# Therapeutic potential of combining PARP inhibitors and immune checkpoint inhibitors in cancer treatment

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*Abstract*

Breast cancer is one of the most common cancers in women worldwide. Both environmental and genetic factors play a role in the development of the disease. Environmental factors that have been shown to increase the risk of breast cancer include increased age, obesity and alcohol use. The main genes responsible for hereditary breast cancer are *BRCA1* and *BRCA2*. These genes are active during the cell cycle and important for DNA damage repair, necessary to allow cells to repair damaged DNA. There are various ways to treat breast cancer, however, they lack specificity, attacking normal cells in the process. One specific treatment type is the use of hormone receptors, however, not all breast cancer types benefit from this treatment. The BRCA proteins activate DNA repair pathways when DNA damage is induced. Furthermore, they play roles in stabilising replication forks, preventing DNA damage. Mutation of these genes causes failure of DNA repair mechanisms. Based on synthetic lethality, inactivation of another gene, in addition to *BRCA*, results in cell death. *BRCA1/*2-deficient breast cancer is mainly treated with DNA repair inhibitors. Recently, researchers have been focussing the use of poly(adenosine diphosphate [ADP]-ribose) polymerase (PARP) inhibitors to treat *BRCA1/2* pathogenic breast cancer. PARP is also a protein involved in DNA damage repair. PARP1, one of the PARP proteins, functions as a key part in DNA repair through the cGAS/STING pathway and mediates transcription of proinflammatory proteins. Treatment of *BRCA*-deficient breast cancer with PARP inhibitors creates synthetic lethality and has shown promising results in clinical studies. However, many patients develop resistance to the inhibitor. Various resistance pathways have been hypothesised; reactivation of the *BRCA1/2* gene, restoration of stable replication forks and reduction of PARP trapping. Nevertheless, these mechanisms are not easy to work around. One of the most recent discoveries is the combination of PARPi with immune checkpoint inhibitors. PARPi upregulates PD-L1, an immune checkpoint ligand. However, the constant PD-1/PD-L1 activation results in exhausted T-cells. Inhibiting PD-L1 restores T-cells. The combination of PARPi and immune checkpoint blockers could therefore promote anti-tumour immune response and provide a promising new therapy to treat cancer.

## *Introduction*

Damage to our genetic material happens regularly from external and internal causes. This affects the survival and transmission of the genetic material in our body to our descendants. Our cells are exposed to an approximate 105 DNA lesions each day.[1] These lesions can happen spontaneously in various ways; due to the disconnection of deoxynucleotides while replicating, alkylation, de-amination, and loss of bases caused by depurination.[2] Breast cancer (BC) is one of the most common diseases, caused by an alteration in genetic material, in women worldwide. At least 2 million new BC cases are diagnosed yearly, with nearly 600,000 deaths around the world.[3] Men are less likely to develop BC, as only up to 1% of all BC occur in men.[4] BC, like other forms of cancer, is thought to be the result of both environmental and hereditary circumstances, which alter cellular physiology.[5] Environmental determinants that have been shown to increase the risk of BC include increased age, obesity and alcohol use. With evidence suggesting that breastfeeding and increasing live births reduce the risk of developing BC.[4] Hereditary predisposition is estimated to cause 5-10% of all BC.[6]

The main genes responsible for hereditary BC are breast cancer susceptibility gene 1 (*BRCA1*) and breast cancer susceptibility gene 2 (*BRCA2*). These genes encode for key proteins involved in an essential DNA repair mechanism.[7] The identification of *BRCA1/*2 gene mutations causing familial BC has led to major improvement of prevention of ovarian and breast cancer.[8] For patients with a family history of BC, genetic screening is possible with which mutations in the *BRCA* genes can be established. Women with a germline mutation in the *BRCA1/2* genes might have an increased risk of developing fallopian tube and ovarian cancer. Other cancer types are also associated with a *BRCA1/2* mutation, including male breast cancer, pancreatic cancer, prostate cancer, melanoma, and gastrointestinal cancer.[9]

## *Diagnosis and treatment*

The initial diagnosis of BC is done with a mammogram, often done before symptoms occur of a lump is felt. When a tumour is suspected a biopsy is done to analyse the nature of the tumour.[10] There are two important molecular targets in the pathogenesis of BC. The first is the oestrogen receptor alpha (ER$\alpha$), a steroid hormone receptor and transcription factor. When activated by oestrogen, this receptor activates oncogenic pathways in breast cells. The progesterone receptor is another marker for ER$\alpha$ signalling. Downregulation of this receptor and its activation is one of the main therapies for ER- and PR-positive BCs.[11] The second important molecular target is the epidermal growth factor 2 (HER2). This receptor is a transmembrane receptor tyrosine kinase and is associated with a poor prognosis when not treated. However, HER2-positive BC cells are less sensitive to hormone therapy and are often treated in other ways, including surgery and chemotherapy.[12] The absence of these three receptors simultaneously occurs in approximately 15% of all BC[13], and has a high probability of relapse.[14]

Based on the stage and characteristics of the BC a decision on the type of treatment is made. Additionally, the age, risks and benefits are taken into consideration. The primary goal of non-metastatic BC is to remove the tumour from the breast and surrounding tissue. Patients with metastatic tumours are mostly treated to prolong life and lessen symptoms. Early-stage hormone-negative BC is most often treated with surgery combined with radiation or chemotherapy.[15]

There are two main surgical procedures that can be done to treat BC. The first is a mastectomy, here the whole affected breast is removed. The second is breast conserving surgery, only the cancerous and a little of the surrounding tissue is taken away. Although a mastectomy removes the entire breast, there is a lower risk or recurrence compared to breast conserving surgery.[16] Additionally to surgery, radiotherapy can be done in order to eliminate the cancer cells that persist in the surrounding tissue. The use of radiation therapy after breast conserving therapy can reduce the risk of recurrence by 50%. Furthermore, radiation therapy can also be used to treat the symptoms of more advanced BC.[17] Chemotherapy can also be used following surgery, however, it is most often used to treat triple negative and HER2-positive BC as these are more sensitive to chemotherapy compared to ER- and PR-positive BC. Whether this therapy is beneficial depends on various factors, including the size of the tumour, number of lymph nodes affected, the presence of receptors and the amount of HER protein cancer cells produce.[18] Lastly, hormone therapy is another type of treatment that might be done before or after surgery. Hormone therapy is used to block or lower the expression of hormones, slowing or halting the growth of tumour cells. ER- and PR-positive BCs are mainly treated with this type of therapy.

In addition to the use of hormone receptors as targets for BC therapy, the loss-of-function mutations in the *BRCA* gene have been used to develop improved precision treatment. Currently, the testing of germline *BRCA1/2* mutations, along with hormone receptor status, is used to select the correct therapy for patients that have already been diagnosed with BC.[19] Approximately 5% of BC patients carry germline mutations in *BRCA1* or *BRCA2*. *BRCA* germline mutation carriers have 1 wildtype *BRCA* allele and 1 mutation. When a mutation occurs in the wildtype allele the function of the gene is lost, resulting in *BRCA* deficiency and subsequently genomic instability.[20] If mutations are found during screening, preventive measures can be taken, such as mastectomy which can reduce the risk of developing BC with 90%.[21]

Currently, *BRCA1* or *BRCA2* pathogenic BC is primarily treated with DNA repair inhibitors. In *BRCA*-deficient cells, drug specificity can be enhanced because DNA repair deficiency only occurs in tumours.[22] Since other repair systems can compensate for the loss of DNA repair mechanisms in tumours, it is important to identify and inhibit those repair pathways to kill tumour cells. Based on the synthetic lethality principle, a situation where only the simultaneous perturbation of two genes results in a deadly combination[22], targeting one or more key DNA repair pathway(s) could lead to improvement in *BRCA*-deficient BC treatment outcomes.

## *Structure and function BRCA1 and BRCA2*

The *BRCA1* and *BRCA2* genes are expressed ubiquitously in the late G2 and S phase of the cell cycle and are located on chromosomes 17q21 and 13q12-13 respectively. BRCA1 is a protein with a RING finger domain at its N-terminus, a nuclear export signal, nuclear localisation signal (NLS) and two BRCA1 C-terminal repeat (BRCT) domains at its C-terminus, see Figure 1. The BRCT domain promotes the binding of BRCA1 to other proteins and is involved in transcription activation.[23] The NLS mediates the relocation of BRCA1 from the cytosol to the nucleus.[24] The RING finger domain binds to BRCA1-associated RING domain protein 1 (BARD1). This heterodimer is hypothesised to play a role in protein ubiquitination, because the dimer contains ubiquitin ligase activity.[25] This domain also binds other proteins including c-MYC and cyclin D1, involved in the regulation of cell cycle progression. These domains also mediate

binding with protein complexes involved in the repair of double stranded DNA breaks (DSB).[26] DSBs can be caused by multiple factors, for example exogenous agents such as ionizing radiation or endogenous agents such as reactive oxygen species.[27]

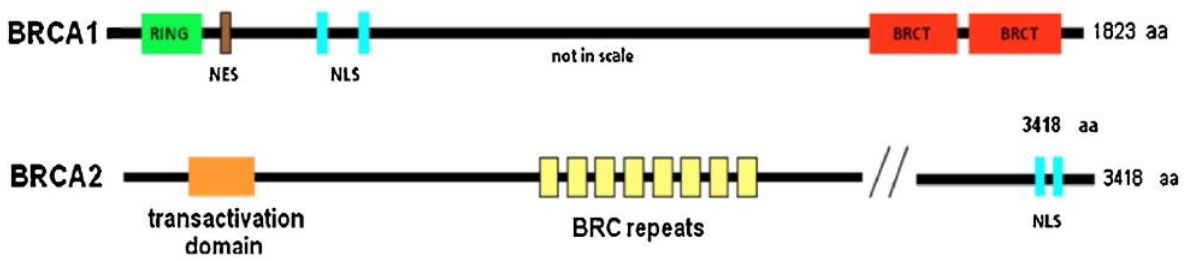


*Figure 1. Structure of BRCA1 and BRCA2 proteins. RING finger domain (green), BRCT (red) domains, nuclear export signal (NES, brown), and nuclear localisation signals (NLS, light blue) are indicated. The third NLS (at amino acid reside 321) of BARD1 is most important for nuclear localisation of BARD1. BRCA2 has a conserved transactivation domain, and 8 copies of a 70 amino acid motif called the BRC repeats. Figure adapted from source: Irminger-Finger, Ratajska, and Pilyugin 2016.*[80]

BRCA2 binds the recombinase RAD51. Using nuclear localisation signals, the BRCA-RAD51 moves from the cytoplasm to the nucleus where is binds other components, forming a complex responsible for further processing of DSB.[26] Besides their function in DNA repair, both BRCA1 and BRCA2 are also involved in the stabilisation of stalled replication forks to prevent their degradation and subsequent DNA damage.[28] Furthermore, BRCA1 regulates the checkpoint arrest in the S-phase and G2-phase, a function also important to maintain chromosomal stability.[29]

In response to DNA damage, BRCA1 and BRCA2 mediate homologous recombination repair (HRR) to repair DNA using the sister chromatid as a template, which results in error-free recovery of DSB.[30] DSBs are the more severe type of DNA damage, as aggregation of DSBs results in genetic translocation and ultimately cell death.[31]

When a mutation occurs in the *BRCA* gene, HRR deficiency ensues, DSBs cannot be repaired by HRR anymore. DSBs then get repaired through non-homologous end joining, an error-prone mechanism. The resulting genomic instability might induce alterations in essential cell cycle checkpoints or proliferation signals. This in turn promotes the development and progression of BRCA-associated cancers.[32,33] The *BRCA1* gene can be reactivated through demethylation of the promotor region, however, this is rare.[34] Based on the synthetic lethality principle, a situation where only the simultaneous perturbation of two genes results in a deadly combination[22], targeting one or more key DNA repair pathway(s) could lead to improvement in *BRCA*-deficient BC treatment outcomes.

## *Poly(ADP-ribose) polymerase*

Recently, studies have explored the use of poly(adenosine diphosphate [ADP]-ribose) polymerase (PARP) inhibitors to treat *BRCA1/2* pathogenic BC.[35] Multiple PARP proteins have been identified, of which PARP1 is the most abundant and best understood.[36] The *PARP1* gene is located on chromosome 1q41-42 and encodes for the nuclear protein PARP1. This protein

contains 4 functional domains, a DNA binding domain, a NLS domain, an auto modification domain, and a catalytic domain, see Figure 2. The DNA binding domain consist of 3 zinc fingers, of which 2 bind to breaks in the DNA strand and the third binds the changes induced by the DNA break to catalytic activity. The auto modification domain has the means to receive ADP-ribose units, this results in self poly(APD-ribosyl)ation (PARylation). This domain also contains the BRCT, like the BRCA1 protein and other proteins with a role in DNA repair.[37] With these domains PARP1 is able to execute its role in the detection and repair of DNA damage.[38] Additionally, depending on the promotor context and cell type, PARP1 functions as an activator or repressor of the transcription of proinflammatory proteins.[39,40] Furthermore, PARP1 is also involved in the cell proliferation, cell differentiation, and cell death.[38] Specifically, in the cell cycle phases, such as the regulation of the cell cycle, DNA replication and mitosis.[40]

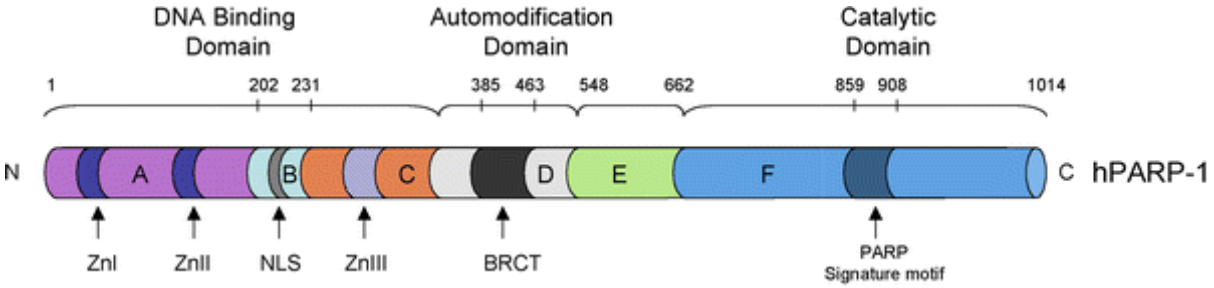


*Figure 2. Structure of the PARP1 protein. The DNA binding domain, with 3 zinc fingers (purple and dark blue respectively), a nuclear localisation signal domain (light blue), the auto modification domain with the BRCT domain (light grey and dark grey respectively) and the catalytic domain (blue) are indicated. Figure adapted from source: Mégnin-Chanet, Bollet, and Hall 2010.*[81]

## *PARP activation*

The induction of DNA damage results in the recruitment of PARP1, which transfers to the DNA damage sites through the DNA binding domain. PARP1 then binds to the site of damage and attaches poly(ADP)ribose (PAR) chains to itself and (non-)histone proteins.[40,41] After activation, PARP1 catalyses the formation of PAR by using $NAD^+$ as a substrate. By cleavage of the $NAD^+$, ADP-ribose can be transferred to amino acid side chains of target proteins, and leads to the recruitment of proteins involved in DNA repair.[42] PARP1 is a tightly regulated protein. When PARP1 is activated, the protein becomes PARylated by itself, another PARP1 protein, or by other PARPs.[43] This posttranslational modification regulates the activity and stability of PARP1 and antagonises PARP1 activity when hydrolysed by poly(ADP-ribose) glycohydrolases.[44] Due to the negative charge of PAR, PARP1 is repelled from DNA damage sites.[45] PARP1 activation can also be modulated by other posttranslational modifications, such as ubiquitination, phosphorylation, and acetylation.[46]

PARP1 has been associated with an important role in various DNA repair mechanisms, including single-strand DNA break (SSB) repair, base excision repair (BER), nucleotide excision repair (NER), and double-strand DNA break (DSB) repair.[42] PARPs are involved in DSB repair through the assistance of NHEJ or HRR. PARP1 recognises the DSB and recruits various repair proteins, such as BRCA1.[47] PARP1, like BRCA1 and BRCA2, is also involved in stalled replication fork stability.[48] Therefore, PARP has a crucial function in maintaining genome stability.

## *Mechanisms of PARP inhibitors*

In cancer treatment with PARP inhibitors (PARPi), the function of PARP1 in DNA repair is hindered and creates synthetic lethality.[22] The simultaneous loss of function of *BRCA1* or *BRCA2* and PARP results in cell death. Cells that are already *BRCA1* or *BRCA2* deficient are therefore more sensitive to the loss of PARP function. This loss of function can be achieved with the use of PARPi. PARPi work via (1) the inactivation of BER. If SSBs are not repaired, they can turn into DSBs during replication. In *BRCA*-deficient cells, however, the repair of DSBs is inhibited, as BRCA proteins are crucial to HRR. So when both BER and HRR are repressed, through PARPi and *BRCA* mutation respectively, it could lead to cell death.[49] Or (2) by trapping PARP1 on the DNA. In the normal circumstance of DNA damage repair, PARP1 is able to free itself from the DNA. However, by use of PARPi, PARP1 cannot dissociate itself and is stuck on the DNA.[50] This last aspect of PARP inhibition negatively affects the functions of PARP1, as it is trapped. Resulting in destabilisation of replication forks and subsequently forms DSBs.

Even though PARPi have shown to be a promising new therapy to treat *BRCA*-deficient cancers, many patients become resistant to the treatment. Resistance to PARPi can be caused by multiple factors. Firstly, it can be caused by restoration of the HRR mechanism in tumour cells. The use of PARPi provides an advantage for tumour cells that are HR-proficient.[34] These mutations are called reversion mutations and take place in affected genes, such as *BRCA1*, and *BRCA2*.

Furthermore, reactivation of *BRCA1* can occur through demethylation of the promotor region.[34] The promotor of *BRCA1* is often hypermethylated in ovarian cancers, which leads to silencing of the gene.[51] 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.[52] Together 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.[53] 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 various pathways, leading to DNA damage. In *BRCA1/2*-deficient cells, nucleases, including MRE11, harm stalled replication forks. This results in shortening of newly formed DNA strands, fork collapse, and chromosomal abnormalities. PTIP is involved in the recruitment of MRE11 to stalled replication forks, and has been observed to regulate the sensitivity of cells to PARPi. When PTIP is deactivated, the recruitment of MRE11 is inhibited. MRE11 then cannot transfer to the stalled replication forks. This inhibition can protect DNA from degradation. However, it does not repair the HRR activity of DSBs.[53] Another 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.[54] Lastly, SLFN11 plays an indirect role in the stability of replication forks. This protein mediates irreversible, and prolonged stalling of replication forks, and subsequently delayed the arrest of the S-phase of the cell cycle when replication stress is induced. The exact mechanisms underlying the

replication fork stalling due to SLFN11 is not completely understood. However, the ATPase activity of SLFN11 might play a role in cleaving the chromatin when stress is induced, resulting in the deformity and stalling of replication forks. The stalling of replication forks might result in forks breaking. This then causes increased sensitivity to PARPi. Therefore, the inactivity of SLFN11, due to loss, inhibits the prolonged arrest of the S-phase when exposed to PARPi, resulting in resistance to PARPi.[55]

Finally, resistance is thought to be caused by mechanisms that reduce PARP trapping. Research by Pettitt et al.[56] 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.[57] PARG deficiency leads to a partial restorage of PARP1 function, which could therefore counteract PARP inhibitors. 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 PARPi 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. In addition to the functions of PARPi already mentioned, another function was recently found. The use of the PARP inhibitor olaparib resulted in an increase of immune-stimulating cytokines which in turn increased the number of T-cells. This function, which is dependent on the STING pathway, showed strong antitumour immunity.[58] The numerous roles of PARP in maintaining genomic stability result in significant deleterious effects of PARPi on the genome. Expectedly, the use of PARPi is associated with increased levels of tumour-derived double-strand DNA in the cytoplasm. This cytosolic DNA is detected by cyclic GMP-AMP synthase (cGAS).[59] cGAS in turn activates the STING pathway via the generation of cyclic GMP-AMP (cGAMP). The STING pathway induces phosphorylation and nuclear translocation of type 1 IFN transcriptional regulatory factors, including TANK-binding kinase 1 (TBK1) and activation of IFN regulatory factor 3 (IRF3) and NF-kB. Additionally the activation of the STING pathway promotes the expression of T-cell-recruiting cytokines.[59–61] This upregulation induced systemic immune response and regulates various components in the anti-tumour community, T-cells in particular. Mutations in the *BRCA1/2* gene are also associated with cytoplasmic DNA[62] and also lead to activation of the cGAS/STING-mediated innate immune response.[63]

What the specific phenotypes of the recruited T-cells are, is still unknown. The current observations are conflicting, increased levels of CD4-, CD8 T-cells, and CD4/Foxp3 T-regs have been detected.[59] Downstream markers of cGAS/STING activation were increased in *BRCA*-deficient cells, due to greater genomic instability.[64] Furthermore, PARP1, when sensing DNA damage, is also a mediator of the non-canonical pathway of STING activation.[65] Upon binding to DSBs, PARP1 recruits and activates ATM, which in turn activates an ubiquitin ligase, TRAF6. Following activation, TRAF6 translocates to the cytosol and in association with IFN

transcriptional regulatory factors activates the STING pathway. The non-canonical pathway, does not depend on cGAS and primarily generates NF-kB, and to a lesser extend IRF3.[65]

## *Regulation of immune-checkpoint ligands by PARPi*

Researchers found that inhibiting PARP1 resulted in increased levels of PD-L1 expression in BC cells, both *in vitro* and *in vivo*.[66] The inhibition of PARP1 leads to the activation of the cGAS/STING pathway, which results in the increased expression of T-cell-related chemokines, including PD-L1 (Figure 3).[67] Programmed cell death ligand 1 (PD-L1) is a protein that binds to programmed cell death 1 (PD-1). PD-1 is an immune-checkpoint protein and is expressed on the surface of T-cells. The PD-1/PD-L1 pathway plays a role in maintaining peripheral T-cell tolerance and regulates inflammation.[68] Activation of the PD-1/PD-L1 pathway induces downregulation of T-cell activity, reduced cytokine production and induces tolerance to antigens.[69] In normal cells, this pathway is crucial to maintain the homeostasis of the immune system and prevention from autoimmune reactions during infection or inflammation. However, in tumour cells this pathway provides an escape for the cells from the immune system, by inhibiting the activation of T-cells.[70] Lasting antigen exposure results in permanent PD-1 expression, which exhausts T-cells and limits the clearance of degenerated cells.[71]

There are several possible mechanisms of PD-L1 upregulation in BC cells treated with PARPi. NF-kB, generated in response to the activation of the STING pathway, binds the PD-L1 promotor, upregulating transcription.[72] However, inhibition of IRF3, also crucial to the STING pathway, has been found to be sufficient to negate the upregulation of PD-L1 in response to PARPi.[73] This suggests that there are multiple mechanisms of PD-L1 regulation. JAK1 or JAK2 activation in tumour cells can also induce PD-L1 expression[74], suggesting that PD-L1 might be upregulated by JAK/STAT signalling, a downstream pathway of the STING pathway. Furthermore, IFN-$\gamma$ is also able to induce PD-L1 expression[75], PD-L1 expression may be induced in response to IFN-$\gamma$ secretion by T-cells following the activation of the STING pathway. Lastly, PD-L1 expression is regulated by GSK3$\beta$, which induces phosphorylation-dependent degradation of PD-L1.[76] It was observed that the inhibition of GSK3$\beta$ was associated with a stabilised expression of PD-L1. The inhibition of PARP1 results in the inactivation of GSK3$\beta$, preventing the degradation of PD-L1[66], however, the exact mechanism underlying this event is not yet known. Recently, it was observed that the inhibition of PARP in CD8 T-cells downregulated PD-1, suggesting decreased inclination for T-cell inhibition, even though PARPi increased the expression of PD-L1 in tumour cells.[60] This suggests that PARPi might stimulate an immunogenic tumour microenvironment.

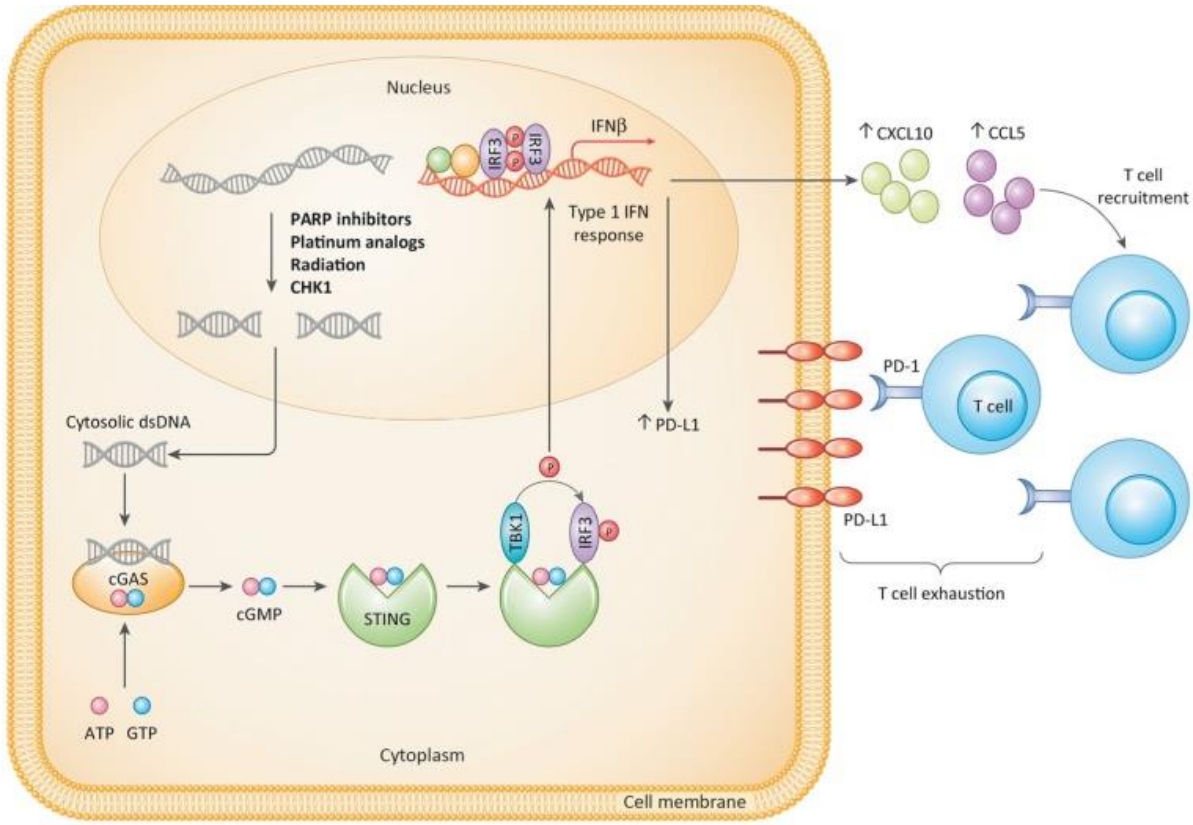


*Figure 3. Anti-tumour effects of PARP inhibition. DSBs induced by PARPi resulting in cytosolic DNA fragments. This leads to cGAS/STING pathway activation and generation of IFN response. Upregulating PD-L1 expression leading to T-cell exhaustion. Figure adapted from Lee and Konstantinopoulos 2019.*[67]

## *Combining PARPi and immune-checkpoint blockers*

The combination of DNA damaging agents, such as PARPi, and immune checkpoint blockers, might provide a bridge to the use of immunotherapies in an extensive number of cancer types. In tumour cells the PD-L1 expression is often upregulated, interfering with the surveillance of the immune system. The upregulated PD-L1 result in exhausted T-cells, whose effector function has been lost due to prolonged stimulation. Immune checkpoint inhibitors restore the T-cells from their exhausted state and subsequently stimulate anti-tumour response.[77] The combination of PARPi and immune checkpoint blockers could therefore promote anti-tumour immune response, through PARPi, and counteract the upregulation of PD-L1 induced by PARPi, through the use of anti-PD-1/PD-L1 (Figure 4). There are various preclinical studies that explore this combination. One study found that the use of PARPi and anti-PD-1/PD-L1 induced an increase of immune cells which infiltrated the tumour microenvironment.

Furthermore, these anti-tumour effects were seen in both *BRCA*-proficient and *BRCA*-deficient animal models.[66,78] In another study, the use of Olaparib, a PARP inhibitor, and PD-L1 blocker resulted in regression of the tumour in mice models. The combination lead to more regression of the tumour compared to either of the two agents alone.[73] One study even observed inhibition of tumour growth in an animal model resistant to immune checkpoint blockers. Suggesting that the use of PARPi and immune checkpoint inhibitors could induce an

inflammatory response enough to overcome immune checkpoint blocker resistance.[67] Additionally, one study showed an increase in T-cell levels after treatment with olaparib and anti-PD-1/PD-L1. Moreover, they observed the crucial role of the cGAS/STING pathway, which, when inhibited, stopped the anti-tumour effects described previous.[58]

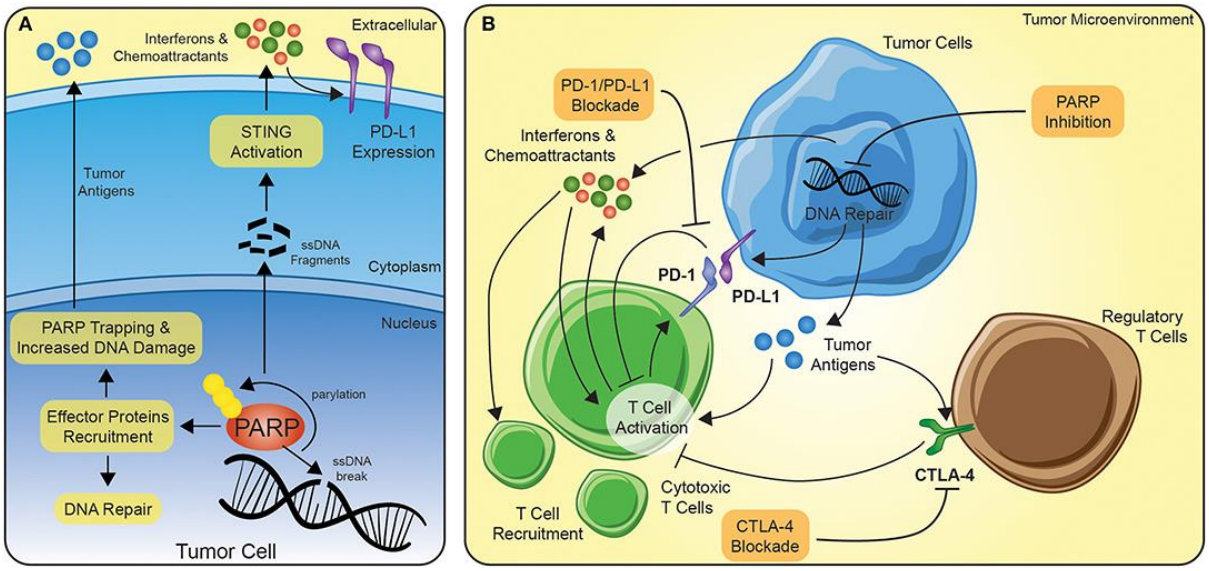


*Figure 4. Synergy between PARPi and immune checkpoint inhibition.* ***(A)*** *When PARP is inhibited, tumour cells secrete interferons which act as signals for immune cells. Interferons promote the activation of T-cells by the immune system.* ***(B)*** *Blockage of immune checkpoints. PD-1 act as a restrictor of T-cell activation. By blocking the PD-1/PD-L1 pathway activation the anti-tumour response is inhibited. CTLA-4, another protein found on T cells regulating the immune response, blocking this protein functions as an inhibitor of tumour growth.*[82]

Although, there are also a few studies that did not observe an improved effect on tumour regression when using both PARPi and anti-PD-1/PD-L1.[79] However, these contradictory observations might be explained by the use of different animal models, which might have varying immune structures. Regardless, the available preclinical data back the notion that the use of PARPi and immune checkpoint inhibitors together provides a therapy that can be used for more cancer types, than PARPi alone.

## *Conclusion*

The use of PARP inhibitors to treat *BRCA*-deficient BC and ovarian cancer has been the focus of many studies in the last decade. PARP inhibitors work mainly through synthetic lethality and PARP trapping.[50] Studies have observed that PARPi modifies the immune response, primarily in context of T-cell recruitment. PARPi alter the production of neoantigens and secretion of cytokines and chemokines, which regulate T-cell activation.[59] The combination of PARPi and immune checkpoint blockers might be able to extend the efficacy and duration of the effectiveness of these agents. Preclinical studies have shown that PARPi and immune checkpoint inhibitors act via the cGAS/STING pathway.[59] This pathway is responsible for releasing IFN-γ which recruits T-cells and upregulated PD-L1 expression in tumour cells.[60] These studies also observed that this combination improves the anti-tumour immune response compared to either agent alone.

However, before this combination treatment can be used in humans more information is needed. Various questions still need to be answered. What drug combination for which patient? Which patients can benefit from this therapy? What is the optimal timeframe to administer the treatment? In order to answer the first question, more research needs to be done to possible combinations of PARPi and various immune checkpoint inhibitors. Currently, research has been generally focussed on PD-L1. Regarding the second question, the patients with DNA damage repair deficiency are more sensitive to PARPi. However, DNA damage repair genes may be mutated differently and therefore cause different responses to treatment. It might be beneficial to discover biomarkers of DNA damage repair and immune responsiveness to personalise and guide treatment. In addition to the last question, the timing of administration might also be linked to personalisation of treatment, as the treatment might operate different for each person due to varying immune structures and mutations. However, more comprehensive research needs to be done, before the treatment can be of use in humans.

The combination of PARPi and immune checkpoint inhibitors might have the potential to improve cancer patient response. The various preclinical studies exploring this combination show promising preliminary results. However, knowledge of the optimal biomarkers, timing and patients will take time. Answers to the previously mentioned questions will provide better insight for the development of the ideal combination therapy to treat cancer patients.

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