes, mutations in NHEJ-involved genes might also increase HR functionality. A protein complex called the Shieldin (SHLD) complex has a role in NHEJ, but also inhibits resection, a process involved in HR (Dev et al. 2018; Noordermeer et al. 2018). This complex is recruited to the site of DNA damage by 53BP1 and RIF1. Mutations in 53BP1, RIF1 and components of the Shieldin complex in BRCA1 deficient cells have been associated with upregulation of resection and consequently an increase in HR (Bunting et al. 2010; Chapman et al. 2013; Xu et al. 2015). Together all these mechanisms restore homologous recombination repair. When HR is no longer deficient, the mechanism of synthetic lethality is compromised, since both PARP inhibition and HR deficiency are necessary for causing lethality. The cells that have become HR proficient are resistant to the PARP inhibitors and will proliferate despite the treatment.

Secondly, resistance to PARP inhibitors can be acquired by the restoration of stable replication forks (Chaudhuri et al. 2016). Normally, during cell cycle arrest, BRCA1 and BRCA2 are involved in the stabilisation of stalled replication forks. BRCA-deficiency results in replication fork degradation via three pathways, leading to DNA damage (See figure 8).

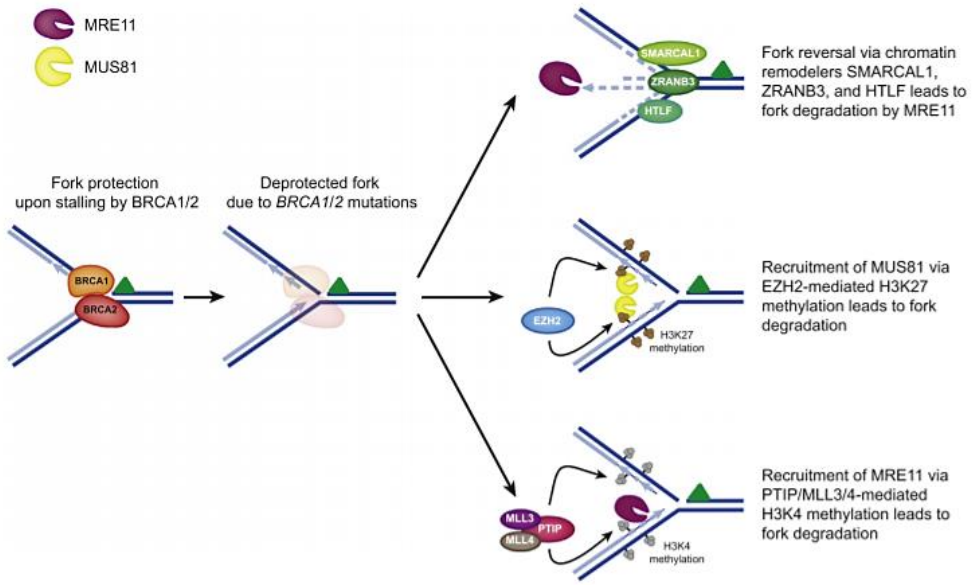


Figure 8: An overview of replication fork degradation pathways that are activated in case of ineffective BRCA1/2 proteins. Source: Noordermeer and van Attikum 2019.

The first pathway of fork degradation is activated through MRE11 recruitment by SMARCAL1, ZRANB3 and HTLF. Depletion of one of these three proteins results in more chromosomal stability and therefore loss of either one could lead to increased PARP inhibitor resistance (Taglialatela et al. 2017). Another pathway that promotes fork degradation is mediated by MUS81, after is has been recruited by H3K27 methylation. This methylation is done by EZH2, depletion of which has also been linked to PARP inhibitor resistance (Rondinelli et al. 2017). The third pathway is activated through methylation of H3K4 by a complex consisting of PTIP, MLL3 and MLL4. This methylation again recruits MRE11 to activate replication fork degradation. PTIP deficiency has been linked to DNA protection and is therefore possibly involved in resistance. Altogether, reduced recruitment of MRE11 and MUS81 leads to more stable replication forks, compromising the synthetic lethality of PARP inhibitors.

A third proposed mechanism of resistance involves the enhanced expression of drug efflux pumps. Indeed, research has shown that ABCB1, an ATP-dependant efflux pump, is upregulated in resistant ovarian cancers (Vaidyanathan et al. 2016). This upregulation increases the cell's ability to pump PARP inhibitors out of the cell and escape cell death, leading to resistance.

Finally, resistance is thought to be caused by mechanisms that reduce PARP trapping. Research by Pettitt et al. (2018) found a PARP1 mutation in a case of PARP resistance and has shown that this mutation reduces PARP trapping by the inhibitors. Furthermore, the loss of PAR glycohydrolase (PARG), an enzyme that reverses PARylation, could contribute to resistance (Gogola et al. 2018). PARG deficiency leads to a partial restorage of PARP1 function, which could therefore counteract PARP inhibitors. In addition, the MET receptor, also known as the hepatocyte growth factor receptor (HGFR), can phosphorylate PARP1, resulting in enhanced activity, but also reduction of its binding affinity to PARP inhibitors (Du et al. 2016). Expression of the MET receptor has been shown to be increased in ovarian cancer, suggesting it plays a role in resistance.

All in all, many different processes are enhanced or interrupted by cancer cells to avoid apoptosis and acquire resistance. Knowing the mechanisms behind this resistance could help to develop additional treatment or drug combinations to prevent it.

Solutions to PARP inhibitor resistance

Both the mechanisms behind PARP inhibitors and resistance against them have led to ideas of how to overcome or reduce resistance. Many new treatment concepts are based on increasing DNA damage to restore synthetic lethality, after it was lost by resistance mechanisms.

One of the treatment suggestions is combining PARP inhibitors with inhibitors of cell cycle regulation proteins. Examples of such cell cycle regulators are CDK12 and WEE1. CDK12 normally upregulates DNA repair proteins involved in HR and inhibition has shown to result in increased genomic instability (Bajrami et al. 2014; Ira et al. 2004). Studies that investigated CDK12 inhibitors in breast cancer and myeloma cells have demonstrated that they increase sensitivity of the cancer cells to PARP inhibitors (Alagpulinsa et al. 2016; Johnson et al. 2016) WEE1 is also a regulator that induces cell cycle arrest to, for example, repair DNA breaks. Combining PARP inhibitor treatment with AZD1775, a WEE1 inhibitor, also showed an increased sensitivity to the PARP inhibitors in acute Leukaemia (Garcia et al. 2017).

Another proposed drug combination is PARP inhibitors with MET inhibitors. As mentioned before, the MET receptor is amplified in certain ovarian cancers and reduces binding of PARP1 to PARP inhibitors. Cases showing increased expression of the MET receptor can

benefit from a treatment with MET inhibitors. Du et al. (2016) demonstrated that combining MET inhibitors and PARP inhibitors has a synergetic effect in BRCA-deficient breast and lung cancer xenografts.
In addition, some other inhibitors, that have already been tested in ovarian cancers, could reduce resistance. One of these inhibitors is AT13387, which inhibits HSP90. HSP90 has been discussed in the previous paragraph and is responsible for the partial restorage of HR trough stabilisation of mutated *BRCA1*. Konstantinopoulos et al. (2016) has shown that AT13387 together with Olaparib has a synergistic effect in HGSOC patient derived xenografts. Interestingly, they also demonstrate this effect in HR-proficient ovarian cancers. Another addition to PARP inhibitors with a positive effect on the response in ovarian cancer is ATR-inhibition (Huntoon et al. 2013). Normally, ATR phosphorylates histone H2A to recruit repair proteins that are involved in HR. Inhibition of ATR leads to a reduction in HR, which could restore synthetic lethality in PARP inhibitor resistant ovarian cancers.
Finally, a treatment option, other than combination of PARP inhibitors with other inhibitors, has been suggested. The function of PARP inhibitors on the immune system is partially counteracted by an increase in the PD-L1 ligand (Ding et al. 2018). This ligand inhibits the immune response, but can be blocked by a PD-1 antibody. The combination of Olaparib with the PD-1 antibody has shown a prolonged survival and tumour regression in mouse models resembling HGSOC.
Besides these suggested treatment combinations, one solution to resistance has already been implemented. Some PARP inhibitors are not a substrate to the ABCB1 efflux pump and are therefore not sensitive to this pathway of resistance (Vaidyanathan et al. 2016).
All in all, multiple additional drugs have been proposed to avoid resistance, but further research is necessary to proof their effectiveness in human ovarian tumours.

Conclusion

From all the literature that has been discussed, it can be concluded that ovarian cancer is a heterogenous disease that is difficult to diagnose and has a low survival rate despite the current treatment efforts. Fortunately, a promising new treatment option has been developed: PARP inhibitors. PARP inhibitors are a relatively new type of medication, with the first clinical PARP inhibitor being FDA-approved in 2014. Research into the mechanisms behind PARP inhibitors has demonstrated that it induces cell death through the concept of synthetic lethality in HR-deficient tumours. Furthermore, they also show a response rate in HR-proficient tumours, for which a mechanism is yet to be determined. In addition, PARP inhibitors appear to stimulate the immune response against the tumour cells. However, the hopeful results of PARP inhibitors have been partially diminished by the development of resistance. Tumour cells seem to be able to avoid cell death by restoring HR, stabilizing replication forks, upregulating efflux pump expression and reducing the ability of PARP inhibitors to trap PARP1. All of these resistance mechanisms undermine the concept of synthetic lethality and therefore weaken the effect of PARP inhibitors. To avoid resistance, several combinations of PARP inhibitors with other kinds of inhibitors have been proposed. A combination with inhibitors of for example cell cycle regulating proteins or proteins involved in the upstream regulation of HR could increase sensitivity to PARP inhibitors. All in all, the results of PARP inhibitor treatment are hopeful and combination with other inhibitors could reduce resistance and enhance the effect of PARP inhibitors.

Discussion

Although the functions of PARP inhibitors and the mechanisms behind resistance have been studied extensively, some of them are put into question. For example, the concept of synthetic lethality is thought to be accomplished by the combination of base excision repair defects and homologous recombination deficiency, but some studies suggest that BER defects are not essential to the function of PARP inhibitors. Research in PARP1 knockout mice has indicated that BER function is similar to that in wildtype mice, suggesting that PARP1 does not perform a function in BER (Vodenicharov 2000). Furthermore, active PARP1 has been shown to reduce BER activity (Allinson, Dianova, and Dianov 2003). Additionally, a study by Patel, Sarkaria, and Kaufmann (2011) has shown that knockdown of XRCC1, an important protein in the BER pathway, does not affect the viability in BRCA2-deficient cells, nor BRCA2-proficient cells. However, Strom et al. (2011) did observe synthetic lethality in cells where PARP-inhibition is combined with XRCC1 deficiency. They insinuate that PARP is not directly involved in BER, but that PARP is trapped on an SSB-intermediate that is formed during BER. Another study has suggested that PARP1 only plays a role in a sub-pathway of BER that repairs damage to purine bases, meaning PARP1 is not involved in the repair of pyrimidine base damage (Reynolds et al. 2015). This could explain the differences in outcomes of the different studies.

Besides the discussion around BER in particular, is has also been questioned whether the conversion of SSBs to DSBs in general is the main cause of synthetic lethality. Experiments by several research groups have demonstrated that the amount of single-strand breaks does not increase upon PARP-inhibition (Gottipati et al. 2010; Strom et al. 2011). It has been suggested that the increase in non-homologous end joining is the primary factor responsible for the function of PARP inhibitors (Patel et al. 2011). Furthermore, the possibility exists that PARP inhibitors work through other undiscovered functions. Research has demonstrated that PARP inhibitors also have several off-targets, targets other than PARP proteins (Antolin et al. 2020).

Additionally, it is important to critically view the contribution of the different resistance mechanisms. Although many different pathways have been demonstrated to be involved in PARP inhibitor resistance, not all pathways are equally involved. Reversion mutations have been studied most extensively, with larger sample sizes and their involvement has been demonstrated in 13 to 21% of all resistant cases (Dhillon, Swisher, and Taniguchi 2011; Lin et al. 2019; Weigelt et al. 2017). Therefore reversion mutations seem to be the most abundant mechanism of resistance. Furthermore, stabilization of replication forks has been examined in large samples. However, this mechanism might be less clinically relevant, since the inhibition of certain factors involved in more stable replication forks did not show a positive effect on survival (Rondinelli et al. 2017). Other mechanisms, such as BRCA1 stabilization and PARP1 mutations, need more investigation to determine their contribution to resistance, because they have been examined in relatively small samples (Johnson et al. 2013; Pettitt et al. 2018). Knowing which mechanisms are most often involved in resistance is important to be able to develop the right treatment combinations and to direct future research.

When looking at all the current studies on PARP inhibitors, it has become clear that some mechanisms are well understood, while others have been broadly discussed. In addition, recent research has demonstrated a quite unexpected function of PARP inhibitors: the stimulation of the immune response against cancer cells. Furthermore, understanding the mechanisms behind PARP inhibitors and resistance has already led to treatment adaptations that have improved PARP inhibitor function, such as the development of PARP inhibitors that

are not a substrate to efflux pumps. These outcomes highlight the importance of knowing the cellular mechanisms behind resistance. Therefore, focussing on further research into the pathways that are involved in PARP inhibitor function and resistance could have more impact on ovarian cancer treatment than the development of new drugs. After all, a 1% increase in the response rate already influences the lives of thousands of patients. New PARP inhibitors, such as Rucaparib, have shown that response rates can be increased as compared to Olaparib and sensitivity to PARP inhibitors can be increased by treatment combinations with other inhibitors. Even though resistance poses a great clinical challenge to PARP inhibitors, they remain a promising new type of drugs for ovarian cancer patients.

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References

Aarnio, Markku, Risto Sankila, Eero Pukkala, Reijo Salovaara, Lauri A. Aaltonen, Albert de la Chapelle, Päivi Peltomäki, Jukka-Pekka Mecklin, and Heikki J. Järvinen. 1999. “Cancer Risk in Mutation Carriers of DNA-mismatch-repair Genes.” *International Journal of Cancer* 81(2):214–18.

Abkevich, V., K. M. Timms, B. T. Hennessy, J. Potter, M. S. Carey, L. A. Meyer, K. Smith-McCune, R. Broaddus, K. H. Lu, J. Chen, T. V Tran, D. Williams, D. Iliev, S. Jammulapati, L. M. FitzGerald, T. Krivak, J. A. DeLoia, A. Gutin, G. B. Mills, and J. S. Lanchbury. 2012. “Patterns of Genomic Loss of Heterozygosity Predict Homologous Recombination Repair Defects in Epithelial Ovarian Cancer.” *British Journal of Cancer* 107(10):1776–82.

Ahmed, Ahmed Ashour, Dariush Etemadmoghadam, Jillian Temple, Andy G. Lynch, Mohamed Riad, Raghwa Sharma, Colin Stewart, Sian Fereday, Carlos Caldas, Anna DeFazio, David Bowtell, and James D. Brenton. 2010. “Driver Mutations in TP53 Are Ubiquitous in High Grade Serous Carcinoma of the Ovary.” *The Journal of Pathology* 221(1):49–56.

Alagpulinsa, D. A., S. Ayyadevara, S. Yaccoby, and R. J. Shmookler Reis. 2016. “A Cyclin-Dependent Kinase Inhibitor, Dinaciclib, Impairs Homologous Recombination and Sensitizes Multiple Myeloma Cells to PARP Inhibition.” *Molecular Cancer Therapeutics* 15(2):241–50.

Allinson, Sarah L., Irina I. Dianova, and Grigory L. Dianov. 2003. “Poly (ADP-Ribose) Polymerase in Base Excision Repair: Always Engaged, but Not Essential for DNA Damage Processing.” *Acta Biochimica Polonica* 50(1):169–79.

Amé, Jean-Christophe, Catherine Spenlehauer, and Gilbert de Murcia. 2004. “The PARP Superfamily.” *Bioessays* 26(8):882–93.

Antolin, Albert A., Malaka Ameratunga, Udai Banerji, Paul A. Clarke, Paul Workman, and Bissan Al-Lazikani. 2020. “The Kinase Polypharmacology Landscape of Clinical PARP Inhibitors.” *Scientific Reports* 10(1):2585.

Antoniou, Anthony, Paul D. P. Pharoah, Steven Narod, Harvey A. Risch, Jorunn E. Eyfjord, John L. Hopper, Niklas Loman, Håkan Olsson, O. Johannsson, and Åke Borg. 2003. “Average Risks of Breast and Ovarian Cancer Associated with BRCA1 or BRCA2 Mutations Detected in Case Series Unselected for Family History: A Combined Analysis of 22 Studies.” *The American Journal of Human Genetics* 72(5):1117–30.

Ashworth, Alan. 2008. “A Synthetic Lethal Therapeutic Approach: Poly (ADP) Ribose Polymerase Inhibitors for the Treatment of Cancers Deficient in DNA Double-Strand Break Repair.” *Journal of Clinical Oncology* 26(22):3785–90.

AstraZeneca Pharmaceuticals LP (2019). Medication guide Lynparza. Retrieved at April 28th 2020 from https://www.accessdata.fda.gov/scripts/cder/daf/index.cfm?event=overview.process&ApplNo=208558

Audeh, M. William, James Carmichael, Richard T. Penson, Michael Friedlander, Bethan Powell, Katherine M. Bell-McGuinn, Clare Scott, Jeffrey N. Weitzel, Ana Oaknin, and Niklas Loman. 2010. “Oral Poly (ADP-Ribose) Polymerase Inhibitor Olaparib in Patients with BRCA1 or BRCA2 Mutations and Recurrent Ovarian Cancer: A Proof-of-Concept Trial.” *The Lancet* 376(9737):245–51.

Bajrami, I., J. R. Frankum, A. Konde, R. E. Miller, F. L. Rehman, R. Brough, J. Campbell, D. Sims, R. Rafiq, S. Hooper, L. Chen, I. Kozarewa, I. Assiotis, K. Fenwick, R. Natrajan, C. J. Lord, and A. Ashworth. 2014. “Genome-Wide Profiling of Genetic Synthetic Lethality Identifies CDK12 as a Novel Determinant of PARP1/2 Inhibitor Sensitivity.” *Cancer Research* 74(1):287–97.

Baker, Vicki V. 2001. “Treatment Options for Ovarian Cancer.” *Clinical Obstetrics and Gynecology* 44(3):522–30.

Barnholtz-Sloan, Jill S., Michael A. Tainsky, Judith Abrams, Richard K. Severson, Faisal Qureshi, Suzanne M. Jacques, Nancy Levin, and Ann G. Schwartz. 2002. “Ethnic Differences in Survival among Women with Ovarian Carcinoma.” *Cancer* 94(6):1886–93.

Birkbak, Nicolai J., Zhigang C. Wang, Ji-Young Kim, Aron C. Eklund, Qiyuan Li, Ruiyang Tian, Christian Bowman-Colin, Yang Li, April Greene-Colozzi, J. Dirk Iglehart, Nadine Tung, Paula D. Ryan, Judy E. Garber, Daniel P. Silver, Zoltan Szallasi, and Andrea L. Richardson. 2012. “Telomeric Allelic Imbalance Indicates Defective DNA Repair and Sensitivity to DNA-Damaging Agents.” *Cancer Discovery* 2(4):366–75.

Bonome, Tomas, Ji-Young Lee, Dong-Choon Park, Mike Radonovich, Cindy Pise-Masison, John Brady, Ginger J. Gardner, Ke Hao, Wing H. Wong, and J. Carl Barrett. 2005. “Expression Profiling of Serous Low Malignant Potential, Low-Grade, and High-Grade Tumors of the Ovary.” *Cancer Research* 65(22):10602–12.

Bowtell, David D., Steffen Böhm, Ahmed A. Ahmed, Paul-Joseph Aspuria, Robert C. Bast Jr, Valerie Beral, Jonathan S. Berek, Michael J. Birrer, Sarah Blagden, and Michael A. Bookman. 2015. “Rethinking Ovarian Cancer II: Reducing Mortality from High-Grade Serous Ovarian Cancer.” *Nature Reviews Cancer* 15(11):668–79.

Bowtell, David D. L. 2010. “The Genesis and Evolution of High-Grade Serous Ovarian Cancer.” *Nature Reviews Cancer* 10(11):803–8.

Bray, Freddie, Jacques Ferlay, Isabelle Soerjomataram, Rebecca L. Siegel, Lindsey A. Torre, and Ahmedin Jemal. 2018. “Global Cancer Statistics 2018: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries.” *CA: A Cancer Journal for Clinicians* 68(6):394–424.

Bryant, Helen E., Niklas Schultz, Huw D. Thomas, Kayan M. Parker, Dan Flower, Elena Lopez, Suzanne Kyle, Mark Meuth, Nicola J. Curtin, and Thomas Helleday. 2005. “Specific Killing of BRCA2-Deficient Tumours with Inhibitors of Poly(ADP-Ribose) Polymerase.” *Nature* 434(7035):913–17.

Bunting, Samuel F., Elsa Callén, Nancy Wong, Hua-Tang Chen, Federica Polato, Amanda Gunn, Anne Bothmer, Niklas Feldhahn, Oscar Fernandez-Capetillo, and Liu Cao. 2010. “53BP1 Inhibits Homologous Recombination in Brca1-Deficient Cells by Blocking Resection of DNA Breaks.” *Cell* 141(2):243–54.

Buys, Saundra S., Edward Partridge, Amanda Black, Christine C. Johnson, Lois Lamerato, Claudine Isaacs, Douglas J. Reding, Robert T. Greenlee, Lance A. Yokochi, and Bruce Kessel. 2011. “Effect of Screening on Ovarian Cancer Mortality: The Prostate, Lung, Colorectal and Ovarian (PLCO) Cancer Screening Randomized Controlled Trial.” *Jama* 305(22):2295–2303.

Calle, Eugenia E., Carmen Rodriguez, Kimberly Walker-Thurmond, and Michael J. Thun. 2003. “Overweight, Obesity, and Mortality from Cancer in a Prospectively Studied Cohort of US Adults.” *New England Journal of Medicine* 348(17):1625–38.

Carcangiu, M L, Robert J. Kurman, Maria Luisa Carcangiu, and C. Simon Herrington. 2014. *WHO Classification of Tumours of Female Reproductive Organs*. International Agency for Research on Cancer.

Celeste, Arkady, Oscar Fernandez-Capetillo, Michael J. Kruhlak, Duane R. Pilch, David W. Staudt, Alicia Lee, Robert F. Bonner, William M. Bonner, and André Nussenzweig. 2003. “Histone H2AX Phosphorylation Is Dispensable for the Initial Recognition of DNA Breaks.” *Nature Cell Biology* 5(7):675–79.

Chapman, J. Ross, Patricia Barral, Jean-Baptiste Vannier, Valérie Borel, Martin Steger, Antonia Tomas-Loba, Alessandro A.

Sartori, Ian R. Adams, Facundo D. Batista, and Simon J. Boulton. 2013. “RIF1 Is Essential for 53BP1-Dependent Nonhomologous End Joining and Suppression of DNA Double-Strand Break Resection.” *Molecular Cell* 49(5):858–71.
Chaudhuri, Arnab Ray, Elsa Callen, Xia Ding, Ewa Gogola, Alexandra A. Duarte, Ji-Eun Lee, Nancy Wong, Vanessa Lafarga, Jennifer A. Calvo, and Nicholas J. Panzarino. 2016. “Replication Fork Stability Confers Chemoresistance in BRCA-Deficient Cells.” *Nature* 535(7612):382–87.
Chaudhuri, Arnab Ray, Yoshitami Hashimoto, Raquel Herrador, Kai J. Neelsen, Daniele Fachinetti, Rodrigo Bermejo, Andrea Cocito, Vincenzo Costanzo, and Massimo Lopes. 2012. “Topoisomerase I Poisoning Results in PARP-Mediated Replication Fork Reversal.” *Nature Structural & Molecular Biology* 19(4):417.
Chaudhuri, Ray A., and A. Nussenzweig. 2017. “The Multifaceted Roles of PARP1 in DNA Repair and Chromatin Remodelling.” *Nature Reviews. Molecular Cell Biology TA - TT* - 18(10):610–21.
Chen, Junjie, Daniel P. Silver, Deepika Walpita, Sharon B. Cantor, Adi F. Gazdar, Gail Tomlinson, Fergus J. Couch, Barbara L. Weber, Terry Ashley, and David M. Livingston. 1998. “Stable Interaction between the Products of the BRCA1 and BRCA2 Tumor Suppressor Genes in Mitotic and Meiotic Cells.” *Molecular Cell* 2(3):317–28.
Chiaffarino, F., C. Pelucchi, F. Parazzini, E. Negri, S. Franceschi, R. Talamini, E. Conti, M. Montella, and C. La Vecchia. 2001. “Reproductive and Hormonal Factors and Ovarian Cancer.” *Annals of Oncology* 12(3):337–41.
Cibula, D., A. Gompel, A. O. Mueck, C. La Vecchia, P. C. Hannaford, S. O. Skouby, M. Zikan, and L. Dusek. 2010. “Hormonal Contraception and Risk of Cancer.” *Human Reproduction Update* 16(6):631–50.
Ciriello, Giovanni, Martin L. Miller, Bülent Arman Aksoy, Yasin Senbabaoglu, Nikolaus Schultz, and Chris Sander. 2013. “Emerging Landscape of Oncogenic Signatures across Human Cancers.” *Nature Genetics* 45(10):1127–33.
Coleman, Robert L., Amit M. Oza, Domenica Lorusso, Carol Aghajanian, Ana Oaknin, Andrew Dean, Nicoletta Colombo, Johanne I. Weberpals, Andrew Clamp, and Giovanni Scambia. 2017. “Rucaparib Maintenance Treatment for Recurrent Ovarian Carcinoma after Response to Platinum Therapy (ARIEL3): A Randomised, Double-Blind, Placebo-Controlled, Phase 3 Trial.” *The Lancet* 390(10106):1949–61.
Coleman, Robert L., Elizabeth M. Swisher, Amit M. Oza, Clare L. Scott, Heidi Giordano, Kevin K. Lin, Gottfried E. Konecny, Anna Tinker, David M. O’Malley, and Rebecca Sophie Kristeleit. 2016. “Refinement of Prespecified Cutoff for Genomic Loss of Heterozygosity (LOH) in ARIEL2 Part 1: A Phase II Study of Rucaparib in Patients (Pts) with High Grade Ovarian Carcinoma (HGOC).”
Cramer, Daniel W., George B. Hutchison, William R. Welch, Robert E. Scully, and Kenneth J. Ryan. 1983. “Determinants of Ovarian Cancer Risk. I. Reproductive Experiences and Family History23.” *JNCI, Journal of the National Cancer Institute* 71:711.
Davies, Adelina A., Jean-Yves Masson, Michael J. McIlwraith, Alicja Z. Stasiak, Andrzej Stasiak, Ashok R. Venkitaraman, and Stephen C. West. 2001. “Role of BRCA2 in Control of the RAD51 Recombination and DNA Repair Protein.” *Molecular Cell* 7(2):273–82.
Dev, Harveer, Ting-Wei Will Chiang, Chloe Lescale, Inge de Krijger, Alistair G. Martin, Domenic Pilger, Julia Coates, Matylda Sczaniecka-Clift, Wenming Wei, Matthias Ostermaier, Mareike Herzog, Jonathan Lam, Abigail Shea, Mukerrem Demir, Qian Wu, Fengtang Yang, Beiyuan Fu, Zhongwu Lai, Gabriel Balmus, Rimma Belotserkovskaya, Violeta Serra, Mark J. O’Connor, Alejandra Bruna, Petra Beli, Luca Pellegrini, Carlos Caldas, Ludovic Deriano, Jacqueline J. L. Jacobs, Yaron Galanty, and Stephen P. Jackson. 2018. “Shieldin Complex Promotes DNA End-Joining and Counters Homologous Recombination in BRCA1-Null Cells.” *Nature Cell Biology* 20(8):954–65.
Dhillon, Kiranjit K., Elizabeth M. Swisher, and Toshiyasu Taniguchi. 2011. “Secondary Mutations of BRCA1/2 and Drug Resistance.” *Cancer Science* 102(4):663–69.
Ding, Liya, Hye-Jung Kim, Qiwei Wang, Michael Kearns, Tao Jiang, Carolynn E. Ohlson, Ben B. Li, Shaozhen Xie, Joyce F. Liu, Elizabeth H. Stover, Brooke E. Howitt, Roderick T. Bronson, Suzan Lazo, Thomas M. Roberts, Gordon J. Freeman, Panagiotis A. Konstantinopoulos, Ursula A. Matulonis, and Jean J. Zhao. 2018. “PARP Inhibition Elicits STING-Dependent Antitumor Immunity in Brca1-Deficient Ovarian Cancer.” *Cell Reports* 25(11):2972-2980.e5.
Du, Yi, Hirohito Yamaguchi, Yongkun Wei, Jennifer L. Hsu, Hung-Ling Wang, Yi-Hsin Hsu, Wan-Chi Lin, Wen-Hsuan Yu, Paul G. Leonard, Gilbert R. Lee, Mei-Kuang Chen, Katsuya Nakai, Ming-Chuan Hsu, Chun-Te Chen, Ye Sun, Yun Wu, Wei-Chao Chang, Wen-Chien Huang, Chien-Liang Liu, Yuan-Ching Chang, Chung-Hsuan Chen, Morag Park, Philip Jones, Gabriel N. Hortobagyi, and Mien-Chie Hung. 2016. “Blocking C-Met–Mediated PARP1 Phosphorylation Enhances Anti-Tumor Effects of PARP Inhibitors.” *Nature Medicine* 22(2):194–201.
El-Khamisy, Sherif F., Mitsuko Masutani, Hiroshi Suzuki, and Keith W. Caldecott. 2003. “A Requirement for PARP-1 for the Assembly or Stability of XRCC1 Nuclear Foci at Sites of Oxidative DNA Damage.” *Nucleic Acids Research* 31(19):5526–33.
Esteller, Manel, Jose Maria Silva, Gemma Dominguez, Felix Bonilla, Xavier Matias-Guiu, Enrique Lerma, Elena Bussaglia, Jaime Prat, I. Clara Harkes, and Elizabeth A. Repasky. 2000. “Promoter Hypermethylation and BRCA1 Inactivation in Sporadic Breast and Ovarian Tumors.” *JNCI: Journal of the National Cancer Institute* 92(7):564–69.
Fairfield, Kathleen M., Walter C. Willett, Bernard A. Rosner, JoAnn E. Manson, Frank E. Speizer, and Susan E. Hankinson. 2002. “Obesity, Weight Gain, and Ovarian Cancer.” *Obstetrics & Gynecology* 100(2):288–96.
Farmer, Hannah, Nuala McCabe, Christopher J. Lord, Andrew N. J. Tutt, Damian A. Johnson, Tobias B. Richardson, Manuela Santarosa, Krystyna J. Dillon, Ian Hickson, and Charlotte Knights. 2005. “Targeting the DNA Repair Defect in BRCA Mutant Cells as a Therapeutic Strategy.” *Nature* 434(7035):917–21.
Fathalla, M. F. 1971. “Incessant Ovulation—a Factor in Ovarian Neoplasia.” *Lancet* 2(7716):163.
Fong, Peter C., David S. Boss, Timothy A. Yap, Andrew Tutt, Peijun Wu, Marja Mergui-Roelvink, Peter Mortimer, Helen Swaisland, Alan Lau, and Mark J. O’Connor. 2009. “Inhibition of Poly (ADP-Ribose) Polymerase in Tumors from BRCA Mutation Carriers.” *New England Journal of Medicine* 361(2):123–34.
Garcia, Tamara B., Jonathan C. Snedeker, Dmitry Baturin, Lori Gardner, Susan P. Fosmire, Chengjing Zhou, Craig T. Jordan, Sujatha Venkataraman, Rajeev Vibhakar, and Christopher C. Porter. 2017. “A Small-Molecule Inhibitor of WEE1, AZD1775, Synergizes with Olaparib by Impairing Homologous Recombination and Enhancing DNA Damage and Apoptosis in Acute Leukemia.” *Molecular Cancer Therapeutics* 16(10):2058–68.
Gelmon, Karen A., Marc Tischkowitz, Helen Mackay, Kenneth Swenerton, André Robidoux, Katia Tonkin, Hal Hirte, David Huntsman, Mark Clemons, and Blake Gilks. 2011. “Olaparib in Patients with Recurrent High-Grade Serous or Poorly Differentiated Ovarian Carcinoma or Triple-Negative Breast Cancer: A Phase 2, Multicentre, Open-Label, Non-Randomised Study.” *The Lancet Oncology* 12(9):852–61.
Gogola, Ewa, Alexandra A. Duarte, Julian R. de Ruiter, Wouter W. Wiegant, Jonas A. Schmid, Roebi de Bruijn, Dominic I. James, Sergi Guerrero Llobet, Daniel J. Vis, and Stefano Annunziato. 2018. “Selective Loss of PARG Restores

PARylation and Counteracts PARP Inhibitor-Mediated Synthetic Lethality." *Cancer Cell* 33(6):1078–93.
Gottipati, Ponnari, Barbara Vischioni, Niklas Schultz, Joyce Solomons, Helen E. Bryant, Tatjana Djureinovic, Natalia Issaeva, Kate Sleeth, Ricky A. Sharma, and Thomas Helleday. 2010. "Poly(ADP-Ribose) Polymerase Is Hyperactivated in Homologous Recombination–Defective Cells." *Cancer Research* 70(13):5389–98.
Grulich, A. E., A. J. Swerdlow, J. Head, and M. G. Marmot. 1992. "Cancer Mortality in African and Caribbean Migrants to England and Wales." *British Journal of Cancer* 66(5):905–11.
Hilton, Jeffrey L., John P. Geisler, Jennifer A. Rathe, Melanie A. Hattermann-Zogg, Barry DeYoung, and Richard E. Buller. 2002. "Inactivation of BRCA1 and BRCA2 in Ovarian Cancer." *Journal of the National Cancer Institute* 94(18):1396–1406.
Howlader, NNAM, A. M. Noone, M. Krapcho, D. Miller, K. Bishop, C. L. Kosary, M. Yu, J. Ruhl, Z. Tatalovich, and A. Mariotto. 2017. "SEER Cancer Statistics Review, 1975-2014, National Cancer Institute." *Bethesda, MD* 1–12.
Hsu, Chih-Yi, Robert Bristow, Moon Seok Cha, Brant G. Wang, Chung-Liang Ho, Robert J. Kurman, Tian-Li Wang, and Ie-Ming Shih. 2004. "Characterization of Active Mitogen-Activated Protein Kinase in Ovarian Serous Carcinomas." *Clinical Cancer Research* 10(19):6432–36.
Hunn, Jessica, and Gustavo C. Rodriguez. 2012. "Ovarian Cancer: Etiology, Risk Factors, and Epidemiology." *Clinical Obstetrics and Gynecology* 55(1):3–23.
Huntoon, C. J., K. S. Flatten, A. E. Wahner Hendrickson, A. M. Huehls, S. L. Sutor, S. H. Kaufmann, and L. M. Karnitz. 2013. "ATR Inhibition Broadly Sensitizes Ovarian Cancer Cells to Chemotherapy Independent of BRCA Status." *Cancer Research* 73(12):3683–91.
Ira, Grzegorz, Achille Pellicioli, Alitukiriza Balijja, Xuan Wang, Simona Fiorani, Walter Carotenuto, Giordano Liberi, Debra Bressan, Lihong Wan, Nancy M. Hollingsworth, James E. Haber, and Marco Foiani. 2004. "DNA End Resection, Homologous Recombination and DNA Damage Checkpoint Activation Require CDK1." *Nature* 431(7011):1011–17.
Jemal, Ahmedin, R. Siegel, E. Ward, Y. Hao, Jand Xu, and M. J. Thun. 2009. "Cancer Statistics." *Ca Cancer J Clin* 59(4).
Johnson, Neil, Shawn F. Johnson, Wei Yao, Yu-Chen Li, Young-Eun Choi, Andrea J. Bernhardy, Yifan Wang, Marzia Capelletti, Kristopher A. Sarosiek, and Lisa A. Moreau. 2013. "Stabilization of Mutant BRCA1 Protein Confers PARP Inhibitor and Platinum Resistance." *Proceedings of the National Academy of Sciences* 110(42):17041–46.
Johnson, Shawn F., Cristina Cruz, Ann Katrin Greifenberg, Sofia Dust, Daniel G. Stover, David Chi, Benjamin Primack, Shiliang Cao, Andrea J. Bernhardy, Rhiannon Coulson, Jean-Bernard Lazaro, Bose Kochupurakkal, Heather Sun, Christine Unitt, Lisa A. Moreau, Kristopher A. Sarosiek, Maurizio Scaltriti, Dejan Juric, José Baselga, Andrea L. Richardson, Scott J. Rodig, Alan D. D'Andrea, Judith Balmaña, Neil Johnson, Matthias Geyer, Violeta Serra, Elgene Lim, and Geoffrey I. Shapiro. 2016. "CDK12 Inhibition Reverses De Novo and Acquired PARP Inhibitor Resistance in BRCA Wild-Type and Mutated Models of Triple-Negative Breast Cancer." *Cell Reports* 17(9):2367–81.
Joly, Daniel J., Abraham M. Lilienfeld, Earl L. Diamond, and Irwin D. J. Bross. 1974. "An Epidemiologic Study of the Relationship of Reproductive Experience to Cancer of the Ovary." *American Journal of Epidemiology* 99(3):190–209.
Kaufman, Bella, Ronnie Shapira-Frommer, Rita K. Schmutzler, M. William Audeh, Michael Friedlander, Judith Balmaña, Gillian Mitchell, Georgeta Fried, Salomon M. Stemmer, Ayala Hubert, Ora Rosengarten, Mariana Steiner, Niklas Loman, Karin Bowen, Anitra Fielding, and Susan M. Domchek. 2015. "Olaparib Monotherapy in Patients With Advanced Cancer and a Germline BRCA1/2 Mutation." *Journal of Clinical Oncology* 33(3):244–50.
Kim, Jaeyeon, Donna M. Coffey, Chad J. Creighton, Zhifeng Yu, Shannon M. Hawkins, and Martin M. Matzuk. 2012. "High-Grade Serous Ovarian Cancer Arises from Fallopian Tube in a Mouse Model." *Proceedings of the National Academy of Sciences* 109(10):3921–26.
Kim, Mi Young, Tong Zhang, and W. Lee Kraus. 2005. "Poly (ADP-Ribosyl) Ation by PARP-1:PAR-Laying'NAD+ into a Nuclear Signal." *Genes & Development* 19(17):1951–67.
Knudson, Alfred G. 1971. "Mutation and Cancer: Statistical Study of Retinoblastoma." *Proceedings of the National Academy of Sciences* 68(4):820–23.
Kondrashova, Olga, Minh Nguyen, Kristy Shield-Artin, Anna V Tinker, Nelson N. H. Teng, Maria I. Harrell, Michael J. Kuiper, Gwo-Yaw Ho, Holly Barker, and Maria Jasin. 2017. "Secondary Somatic Mutations Restoring RAD51C and RAD51D Associated with Acquired Resistance to the PARP Inhibitor Rucaparib in High-Grade Ovarian Carcinoma." *Cancer Discovery* 7(9):984–98.
Kondrashova, Olga, Monique Topp, Ksenija Nesic, Elizabeth Lieschke, Gwo-Yaw Ho, Maria I. Harrell, Giada V Zapparoli, Alison Hadley, Robert Holian, and Emma Boehm. 2018. "Methylation of All BRCA1 Copies Predicts Response to the PARP Inhibitor Rucaparib in Ovarian Carcinoma." *Nature Communications* 9(1):1–16.
Konecny, G. E., and R. S. Kristeleit. 2016. "PARP Inhibitors for BRCA1/2-Mutated and Sporadic Ovarian Cancer: Current Practice and Future Directions." *British Journal of Cancer* 115(10):1157–73.
Konstantinopoulos, Panagiotis, Sangeetha Palakurthi, Qing Zeng, Shan Zhou, Joyce F. Liu, Elena Ivanova, Cloud Paweletz, Naveen Kommajosyula, Alan D. D'Andrea, and Geoffrey Shapiro. 2016. "In Vivo Synergism between PARP-Inhibitor Olaparib and HSP90-Inhibitor AT13387 in High Grade Serous Ovarian Cancer Patient Derived Xenografts."
Lee, Y., A. Miron, R. Drapkin, M. R. Nucci, F. Medeiros, A. Saleemuddin, J. Garber, C. Birch, H. Mou, and R. W. Gordon. 2007. "A Candidate Precursor to Serous Carcinoma That Originates in the Distal Fallopian Tube." *The Journal of Pathology: A Journal of the Pathological Society of Great Britain and Ireland* 211(1):26–35.
Li, Mo, and Xiaochun Yu. 2013. "Function of BRCA1 in the DNA Damage Response Is Mediated by ADP-Ribosylation." *Cancer Cell* 23(5):693–704.
Lin, Kevin K., Maria I. Harrell, Amit M. Oza, Ana Oaknin, Isabelle Ray-Coquard, Anna V. Tinker, Elena Helman, Marc R. Radke, Carmen Say, Lan-Thanh Vo, Elaina Mann, Jeffrey D. Isaacson, Lara Maloney, David M. O'Malley, Setsuko K. Chambers, Scott H. Kaufmann, Clare L. Scott, Gottfried E. Konecny, Robert L. Coleman, James X. Sun, Heidi Giordano, James D. Brenton, Thomas C. Harding, Iain A. McNeish, and Elizabeth M. Swisher. 2019. "BRCA Reversion Mutations in Circulating Tumor DNA Predict Primary and Acquired Resistance to the PARP Inhibitor Rucaparib in High-Grade Ovarian Carcinoma." *Cancer Discovery* 9(2):210–19.
Litton, Jennifer K., Hope S. Rugo, Johannes Ettl, Sara A. Hurvitz, Anthony Gonçalves, Kyung-Hun Lee, Louis Fehrenbacher, Rinat Yerushalmi, Lida A. Mina, and Miguel Martin. 2018. "Talazoparib in Patients with Advanced Breast Cancer and a Germline BRCA Mutation." *New England Journal of Medicine* 379(8):753–63.
Luan, Nan-Nan, Qi-Jun Wu, Ting-Ting Gong, Emily Vogtmann, Yong-Lai Wang, and Bei Lin. 2013. "Breastfeeding and Ovarian Cancer Risk: A Meta-Analysis of Epidemiologic Studies." *The American Journal of Clinical Nutrition* 98(4):1020–31.
Luijsterburg, Martijn S., Michael Lindh, Klara Acs, Mischa G. Vrouwe, Alex Pines, Haico van Attikum, Leon H. Mullenders, and Nico P. Dantuma. 2012. "DDB2 Promotes Chromatin Decondensation at UV-Induced DNA Damage." *Journal of Cell*

Biology 197(2):267–81.
Mallery, Donna L., Cassandra J. Vandenberg, and Kevin Hiom. 2002. “Activation of the E3 Ligase Function of the BRCA1/BARD1 Complex by Polyubiquitin Chains.” *The EMBO Journal* 21(24):6755–62.
Malvezzi, M., G. Carioli, T. Rodriguez, E. Negri, and La Vecchia C. 2016. “Global Trends and Predictions in Ovarian Cancer Mortality. LK - Https://Rug.on.Worldcat.Org/Oclc/6924386903.” *Annals of Oncology : Official Journal of the European Society for Medical Oncology TA - TT* - 27(11):2017–25.
McAlpine, JN, H. Porter, M. Köbel, BH Nelson, LM Prentice, SE Kalloger, J. Senz, K. Milne, J. Ding, SP Shah, DG Huntsman, and CB Gilks. 2012. “BRCA1 and BRCA2 Mutations Correlate with TP53 Abnormalities and Presence of Immune Cell Infiltrates in Ovarian High-Grade Serous Carcinoma.” *Modern Pathology : An Official Journal of the United States and Canadian Academy of Pathology, Inc TA - TT* - 25(5):740–50.
Mirza, Mansoor R., Bradley J. Monk, Jørn Herrstedt, Amit M. Oza, Sven Mahner, Andrés Redondo, Michel Fabbro, Jonathan A. Ledermann, Domenica Lorusso, Ignace Vergote, Noa E. Ben-Baruch, Christian Marth, Radosław Mądry, René D. Christensen, Jonathan S. Berek, Anne Dørum, Anna V. Tinker, Andreas du Bois, Antonio González-Martín, Philippe Follana, Benedict Benigno, Per Rosenberg, Lucy Gilbert, Bobbie J. Rimel, Joseph Buscema, John P. Balser, Shefali Agarwal, and Ursula A. Matulonis. 2016. “Niraparib Maintenance Therapy in Platinum-Sensitive, Recurrent Ovarian Cancer.” *New England Journal of Medicine* 375(22):2154–64.
Montoni, A., M. Robu, E. Pouliot, and Shah GM. 2013. “Resistance to PARP-Inhibitors in Cancer Therapy.” *Frontiers in Pharmacology TA - TT* - 4:18.
Moynahan, ME, JW Chiu, BH Koller, and M. Jasin. 1999. “Brca1 Controls Homology-Directed DNA Repair. LK - Https://Rug.on.Worldcat.Org/Oclc/122154464.” *Molecular Cell TA - TT* - 4(4):511–18.
Moynahan, ME, AJ Pierce, and M. Jasin. 2001. “BRCA2 Is Required for Homology-Directed Repair of Chromosomal Breaks. LK - Https://Rug.on.Worldcat.Org/Oclc/118264221.” *Molecular Cell TA - TT* - 7(2):263–72.
Murai, J., S. Y. N. Huang, A. Renaud, Y. Zhang, J. Ji, S. Takeda, J. Morris, B. Teicher, J. H. Doroshow, and Y. Pommier. 2014. “Stereospecific PARP Trapping by BMN 673 and Comparison with Olaparib and Rucaparib.” *Molecular Cancer Therapeutics* 13(2):433–43.
Murai, J., Huang SY, Das BB, A. Renaud, Y. Zhang, Doroshow JH, J. Ji, S. Takeda, and Y. Pommier. 2012. “Trapping of PARP1 and PARP2 by Clinical PARP Inhibitors.” *Cancer Research TA - TT* - 72(21):5588–99.
Nakamura, TM, Du LL, C. Redon, and P. Russell. 2004. “Histone H2A Phosphorylation Controls Crb2 Recruitment at DNA Breaks, Maintains Checkpoint Arrest, and Influences DNA Repair in Fission Yeast. LK - Https://Rug.on.Worldcat.Org/Oclc/111320712.” *Molecular and Cellular Biology TA - TT* - 24(14):6215–30.
Nakayama, Kentaro, Naomi Nakayama, Natini Jinawath, Ritu Salani, Robert J. Kurman, Ie-Ming Shih, and Tian-Li Wang. 2007. “Amplicon Profiles in Ovarian Serous Carcinomas.” *International Journal of Cancer TA - TT* - 120(12):2613–17.
Noordermeer, Sylvie M., Salomé Adam, Dheva Setiaputra, Marco Barazas, Stephen J. Pettitt, Alexanda K. Ling, Michele Olivieri, Alejandro Álvarez-Quilón, Nathalie Moatti, Michal Zimmermann, Stefano Annunziato, Dragomir B. Krastev, Feifei Song, Inger Brandsma, Jessica Frankum, Rachel Brough, Alana Sherker, Sébastien Landry, Rachel K. Szilard, Meagan M. Munro, Andrea McEwan, Théo Goullet de Rugy, Zhen-Yuan Lin, Traver Hart, Jason Moffat, Anne-Claude Gingras, Alberto Martin, Haico van Attikum, Jos Jonkers, Christopher J. Lord, Sven Rottenberg, and Daniel Durocher. 2018. “The Shieldin Complex Mediates 53BP1-Dependent DNA Repair.” *Nature* 560(7716):117–21.
Paik, Daniel Y., Deanna M. Janzen, Amanda M. Schafenacker, Victor S. Velasco, May S. Shung, Donghui Cheng, Jiaoti Huang, Owen N. Witte, and Sanaz Memarzadeh. 2012. “Stem-Like Epithelial Cells Are Concentrated in the Distal End of the Fallopian Tube: A Site for Injury and Serous Cancer Initiation.” *STEM CELLS TA - TT* - 30(11):2487–97.
Parazzini, F., La Vecchia C, E. Negri, and A. Villa. 1994. “Estrogen Replacement Therapy and Ovarian Cancer Risk. LK - Https://Rug.on.Worldcat.Org/Oclc/118092370.” *International Journal of Cancer TA - TT* - 57(1):135–36.
Parazzini, Fabio, Carlo La Vecchia, Eva Negri, and Antonella Gentile. 1989. “Menstrual Factors and the Risk of Epithelial Ovarian Cancer.” *Journal of Clinical Epidemiology TA - TT* - 42(5):443–48.
Patel, A. G., J. N. Sarkaria, and S. H. Kaufmann. 2011. “Nonhomologous End Joining Drives Poly(ADP-Ribose) Polymerase (PARP) Inhibitor Lethality in Homologous Recombination-Deficient Cells.” *Proceedings of the National Academy of Sciences* 108(8):3406–11.
Pettitt, Stephen J., Dragomir B. Krastev, Inger Brandsma, Amy Dréan, Feifei Song, Radoslav Aleksandrov, Maria I. Harrell, Malini Menon, Rachel Brough, James Campbell, Jessica Frankum, Michael Ranes, Helen N. Pemberton, Rumana Rafiq, Kerry Fenwick, Amanda Swain, Sebastian Guettler, Jung-Min Lee, Elizabeth M. Swisher, Stoyno Stoynov, Kosuke Yusa, Alan Ashworth, and Christopher J. Lord. 2018. “Genome-Wide and High-Density CRISPR-Cas9 Screens Identify Point Mutations in PARP1 Causing PARP Inhibitor Resistance.” *Nature Communications* 9(1):1849.
Popova, T., E. Manie, G. Rieunier, V. Caux-Moncoutier, C. Tirapo, T. Dubois, O. Delattre, B. Sigal-Zafrani, M. Bollet, M. Longy, C. Houdayer, X. Sastre-Garau, A. Vincent-Salomon, D. Stoppa-Lyonnet, and M. H. Stern. 2012. “Ploidy and Large-Scale Genomic Instability Consistently Identify Basal-like Breast Carcinomas with BRCA1/2 Inactivation.” *Cancer Research* 72(21):5454–62.
Pujade-Lauraine, Eric, Jonathan A. Ledermann, Frédéric Selle, Val Gebski, Richard T. Penson, Amit M. Oza, Jacob Korach, Tomasz Huzarski, Andrés Poveda, Sandro Pignata, Michael Friedlander, Nicoletta Colombo, Philipp Harter, Keiichi Fujiwara, Isabelle Ray-Coquard, Susana Banerjee, Joyce Liu, Elizabeth S. Lowe, Ralph Bloomfield, Patricia Pautier, Jacob Korach, Tomasz Huzarski, Tomasz Byrski, Patricia Pautier, Michael Friedlander, Philipp Harter, Nicoletta Colombo, Sandro Pignata, Giovanni Scambia, Maria Nicoletto, Fiona Nussey, Andrew Clamp, Richard Penson, Amit Oza, Andrés Poveda Velasco, Manuel Rodrigues, Jean-Pierre Lotz, Frédéric Selle, Isabelle Ray-Coquard, Diane Provencher, Aleix Prat Aparicio, Laura Vidal Boixader, Clare Scott, Kenji Tamura, Mayu Yunokawa, Alla Lisyanskaya, Jacques Medioni, Nicolas Pécuchet, Coraline Dubot, Thibault de la Motte Rouge, Marie-Christine Kaminsky, Béatrice Weber, Alain Lortholary, Christine Parkinson, Jonathan Ledermann, Sarah Williams, Susana Banerjee, Jonathan Cosin, James Hoffman, Richard Penson, Marie Plante, Allan Covens, Gabe Sonke, Florence Joly, Anne Floquet, Susana Banerjee, Holger Hirte, Amnon Amit, Tjoung-Won Park-Simon, Koji Matsumoto, Sergei Tjulandin, Jae Hoon Kim, Laurence Gladieff, Roberto Sabbatini, David O’Malley, Patrick Timmins, Daniel Kredentser, Nuria Laínez Milagro, Maria Pilar Barretina Ginesta, Ariadna Tibau Martorell, Alfonso Gómez de Liaño Lista, Belén Ojeda González, Linda Mileshkin, Masaki Mandai, Ingrid Boere, Petronella Ottevanger, Joo-Hyun Nam, Elias Filho, Salima Hamizi, Francesco Cognetti, David Warshal, Elizabeth Dickson-Michelson, Scott Kamelle, Nathalie McKenzie, Gustavo Rodriguez, Deborah Armstrong, Eva Chalas, Paul Celano, Kian Behbakht, Susan Davidson, Stephen Welch, Limor Helpman, Ami Fishman, Ilan Bruchim, Magdalena Sikorska, Anna Słowińska, Wojciech Rogowski, Mariusz Bidziński, Beata Śpiewankiewicz, Antonio Casado Herraez, César Mendiola Fernández, Martina Gropp-Meier, Toshiaki Saito, Kazuhiro Takehara,

Takayuki Enomoto, Hidemichi Watari, Chel Hun Choi, Byoung-Gie Kim, Jae Weon Kim, Roberto Hegg, and Ignace Vergote. 2017. “Olaparib Tablets as Maintenance Therapy in Patients with Platinum-Sensitive, Relapsed Ovarian Cancer and a BRCA1/2 Mutation (SOLO2/ENGOT-Ov21): A Double-Blind, Randomised, Placebo-Controlled, Phase 3 Trial.” *The Lancet Oncology* 18(9):1274–84.

Ramus, Susan J., and Simon A. Gayther. 2009. “The Contribution of BRCA1 and BRCA2 to Ovarian Cancer.” *Molecular Oncology* 3(2):138–50.

Reynolds, Pamela, Sarah Cooper, Martine Lomax, and Peter O’Neill. 2015. “Disruption of PARP1 Function Inhibits Base Excision Repair of a Sub-Set of DNA Lesions.” *Nucleic Acids Research* 43(8):4028–38.

Risch, H. A., J. R. McLaughlin, D. E. Cole, B. Rosen, L. Bradley, I. Fan, J. Tang, S. Li, S. Zhang, and P. A. Shaw. 2006. “Population BRCA1 and BRCA2 Mutation Frequencies and Cancer Penetrances: A Kin-Cohort Study in Ontario, Canada LK - Https://Rug.on.Worldcat.Org/Oclc/203285772.” *JOURNAL- NATIONAL CANCER INSTITUTE TA - TT -* 98(23):1694–1706.

Rodriguez, GC, NP Nagarsheth, KL Lee, RC Bentley, DK Walmer, M. Cline, RS Whitaker, P. Isner, A. Berchuck, RK Dodge, and CL Hughes. 2002. “Progestin-Induced Apoptosis in the Macaque Ovarian Epithelium: Differential Regulation of Transforming Growth Factor-Beta. LK - Https://Rug.on.Worldcat.Org/Oclc/120705055.” *Journal of the National Cancer Institute TA - TT* - 94(1):50–60.

Rondinelli, Beatrice, Ewa Gogola, Hatice Yücel, Alexandra A. Duarte, Marieke van de Ven, Roxanne van der Sluijs, Panagiotis A. Konstantinopoulos, Jos Jonkers, Raphaël Ceccaldi, Sven Rottenberg, and Alan D. D’Andrea. 2017. “EZH2 Promotes Degradation of Stalled Replication Forks by Recruiting MUS81 through Histone H3 Trimethylation.” *Nature Cell Biology* 19(11):1371–78.

SALANI, R., R. J. KURMAN, R. GIUNTOLI, G. GARDNER, R. BRISTOW, T. L. WANG, and I. M. SHIH. 2008. “Assessment of *TP53* Mutation Using Purified Tissue Samples of Ovarian Serous Carcinomas Reveals a Higher Mutation Rate than Previously Reported and Does Not Correlate with Drug Resistance.” *International Journal of Gynecological Cancer TA - TT* - 18(3):487–91.

Schildkraut, JM, N. Risch, and WD Thompson. 1989. “Evaluating Genetic Association among Ovarian, Breast, and Endometrial Cancer: Evidence for a Breast/Ovarian Cancer Relationship. LK - Https://Rug.on.Worldcat.Org/Oclc/120047549.” *American Journal of Human Genetics TA - TT* - 45(4):521–29.

Schlacher, Katharina, Hong Wu, and Maria Jasin. 2012. “A Distinct Replication Fork Protection Pathway Connects Fanconi Anemia Tumor Suppressors to RAD51-BRCA1/2.” *Cancer Cell* 22(1):106–16.

Schmid, Bernd C., and Martin K. Oehler. 2014. “New Perspectives in Ovarian Cancer Treatment.” *Maturitas* 77(2):128–36.

Singer, G., R. Oldt, Y. Cohen, B. G. Wang, D. Sidransky, R. J. Kurman, and I. M. Shih. 2003. “Mutations in BRAF and KRAS Characterize the Development of Low-Grade Ovarian Serous Carcinoma.” *JNCI Journal of the National Cancer Institute* 95(6):484–86.

Strom, C. E., F. Johansson, M. Uhlen, C. A. K. Szigyarto, K. Erixon, and T. Helleday. 2011. “Poly (ADP-Ribose) Polymerase (PARP) Is Not Involved in Base Excision Repair but PARP Inhibition Traps a Single-Strand Intermediate.” *Nucleic Acids Research* 39(8):3166–75.

Taglialatela, Angelo, Silvia Alvarez, Giuseppe Leuzzi, Vincenzo Sannino, Lepakshi Ranjha, Jen-Wei Huang, Chioma Madubata, Roopesh Anand, Brynn Levy, and Raul Rabadan. 2017. “Restoration of Replication Fork Stability in BRCA1-and BRCA2-Deficient Cells by Inactivation of SNF2-Family Fork Remodelers.” *Molecular Cell* 68(2):414–30.

Thomas, Anish, Junko Murai, and Yves Pommier. 2018. “The Evolving Landscape of Predictive Biomarkers of Response to PARP Inhibitors.” *The Journal of Clinical Investigation* 128(5):1727–30.

Tutt, Andrew, David Bertwistle, Janet Valentine, Anastasia Gabriel, Sally Swift, Gillian Ross, Carol Griffin, John Thacker, and Alan Ashworth. 2001. “Mutation in *Brca2* Stimulates Error-Prone Homology-Directed Repair of DNA Double-Strand Breaks Occurring between Repeated Sequences.” *The EMBO Journal TA - TT* - 20(17):4704–16.

Vaidyanathan, Aparajitha, Lynne Sawers, Anne-Louise Gannon, Probir Chakravarty, Alison L. Scott, Susan E. Bray, Michelle J. Ferguson, and Gillian Smith. 2016. “ABCB1 (MDR1) Induction Defines a Common Resistance Mechanism in Paclitaxel- and Olaparib-Resistant Ovarian Cancer Cells.” *British Journal of Cancer* 115(4):431–41.

Vodenicharov, M. D. 2000. “Base Excision Repair Is Efficient in Cells Lacking Poly(ADP-Ribose) Polymerase 1.” *Nucleic Acids Research* 28(20):3887–96.

Weigelt, Britta, Iñaki Comino-Méndez, Ino de Bruijn, Lei Tian, Jane L. Meisel, Isaac García-Murillas, Charlotte Fribbens, Ros Cutts, Luciano G. Martelotto, and Charlotte K. Y. Ng. 2017. “Diverse BRCA1 and BRCA2 Reversion Mutations in Circulating Cell-Free DNA of Therapy-Resistant Breast or Ovarian Cancer.” *Clinical Cancer Research* 23(21):6708–20.

Welcsh, Piri L., Kelly N. Owens, and Mary-Claire King. 2000. “Insights into the Functions of BRCA1 and BRCA2.” *Trends in Genetics* 16(2):69–74.

Wong, AK, R. Pero, PA Ormonde, SV Tavtigian, and PL Bartel. 1997. “RAD51 Interacts with the Evolutionarily Conserved BRC Motifs in the Human Breast Cancer Susceptibility Gene Brca2. LK - Https://Rug.on.Worldcat.Org/Oclc/121343194.” *The Journal of Biological Chemistry TA - TT* - 272(51):31941–44.

Wu, AH, CL Pearce, CC Tseng, and MC Pike. 2015. “African Americans and Hispanics Remain at Lower Risk of Ovarian Cancer Than Non-Hispanic Whites after Considering Nongenetic Risk Factors and Oophorectomy Rates.” *Cancer Epidemiology, Biomarkers & Prevention : A Publication of the American Association for Cancer Research, Cosponsored by the American Society of Preventive Oncology TA - TT* - 24(7):1094–1100.

Wu, ML, AS Whittemore, R. S. Jr Paffenbarger, DL Sarles, JB Kampert, S. Grosser, JL Jung, S. Ballon, M. Hendrickson, and J. Mohle-Boetani. 1988. “Personal and Environmental Characteristics Related to Epithelial Ovarian Cancer. I. Reproductive and Menstrual Events and Oral Contraceptive Use. LK - Https://Rug.on.Worldcat.Org/Oclc/115308298.” *American Journal of Epidemiology TA - TT* - 128(6):1216–27.

Xu, B., St Kim, and MB Kastan. 2001. “Involvement of Brca1 in S-Phase and G(2)-Phase Checkpoints after Ionizing Irradiation. LK - Https://Rug.on.Worldcat.Org/Oclc/119068341.” *Molecular and Cellular Biology TA - TT* - 21(10):3445–50.

Xu, Guotai, J. Ross Chapman, Inger Brandsma, Jingsong Yuan, Martin Mistrik, Peter Bouwman, Jirina Bartkova, Ewa Gogola, Daniël Warmerdam, Marco Barazas, Janneke E. Jaspers, Kenji Watanabe, Mark Pieterse, Ariena Kersbergen, Wendy Sol, Patrick H. N. Celie, Philip C. Schouten, Bram van den Broek, Ahmed Salman, Marja Nieuwland, Iris de Rink, Jorma de Ronde, Kees Jalink, Simon J. Boulton, Junjie Chen, Dik C. van Gent, Jiri Bartek, Jos Jonkers, Piet Borst, and Sven Rottenberg. 2015. “REV7 Counteracts DNA Double-Strand Break Resection and Affects PARP Inhibition.” *Nature* 521(7553):541–44.