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Commentary

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The evolving landscape of predictive biomarkers of response to PARP inhibitors

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Poly(ADP-ribose) polymerase inhibitors (PARPis) are DNA-damaging agents that trap PARP-DNA complexes and interfere with DNA replication. Three PARPis — olaparib, niraparib, and rucaparib — were recently approved by the FDA for the treatment of breast and ovarian cancers. These PARPis, along with 2 others (talazoparib and veliparib), are being evaluated for their potential to treat additional malignancies, including prostate cancers. While lack of PARP-1 confers high resistance to PARPis, it has not been established whether or not the levels of PARP-1 directly correlate with tumor response. In this issue of the *JCI*, Makvandi and coworkers describe an approach to address this question using [^{18}F]FluorThanatrace, an [^{18}F]-labeled PARP-1 inhibitor, for PET. The tracer was taken up by patient tumor tissue and appeared to differentiate levels of PARP-1 expression; however, future studies should be aimed at determining if this tracer can be used to stratify patient response to PARPi therapy.

PARPs, PARP trapping, and PARPis

PARPs attach poly(ADP-ribose) (PAR) polymers to proteins, including themselves. Glu, Asp, Lys, Arg, and Ser have been indicated as the major ADP-ribosylation target residues (1, 2). PARPs belong to a large, 17-member family of enzymes that share a common ADP-ribosyl transferase (ART) motif. PARP-1 and PARP-2 are the relevant targets of clinical PARPis (reviewed in refs. 3, 4) and are directly activated by binding to DNA breaks. NAD^+ serves as the building block for the PAR polymers (PARylation). An important salvage pathway for cellular NAD^+ relies on nicotinamide phosphoribosyltransferase (NAMPT). Upon PARylation, nuclear proteins acquire a highly negative charge, which changes their overall structure and binding to their nuclear partners. PARylation of histones and other chromatin proteins leads to their dissociation from DNA, as it is also highly negatively charged. Auto-PARylation of PARP-1 and PARP-2 leads to their dissociation from DNA, a critical step that provides access to the broken DNA for the repair enzymes that are initially recruited by PARP-1 and PARP-2. Hydrolysis of the PAR polymers by PAR glycohydrolase (PARG) regenerates unmodified PARP (Figure 1).

PARPis are selective inhibitors of the PARP nuclear proteins that detect DNA damage and promote repair. Hence, these inhibitors were initially developed to prevent DNA repair based on the observation that genetic deletion of the genes encoding PARP-1 sensitizes cells to ionizing radiation, alkylating agents, and topoisomerase I (TOP1) inhibitors. However, PARPis primarily act as single agents by PARP trapping (5). Specifically, PARPis act as DNA-damaging agents by trapping PARP-DNA complexes on endogenous DNA breaks. This explains why PARP-1 is required for PARPi cytotoxicity and why lack of PARP-1 confers high resistance to PARPis (Figure 1).

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Three PARPis are now licensed for use as single agents: olaparib for patients with germline *BRCA*-mutated breast and advanced ovarian cancers who have previously been treated with chemotherapy (6); rucaparib for patients with germline and/or somatic *BRCA*-mutated advanced ovarian cancer treated previously with chemotherapy (7); and niraparib, which along with olaparib is approved as maintenance therapy regardless of *BRCA* mutation in patients with recurrent epithelial ovarian, fallopian tube, or primary peritoneal cancer who are in a complete or partial response to platinum-based chemotherapy (8, 9). Two additional PARPis, talazoparib and veliparib, are currently in advanced phase clinical trials.

PARPis differ in their PARP-trapping potency (Figure 1). While talazoparib is the most potent PARP trapper (nanomolar potency), veliparib is primarily a catalytic inhibitor with only weak PARP-trapping ability (5, 10). Hence, the antitumor activity of veliparib is limited as a single agent, and this PARPi is mostly active in combination with TOP1 inhibitors (11) and radiotherapy (12). The other PARPis primarily act as DNA-damaging agents by PARP trapping, with talazoparib having the greatest potency, followed by niraparib, olaparib, and rucaparib, which have similar potency (Figure 1A) (5, 10).

Although many patients are benefiting from PARPi therapy, predicting an individual's response to treatment remains imprecise. Mutations in *BRCA* genes, which are essential for high-fidelity repair of DNA double-strand breaks through the homologous recombination (HR) repair pathway, do not entirely account for the treatment benefit associated with PARPis. Measures of HR deficiency (HRD) also are not sufficiently precise to predict which patients will respond, and recent trials have shown that PARPi therapy can be beneficial to patients without known HRD (13). It is clear from studies in cancer cell lines and

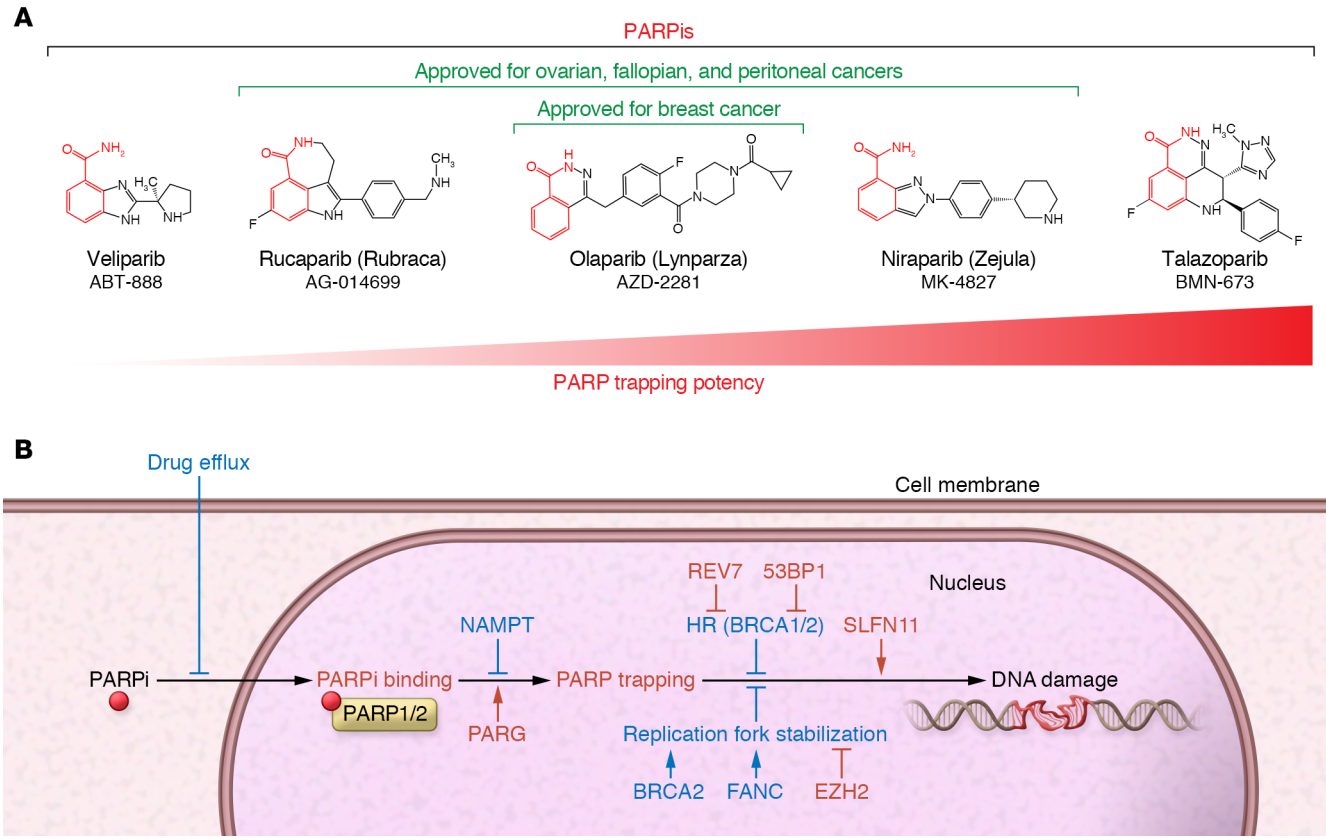

Figure 1. Clinical PARP inhibitors (PARPis) and determinants of response and resistance to PARPis. (**A**) Three PARPis are approved for ovarian and/or breast cancers. All PARPis comprise a nicotinamide moiety (red), which binds the β-NAD^+ acceptor site in PARP-1 and PARP-2. PARPis differ by their PARP-trapping potency. (**B**) PARPis are potential substrates for the drug efflux pumps (PgP/ABCB1), which limit cellular accumulation. Binding of the PARPis to the NAD^+ site of PARP-1 and PARP-2 (red circle) results both in catalytic inhibition by competitive inhibition of β-NAD^+ binding and in PARP trapping by a proposed reverse allosteric mechanism (5). Trapping can be mitigated by increased PARP-1 and PARP-2 auto-PARylation due to high NAD^+ synthesis by nicotinamide phosphoribosyltransferase (NAMPT) or enhanced by PAR removal by poly(ADP-ribose) glycohydrolase (PARG). Trapping of PARP-1 and PARP-2 damages replicating cells. Cells can alleviate this effect by stabilizing replication forks through BRCA2 and other factors of the Fanconi anemia pathway (FANC) or suppression of the polycomb complex (EZH2). Damaged cells can also repair the broken forks by homologous recombination (HR) involving BRCA1 or BRCA2 or commit themselves to death through Schlafen 11 (SLFN11). Inactivation of 53BP1 and REV7 reactivates HR. Red symbols define determinants of response to the PARPis. Blue symbols signify resistance to PARPis. Synthetic lethality (4) occurs in cancer cells deficient for the resistance factors highlighted in blue. BRCA1 and BRCA2 were the first resistance factors identified and led to the approval of PARPis as monotherapy.

clinical responses that sensitivity and resistance to PARPis is determined by factors beyond tumor DNA repair status (Figure 1).

Determinants of response and resistance to monotherapy PARPis

The first identified determinants of response to PARPi were the presence of deleterious *BRCA1* and *BRCA2* mutations (14, 15), which substantially sensitize cancer cells to PARPis (5, 14, 15) and render such cells defective in HR, such as occurs in HRD cancers. It is increasingly clear that HR repair is a multifactorial process that involves many proteins beyond BRCA1 and BRCA2. Indeed, at the beginning of HR, the ends of the DNA double-strand breaks need to be processed by nucleases (MRE11, CtIP, DNA2, exonuclease 1) to generate 3′ single-stranded DNA (ssDNA) tails. These ssDNA tails need to be coated by RAD51 to form presynaptic filaments, which then invade a duplex region of intact DNA that is homologous to the broken DNA, forming D-loops that require chromatin remodeling and adjustment of DNA supercoiling. The invading DNA needs to be copied by DNA polymerase(s) and ligated before restoration of the original DNA sequence. Finally, after dissociation, the repaired DNA can move back to its normal nuclear position (territory). The precise regulation and factors involved in many of these steps are still unknown, explaining why it remains difficult to comprehensively identify HRD based on current genomic analyses. Furthermore, multiple different mutations can lead to loss of *BRCA1* or *BRCA2* function, and scoring such mutations can be ambiguous. An alternative to single gene mutation, deletion, or methylation analyses is to use HRD genomic signatures. However, HRD genomic signatures may only represent so-called mutational genomic scars, which persist while the cancer cells reactivate HR. For instance, inactivation of 53BP1 (16), REV7 (17), and EZH2 (18) in *BRCA1*- or *BRCA2*-deficient cells can reactivate HR and confer resistance to PARPis (Figure 1).

Other important determinants of the response downstream of PARP trapping are the ability of cells to stabilize replication forks (5) or, alternatively, to irrevers-

ibly arrest replication in response to replication stress (19). In addition to their role in HR repair, *BRCA2* and other Fanconi anemia–associated genes act to stabilize replication forks, whereas EZH2 tends to promote replication fork breakage (collapse) by engaging MUS81 endonuclease at such forks (Figure 1) (18). In addition, recent studies demonstrate that stressed replication forks can induce irreversible cell-cycle arrest by engaging Schlafen 11 (SLFN11) with the replication helicase complexes, and blocking replication by opening chromatin through the ATPase activity of SLFN11 (20).

Implications of a PARP-1 tracer

Protein levels of PARP-1 and PARP-2 are also determinants for PARPi sensitivity, because PARP-1 and PARP-2 are required as substrates for PARP trapping. In this issue, a study by Makvandi and coworkers (21) confirms the requirement of PARP-1 for the cytotoxicity of PARPis (Figure 1) and shows that low PARP-1 expression is among the potential causes of resistance to PARPis. The Makvandi et al. study (21) is primarily aimed at providing a way to determine the level of PARP-1 in tumors by a novel imaging procedure. The authors used [^{18}F]FluorThanatrace ([^{18}F]FTT) for PET. [^{18}F]FTT has previously been demonstrated to result in highly specific tracer uptake in animal models and humans (22, 23). MicroPET studies in a preclinical patient-derived xenograft (PDX) mouse model showed high tracer uptake in tumors relative to normal tissue. Blockade of radiotracer uptake following pretreatment with olaparib was used to confirm specificity of [^{18}F]FTT for PARP-1. In a clinical trial of patients with epithelial ovarian cancer who underwent surgical debulking or biopsy, [^{18}F]FTT localized to areas of known cancer. Moreover, [^{18}F]FTT demonstrated a wide dynamic range of uptake, correlating with in vitro measures of PARP-1 expression.

The study by Makvandi et al. provides proof-of-principle that a noninvasive imaging procedure can assess PARP-1 levels and/or activity. Whether this approach will be useful in determining which patients may benefit from PARPi therapy warrants further study. PARP-1 is an abundant protein in the nucleus and a fraction of it is trapped by PARPis. Hence, it is not clear how much reduced expression of PARP-1 could contribute to the reduction of PARP trapping and resistance to PARPis. In the cancer cell line databases CellMiner CDB (http://discover.nci.nih.gov/cellminercdb), NCI-60 (https://dtp.cancer.gov/discovery_development/nci-60/), Cancer Cell Line Encyclopedia (https://portals.broadinstitute.org/ccle), and Genomics of Drug Sensitivity in Cancer (http://www.cancerrxgene.org/), none of the approximately 1,000 cell lines is null for PARP-1, suggesting that PARPi resistance due to PARP-1 deficiency is not expected in tumors. Data showing a positive correlation between PARP-1 expression level and sensitivity to PARPis are necessary to utilize the methods of Makvandi et al. in the clinic. Archived tumors could be retrospectively examined for PARP-1 expression and response to PARPis. It would also be important to test [^{18}F]FTT PET in parallel with PARPis in clinical trials that stratify patients based on other known determinants of PARPi response. The effect of heterogeneity of [^{18}F]FTT-based PARP-1 expression in terms of tumor responses also warrants further investigation. [^{18}F]FTT-based determination of PARP-1 expression could also allow monitoring for the emergence of resistance mediated by downregulation of PARP-1.

In conclusion, multiple effectors and molecular signatures should be considered for the prediction of responders and nonresponders to PARPis, including DNA repair and replication defects, expression of SLFN11, and hyperactive drug efflux pumps. The novel approach described by Makvandi and coworkers will enable us to monitor not only PARP-1 expression levels in patient tumors but also the penetration of PARPis into tumors, which can provide us with useful information for the selection of patients who may benefit from PARPi therapy.

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