PARP inhibition and immune modulation: scientific rationale and perspectives for the treatment of gynecologic cancers

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Abstract: Poly[adenosine diphosphate (ADP) ribose]polymerase (PARP) has multifaceted roles in the maintenance of genomic integrity, deoxyribonucleic acid (DNA) repair and replication, and the maintenance of immune-system homeostasis. PARP inhibitors are an attractive oncologic therapy, causing direct cancer cell cytotoxicity by propagating DNA damage and indirectly, by various mechanisms of immunostimulation, including activation of the cGAS/STING pathway, paracrine stimulation of dendritic cells, increased T-cell infiltration, and upregulation of death-ligand receptors to increase susceptibility to natural-killer-cell killing. However, these immunostimulatory effects are counterbalanced by PARPi-mediated upregulation of programmed cell-death-ligand 1 (PD-L1), which leads to immunosuppression. Combining PARP inhibition with immune-checkpoint blockade seeks to exploit the immune stimulatory effects of PARP inhibition while negating the immunosuppressive effects of PD-L1 upregulation.

Keywords: gynecologic cancer, immune-checkpoint inhibition, immune modulation, ovarian cancer, PARP inhibitor, PD-L1, STING

Introduction
Poly[adenosine diphosphate (ADP) ribose]polymerase (PARP) inhibitors (PARPi) and immune-checkpoint inhibitors have revolutionized the treatment paradigm for many cancer types. This is particularly notable in gynecologic cancers, with multiple US Food and Drug Administration (FDA) approvals for PARPi in recent years across all lines of treatment in ovarian cancer. Here, we review the mechanisms of PARP inhibition, the connection between deoxyribonucleic acid (DNA) damage, PARP, and immunogenicity, and the rationale for combined PARP inhibition and immune-checkpoint blockade (ICB) within the context of gynecologic cancers.

The PARP superfamily is composed of 17 proteins with varying catalytic abilities and functions. Though the majority of PARP proteins generate mono(ADP ribose; MAR) modifications,1 a few (PARP1, PARP2, and PARP5A/B) generate poly(ADP ribose; PAR) moieties. As the majority of available data focuses on PARP1 and PARP2, this review will also focus on these PARP proteins. PARP1 and PARP2 share a common Trp-Gly-Arg (WGR) domain, which interacts with DNA and regulates catalytic activity.2 PARP1 differs in having zinc-finger domains, a BRCA-C-terminus (BRCT) domain, and a WGR domain that is activated by DNA breaks, irrespective of the presence of phosphorylation groups. PARP2 has only a short N-terminal domain and a WGR domain that preferentially binds phosphorylated DNA breaks. These variances may underlie some differences in these protein’s functions, though globally, both PARP1 and PARP2 play roles in gene expression, cell signaling, and genome integrity. Overall, PARP1 is thus far considered to contribute the majority of known PARP function.

To date, the PARP inhibitors olaparib, rucaparib, and niraparib are FDA approved for the
treatment of epithelial ovarian cancer (EOC). All three drugs potently inhibit PARP1 and PARP2, with nanomolar half-maximal inhibitory concentration values. They are generally considered of comparable clinical efficacy, though harbor some differences in toxicity profiles. Currently, there are no approved indications for PARPi in the treatment of other gynecologic malignancies, though PARPi are under active study in endometrial and cervical cancers.

DNA repair and the role of PARP

High-fidelity repair of DNA damage is critical for cell survival. If DNA damage or replicative errors are unrepaired and propagated forward, the accumulation of progressive genomic instability can lead to cell death. PARPi are used in the treatment of gynecologic cancers, particularly in the context of existing DNA damage-repair deficiencies, for this purpose.

In response to particular types of DNA damage, PARP1 is recruited to and binds the sites of damage. DNA binding triggers a conformational change and PARP1 activation, leading to the addition of PAR moieties to itself and other proteins. PAR chains act as binding sites, recruiting and activating proteins involved in the DNA repair process upon PAR binding. This displaces PARP1 from DNA and catalyzes the repair process. In parallel, PARP1 will stall and protect replication forks, allowing time for DNA repair.

PARP1 is crucial to several repair pathways. Single-strand breaks (SSBs) in DNA, generated spontaneously or through base modifications, are rapidly recognized by PARP1. Upon binding, PARP1 recruits target proteins including the scaffold protein XRCC1, DNA ligase-3 and DNA polymerase β, catalyzing SSB repair. Base excision repair (BER), in which SSBs are generated in the process of an endonuclease removing a damaged base, therefore may also rely on PARP1, though data is conflicting as to whether PARP1 is essential for BER. Additionally, PARP1 may function in the nucleotide excision repair (NER) pathway, which removes stretches of damaged single-strand DNA (ssDNA), fills in the resultant gap, and ligates the repaired strand.

Double-strand breaks (DSBs), generated by DNA-damaging agents or from the collapse of replication forks, rely on PARP1 in multiple roles in several repair pathways. Firstly, PARP1 recognizes, and is recruited to, sites of DSBs, where PARylation recruits additional repair proteins, including ataxia–telangiectasia mutated (ATM) and the nuclease MRE11/RAD50/NBS1 (MRN) complex. DSBs can be repaired through homologous recombination (HR) or non-homologous end joining (NHEJ), a branchpoint influenced by the balance between functional BRCA1 and 53BP1. Evidence suggests that PARP2 limits 53BP1 accumulation, thereby promoting end-resection and HR. In high-fidelity HR repair, BRCA1 is recruited by PARP1 to sites of DSBs and helps to stabilize end-resected DNA. Alternatively, DSBs can be repaired through the less-fidelitous NHEJ pathway. In both classical and alternative NHEJ, PARP1 remains important for the appropriate recruitment of NHEJ-associated factors. NHEJ is particularly error prone compared with HR, due to the lack of a sister chromatid template and resultant insertions and deletions.

PARP1 additionally plays a vital role in DNA synthesis by engaging with DNA replication-associated proteins and stabilizing replication forks in the setting of replication stress. Importantly, PARP1 interaction with the DNA helicase RECQ1 slows and reverses replication forks, preventing inappropriate fork movement into unrepaired DNA lesions, which would cause replication fork collapse and formation of DSBs. Loading of RAD51 to damaged replication forks is regulated by both PARP1 and PARP2.

PARP1 impacts chromatin structure to better facilitate DNA accessibility and allow DNA damage repair. After recruitment to sites of DNA damage, PARP1 PARylates histones, which promote nucleosome disassociation and recruit chromatin remodelers to further induce chromatin relaxation so damaged DNA is more accessible for repair.

Therefore, PARPi can be highly deleterious due to effects at multiple points in the DNA repair and synthesis process. The negative effects of PARPi are amplified in the context of a cancer cell that may have underlying DNA repair- or cell-cycle-associated alterations. For example, high grade serous ovarian carcinoma (HGSOC), the most common subtype of EOC, is typified by high copy number variation, uncontrolled cell proliferation (most commonly due to p53 loss of function), and defective homologous recombination, all contributing to a high baseline degree of
genomic instability. Therefore, HGSOC is exquisitely predisposed to additional perturbation in the DNA repair process, such as by PARPi.

This forms the basis for synthetic lethality by PARPi in BRCA1/2-mutated cancers. PARP inhibition leads to the accumulation of unrepairred SSBs, which, in the setting of replication, are processed into DSBs that, due to the lack of a functional BRCA1/2, cannot be repaired through HR. DSBs are additionally unable to be repaired through alternative NHEJ, despite BRCA-deficient cells relying on polymerase-θ-mediated alternative NHEJ, as this process also relies on PARP. PARPi also disrupt the carefully controlled action of the PARP1-recruited MRE11 nuclease at the sites of replication-fork restart, which requires PARP1 and BRCA2 to appropriately disengage. In the setting of PARP inhibition and BRCA2 deficiency, the nuclease remains engaged on the DNA strand, leading to uncontrolled strand degradation, replication fork collapse, and DSBs that cannot be repaired. Lastly, PARP inhibition traps PARP on DNA, generating a DNA-protein complex that stalls replication forks; the process of replication-fork restart requires BRCA1/2 and functional PARP.

The immune system: role of PARP and effects of PARP deficiency or inhibition

It is now well recognized that the interface between the immune system and cancer is a dynamic, complex process. The immune system is engaged throughout tumorigenesis, including recognition of malignant cells and inflammation, immune exhaustion and pruning, and immune surveillance. It is therefore important to understand what roles PARP plays in the functioning immune system and what effects PARP deficiency and/or inhibition may have on specific immune cells.

Effects on T-cells

T-cell development is highly regulated, occurring through several steps of maturation and involving a complex system of transcription factors and cytokines. T-cells play numerous roles in cancer development, shaped by the signaling milieu in the tumor microenvironment.

PARP2 appears to be involved early in T-cell maturation, specifically in the development of CD4/CD8 double-positive thymocytes. PARP2 deficiency was associated with reduced total and double-positive populations of thymocytes; this was not seen with PARP1 deficiency. In double-positive thymocytes, PARP2 is theorized to be critical for repair of DNA strand breaks generated in the process of T-cell-receptor-α rearrangement. Without PARP2, unrepairred DNA breaks initiate a pro-apoptotic cascade.

In a murine model of dually PARP1/2-deficient T-cells, total populations of CD4 single-positive and CD8 single-positive T-cells were reduced, with greater reduction in the CD8 lineage. These results were seen only in dual PARP1/2 deficiency and not in singular PARP1 or singular PARP2 deficiency, with concurrently elevated markers of DNA damage and apoptosis suggesting that reduced T-cell populations were due to accumulation of genomic instability precipitating cell death and not solely a block in maturation. In the same murine model of background PARP1 deficiency and selective PARP2 deficiency in T-cells, implanted breast cancer cells grew larger and more rapidly compared with single PARP1- or single PARP2-deficient cells; intratumoral CD4 and CD8 T-cell infiltration was decreased, likely related to dual PARP1/2-deficiency-related lymphocyte cell death.

Expression of Foxp3 marks the differentiation of CD4 single-positive T-cells in regulatory T-cells (Tregs) and imparts the immunosuppressive capability of Tregs. The expression of Foxp3 is regulated by PARP1, which acts post-translationally to PARylate Foxp3, marking it for ubiquitination by the Stub1 E3 ubiquitin ligase and subsequent degradation. The role of PARP1 in indirectly modulating Foxp3 transcription via Smad3 binding at the Foxp3 enhancer is debated. Overall, in the setting of PARP1 deficiency, the population of CD4/Foxp3-positive Tregs increased, due to persistence of Foxp3. Consequently, expression of genes downstream of Foxp3 was increased, including of CD25, CTLA-4, and interleukin 10 (IL-10). Though one study noted that the increase in expression was associated with greater suppressive function of Tregs on peripheral blood mononuclear cells, this may not wholly reflect a tumor microenvironment. For example, the role of secreted IL-10 has been shown to be immunostimulatory, rather than suppressive, in different tumor contexts.
(nuclear factor of activated T-cells) family of transcription factors.\textsuperscript{32} NFAT activity is itself modulated by PARP1, whereby PARP1 binds and PARylates NFAT, increasing its DNA binding ability and regulating its nuclear import and export.\textsuperscript{33,34} It is important to note that this activity of PARP1 occurred secondary to T-cell stimulation and not due to the presence of DNA damage.\textsuperscript{34} Therefore, PARP1 directly impacts T-cell differentiation. PARP1 deficiency in T-cells resulted in reduced expression of cytokines reliant on NFAT, including IL-2 and IL-4, suggesting further downstream effects on immune-cell differentiation.\textsuperscript{33} Furthermore, PARP1 deficiency and/or inhibition may bias CD4 T-cell differentiation to a Th1 phenotype rather than a Th2 phenotype,\textsuperscript{35–37} though conflicting data may underscore context-specific differences. In a model of airway inflammation, olaparib treatment yielded increases in the Th1-associated cytokine interferon-γ (IFNγ) and expression of T-bet, a Th1-associated T-box transcription factor, while suppressing expression of the Th2-associated cytokines IL-4, IL-5, IL-6, IL-13, and M-CSF,\textsuperscript{36} suggesting a skew toward a Th1 phenotype. Conversely, in a model of inflammatory arthritis, PARP inhibition was associated with reduced expression of Th1-associated cytokines TNFα and IFNγ and partially inhibited Th1-cell clonal expansion.\textsuperscript{38}

Furthermore, PARP1 modulates transforming growth factor β (TGFβ)-receptor expression on CD4 T-cells. At least for TGFβ-receptor 2, this appears to be through direct binding of PARP on the \textit{tgfbr2} promoter to affect its transcription.\textsuperscript{28} Interestingly, PARP1 deficiency was associated with higher expression of TGFβ receptors, but inhibition of PARP1 enzymatic activity was associated only with increased TGFβ-receptor-1 expression, suggesting differential regulation. PARP inhibition also predisposed T-cells to greater sensitivity to TGFβ, and PARP1 deficiency with concurrent TGFβ treatment was associated with an increased Th17 population, which requires TGFβ for differentiation,\textsuperscript{28} suggesting that PARP1 plays this additional role in T-cell differentiation.

In addition to affecting T-cell differentiation, PARP1 and PARP2 affect T-cell function. In a murine model of background PARP1 deficiency with selective PARP2 deficiency in T-cells, the populations of activated CD4 and CD8 T-cells secreting IL-2 and IFNγ in response to viral inoculation were diminished.\textsuperscript{23} Dual PARP1/2 deficient models had a more dramatic reduction compared with models of singular PARP1 or singular PARP2 deficiency, suggesting additive roles in effector T-cell function. Furthermore, in the same murine model, CD4 and CD8 T-cells infiltrating implanted breast cancer tumors had reduced expression of genes associated with chemotaxis, T-cell activation, and T-cell-mediated cytotoxicity.\textsuperscript{25} Notably, gene expression was not changed in either PARP1 or PARP2 deficiency.

\textbf{In vivo models of BRCA1-deficient ovarian cancer} demonstrated that PARP inhibition using olaparib significantly increased the number of effector CD4 and CD8 T-cells intratumorally and peripherally, demonstrating the global effects of PARP inhibition.\textsuperscript{39} Moreover, olaparib-treated CD8 T-cells had reduced expression of the immune-checkpoint receptors PD-1, Tim-3, and Lag-3, associated with T-cell inhibition and exhaustion, and produced significantly higher levels of TNFα and IFNγ. Interestingly, intratumoral CD4/Foxp3 Tregs were not increased following olaparib treatment, counter to expectations in considering PARP1 modulation of Foxp3 expression. These results suggest overall that PARP inhibition was associated with an activated effector T-cell response with alteration in immune-checkpoint receptor expression that could predispose to response to immune-checkpoint blockade (ICB).

\textbf{Effects on B cells}

There is increasing evidence for a role of B-cells in malignancy, including both pro- and antitumor functions depending on the tumor microenvironment.\textsuperscript{40,41} B-cells play important roles in generating antibodies, but also modulate immunity independently of antibody generation, \textit{via} interactions with effector cells and antigen-presenting cells.

\textit{V(D)J} gene recombination is critical for the appropriate generation of immunoglobulins, occurring in the pre-B-cell stage. The generation and pairing of \textit{V}_{\text{JL}} and \textit{V}_{\text{H}}\textit{DJ}_{\text{H}} generate immunoglobulin M (IgM) in immature B-cells. Later on, mature B-cells undergo class-switching recombination, altering the immunoglobulin isotype, for example to IgG. Both the \textit{V(D)J} and class-switching recombination processes generate DSBs which are repaired through the PARP1-mediated NHEJ pathway, thus giving rise to the question of whether PARPi may impact humoral immunity. In steady-state conditions without...
introduction of an antigen stimulus, serum IgM and IgG levels were comparable between PARP1/2-proficient, singular PARP1 deficient, singular PARP2 deficient, and dual PARP1/2 deficient mice. Therefore, despite the role of PARP in NHEJ, PARP1/2 did not appear to be critical for V(D)J recombination nor class switching. Interestingly, dual PARP1/2 deficiency in B-cells did not impact Ig V(D)J recombination, baseline serum levels of IgM and IgG, or antibody responses to T-cell-dependent antigens, but led to reduced serum IgG levels in response to T-cell-independent antigens.

PARP plays a role in maintaining B-cell homeostasis, most notably in mediating the differentiation of transitional B-cells into follicular B-cells. Bone marrow B-cell progenitors and peripheral mature B-cells were preserved in a genetically engineered mouse model of dual PARP1/2 deficiency; however, peripheral transitional and follicular B-cell populations were significantly depleted. This suggests a crucial role of PARP at that level of B-cell differentiation. Notably, dual PARP1/2-deficient B-cells accumulated DNA damage and apoptosed at a faster rate compared with control, leading to B-cell lymphopenia, and underscoring the important role of PARP1/2 in maintaining genomic stability, even in immune cells.

PARP may additionally play a role in the expression of Bcl-6, a transcription factor essential for the generation of germinal centers and high-affinity antibodies. PARP1 binds the first intron of Bcl-6 and suppresses its transcription. PARP inhibition and PARP knockdown in vitro induced expression of Bcl-6, corroborating the inhibitory role of PARP1 in B-cell differentiation into germinal-center B-cells. It remains unknown at this time how B-cell homeostasis and immunoglobulin responses are affected by PARP-inhibition treatment in solid malignancies.

**Effects on dendritic cells**

Dendritic cells are pivotal antigen-presenting cells with the ability to activate and induce differentiation of T-cells, conferring an antitumor microenvironment.

PARP1 has been shown in several contexts to be important for the recruitment of dendritic cells to sites of inflammation, possibly through regulation of VCAM-1 expression. In contrast, in a murine model of PARP1 deficiency and selective PARP2 deficiency in T-cells, intratumoral infiltration by CD11b dendritic cells was higher compared with settings of PARP1 deficiency, PARP2 deficiency, or control. Whether PARP is critical to the function of dendritic cells remains unclear, as some studies suggest that PARPi impaired the maturation and antigen presenting function of DCs, while other studies did not.

There is evidence for an indirect role of PARP1 in activating dendritic cells (Figure 1). The synthetic lethality of PARP1 in an HR-deficient setting generates DNA damage and genomic instability, leading to micronuclei and cytosolic DNA. Cytosolic DNA activates the cyclic guanosine monophosphate (GMP)–adenine monophosphate synthase (cGAS)/stimulator of interferon genes (STING) pathway within the tumor cell, but is also exocytosed to act in a paracrine fashion, activating the cGAS/STING pathway in neighboring dendritic cells. In a BRCA1-deficient model of triple-negative breast cancer, olaparib was associated with significantly upregulated levels of tank-binding kinase 1 (TBK1) and interferon regulatory factor 3 (IRF3), markers of activated cGAS/STING. However, olaparib treatment of dendritic cells alone did not induce cGAS/STING pathway activation, implying that the action of PARPi on dendritic cells was indirect. Ultimately, the in vivo dendritic cell population increased and demonstrated increased antigen presentation and recruitment of CD8 T-cells. These findings were replicated in a BRCA1-deficient model of ovarian cancer.

In response to treatment with olaparib, tumor-associated dendritic cells were increased in number, with upregulated cell-surface costimulatory CD80 and CD86 and antigen-presenting major histocompatibility complex class II. Additionally, co-culturing olaparib-treated ovarian cancer cells with naïve dendritic cells led to increased levels of TBK1, IRF3, CXCL10, and IFNβ, indicating cGAS/STING activation and downstream gene expression, confirming the paracrine effect of PARPi on dendritic cells. Dendritic cells activated by cytosolic DNA stimulated CD4 T-cells to generate Th1-type cytokines, and induced formation of cytotoxic CD4 and CD8 T-cells.

**Effects on macrophages**

Macrophage phenotype is influenced by exposure to specific antigens and cytokines. Stimulation by Th1-associated cytokines promote a pro-inflammatory M1 phenotype, while stimulation...
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by Th2-associated cytokines promote an anti-inflammatory M2 phenotype. However, macrophage polarization and functions exist on a continuum between M1 and M2 and therefore, in turn, may be tumoricidal or tumorigenic.52,53

PARP1 may modulate macrophage phenotype polarization through its regulation of high-mobility group box protein 1 (HMGB1), an inflammatory mediator with macrophage-differentiating effects. In the setting of lipopolysaccharide stimulation, PARP1 PARylates HMGB1, facilitating its acetylation and subsequent displacement from chromatin, inducing migration of HMGB1 from the nucleus to the cytoplasm.54 This is paralleled by HMGB1 cytosolic translocation due to PARP1 activation in the setting of alkylating DNA damage.55 Cytosolic HMGB1 can be exocytosed through a lysosomal pathway and secreted into the extracellular space as a damage-associated molecular pattern (DAMP) with cytokine and chemokine functions. Though HMGB1 can signal polarization into an M1 phenotype via interaction with the receptor for advanced glycation products (RAGE),56 it can also direct M2 polarization through interaction with C1q complement.57 Under oxidative stress, PARP1 appears to protect M1 macrophages from cell death.58 In a pancreatic cancer model, PARP1 deficiency was associated with significantly fewer tumor-infiltrating macrophages, thought to be related to the concomitant decrease in vascular endothelial growth-factor receptor (VEGFR) expression.59,60 Ultimately, the effects of PARP and PARPi on macrophages will be directed by the immediate microenvironment and signaling milieu.
**The cancer cell: role of PARP and effects of PARP inhibition**

**DNA damage and the immune response**

Maintenance of genomic stability requires careful coordination of DNA damage repair, DNA synthesis, and cell-cycle regulation. Under conditions of genomic stress, double-stranded DNA is released into the cytoplasm, which initiates a cell-intrinsic innate immune response through the well-characterized cGAS/STING pathway.

The numerous roles of PARP in maintaining genomic stability result in significant deleterious effects of PARPi on the genome. Expectedly, PARP inhibition is associated with increased levels of cytosolic DNA, which are detected by cGAS, leading to production of the secondary messenger cyclic GMP. cGMP activates STING, prompting the recruitment of TBK1 and IFI16, activation of the IRF3 and NF-kB transcription factors, nuclear translocation of IRF3 and NF-kB, and expression of several genes that mediate an innate immune response. This includes expression of type 1 interferons and T-cell-recruiting chemokines (CCL5, CXCL10), leading to higher percentages of tumor-infiltrating T-cells. The specific phenotypes of recruited T-cells are an area of active investigation, as there are conflicting data regarding increased levels of CD4 T-cells, CD8 T-cells, and CD4/Foxp3 Tregs, with PARPi alone and PARPi in combination with other treatments.

Additionally, PARP1’s role in sensing DNA damage mediates a non-canonical pathway of STING activation. Upon binding DSBs, PARP1 recruits and activates ATM, which subsequently activates the ubiquitin ligase TRAF6. Translocation of TRAF6 to the cytosol and association with IFI16 and p53 results in STING activation. This non-canonical pathway, independent of cGAS, preferentially generates the pro-inflammatory transcription factor NF-kB, and to a lesser extent, generates activated IRF3.

**Evasion of NK-cell-mediated cancer cell death**

NK cells possess potent cytolytic abilities, mediated by direct cytotoxicity through release of perforin and granzyme, direct interaction with target cells via TNF-related apoptosis-inducing ligand (TRAIL) and Fas ligand, or indirectly through secretion of IFNγ and TNFα to stimulate apoptotic pathways in target cells. NK cells play crucial roles in the antitumor immune response, as they are able to kill tumor cells without prior antigen exposure.

In tumor cells, similar to in immune-system cells, PARP1 is required for HMGB1 localization to the cytoplasm. However, in the tumor-cell context, PARP1 activation occurred via stimulation by TRAIL; subsequent HMGB1 cytoplasmic localization promoted an autophagic response, protecting the tumor cell from TRAIL-induced caspase-8-mediated apoptosis.

Suppression of the PARP1/HMGB1 pathway via PARP inhibition and PARP deficiency reversed this resistance and sensitized cancer cells to TRAIL-mediated cell death. This suggests that antitumor effects of PARP inhibition may include enhanced tumor-cell sensitivity to NK-cell-mediated TRAIL activation and apoptosis.

PARPi-modulated susceptibility to NK-cell-mediated cytotoxicity may also occur through upregulation of death receptors on tumor cells. Olaparib and veliparib upregulated transcription and protein expression of the death receptors Fas and death receptor 5, sensitizing several cell lines, including ovarian cancer, lung cancer, and leukemic cells, to TRAIL-induced cell death. The effects of PARPi appeared to be specific to inhibition of PARP1 and PARP2 and not of the other PARP isoforms. These findings were recapitulated in prostate cancer cells, wherein NK cell killing was greater in cells pretreated with olaparib compared with control. These findings demonstrate that PARPi-induced antitumor effects are at least partially mediated by NK cells.

Furthermore, cancer-cell-surface expression of NKG2D ligands, which interact with NKG2D on NK cells to effect tumor-cell death, is downregulated by PARP1. In vitro and in vivo AML models demonstrated PARP1-mediated suppression of NKG2D ligands, and PARP inhibition was sufficient to suppress leukemogenesis. Interestingly, in addition to immune cells, cancer cells can also express the NKG2D receptor, co-opting it for other purposes.
autonomous stimulation and oncogenic signaling. In ovarian cancer, NKG2D expression was associated with increased cancer cell self-renewal capacity and tumor spheroid formation, though it is unclear how PARP may regulate autonomous NKG2D signaling.

**Regulation of immune-checkpoint ligands**

Olaparib, talazoparib, rucaparib, and PARPi knockdown resulted in higher levels of cancer cell PD-L1 expression in breast cancer cells in vitro and in vivo, an effect seen regardless of BRCA proficiency or deficiency. There are several possible mechanisms of PD-L1 upregulation. Transcription factor NF-kB, generated in response to activated IFI16/STING/TBK1 in human papillomavirus (HPV)-positive cervical cancer, binds the PD-L1 promoter, upregulating transcription. However, knockdown of IRF3, another crucial transcription factor in the cGAS/STING pathway, was sufficient to abrogate upregulation of PD-L1 in response to PARPi, suggesting multiple mechanisms of PD-L1 regulation. JAK1/2 activation in tumor cells is also sufficient to induce PD-L1, and therefore it is possible that PD-L1 is upregulated due to autocrine or paracrine JAK/STAT signaling downstream of a cGAS/STING/type 1 IFN response. IFN-γ was sufficient to induce PD-L1 expression in a non-small-cell lung cancer cell line, and therefore PD-L1 may be upregulated in response to IFNγ secretion by T-cells or NK cells recruited following the cGAS/STING/type 1 interferon response. PD-L1 expression is additionally modulated by glycoconjugate synthase kinase 3β (GSK3β), which induces phosphorylation-dependent degradation of PD-L1. Inactivation of GSK3β was associated with stabilized expression of PD-L1. PARP inhibition generates inactivated GSK3β, thereby preventing PD-L1 degradation, though the exact mechanism linking PARP and GSK3β is not yet known.

**Rationale for combined immune-checkpoint blockade and PARP inhibition**

Preclinical and correlative data provide strong support for the combination of PARP inhibition and ICB.

PARP inhibition has widespread effects on cells in both innate and adaptive immune responses (Figure 1). DNA damage and genomic instability generated by PARPi activates the cytosolic DNA sensing cGAS/STING pathway, culminating in a type 1 interferon response with several immunogenic effects. T-cell-associated chemokines increase T-cell recruitment and tumoral infiltration. PARP inhibition, via its effect on NFAT, may bias CD4 T-cell differentiation into pro-inflammatory Th1 cells, and may promote pro-inflammatory Th17-cell differentiation via its effects on the TGFβ receptor. PARPi-treated CD8 T-cells downregulated the immune-checkpoint receptors PD-1, Tim-3, and Lag-3, suggesting decreased propensity for T-cell inhibition and exhaustion, despite PARPi-associated increases in tumor-cell expression of PD-L1. Additionally, tumoral cytosolic DNA can act in a paracrine manner to activate the cGAS/STING pathway in dendritic cells, leading to increased antigen-presenting ability. Furthermore, PARP inhibition increases the sensitivity of tumor cells to NK-cell-mediated TRAIL-induced apoptosis. Taken together, PARPi may stimulate an immunogenic tumor microenvironment.

Disruption of immune-checkpoint interactions between tumor cells and T-cells has been the primary focus of immuno-oncologic development. In view of the numerous mechanisms of PARPi-associated PD-L1 upregulation, ICB is a logical pairing. In a murine model of small-cell lung cancer, PARP inhibition significantly increased PD-L1 expression, and dual inhibition with olaparib and an anti-PD-L1 agent induced significant tumor regression, greater than either agent alone. Correlative tumor analysis demonstrated markedly increased populations of CD3 T-cells and cytotoxic CD8 T-cells, and decreased populations of CD4/Foxp3 Tregs and exhausted PD-1/Tim-3 CD8 T-cells. The cGAS/STING pathway was directly implicated, as cGAS knockdown abolished the antitumor effect of combination olaparib/anti-PD-L1 therapy. In a BRCA1-deficient ovarian cancer model, combined olaparib and anti-PD-1 therapy significantly extended the survival of tumor-bearing mice compared with olaparib alone. In a breast cancer model, olaparib upregulated PD-L1 in both BRCA-deficient and -proficient cells, and combined olaparib and anti-PD-L1 treatment resulted in T-cell-mediated tumor cell death that was greater than olaparib alone. A preclinical study of niraparib and pembrolizumab in BRCA-mutated breast cancer, BRCA-mutated ovarian cancer, and BRCA wild-type skin-cancer cells demonstrated that niraparib induced T-cell infiltration regardless of BRCA status. Combination niraparib/pembrolizumab yielded better antitumor activity than...
either agent alone. There is early evidence that dual inhibition may reprogram the tumor micro-environment in a durable fashion. One mouse achieved a complete response with niraparib/pembrolizumab and growth of a second implanted tumor was prevented, even in the absence of active treatment, indicating an enduring antitumor response. This study also reported that niraparib/pembrolizumab treatment inhibited tumor growth in a sarcoma xenograft previously refractory to anti-PD-1 treatment. This suggests that the addition of PARPi was able to induce an inflammatory immune response sufficient to overcome prior ICB resistance.

**Perspectives for gynecologic cancers**

In theory, the combination of PARPi and ICB may provide benefit in two specific situations: to induce a greater or more durable response in settings of PARPi sensitivity or HR deficiency, or to gain antitumor effect in the setting of PARPi resistance or HR proficiency.

**Combination PARPi + ICB in HR deficiency**

In the setting of HR deficiency, and therefore assumed PARPi sensitivity, the combination of PARPi with ICB would presumably capitalize on the synthetic lethality of PARPi, a potentially higher neoantigen load, and the immunogenic effects of both. For example, HGSOC accounts for the majority of epithelial ovarian cancer (EOC). Within HGSOC, approximately 50% are HR deficient, harboring germline (~14%) and somatic (6%) BRCA1/2 mutations, BRCA1 promoter methylation (10%), and alterations in other HR-associated genes, such as RAD51C. In trials of PARPi as a single agent and in combinations in frontline maintenance, recurrence monotherapy, and recurrence maintenance settings, almost all of which were restricted to HGSOC, the subgroup of HR-deficient EOC has consistently achieved greater benefit than HR-proficient subgroups, underscoring the significant effect of synthetic lethality of PARPi, a potentially higher neoantigen load, increased CD3 and CD8 T-cell infiltration, a higher CD8:CD4 T-cell ratio, and higher PD-L1 expression compared with HR-proficient HGSOC, emphasizing the inherent immunogenicity of tumors with defective DNA repair. The phase II MEDIOLA trial [ClinicalTrials.gov identifier: NCT02734004] evaluating the combination of olaparib and durvalumab in 32 patients with germline BRCA1/2-mutated platinum-sensitive recurrent EOC found an overall response rate (ORR) of 71.9%, 28-week disease control rate (DCR) of 65.9%, median progression-free survival (PFS) of 11.1 months, and median duration of response of 10.2 months. There were seven patients (21.8%) with complete responses (CRs) and median overall survival (OS) was not yet reached at time of data presentation. In comparison, in the randomized phase III SOLO3 trial comparing olaparib alone with physician’s choice non-platinum chemotherapy in patients with germline BRCA1/2-mutated platinum-sensitive recurrent EOC, treatment with olaparib yielded an ORR of 72.2%, including 14 patients (9.3%) achieving a CR, and median PFS of 13.4 months, by blinded independent central review. Of olaparib-treated patients with a partial response (PR) or CR, median duration of response was 9.4 months. Data immaturity precluded OS estimation. Acknowledging the limitations of cross-trial comparisons, the MEDIOLA and SOLO3 trials appear to have similar response rates, median PFS estimates, and durations of response. This prompts the questions of whether and to what extent preclinical evidence of combination PARPi and ICB is borne out in clinical practice, and whether HR deficiency is the best context in which to visualize possible benefits of combined PARPi and ICB. Ongoing clinical trials (FIRST trial of niraparib/dostarlimab [ClinicalTrials.gov identifier: NCT03602859], ATHENA trial of rucaparib/nivolumab [ClinicalTrials.gov identifier: NCT03522246]) in the maintenance setting after first-line therapy will hopefully address whether addition of ICB is superior to PARPi alone in HR-deficient ovarian cancer.

This has bearing on other gynecologic malignancies in which subsets of disease are also HR deficient. For example, in endometrial cancer, alterations in HR-related genes as detected by next-generation sequencing was found in 20–30% of cases, occurring primarily in non-endometrioid, p53-mutant endometrial cancers, likely reflecting the copy-number-high/serous-like molecular subgroup. In one study, 46% of non-endometrioid endometrial cancer specimens were HR deficient by functional assay. Loss or deficiency of MRE11, part of the MRN complex crucial for end resection in HR, sensitized an endometrial carcinoma cell line to talazoparib. Furthermore, PTEN-deficient endometrial cancer cells predispose to PARPi sensitivity in vitro, theorized to be due to transcriptional downregulation of RAD51. Case reports describe PARPi
inducing clinical responses in endometrial carcinomas, one case involving a patient with PTEN-deficient disease\textsuperscript{102} and another involving a patient with \textit{BRCA}-mutated endometrial carcinoma;\textsuperscript{103} however, to date there are no published clinical trial data of PARPi in HR-deficient endometrial carcinoma. Nonetheless, trials of combination PARPi and ICB in endometrial carcinoma are ongoing, including combinations of olaparib and durvalumab [ClinicalTrials.gov identifier: NCT03951415], rucaparib and nivolumab [ClinicalTrials.gov identifier: NCT03572478], and niraparib and TSR-042 (dostarlimab, anti-PD-1) [ClinicalTrials.gov identifier: NCT03016338]. The combination of avelumab and talazoparib [ClinicalTrials.gov identifier: NCT02912572] is being investigated in microsatellite-stable (MSS) endometrial carcinoma, a population also typified by genomic stability.

HPV-positive cervical cancer, accounting for nearly all cervical cancer cases, may also be deficient in effective HR. While the prevailing understanding of HPV-induced carcinogenesis involves E6- and E7-mediated cell-cycle dysregulation through their well-documented effects on p53 and Rb, evidence is emerging for virally mediated upregulation of HR-related genes\textsuperscript{105,107} and by E7-mediated interaction with the E3 ubiquitin ligase RNF168,\textsuperscript{110} and suppression of NHEJ and shunting of DSB repair toward the more error-prone microhomology-mediated end joining.\textsuperscript{111} Interestingly, \textit{in vitro} analysis of nine patient-derived cervical-cancer cell lines, eight of which were HPV 16- or HPV 18-positive, found that none met the criteria of HR deficiency, defined using log2-ratios and allele frequencies to generate a loss of heterozygosity score in a method used previously in EOC trials.\textsuperscript{112} Despite this, olaparib inhibited tumor growth and induced apoptosis in three of the cell lines and suppressed xenograft tumor growth from a sensitive cell line. Therefore, a subset of cervical cancer may be amenable to synthetic lethality using PARPi; adding ICB may take further advantage of PD-L1 expression and/or amplification.\textsuperscript{113,114} Though there are trials of PARPi combined with chemotherapy and/or radiotherapy in cervical cancer, there are thus far no published clinical trials of PARPi monotherapy nor, to our knowledge, ongoing trials of PARPi and ICB.

\textbf{Combination PARPi + ICB in HR proficiency}

The ability of combination PARPi and ICB to inhibit tumor growth regardless of \textit{BRCA} status (i.e. regardless of HR repair status) suggests that this combination should not be limited only to states of HR deficiency. Indeed, the benefit of PARPi/ICB regardless of HR status is exemplified in the phase I/II TOPACIO/KEYNOTE-162 trial of combined niraparib and pembrolizumab.\textsuperscript{115} In the pooled platinum-resistant EOC cohort of 62 patients, response rates were similar regardless of tumor \textit{BRCA} or HR status. The majority of patients with EOC had \textit{BRCA} wild-type (79\%) or HR-proficient (53\%) disease. An ORR of 19\% was seen in subgroup analysis in both the \textit{BRCA} wild-type and HR-proficient groups, similar to the ORR of 18\% in the \textit{BRCA}-mutated group, and ORR of 14\% in the HR-deficient group. Intriguingly, five of eight patients achieving a PR or CR lasting longer than 6 months had tumors that were \textit{BRCA} wild type. Correlative work, which included immunogenomic profiling and highly multiplexed single-cell imaging on tumor samples from patients enrolled the study, identified two determinants of response; mutational signature 3 reflecting defective HR, and positive immune score as a surrogate of interferon-primed exhausted CD8+T-cells in the tumor microenvironment.\textsuperscript{116} Absence of both features yielded no responses, while presence of one or both features captured all objective responses. Single-cell spatial analysis revealed prominent interactions of exhausted CD8+T-cells with PD-L1+ macrophages and/or PD-L1+ tumor cells as mechanistic determinants of response. Of note, two extreme responders showed differential clustering of exhausted CD8+T-cells either with PD-L1+ macrophages in the first patient, or with cancer cells harboring genomic PD-L1 and PD-L2 amplification in the second patient.
Similarly, a phase II trial of olaparib and durvalumab [ClinicalTrials.gov identifier: NCT02484404] in 35 patients with predominantly platinum-resistant (86%), BRCA wild-type (77%) recurrent EOC yielded an ORR of 14%. However, a subset of patients experienced a durable benefit, as evidenced by a clinical benefit rate (PR + SD ⩾6 months) of 34%, including 10 platinum resistant patients, of whom three attained a PR with a median duration on study of 17.2 months, and of whom seven patients achieved a median of 7.3 months’ disease stabilization. Correlative studies of paired pre- and on-treatment tissue and blood specimens indicated that the combination of olaparib and durvalumab promoted an immune-inflamed environment, with increased tumoral IFNγ and CXCL9/CXCL10 expression and increased systemic IFNγ and TNFα production. Increased systemic IFNγ was associated with clinical benefit and improved PFS (HR 0.37, p = 0.023). Treatment significantly increased tumor-infiltrating lymphocytes (TILs) and increased PD-L1 expression compared with pre-treatment specimens. Interestingly, PARPi may not induce an effect through the STING pathway. Only 4 of 14 patients with available samples had increased expression of STING following treatment, none of whom experienced a response. However, in patients without increased STING expression but who achieved clinical benefit, the type 1 interferon downstream inflammatory chemokines CCL4 and CCL5 were still increased, suggesting an alternative pathway of activation. Additionally, increased post-treatment levels of VEGF3R3 were associated with worse PFS, suggesting that activation of the VEGF/VEGFR pathway may arise to compensate for treatment-related immunostimulatory changes. Accepting the limitations of cross-trial comparisons, response rates in this and the TOPACIO trial are in line with responses to PARPi monotherapy in similar populations.

**Conflict of interest statement**

EKL declares no conflicting interests.

PAK reports participation in advisory boards from GSK/Tesaro, Merck, AstraZeneca, and Bayer, outside the submitted work.

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