VOLUME 33 · NUMBER 12 · APRIL 20 2015

JOURNAL OF CLINICAL ONCOLOGY

BIOLOGY OF NEOPLASIA

Poly (ADP-Ribose) Polymerase Inhibitors: Recent Advances and Future Development

Clare L. Scott, Elizabeth M. Swisher, and Scott H. Kaufmann

Clare L. Scott, Walter and Eliza Hall Institute of Medical Research and Royal Melbourne Hospital, Parkville, Victoria, Australia; Elizabeth M. Swisher, University of Washington, Seattle, WA; and Scott H. Kaufmann, Mayo Clinic, Rochester, MN.

Published online ahead of print at www.jco.org on March 16, 2015.

Supported by grants from the National Health and Medical Research Council Australia (Project Grant No. 1062702) and Independent Medical Research Institutes Infrastructure Support Scheme, by Cancer Council Victoria (Sir Edward Dunlop Fellowship in Cancer Research), by the Australian Cancer Research Foundation, and by an Operational Infrastructure Support Grant from the Victorian State Government (C.L.S.); by a V Foundation Translation Research Award (E.M.S.); by Grant No. P50 CA136393 from the National Cancer Institute (S.H.K.); and by an Ovarian Cancer Research Fund Program Project Development Grant and a Department of Defense Synergistic Translational Leverage Award (E.M.S., S.H.K.).

C.L.S., E.M.S., and S.H.K. contributed equally to this work.

Terms in blue are defined in the glossary, found at the end of this article and online at www.jco.org.

Authors' disclosures of potential conflicts of interest are found in the article online at www.jco.org. Author contributions are found at the end of this article.

Corresponding author: Scott H. Kaufmann, MD, PhD, Division of Oncology Research, Gonda 19-212, Mayo Clinic, 200 First St SW, Rochester, MN 55905; e-mail: kaufmann.scott@mayo.edu.

0732-183X/15/3312w-1397w/$20.00

DOI: 10.1200/JCO.2014.58.8848

ABSTRACT

Poly (ADP-ribose) polymerase (PARP) inhibitors have shown promising activity in epithelial ovarian cancers, especially relapsed platinum-sensitive high-grade serous disease. Consistent with preclinical studies, ovarian cancers and a number of other solid tumor types occurring in patients with deleterious germline mutations in *BRCA1* or *BRCA2* seem to be particularly sensitive. However, it is also becoming clear that germline *BRCA1/2* mutations are neither necessary nor sufficient for patients to derive benefit from PARP inhibitors. We provide an update on PARP inhibitor clinical development, describe recent advances in our understanding of PARP inhibitor mechanism of action, and discuss current issues in the development of these agents.

J Clin Oncol 33:1397-1406. © 2015 by American Society of Clinical Oncology

POLY (ADP-RIBOSE) POLYMERASE INHIBITORS IN THE CLINIC

Since last reviewed in *Journal of Clinical Oncology*,[1] poly (ADP-ribose) polymerase (PARP) inhibitors have demonstrated efficacy in a number of settings, including platinum-sensitive epithelial ovarian cancer (OC)[2,3] and breast cancer (BC) with mutation in *BRCA1* or *BRCA2*.[4]

OC

PARP inhibitors have been studied most extensively in high-grade serous OC, with efficacy noted particularly in platinum-sensitive high-grade serous OC. A pivotal phase II study demonstrated that olaparib induces responses in *BRCA1/2* mutation carriers with progressive high-grade OC, with efficacy greater in, but not restricted to, platinum-sensitive OC.[5] A subsequent study comparing olaparib maintenance therapy versus placebo after response of relapsed high-grade serous OC to platinum-based therapy demonstrated progression-free survival (PFS) of 8.4 months with olaparib versus 4.8 months without (hazard ratio, 0.35; $P < .001$).[6] A preplanned subset analysis showed greatest benefit in OC with *BRCA1/2* mutations (either germline or somatic), with PFS extended from 4.3 to 11.2 months (hazard ratio, 0.18; $P < .001$).[7] These data and additional results led to approval of olaparib by the European Commission as maintenance therapy for platinum-responsive advanced OC and by the US Food and Drug Administration as fourth-line monotherapy, with both approvals limited to the subset of cases with *BRCA1/2* mutations.

Importantly, women whose OC lacked *BRCA1/2* mutations also derived benefit in the randomized olaparib maintenance trial (hazard ratio, 0.53; 95% CI, 0.33 to 0.84; $P < .001$),[7] suggesting a sensitive non–*BRCA1/2*-mutation subgroup, as predicted from preclinical studies.[8] Excitingly, a large subset of patients derived long-term benefit from olaparib, with approximately 40% and approximately 20% of women with *BRCA1/2*-mutant or *BRCA1/2*–wild type high-grade serous OC, respectively, not requiring a different therapy within 3 years after random assignment, compared with only approximately 10% and approximately 1% of those receiving placebo.[9] Olaparib also prolonged time to second subsequent therapy in both *BRCA1/2*-mutated OC (hazard ratio, 0.44; $P < .001$) and non–*BRCA1/2*-mutated OC (hazard ratio, 0.64; $P < .034$), suggesting that PARP inhibitor treatment did not make OC less responsive to platinum or other therapies, a conclusion supported by additional studies.[10] Olaparib in combination with carboplatin[11] or cediranib[12] has also shown efficacy against OC in phase I and II studies. Notably, however, hematologic toxicity prevented continuous dosing of olaparib when combined with typical carboplatin doses (area under curve of 5 every 3 weeks).[11]

A number of additional PARP inhibitors, including veliparib, rucaparib, niraparib, and BMN-673, have also shown efficacy in high-grade serous OC.[13] On the basis of the encouraging results of the phase II olaparib maintenance trial,[6,7] phase III trials

Table 1. Open and Soon-to-Open Phase III Trials of PARP Inhibitors in Ovarian Cancer

Drug	Sponsor	ClinicalTrials.gov Identifier	Trial	First Line or Relapsed	Ovarian Cancer Population*	*BRCA1/2* WT Allowed?	Platinum-Resistant Patients Allowed?
Olaparib	AstraZeneca	NCT01844986	SOLO1; GOG3004	First line	FIGO stage IIIC or IV; high-grade serous/endometrioid; deleterious *BRCA1/2* mutation†; CR or PR to initial platinum	No	No
Veliparib	Abbvie		GOG3005	First line	High-grade serous/endometrioid; genomic testing at enrollment	Yes	NA
Olaparib	AstraZeneca	NCT01874363	SOLO2; ENGOT-OV21	Relapsed	High-grade serous/endometrioid; deleterious *BRCA1/2* mutation†; sensitive to penultimate platinum regimen; CR or PR to current platinum	No	No
Rucaparib	Clovis	NCT01968213	ARIEL3	Relapsed	High-grade serous/endometrioid; sensitive to penultimate platinum regimen; CR or PR to current platinum	Yes	No
Niraparib	Tesaro	NCT01847274	ENGOT-OV16; NOVA; US Oncology; others	Relapsed	Deleterious *BRCA1/2* mutation or high-grade serous with CR or PR to current platinum	Yes	No

Abbreviations: ARIEL3, Assessment of Rucaparib in Ovarian Cancer Phase 3 Trial; CR, complete response; ENGOT-OV, European Network for Gynaecological Oncological Trial Groups-Ovarian Cancer; FIGO, International Federation of Gynecology and Obstetrics; GOG, Gynecologic Oncology Group; NA, not applicable; NOVA, Niraparib in Ovarian Cancer; PARP, poly (ADP-ribose) polymerase; SOLO, Studies of Olaparib in Ovarian Cancer; WT, wild type.
*Ovarian, fallopian tube, and peritoneal cancers.
†Deleterious *BRCA1/2* mutation includes germline or somatic.

with the same design are ongoing in OC (Table 1). Each of these is also attempting to improve identification of responsive patients through analysis of biospecimens (eg, examining biomarkers of homologous recombination [HR] deficiency [HRD]).[14]

BC

Overall, PARP inihibitors have been less efficacious in BC than in high-grade serous OC,[13] perhaps reflecting the biologic heterogeneity[15,16] and low *BRCA1/2* somatic mutation rate[17] in triple-negative BC. Responses were observed in 11 (41%) of 27 patients in an initial phase II trial of olaparib in *BRCA1/2*-mutated BC.[4] In contrast, there were no responses in 23 patients with triple-negative BC regardless of *BRCA1/2* mutation status. Other PARP inhibitors, including the potent agent BMN-673,[18] have induced responses in small studies, and phase III trials are ongoing in *BRCA1/2*-mutated BC and triple-negative BC (Table 2).

Other Solid Tumor Types

Additional solid tumors contain subsets that are likely to have HRD and potentially be PARP inhibitor responsive.[19] Five percent of

Table 2. Phase III Trials of PARP Inhibitors in Other Solid Tumors

Sponsor	ClinicalTrials.gov Identifier	Trial	Treatment	Cancer Population	Biomarker
Abbvie	NCT02032277	Brightness	standard NAC plus carboplatin/veliparib or standard NAC plus carboplatin/placebo	Early-stage triple-negative breast cancer	None
AstraZeneca	NCT02032823	OlympiA	Maintenance olaparib or placebo	High-risk early-stage *HER2*-nonamplified breast cancer after adjuvant chemotherapy	*BRCA1/2* mutation
AstraZeneca	NCT02000622	OlympiaD	Olaparib or physician's choice	Advanced breast cancer	*BRCA1/2* mutation
Abbvie	NCT02163694		Paclitaxel/carboplatin plus veliparib or paxlitaxel/carboplatin plus placebo	Advanced *HER2*-nonamplified breast cancer	*BRCA1/2* mutation
Tesaro	NCT01905592	BRAVO	Niraparib or physician's choice	Second-line or beyond *HER2*-nonamplified breast cancer	*BRCA1/2* mutation
AstraZeneca	NCT02184195	POLO	Maintenance olaparib or placebo	Pancreatic cancer after first-line platinum-based chemotherapy	*BRCA1/2* mutation
AstraZeneca	NCT01924533		Paclitaxel/olaparib or paclitaxel/placebo followed by maintenance olaparib or placebo	Progressive gastric cancer, second line	None
Abbvie	NCT02106546		Paclitaxel/carboplatin plus veliparib paclitaxel/carboplatin plus placebo	First-line advanced squamous non–small-cell lung cancer	None
Abbvie	NCT02152982		Temozolamide plus veliparib or temozolomide plus placebo	First-line glioblastoma	MGMT promoter hypermethylation

Abbreviations: BRAVO, Niraparib Versus Physician's Choice in Her2 Negative, Germline BRCA Mutation-Positive Breast Cancer; MGMT, O6-methylguanine-DNA methyltransferase; NAC, neoadjuvant chemotherapy; PARP, poly (ADP-ribose) polymerase; POLO, Olaparib in gBRCA Mutated Pancreatic Cancer Whose Disease Has Not Progressed on First Line Platinum-Based Chemotherapy.

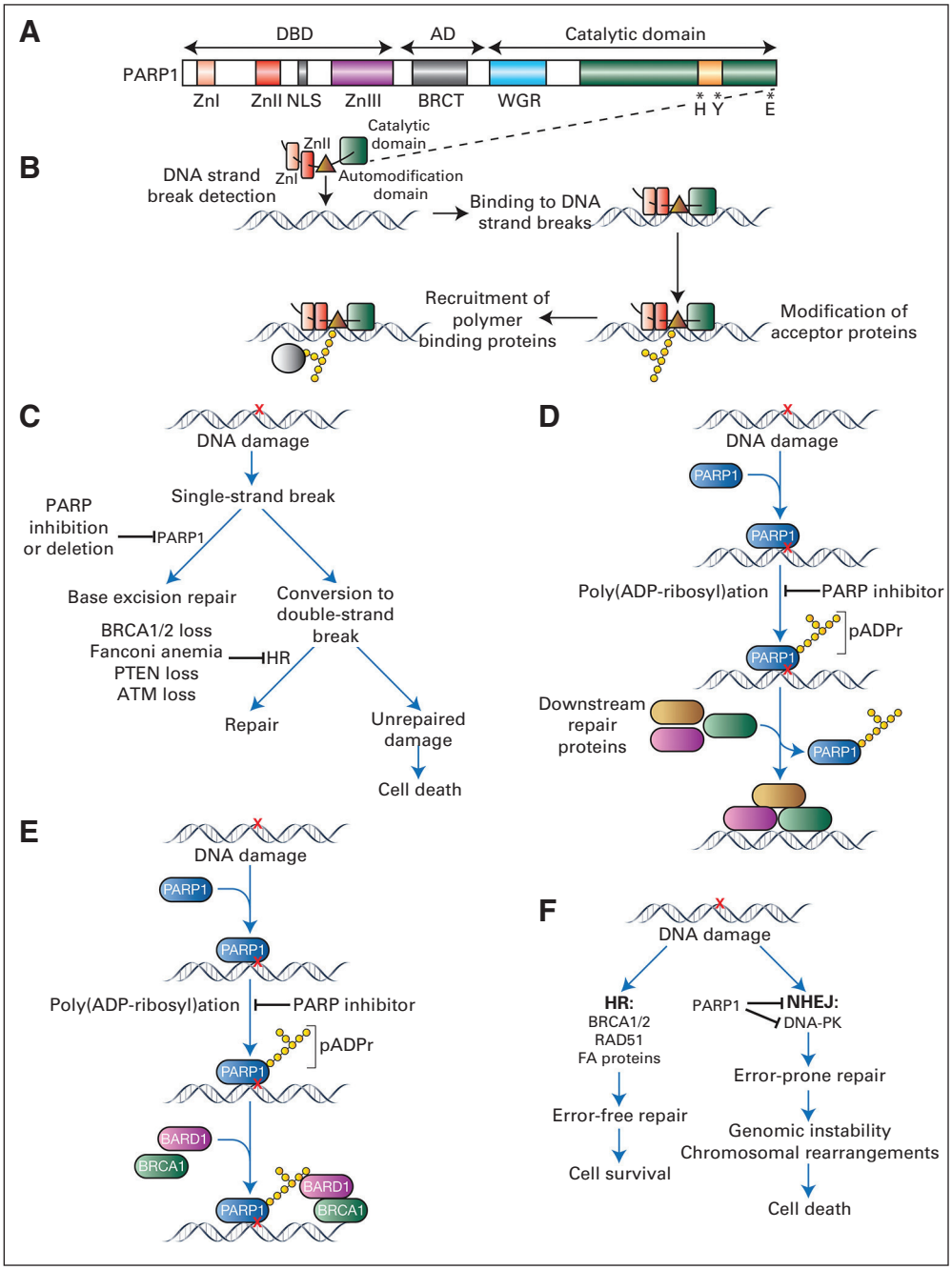

Fig 1. Summary of poly (ADP-ribose) [pADPr] polymerase 1 (PARP1) structure, function, and proposed contribution to synthetic lethality. (A) Schematic of PARP1 structure. (B) On binding to damaged DNA, PARP1 undergoes conformation change that increases its catalytic activity, leading to cleavage of NAD^+ and addition of ADP-ribose units to various proteins, including its own automodification domain. Resulting pADPr polymers (depicted as chains of yellow circles) alter function of proteins that are modified (eg, by decreasing affinity of PARP1 for damaged DNA)[29] and also recruit additional proteins that bind to polymer noncovalently.[30,31] (C-F) Models proposed to explain observed synthetic lethality between homologous recombination (HR) deficiency and PARP inhibition. These models emphasize (C) role of PARP1 in base excision repair, (D) recruitment of DNA repair proteins, (E) recruitment of BARD1-BRCA1 complex, and (F) suppression of nonhomologous end joining (NHEJ). AD, automodification domain; BRCT, *BRCA1* C-terminal domain; DBD, DNA binding domain; FA, Fanconi anemia; NLS, nuclear localization signal; PK, protein kinase; WGR, tyrptophan-glycine-arginine-rich domain; Zn, zinc finger.

cutaneous melanomas and gastric cancers, 5%-19% of familial pancreatic cancers, and 1% of prostate cancers harbor germline *BRCA1/2* mutations, with encouraging reports of responses to olaparib in *BRCA1/2*-mutant pancreatic and prostate cancers.[20] Clinical trials of single-agent PARP inhibitor treatment are ongoing in additional tumor types, with responses reported in melanoma, PTEN-deficient endometrial cancer, and colorectal carcinoma.[19]

Unanswered Questions

At present, it remains unclear how to best identify patients who will respond to PARP inhibitors. Although tumor phenotypes can provide rough predictions, as evidenced by responses of sporadic triple-negative BC[13,21] and high-grade serous OC to PARP inihbitor monotherapy,[21] the response rates are lower than for *BRCA1/2*-mutant BC or OC.[13] Accordingly, it seems that optimal clinical development might be advanced by improved understanding of both the mechanism of action of PARP inhibitors and mechanisms of resistance.

PRIMER ON PARP BIOLOGY

Since the initial description of poly (ADP-ribose) [pADPr] synthesis in the 1960s,[22,23] PARP biology has been extensively studied.[24-28] PARP1 (Fig 1A) is the founding member of a family of enzymes[24-26] that exhibit homology in their active sites, where the dinucleotide

NAD^+ binds and is cleaved during mono- or poly (ADP-ribosyl)ation of protein substrates.[26,32,33] Although 17 PARP family members have been identified in mammalian cells,[26,34] only six synthesize pADPr,[27,34] and only three (PARP1, PARP2, and PARP3) play identified roles in DNA repair.[35,36]

PARP1 is the best understood of these enzymes (Fig 1B). In cells with certain types of DNA damage, particularly nicks and double-strand breaks (DSBs),[37] PARP1 binds to damaged DNA and undergoes a conformational change that realigns critical residues in the enzyme active site,[38-40] producing an up to 500-fold increase in activity.[39,41,42] Once activated, PARP1 synthesizes pADPr chains covalently bound to a variety of chromatin proteins, although PARP1 itself is the acceptor for most of the polymer.[39,43] The resulting pADPr chains not only alter the functions of the covalently modified proteins[29,43-45] but also noncovalently bind a wide variety of additional nuclear proteins.[30,31,39,46-48]

Like other post-translational modifications, pADPr is highly dynamic. After DNA damage, polymers consisting of scores or hundreds of subunits are detectable within seconds,[41,42,49,50] resulting in rapid recruitment of additional DNA repair proteins.[49,50] Once formed, pADPr is also rapidly degraded by pADPr glycohydrolase, assuring that pADPr levels reflect persistent damage, and the response is extinguished as repair ensures.[51-53]

Through its synthesis of pADPr, PARP1 contributes to a number of DNA repair pathways.[27,28] In its most extensively studied role, PARP1 is essential for base excision repair (BER),[54-56] a process that removes a single damaged base and restores DNA integrity.[28,57] In addition, PARP1 binds to DSBs and recruits the proteins MRE11 and NBS1[49] to initiate HR,[58-60] a high-fidelity repair process that allows one copy of a gene to serve as a template for restoration of a second copy of the same gene.[28,61,62] PARP1 also poly (ADP-ribosyl)ates *BRCA1*, further contributing to and fine-tuning HR-mediated DSB repair in HR-competent cells.[63] Moreover, PARP1 prevents binding of the Ku proteins to free DNA ends,[64] thereby preventing activation of the competing but error-prone nonhomologous end-joining (NHEJ) DSB repair pathway. In addition, PARP1 is essential for microhomology mediated (alternative end-joining) repair,[65,66] a third DSB repair pathway.

PARP1 also contributes to additional cellular processes. It helps restart replication forks that stall because of nucleotide depletion or collisions with bulky lesions,[67-70] modulates gene transcription,[71] regulates chromatin structure,[71-73] alters cytoplasmic microRNA processing and action,[74] and affects energy metabolism.[27,75,76] Despite its involvement in all of these processes, however, PARP1 is not essential. *Parp1* knockout mice develop normally[77] and do not exhibit any phenotype until they encounter genotoxic stress.[54] These observations prompted the initial development of PARP inhibitors as agents to enhance targeted DNA damage.[28,78,79]

PARP2 and PARP3 also contribute to DNA repair.[27,36] PARP2 cooperates with PARP1 in synthesizing pADPr after DNA damage.[80,81] PARP3 suppresses error-prone NHEJ[82] while simultaneously partnering with PARP1 to enhance DSB repair.[83] The observation that the PARP inhibitors undergoing clinical testing interact strongly with the active sites of PARP2 and PARP3 in addition to PARP1[84] raises the possibility that effects of PARP inhibitors reflect inhibition of all three family members.

HOW PARP BIOLOGY CONTRIBUTES TO SYNTHETIC LETHALITY

Current development of PARP inhibitors as anticancer agents is motivated by the hypersensitivity of HR-deficient cells to PARP inhibition[85,86] and the ability of PARP inhibitors to sensitize cells to certain types of DNA damage.[27,28] There is emerging evidence that these two effects might reflect different aspects of PARP biology.

The observation that PARP inhibitors selectively kill *BRCA1/2*-deficient cells in preclinical models[85,86] was rapidly followed by the demonstration that additional changes leading to HRD also confer PARP inhibitor hypersensitivity.[8,87,88] At least four different aspects of PARP1 biology have been invoked to explain this so-called synthetic lethality, although each model also has limitations.

Inhibition of BER

Because PARP1 is essential for BER,[36,89] initial explanations suggested that DNA single-strand breaks (SSBs), which arise during normal cellular activity and are ordinarily repaired by BER, persist during PARP inhibitor treatment and are converted to DSBs, which are repaired by HR in HR-proficient cells but remain unrepaired in HRD cells (Fig 1C).[90,91] The inability to detect SSB accumulation during PARP inhibitor treatment,[92] however, casts doubt on this model. Moreover, knockdown of PARP1 kills HRD cells,[85,86,93] whereas knockdown of XRCC1, the protein immediately downstream of PARP1 in BER, does not,[93] suggesting that loss of PARP1 activity is critical for killing of HRD cells, but loss of BER is not.

Trapping of PARP1 on Damaged DNA

When DNA damage activates PARP1,[40,41,94] the resulting pADPr recruits additional repair proteins[30,46,47,55] and simultaneously diminishes the affinity of PARP1 for DNA,[29] allowing its dissociation so other repair proteins can bind. Conversely, PARP1 that cannot synthesize polymer remains bound to damaged DNA and inhibits DNA repair under cell-free conditions (Fig 1D).[29] Moreover, overexpression of the isolated PARP1 DNA binding domain, which also recognizes damaged DNA but cannot synthesize pADPr, potentiates certain types of DNA damage.[95,96] PARP1 that is inactivated by a PARP inhibitor would likewise be expected to bind to damaged DNA and inhibit repair. This trapping mechanism has been implicated in the synergy between PARP inhibitors and certain DNA damaging agents, including temozolomide[97,98] and topotecan.[99] Extrapolating from these observations, it has been suggested that cytotoxicity of PARP inhibitors in HRD cells might result from trapping of PARP1 at sites of endogenous damage,[100] although this mechanism fails to explain the observation that PARP1 knockdown also selectively kills BRCA1/2-deficient cells.[85,86,93]

Defective BRCA1 Recruitment

BRCA1 recruitment to damaged DNA involves two steps[50]: first, an interaction between pADPr at the damage site and the pADPr binding protein BARD1, which brings along its binding partner BRCA1, and second, an interaction of BRCA1 with γ-H2AX, a modified histone formed in response to DNA damage.[101] If *BRCA1* mutation impairs the BRCA1/γ-H2AX interaction, recruitment of the BARD1-BRCA1 complex to pADPr becomes critical for DNA repair (Fig 1E). The ability of PARP inhibitors to diminish recruitment of the BARD1-BRCA1 complex to damaged DNA, thereby

impairing DSB repair, provides an explanation for the PARP inhibitor hypersensitivity of cells with certain *BRCA1* mutations,[50] but it is unclear whether this explains PARP inhibitor hypersensitivity of cells with other HR defects.

NHEJ Activation

A fourth explanation for PARP inhibitor–induced killing focuses on the role of PARP1 in suppressing the error-prone NHEJ repair pathway (Fig 1F).[93,102] Several proteins in this pathway,[103] including Ku70, Ku80, and DNA-PKcs, are pADPr binding proteins.[30,46,47] The interactions of Ku70 and Ku80 with pADPr suppress NHEJ.[64,104,105] Conversely, PARP inhibitors de-repress NHEJ, which then becomes active in HR-deficient cells.[93] Importantly, chromosomal rearrangements and mutations, felt to be hallmarks of error-prone NHEJ,[86] are induced by PARP inhibitors and diminished by simultaneous addition of DNA-PK inhibitors to HR-deficient cells.[93] Moreover, PARP inhibitor cytotoxicity in HR-deficient cells is diminished by manipulations that inhibit NHEJ,[93,106,107] suggesting that activation of error-prone NHEJ contributes to PARPi/HRD synthetic lethality (Fig 1F). Conversely, PARP inhibitor sensitivity of HR-deficient cells is enhanced by changes that inhibit alternative end joining,[108] another DSB repair pathway that functions in parallel with HR and NHEJ. It is unclear, however, what activates the NHEJ pathway in PARP inhibitor–treated cells or how cells survive when HR and NHEJ are both disabled.

Potential Implications for Patient Selection

These models of PARP inhibitor–induced killing make different predictions regarding PARP inhibitor sensitivity and resistance.[102] The PARP trapping model (Fig 1D), for example, predicts that cancers with higher PARP1 expression will be more sensitive to PARP inhibitors (because of increased PARP1 trapping on damaged DNA), whereas the other models predict that cancers with lower PARP1 expression will be more sensitive. Furthermore, the NHEJ model (Fig 1F) predicts that changes affecting the rate of NHEJ will have an impact on PARP inhibitor sensitivity, in agreement with the observation that loss of 53BP1 (protein that facilitates NHEJ) or the NHEJ protein Ku80, DNA-PKcs, or Artemis diminishes PARP inhibitor sensitivity,[93,106,107,109-111] whereas loss of POLQ, the DNA polymerase in the alternative end-joining pathway, enhances PARP inhibitor sensitivity.[108] Accordingly, sorting out which of these models accounts for responses in the clinical setting might help identify patients more likely to respond to PARP inhibitors.

WHICH PATIENTS ARE MOST LIKELY TO RESPOND, AND HOW CAN WE BEST IDENTIFY THEM?

In the absence of more refined understanding of PARP inhibitor action, *BRCA1/2* mutation status has been the most extensively studied predictor of PARP inhibitor sensitivity to date. When PARP inhibitors are administered as single agents in the relapsed setting, *BRCA1/2*-mutated OC has a 30% to 45% objective response rate.[5,112,113] A higher response rate is observed in platinum-sensitive *BRCA1/2*-mutant high-grade serous OC than in platinum-resistant or -refractory groups,[112] but responses in cases of platinum-resistant disease[114] suggest that PARP inhibitors could also be useful in subsets of patients with resistant or refractory disease. Responses to PARP inhibitor therapy in other solid tumors that occur in families with germline *BRCA1/2* mutations, including pancreatic cancer, melanoma, and prostate cancer, have also been reported.[20]

In contrast, not all patients with deleterious *BRCA1* or *BRCA2* mutations at diagnosis respond to PARP inhibitors. In cell lines, secondary somatic mutations in *BRCA1*- or *BRCA2*-mutant cancer cells can restore protein expression, reconstitute HR, and confer resistance to PARP inhibitors and platinum.[115-117] Secondary mutations that restore BRCA1 and BRCA2 also predict platinum and PARP inhibitor resistance in the clinical setting.[118,119] It seems that approximately 45% of recurrent platinum-resistant *BRCA1/2*-mutated OCs have secondary somatic mutations.[118] Interestingly, clinical cancer specimens most commonly sustain secondary somatic mutations that revert the mutant allele to wild-type sequence, making secondary mutations highly predictive of response but technically difficult to identify.[118]

In addition to reversion mutations, HR can be restored in other ways. Some mutant *BRCA1* alleles encode proteins that are potentially functional but degraded rapidly (so-called hypomorphic alleles). Stabilization of these mutant proteins (eg, by elevated expression of heat shock protein 90) can restore HR and confer PARP inhibitor resistance without any secondary *BRCA1* mutation.[120] Likewise, decreased expression of 53BP1, which ordinarily channels DSB repair to NHEJ, restores HR and confers PARP inhibitor resistance in *BRCA1*-mutant cells despite the continued absence of BRCA1 protein.[109,110,121] The extent to which these mechanisms contribute to PARP inhibitor resistance in clinical OC remains to be fully defined.

Despite the current focus on *BRCA1/2* mutation carriers with OC, responses are not limited to this group. OCs with somatic *BRCA1/2* mutations seem to be as likely to benefit from PARP inhibitor maintenance therapy as those with inherited mutations,[7] although the number of treated patients with somatic mutations is small. Moreover, germline or somatic mutations in other genes critical to HR correlate with platinum sensitivity in OC and might also predict PARP inhibitor response.[122] Intriguing efficacy has been reported for olaparib in PTEN-deficient endometrial cancer[123] and in combination with paclitaxel in gastric cancer with *ATM* deficiency.[124] Studies including *PALB2*-mutated OC and pancreatic cancer are also under way.

In addition to mutations, other processes, including epigenetic alterations and changes in expression of microRNAs or transcription factors, could in principle impair HR and confer PARP inhibitor sensitivity. *BRCA1* promoter hypermethylation, which downregulates BRCA1 expression, occurs in 10% to 15% of OCs and has been proposed as a mechanism of HRD.[125-127] However, data from The Cancer Genome Atlas and others fail to correlate *BRCA1* hypermethylation with increased platinum sensitivity or improved survival,[128] suggesting that epigenetic BRCA1 downregulation may have a less profound impact on HR and PARP inhibitor sensitivity than inactivating *BRCA1* mutations. In short, improved understanding of PARP biology and HRD is providing important new clues for predicting PARP inhibitor responders versus nonresponders.

PARP INHIBITOR–CONTAINING COMBINATION THERAPY

Improved understanding of PARP biology is also contributing insights into the design of PARP inhibitor–containing combination

therapy. PARP inhibitors have been combined with standard chemotherapy, such as platinum in OC and BC[13] or temozolomide in melanoma, BC, glioblastoma, and acute leukemia, as well as with signal transduction inhibitors (eg, gefitinib in *EGFR*-mutant non–small-cell lung cancer).[13,19] Mechanisms underlying these combinations fall into two broad categories: first, induction of HRD and PARP inhibitor hypersensitivity in cells that initially contain an intact Fanconi anemia (FA)/HR pathway, or second, enhancement of DNA damage through interference with one of the roles of PARP1.

Previous studies have demonstrated that HRD can be induced by a variety of treatments, including epidermal growth factor receptor inhibitors[129] or cyclin-dependent kinase inhibitors,[130] which promote BRCA1 trafficking from the nucleus to the cytoplasm; phosphatidylinositol 3-kinase inhibitors, which downregulate Rad51[131] or BRCA1/2[132]; ATR inhibitors, which diminish replication stress–induced activation of cell-cycle checkpoints and repair[133]; or even PARP inhibitors themselves.[134] Whether pharmacologic induction of HRD will sensitize clinical cancers to PARP inhibitors as effectively as inactivating mutations in FA/HR pathway genes remains to be determined.

PARP inhibitors also sensitize cells to certain DNA-damaging agents.[27,28,78,79] Different modes of PARP inhibitor action depicted in Figure 1 explain these effects. For example, PARP inhibitors acting as inhibitors of BER (Fig 1C) sensitize cancer cells to the nucleoside analog floxuridine.[135,136] In contrast, sensitization to temozolomide and other methylating agents reflects the PARP trapping mechanism (Fig 1D). Not only do PARP inhibitors increase the amount of PARP1 and PARP2 bound to methylated DNA,[98,100] but diminished PARP1 protein protects cells from methylating agents,[97,137] as predicted by this mechanism. Importantly, complete PARP1 inhibition might not be required to sensitize cells through this mechanism, because trapping of only a small amount of PARP1 on the DNA should impede repair of some of the lesions and enhance cytotoxicity. This might explain the severe hematologic toxicity observed when PARP inhibitors are combined with temozolomide[138] or topoisomerase I poisons,[139] where a similar mechanism of sensitization has been reported.[99] Whether this trapping mechanism can be harnessed to selectively increase the toxicity of DNA damage in cancer cells as compared with normal tissues in the clinical setting remains to be established.

PREVIOUS BARRIERS TO CLINICAL IMPLEMENTATION

Despite the promising clinical results observed thus far, there have been a number of barriers to clinical development of PARP inhibitors, including confusion about what constitutes a bona fide PARP inhibitor as well as problems with predictive biomarkers, pharmacodynamic end points, and ideal trial design.

Implications of Accurate Mechanism of Action

PARP inhibitor development was delayed by inaccurate classification of earlier compounds. In particular, iniparib was classified as a PARP inhibitor based on its inhibition of purified PARP1.[140] When iniparib failed to enhance the efficacy of the gemcitabine/oxaliplatin doublet in triple-negative BC,[141] the entire class of PARP inhibitors was considered by many to have failed.[142] It turned out, however, that iniparib does not inhibit PARP in intact cells.[143,144] Until this was realized, the inaccurate classification of iniparib as a PARP inhibitor slowed pivotal testing of bone fide PARP inhibitors.

Identification of Predictive Biomarkers

At the present time, *BRCA1/2* loss-of-function mutations, either germline or somatic, have been the most extensively studied biomarkers of PARP inhibitor response. However, restricting PARP inhibitor development to *BRCA1/2*-mutated cancers would exclude additional cancers that may benefit. Because not all of the genes that affect DNA repair are currently known, a functional test of DNA repair capability that could be applied in the clinical setting would accelerate the identification of cancers appropriate for PARP inhibitor therapy. Initially, static tests such as immunohistochemistry or immunofluorescence for RAD51 pathway components, including RAD51 itself, were suggested as a way to determine whether DNA repair was occurring. However, antibodies to RAD51 have not proven sufficiently specific, sensitive, or reliable for clinical application.

At present, there is substantial interest in assays of genomic scarring (ie, subchromosomal amplifications and deletions thought to reflect HRD).[128,145-149] Preliminary data from both patient-derived xenografts and the ARIEL2 (Assessment of Rucaparib in Ovarian Cancer Phase 2 Trial) trial suggest that an assay using loss of heterozygosity to identify genomic scarring may be useful to predict PARP inhibitor response in OC without *BRCA1/2* mutations.[150,151] In contrast, it is important to emphasize that genomic scarring will not disappear when HR is restored by these secondary mutations, suggesting that assays of genomic scarring might need to be supplemented with assays for resistance mechanisms.[149]

Limitations of Pharmacodynamic Assays

Most early-phase PARP inhibitor trials have included measurement of pADPr to assess PARP1 inhibition. Because PARP activity can increase up to 500-fold after DNA damage,[39,41,42] it is important that 50% or even 90% PARP inhibition not be viewed as satisfactory suppression of pADPr synthesis. In early reports of failed efficacy, for example, the dose of veliparib guided by pADPr assays was 20 to 60 mg per day, which is much less than the 200 to 400 mg twice daily being delivered in veliparib trials now showing efficacy.

Limitations of Combination Trial Design

Most existing combination trials have started with the premise of adding PARP inhibitors to standard-dose chemotherapy. This has often led to administration of low doses of PARP inhibitors, which is concerning given evidence suggesting a dose-response relationship for PARP inhibitors. The alternative of using a low-dose chemotherapeutic regimen such as oral metronomic cyclophosphamide has been explored, but a standard dose of cyclophosphamide (50 mg daily) was again used, resulting in a relatively low veliparib dose (60 mg twice daily) at the maximum-tolerated dose.[152] An alternative approach of combining a near-maximal PARP inhibitor dose with lower, intermittent doses of a DNA-damaging agent such as oral cyclophosphamide should be considered.

PERSPECTIVE ON FUTURE DEVELOPMENT

With the previous considerations in mind, we offer suggestions that we hope will advance the development of PARP inhibitors.

How Can We Most Efficiently Identify Patients Who Will Benefit From PARP Inhibitors?

Patients are currently considered for PARP inhibitor trials if they have a particular tumor type (eg, high-grade serous OC or triple-negative BC) or their cancer could belong to a relevant molecular subtype (eg, *BRCA1/2*-mutated breast, ovarian, pancreatic, or prostate cancer). Given the known relationship between *BRCA1/2* mutations and PARP inhibitor responsiveness, we suggest that all PARP inhibitor trials enrolling these patients should report *BRCA1/2* mutation status for all participants (both germline and somatic), analogous to trials of any other therapy with a known molecular target.

The current focus on *BRCA1*- and *BRCA2*-mutated BC or OC should also be reexamined. Other cancers (eg, a substantial fraction of *BRCA1/2*–wild type high-grade nonserous OCs) have hallmarks of HRD and might respond to PARP inhibitors. Although it is currently unclear how to best identify PARP inhibitor–responsive cancers, biomarker development trials such as ARIEL2[14] should inform this issue. Patients could then be selected for subsequent trials using promising biomarkers (including FA/HR pathway–mutation testing) rather than cancer type, thereby allowing PARP inhibitors to be tested in various rare cancer subtypes that might never be studied on their own.

Can We Learn More About Drug Resistance in the Clinical Setting?

At present, there is little information about the causes of disease progression after initial clinical response to PARP inhibitors. Optional tumor biopsies on progression that have been incorporated into several PARP inhibitor trials[14,153] should help address this issue. The ability of the off-study biopsies to help guide the next therapy for some patients is an added benefit. Until HRD can be reliably identified through analysis of circulating tumor cells or circulating tumor DNA, we strongly advocate both on- and off-study biopsies in the setting of trials that can productively use them to better understand resistance and ways to circumvent it.

Are Current Expectations Reasonable?

In view of the initial high expectations for PARP inhibitors[90] and disappointment after the negative iniparib phase III trial in BC,[142] it is important to ask what can reasonably be expected of PARP inhibitors. All current models (Fig 1) suggest that these agents kill susceptible cancer cells by perpetuating DNA damage. Thus, their efficacy might be similar to that of other DNA-damaging agents in the same cancers. Accordingly, the similar response rates of olaparib and liposomal doxorubicin in relapsed *BRCA1/2*-mutant OC, albeit with lower toxicity in the olaparib arm,[114] should not be a surprise. Moreover, PARP inhibitors would be expected to select for pre-existing resistant subclones[154,155] just as conventional chemotherapeutic agents do, explaining why the majority of relapsed platinum-responsive OCs progress during PARP inhibitor treatment over the first 18 months.[7] These considerations suggest that PARP inhibitors will benefit suitably chosen patients but will not be curative in advanced disease, even if *BRCA1* or *BRCA2* is mutated. Thus, it will be important to study cancers with prolonged responses to PARP inhibitors[9] to search for even better predictive markers. Moreover, PARP inhibitors will need to be tested in settings of lower disease burden, where their benefit might be even greater (eg, chemoprevention in suitable high-risk groups[156]) as maintenance therapy (Table 1) or in combination with other agents in the advanced-disease setting. Only in this way will the tantalizing activity of these agents be optimized for clinical benefit.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Disclosures provided by the authors are available with this article at www.jco.org.

AUTHOR CONTRIBUTIONS

Conception and design: All authors
Administrative support: All authors
Collection and assembly of data: All authors
Data analysis and interpretation: All authors
Manuscript writing: All authors
Final approval of manuscript: All authors

REFERENCES

1. Ashworth A: A synthetic lethal therapeutic approach: Poly(ADP) ribose polymerase inhibitors for the treatment of cancers deficient in DNA double-strand break repair. J Clin Oncol 26:3785-3790, 2008
2. Reinbolt RE, Hays JL: The role of PARP inhibitors in the treatment of gynecologic malignancies. Front Oncol 3:237, 2013
3. Lee JM, Ledermann JA, Kohn EC: PARP inhibitors for BRCA1/2 mutation-associated and BRCA-like malignancies. Ann Oncol 25:32-40, 2014
4. Tutt A, Robson M, Garber JE, et al: Oral poly(ADP-ribose) polymerase inhibitor olaparib in patients with BRCA1 or BRCA2 mutations and advanced breast cancer: A proof-of-concept trial. Lancet 376:235-244, 2010
5. Audeh MW, Carmichael J, Penson RT, et al: Oral poly(ADP-ribose) polymerase inhibitor olaparib in patients with BRCA1 or BRCA2 mutations and recurrent ovarian cancer: A proof-of-concept trial. Lancet 376:245-251, 2010
6. Ledermann J, Harter P, Gourley C, et al: Olaparib maintenance therapy in platinum-sensitive relapsed ovarian cancer. N Engl J Med 366:1382-1392, 2012
7. Ledermann J, Harter P, Gourley C, et al: Olaparib maintenance therapy in patients with platinum-sensitive relapsed serous ovarian cancer: A preplanned retrospective analysis of outcomes by BRCA status in a randomised phase 2 trial. Lancet Oncol 15:852-861, 2014
8. McCabe N, Turner NC, Lord CJ, et al: Deficiency in the repair of DNA damage by homologous recombination and sensitivity to poly(ADP-ribose) polymerase inhibition. Cancer Res 66:8109-8115, 2006
9. L'heureux S, Ledermann JA, Kaye SB, et al: Characterization of ovarian cancer long-term responders on olaparib. J Clin Oncol 32:359s, 2014 (suppl 15s; abstr 5534)
10. Ang JE, Gourley C, Powell CB, et al: Efficacy of chemotherapy in BRCA1/2 mutation carrier ovarian cancer in the setting of PARP inhibitor resistance: A multi-institutional study. Clin Cancer Res 19:5485-5493, 2013
11. Lee JM, Hays JL, Annunziata CM, et al: Phase I/Ib study of olaparib and carboplatin in BRCA1 or BRCA2 mutation-associated breast or ovarian cancer with biomarker analyses. J Natl Cancer Inst 106:dju089, 2014
12. Liu JF, Barry WT, Birrer M, et al: Combination cediranib and olaparib versus olaparib alone for women with recurrent platinum-sensitive ovarian cancer: A randomised phase 2 study. Lancet Oncol 15:1207-1214, 2014
13. Burgess M, Puhalla S: BRCA 1/2-mutation related and sporadic breast and ovarian cancers: More alike than different. Front Oncol 4:19, 2014
14. Swisher EM, McNeish IA, Coleman RL, et al: ARIEL 2/3: An integrated clinical trial program to assess activity of rucaparib in ovarian cancer and to identify tumor molecular characteristics predictive of response. J Clin Oncol 32:380s, 2014 (suppl 15s; abstr TPS5619)
15. Lehmann BD, Bauer JA, Chen X, et al: Identification of human triple-negative breast cancer subtypes and preclinical models for selection of

targeted therapies. J Clin Invest 121:2750-2767, 2011

16. Balko JM, Giltnane JM, Wang K, et al: Molecular profiling of the residual disease of triple-negative breast cancers after neoadjuvant chemotherapy identifies actionable therapeutic targets. Cancer Discov 4:232-245, 2014

17. Gonzalez-Angulo AM, Timms KM, Liu S, et al: Incidence and outcome of BRCA mutations in unselected patients with triple receptor-negative breast cancer. Clin Cancer Res 17:1082-1089, 2011

18. Shen Y, Rehman FL, Feng Y, et al: BMN 673, a novel and highly potent PARP1/2 inhibitor for the treatment of human cancers with DNA repair deficiency. Clin Cancer Res 19:5003-5015, 2013

19. O'Sullivan CC, Moon DH, Kohn EC, et al: Beyond breast and ovarian cancers: PARP inhibitors for BRCA mutation-associated and BRCA-like solid tumors. Front Oncol 4:42, 2014

20. Kaufman B, Shapira-Frommer R, Schmutzler RK, et al: Olaparib monotherapy in patients with advanced cancer and a germ-line BRCA1/2 mutation: An open-label phase II study. J Clin Oncol 32:701s, 2014 (suppl 15s; abstr 11024)

21. Gelmon KA, Tischkowitz M, Mackay H, et al: 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. Lancet Oncol 12:852-861, 2011

22. Chambon P, Weill JD, Mandel P: Nicotinamide mononucleotide activation of new DNA-dependent polyadenylic acid synthesizing nuclear enzyme. Biochem Biophys Res Commun 11:39-43, 1963

23. Sugimura T, Fujimura S, Hasegawa S, et al: Polymerization of the adenosine 5′-diphosphate ribose moiety of NAD by rat liver nuclear enzyme. Biochim Biochim Biophys Acta 138:438-441, 1967

24. Amé JC, Spenlehauer C, de Murcia G: The PARP superfamily. Bioessays 26:882-893, 2004

25. Hassa PO, Haenni SS, Elser M, et al: Nuclear ADP-ribosylation reactions in mammalian cells: Where are we today and where are we going? Microbiol Mol Biol Rev 70:789-829, 2006

26. Schreiber V, Dantzer F, Ame JC, et al: Poly(ADP-ribose): Novel functions for an old molecule. Nat Rev Mol Cell Biol 7:517-528, 2006

27. Rouleau M, Patel A, Hendzel MJ, et al: PARP inhibition: PARP1 and beyond. Nat Rev Cancer 10:293-301, 2010

28. Curtin NJ: DNA repair dysregulation from cancer driver to therapeutic target. Nat Rev Cancer 12:801-817, 2012

29. Satoh MS, Lindahl T: Role of poly (ADP-ribose) formation in DNA repair. Nature 356:356-358, 1992

30. Ahel I, Ahel D, Matsusaka T, et al: Poly(ADP-ribose)-binding zinc finger motifs in DNA repair/checkpoint proteins. Nature 451:81-85, 2008

31. Zhang F, Chen Y, Li M, et al: The oligonucleotide/oligosaccharide-binding fold motif is a poly(ADP-ribose)-binding domain that mediates DNA damage response. Proc Natl Acad Sci U S A 111:7278-7283, 2014

32. Bürkle A: Physiology and pathophysiology of poly(ADP-ribosyl)ation. Bioessays 23:795-806, 2001

33. Kim MY, Zhang T, Kraus WL: Poly(ADP-ribosyl)ation by PARP-1: "PAR-laying" NAD+ into a nuclear signal. Genes Dev 19:1951-1967, 2005

34. Hassa PO, Hottiger MO: The diverse biological roles of mammalian PARPS, a small but powerful family of poly-ADP-ribose polymerases. Front Biosci 13:3046-3082, 2008

35. Sousa FG, Matuo R, Soares DG, et al: PARPs and the DNA damage response. Carcinogenesis 33:1433-1440, 2012

36. De Vos M, Schreiber V, Dantzer F: The diverse roles and clinical relevance of PARPs in DNA damage repair: Current state of the art. Biochem Pharmacol 84:137-146, 2012

37. de Murcia G, Ménissier de Murcia J: Poly(ADP-ribose) polymerase: A molecular nick-sensor. Trends Biochem Sci 19:172-176, 1994

38. Langelier MF, Planck JL, Roy S, et al: Structural basis for DNA damage-dependent poly(ADP-ribosyl)ation by human PARP-1. Science 336:728-732, 2012

39. Hassler M, Ladurner AG: Towards a structural understanding of PARP1 activation and related signalling ADP-ribosyl-transferases. Curr Opin Struct Biol 22:721-729, 2012

40. Langelier MF, Pascal JM: PARP-1 mechanism for coupling DNA damage detection to poly(ADP-ribose) synthesis. Curr Opin Struct Biol 23:134-143, 2013

41. Juarez-Salinas H, Sims JL, Jacobson MK: Poly(ADP-ribose) levels in carcinogen-treated cells. Nature 282:740-741, 1979

42. Mendoza-Alvarez H, Alvarez-Gonzalez R: Poly(ADP-ribose) polymerase is a catalytic dimer and the automodification reaction is intermolecular. J Biol Chem 268:22575-22580, 1993

43. Althaus FR, Richter C: ADP-ribosylation of proteins: Enzymology and biological significance. Mol Biol Biochem Biophys 37:1-237, 1987

44. Realini CA, Althaus FR: Histone shuttling by poly(ADP-ribosylation). J Biol Chem 267:18858-18865, 1992

45. Malanga M, Althaus FR: Poly(ADP-ribose) reactivates stalled DNA topoisomerase I and induces DNA strand break resealing. J Biol Chem 279:5244-5248, 2004

46. Gagné JP, Isabelle M, Lo KS, et al: Proteome-wide identification of poly(ADP-ribose) binding proteins and poly(ADP-ribose)-associated protein complexes. Nucleic Acids Res 36:6959-6976, 2008

47. Gagné JP, Pic E, Isabelle M, et al: Quantitative proteomics profiling of the poly(ADP-ribose)-related response to genotoxic stress. Nucleic Acids Res 40:7788-7805, 2012

48. Gibson BA, Kraus WL: New insights into the molecular and cellular functions of poly(ADP-ribose) and PARPs. Nat Rev Mol Cell Biol 13:411-424, 2012

49. Haince JF, McDonald D, Rodrigue A, et al: PARP1-dependent kinetics of recruitment of MRE11 and NBS1 proteins to multiple DNA damage sites. J Biol Chem 283:1197-1208, 2008

50. Li M, Yu X: Function of BRCA1 in the DNA damage response is mediated by ADP-ribosylation. Cancer Cell 23:693-704, 2013

51. Bonicalzi ME, Haince JF, Droit A, et al: Regulation of poly(ADP-ribose) metabolism by poly(ADP-ribose) glycohydrolase: Where and when? Cell Mol Life Sci 62:739-750, 2005

52. Meyer-Ficca ML, Meyer RG, Jacobson EL, et al: Poly(ADP-ribose) polymerases: Managing genome stability. Int J Biochem Cell Biol 37:920-926, 2005

53. Min W, Wang ZQ: Poly (ADP-ribose) glycohydrolase (PARG) and its therapeutic potential. Front Biosci (Landmark Ed) 14:1619-1626, 2009

54. de Murcia JM, Niedergang C, Trucco C, et al: Requirement of poly(ADP-ribose) polymerase in recovery from DNA damage in mice and in cells. Proc Natl Acad Sci U S A 94:7303-7307, 1997

55. Masson M, Niedergang C, Schreiber V, et al: XRCC1 is specifically associated with poly(ADP-ribose) polymerase and negatively regulates its activity following DNA damage. Mol Cell Biol 18:3563-3571, 1998

56. Trucco C, Oliver FJ, de Murcia G, et al: DNA repair defect in poly(ADP-ribose) polymerase-deficient cell lines. Nucleic Acids Res 26:2644-2649, 1998

57. Horton JK, Watson M, Stefanick DF, et al: XRCC1 and DNA polymerase beta in cellular protection against cytotoxic DNA single-strand breaks. Cell Res 18:48-63, 2008

58. Schultz N, Lopez E, Saleh-Gohari N, et al: Poly(ADP-ribose) polymerase (PARP-1) has a controlling role in homologous recombination. Nucleic Acids Res 31:4959-4964, 2003

59. Helleday T, Bryant HE, Schultz N: Poly(ADP-ribose) polymerase (PARP-1) in homologous recombination and as a target for cancer therapy. Cell Cycle 4:1176-1178, 2005

60. Haince JF, Kozlov S, Dawson VL, et al: Ataxia telangiectasia mutated (ATM) signaling network is modulated by a novel poly(ADP-ribose)-dependent pathway in the early response to DNA-damaging agents. J Biol Chem 282:16441-16453, 2007

61. Ciccia A, Elledge SJ: The DNA damage response: Making it safe to play with knives. Mol Cell 40:179-204, 2010

62. Roy R, Chun J, Powell SN: BRCA1 and BRCA2: Different roles in a common pathway of genome protection. Nat Rev Cancer 12:68-78, 2012

63. Hu Y, Petit SA, Ficarro SB, et al: PARP1-driven poly-ADP-ribosylation regulates BRCA1 function in homologous recombination-mediated DNA repair. Cancer Discov 4:1430-1447, 2014

64. Wang M, Wu W, Wu W, et al: PARP-1 and Ku compete for repair of DNA double strand breaks by distinct NHEJ pathways. Nucleic Acids Res 34:6170-6182, 2006

65. Robert I, Dantzer F, Reina-San-Martin B: Parp1 facilitates alternative NHEJ, whereas Parp2 suppresses IgH/c-myc translocations during immunoglobulin class switch recombination. J Exp Med 206:1047-1056, 2009

66. Soni A, Siemann M, Grabos M, et al: Requirement for Parp-1 and DNA ligases 1 or 3 but not of Xrcc1 in chromosomal translocation formation by backup end joining. Nucleic Acids Res 42:6380-6392, 2014

67. Yang YG, Cortes U, Patnaik S, et al: Ablation of PARP-1 does not interfere with the repair of DNA double-strand breaks, but compromises the reactivation of stalled replication forks. Oncogene 23:3872-3882, 2004

68. Bryant HE, Petermann E, Schultz N, et al: PARP is activated at stalled forks to mediate Mre11-dependent replication restart and recombination. EMBO J 28:2601-2615, 2009

69. Ying S, Hamdy FC, Helleday T: Mre11-dependent degradation of stalled DNA replication forks is prevented by BRCA2 and PARP1. Cancer Res 72:2814-2821, 2012

70. Min W, Bruhn C, Grigaravicius P, et al: Poly(ADP-ribose) binding to Chk1 at stalled replication forks is required for S-phase checkpoint activation. Nat Commun 4:2993, 2013

71. Krishnakumar R, Kraus WL: The PARP side of the nucleus: Molecular actions, physiological outcomes, and clinical targets. Mol Cell 39:8-24, 2010

72. de Murcia G, Huletsky A, Poirier GG: Modulation of chromatin structure by poly(ADP-ribosyl)ation. Biochem Cell Biol 66:626-635, 1988

73. Quénet D, El Ramy R, Schreiber V, et al: The role of poly(ADP-ribosyl)ation in epigenetic events. Int J Biochem Cell Biol 41:60-65, 2009

74. Leung AK, Vyas S, Rood JE, et al: Poly(ADP-ribose) regulates stress responses and microRNA activity in the cytoplasm. Mol Cell 42:489-499, 2011

75. Bai P, Cantó C: The role of PARP-1 and PARP-2 enzymes in metabolic regulation and disease. Cell Metab 16:290-295, 2012

76. Luo X, Kraus WL: On PAR with PARP: Cellular stress signaling through poly(ADP-ribose) and PARP-1. Genes Dev 26:417-432, 2012

77. Wang ZQ, Auer B, Stingl L, et al: Mice lacking ADPRT and poly(ADP-ribosyl)ation develop normally but are susceptible to skin disease. Genes Dev 9:509-520, 1995

78. Durkacz BW, Omidiji O, Gray DA, et al: (ADP-ribose)n participates in DNA excision repair. Nature 283:593-596, 1980

79. Chatterjee S, Berger SJ, Berger NA: Poly(ADP-ribose) polymerase: A guardian of the genome that facilitates DNA repair by protecting against DNA recombination. Mol Cell Biochem 193:23-30, 1999

80. Amé JC, Rolli V, Schreiber V, et al: PARP-2, A novel mammalian DNA damage-dependent poly(ADP-ribose) polymerase J Biol Chem 274:17860-17868, 1999

81. Schreiber V, Amé JC, Dollé P, et al: Poly(ADP-ribose) polymerase-2 (PARP-2) is required for efficient base excision DNA repair in association with PARP-1 and XRCC1. J Biol Chem 277:23028-23036, 2002

82. Rulten SL, Fisher AE, Robert I, et al: PARP-3 and APLF function together to accelerate nonhomologous end-joining. Mol Cell 41:33-45, 2011

83. Boehler C, Gauthier LR, Mortusewicz O, et al: Poly(ADP-ribose) polymerase 3 (PARP3), a newcomer in cellular response to DNA damage and mitotic progression. Proc Natl Acad Sci U S A 108:2783-2788, 2011

84. Wahlberg E, Karlberg T, Kouznetsova E, et al: Family-wide chemical profiling and structural analysis of PARP and tankyrase inhibitors. Nat Biotechnol 30:283-288, 2012

85. Bryant HE, Schultz N, Thomas HD, et al: Specific killing of BRCA2-deficient tumours with inhibitors of poly(ADP-ribose) polymerase. Nature 434:913-917, 2005

86. Farmer H, McCabe N, Lord CJ, et al: Targeting the DNA repair defect in BRCA mutant cells as a therapeutic strategy. Nature 434:917-921, 2005

87. Lord CJ, McDonald S, Swift S, et al: A high-throughput RNA interference screen for DNA repair determinants of PARP inhibitor sensitivity. DNA Repair (Amst) 7:2010-2019, 2008

88. Dedes KJ, Wetterskog D, Mendes-Pereira AM, et al: PTEN deficiency in endometrioid endometrial adenocarcinomas predicts sensitivity to PARP inhibitors. Sci Transl Med 2:53ra75, 2010

89. Dantzer F, Schreiber V, Niedergang C, et al: Involvement of poly(ADP-ribose) polymerase in base excision repair. Biochimie 81:69-75, 1999

90. Iglehart JD, Silver DP: Synthetic lethality: A new direction in cancer-drug development. N Engl J Med 361:189-191, 2009

91. Yap TA, Sandhu SK, Carden CP, et al: Poly(ADP-ribose) polymerase (PARP) inhibitors: Exploiting a synthetic lethal strategy in the clinic. CA Cancer J Clin 61:31-49, 2011

92. Gottipati P, Vischioni B, Schultz N, et al: Poly(ADP-ribose) polymerase is hyperactivated in homologous recombination-defective cells. Cancer Res 70:5389-5398, 2010

93. Patel A, Sarkaria J, Kaufmann SH: Nonhomologous end-joining drives PARP inhibitor synthetic lethality in homologous recombination-deficient cells. Proc Natl Acad Sci U S A 108:3406-3411, 2011

94. Benjamin RC, Gill DM: Poly(ADP-ribose) synthesis in vitro programmed by damaged DNA: A comparison of DNA molecules containing different types of strand breaks. J Biol Chem 255:10502-10508, 1980

95. Küpper JH, deMurcia G, Bürkle A: Inhibition of poly(ADP-ribosyl)ation by overexpressing the poly(ADP-ribose) polymerase DNA-binding domain in mammalian cells. J Biol Chem 265:18721-18724, 1990

96. Molinete M, Vermeulen W, Bürkle A, et al: Overproduction of the poly(ADP-ribose) polymerase DNA-binding domain blocks alkylation-induced DNA repair synthesis in mammalian cells. EMBO J 12:2109-2117, 1993

97. Liu X, Han EK, Anderson M, et al: Acquired resistance to combination treatment with temozolomide and ABT-888 is mediated by both base excision repair and homologous recombination DNA repair pathways. Mol Cancer Res 7:1686-1692, 2009

98. Murai J, Zhang Y, Morris J, et al: Rationale for PARP inhibitors in combination therapy with camptothecins or temozolomide based on PARP trapping versus catalytic inhibition. J Pharmacol Exp Ther 349:408-416, 2014

99. Patel AG, Flatten KS, Schneider PA, et al: Enhanced killing of cancer cells by poly(ADP-ribose) polymerase inhibitors and topoisomerase inhibitors reflects poisoning of both enzymes. J Biol Chem 287:4198-4210, 2012

100. Murai J, Huang SY, Das BB, et al: Trapping of PARP1 and PARP2 by clinical PARP inhibitors. Cancer Res 72:5588-5599, 2012

101. Bonner WM, Redon CE, Dickey JS, et al: GammaH2AX and cancer. Nat Rev Cancer 8:957-967, 2008

102. De Lorenzo SB, Patel AG, Hurley RM, et al: The elephant and the blind men: Making sense of PARP inhibitors in homologous recombination deficient tumor cells. Front Oncol 3:228, 2013

103. Lieber MR: The mechanism of double-strand DNA break repair by the nonhomologous DNA end-joining pathway. Annu Rev Biochem 79:181-211, 2010

104. Hochegger H, Dejsuphong D, Fukushima T, et al: Parp-1 protects homologous recombination from interference by Ku and ligase IV in vertebrate cells. EMBO J 25:1305-1314, 2006

105. Paddock MN, Bauman AT, Higdon R, et al: Competition between PARP-1 and Ku70 control the decision between high-fidelity and mutagenic DNA repair. DNA Repair (Amst) 10:338-343, 2011

106. Murai J, Yang K, Dejsuphong D, et al: The USP1/UAF1 complex promotes double-strand break repair through homologous recombination. Mol Cell Biol 31:2462-2469, 2011

107. Williamson CT, Kubota E, Hamill JD, et al: Enhanced cytotoxicity of PARP inhibition in mantle cell lymphoma harbouring mutations in both ATM and p53. EMBO Mol Med 4:515-527, 2012

108. Ceccaldi R, Liu JC, Amunugama R, et al: Homologous-recombination-deficient tumours are dependent on Polθ-mediated repair. Nature 518:258-262, 2015

109. Bunting SF, Callén E, Wong N, et al: 53BP1 inhibits homologous recombination in Brca1-deficient cells by blocking resection of DNA breaks. Cell 141:243-254, 2010

110. Jaspers JE, Kersbergen A, Boon U, et al: Loss of 53BP1 causes PARP inhibitor resistance in Brca1-mutated mouse mammary tumors. Cancer Discov 3:68-81, 2013

111. Wang J, Aroumougame A, Lobrich M, et al: PTIP associates with Artemis to dictate DNA repair pathway choice. Genes Dev 28:2693-2698, 2014

112. Fong PC, Yap TA, Boss DS, et al: Poly(ADP)-ribose polymerase inhibition: Frequent durable responses in *BRCA* carrier ovarian cancer correlating with platinum-free interval. J Clin Oncol 28:2512-2519, 2010

113. Sandhu SK, Schelman WR, Wilding G, et al: The poly(ADP-ribose) polymerase inhibitor niraparib (MK4827) in BRCA mutation carriers and patients with sporadic cancer: A phase 1 dose-escalation trial. Lancet Oncol 14:882-892, 2013

114. Kaye SB, Lubinski J, Matulonis U, et al: Phase II, open-label, randomized, multicenter study comparing the efficacy and safety of olaparib, a poly (ADP-ribose) polymerase inhibitor, and pegylated liposomal doxorubicin in patients with *BRCA1* or *BRCA2* mutations and recurrent ovarian cancer. J Clin Oncol 30:372-379, 2012

115. Edwards SL, Brough R, Lord CJ, et al: Resistance to therapy caused by intragenic deletion in BRCA2. Nature 451:1111-1115, 2008

116. Sakai W, Swisher EM, Karlan BY, et al: Secondary mutations as a mechanism of cisplatin resistance in BRCA2-mutated cancers. Nature 451:1116-1120, 2008

117. Swisher EM, Sakai W, Karlan BY, et al: Secondary BRCA1 mutations in BRCA1-mutated ovarian carcinomas with platinum resistance. Cancer Res 68:2581-2586, 2008

118. Norquist B, Wurz KA, Pennil CC, et al: Secondary somatic mutations restoring *BRCA1/2* predict chemotherapy resistance in hereditary ovarian carcinomas. J Clin Oncol 29:3008-3025, 2011

119. Barber LJ, Sandhu S, Chen L, et al: Secondary mutations in BRCA2 associated with clinical resistance to a PARP inhibitor. J Pathol 229:422-429, 2013

120. Johnson N, Johnson SF, Yao W, et al: Stabilization of mutant BRCA1 protein confers PARP inhibitor and platinum resistance. Proc Natl Acad Sci U S A 110:17041-17046, 2013

121. Bouwman P, Aly A, Escandell JM, et al: 53BP1 loss rescues BRCA1 deficiency and is associated with triple-negative and BRCA-mutated breast cancers. Nat Struct Mol Biol 17:688-695, 2010

122. Pennington KP, Walsh T, Harrell MI, et al: Germline and somatic mutations in homologous recombination genes predict platinum response and survival in ovarian, fallopian tube, and peritoneal carcinomas. Clin Cancer Res 20:764-775, 2014

123. Forster MD, Dedes KJ, Sandhu S, et al: Treatment with olaparib in a patient with PTEN-deficient endometrioid endometrial cancer. Nat Rev Clin Oncol 8:302-306, 2011

124. Bang YJ, Im SA, Lee KW, et al: Olaparib plus paclitaxel in patients with recurrent or metastatic gastric cancer: A randomized, double-blind phase II study. J Clin Oncol 31:246s, 2013 (suppl 15s; abstr 4013)

125. Baldwin RL, Nemeth E, Tran H, et al: BRCA1 promoter region hypermethylation in ovarian carcinoma: A population-based study. Cancer Res 60:5329-5333, 2000

126. Esteller M, Silva JM, Dominguez G, et al: Promoter hypermethylation and BRCA1 inactivation in sporadic breast and ovarian tumors. J Natl Cancer Inst 92:564-569, 2000

127. Swisher EM, Gonzalez RM, Taniguchi T, et al: Methylation and protein expression of DNA repair

genes: Association with chemotherapy exposure and survival in sporadic ovarian and peritoneal carcinomas. Mol Cancer 8:48, 2009

128. Cancer Genome Atlas Research Network: Integrated genomic analyses of ovarian carcinoma. Nature 474:609-615, 2011

129. Nowsheen S, Cooper T, Stanley JA, et al: Synthetic lethal interactions between EGFR and PARP inhibition in human triple negative breast cancer cells. PLoS One 7:e46614, 2012

130. Johnson N, Li YC, Walton ZE, et al: Compromised CDK1 activity sensitizes BRCA-proficient cancers to PARP inhibition. Nat Med 17:875-882, 2011

131. Juvekar A, Burga LN, Hu H, et al: Combining a PI3K inhibitor with a PARP inhibitor provides an effective therapy for BRCA1-related breast cancer. Cancer Discov 2:1048-1063, 2012

132. Ibrahim YH, García-García C, Serra V, et al: PI3K inhibition impairs BRCA1/2 expression and sensitizes BRCA-proficient triple-negative breast cancer to PARP inhibition. Cancer Discov 2:1036-1047, 2012

133. Huntoon CJ, Flatten KS, Wahner Hendrickson AE, et al: ATR inhibition broadly sensitizes ovarian cancer cells to chemotherapy independent of BRCA status. Cancer Res 73:3683-3691, 2013

134. Hegan DC, Lu Y, Stachelek GC, et al: Inhibition of poly(ADP-ribose) polymerase down-regulates BRCA1 and RAD51 in a pathway mediated by E2F4 and p130. Proc Natl Acad Sci U S A 107:2201-2206, 2010

135. Huehls AM, Wagner JM, Huntoon CJ, et al: Poly(ADP-ribose) polymerase inhibition synergizes with 5-fluorodeoxyuridine but not 5-fluorouracil in ovarian cancer cells. Cancer Res 71:4944-4954, 2011

136. Geng L, Huehls AM, Wagner JM, et al: Checkpoint signaling, base excision repair, and PARP promote survival of colon cancer cells treated with 5-fluorodeoxyuridine but not 5-fluorouracil. PLoS One 6:e28862, 2011

137. Zong WX, Ditsworth D, Bauer DE, et al: Alkylating DNA damage stimulates a regulated form of necrotic cell death. Genes Dev 18:1272-1282, 2004

138. Hussain M, Carducci MA, Slovin S, et al: Targeting DNA repair with combination veliparib (ABT-888) and temozolomide in patients with metastatic castration-resistant prostate cancer. Invest New Drugs 32:904-912, 2014

139. Kummar S, Chen A, Ji J, et al: Phase I study of PARP inhibitor ABT-888 in combination with topotecan in adults with refractory solid tumors and lymphomas. Cancer Res 71:5626-5634, 2011

140. Mendeleyev J, Kirsten E, Hakam A, et al: Potential chemotherapeutic activity of 4-iodo-3-nitrobenzamide: Metabolic reduction to the 3-nitroso derivative and induction of cell death in tumor cells in culture. Biochem Pharmacol 50:705-714, 1995

141. O'Shaughnessy J, Schwartzberg LA, Danso MA, et al: A randomized phase III study of iniparib (BSI-201) in combination with gemcitabine/carboplatin (G/C) in metastatic triple-negative breast cancer (TNBC). J Clin Oncol 29:81s, 2011 (suppl; abstr 1007)

142. Domchek SM, Mitchell G, Lindeman GJ, et al: Challenges to the development of new agents for molecularly defined patient subsets: Lessons from *BRCA1/2*-associated breast cancer. J Clin Oncol 29:4224-4226, 2011

143. Liu X, Shi Y, Maag DX, et al: Iniparib nonselectively modifies cysteine-containing proteins in tumor cells and is not a bona fide PARP inhibitor. Clin Cancer Res 18:510-523, 2012

144. Patel AG, De Lorenzo SB, Flatten KS, et al: Failure of iniparib to inhibit poly(ADP-ribose) polymerase in vitro. Clin Cancer Res 18:1655-1662, 2012

145. Abkevich V, Timms KM, Hennessy BT, et al: Patterns of genomic loss of heterozygosity predict homologous recombination repair defects in epithelial ovarian cancer. Br J Cancer 107:1776-1782, 2012

146. Birkbak NJ, Wang ZC, Kim JY, et al: Telomeric allelic imbalance indicates defective DNA repair and sensitivity to DNA-damaging agents. Cancer Discov 2:366-375, 2012

147. Popova T, Manié E, Rieunier G, et al: Ploidy and large-scale genomic instability consistently identify basal-like breast carcinomas with BRCA1/2 inactivation. Cancer Res 72:5454-5462, 2012

148. Wang ZC, Birkbak NJ, Culhane AC, et al: Profiles of genomic instability in high-grade serous ovarian cancer predict treatment outcome. Clin Cancer Res 18:5806-5815, 2012

149. Watkins JA, Irshad S, Grigoriadis A, et al: Genomic scars as biomarkers of homologous recombination deficiency and drug response in breast and ovarian cancers. Breast Cancer Res 16:211, 2014

150. Haluska P, Timms KM, AlHilli M, et al: Homologous recombination deficiency score and niraparib efficacy in high grade ovarian cancer. Presented at the 26th European Organisation for Research and Treatment of Cancer–National Cancer Institute–American Association for Cancer Research Symposium, Barcelona, Spain, November 18-21, 2014

151. Swisher E, Brenton J, Kaufmann S, et al: Updated clinical and preliminary correlative results of Ariel2, a phase 2 study to identify ovarian cancer patients likely to respond to rucaparib. Presented at the 26th European Organisation for Research and Treatment of Cancer–National Cancer Institute–American Association for Cancer Research Symposium, Barcelona, Spain, November 18-21, 2014

152. Kummar S, Ji J, Morgan R, et al: A phase I study of veliparib in combination with metronomic cyclophosphamide in adults with refractory solid tumors and lymphomas. Clin Cancer Res 18:1726-1734, 2012

153. Moore KN, DiSilvestro P, Lowe ES, et al: SOLO1 and SOLO2: Randomized phase III trials of olaparib in patients (pts) with ovarian cancer and a BRCA1/2 mutation (BRCAm). J Clin Oncol 32:379s, 2014 (suppl 15s; abstr TPS5616)

154. Lord CJ, Ashworth A: Mechanisms of resistance to therapies targeting BRCA-mutant cancers. Nat Med 19:1381-1388, 2013

155. Bouwman P, Jonkers J: Molecular pathways: How can BRCA-mutated tumors become resistant to PARP inhibitors? Clin Cancer Res 20:540-547, 2014

156. To C, Kim EH, Royce DB, et al: The PARP inhibitors, veliparib and olaparib, are effective chemopreventive agents for delaying mammary tumor development in BRCA1-deficient mice. Cancer Prev Res (Phila) 7:698-707, 2014

GLOSSARY TERMS

base excision repair (BER): one of the major DNA repair pathways that repairs simple DNA base lesions, such as the products of deamination, oxidation, and alkylation. In BER, a damaged base is removed by a DNA glycosylase, followed by excision of the resulting sugar phosphate. The small gap left in the DNA helix is then filled in by the sequential action of DNA polymerase and DNA ligase.

***BRCA1*:** a tumor suppressor gene known to play a role in repairing DNA breaks. Mutations in this gene are associated with increased risks of developing breast or ovarian cancer.

***BRCA2*:** a tumor suppressor gene whose protein product is involved in repairing chromosomal damage. Although structurally different from *BRCA1*, *BRCA2* has cellular functions similar to *BRCA1*. *BRCA2* binds to RAD51 to fix DNA breaks caused by irradiation and other environmental agents. Also known as the breast cancer 2 early onset gene.

homologous recombination: genetic recombination whereby nucleotide sequences are exchanged between two similar or identical strands of DNA to facilitate accurate repair of DNA double-strand breaks.

promoter hypermethylation: methylation of the promoter region of a gene, which can lead to DNA silencing as a consequence of the inability of activating transcriptional factors to bind to the promoter region, a process important in gene transcription. In addition, repressor complexes may be attracted to sites of promoter methylation, leading to the formation of inactive chromatin structures.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Poly (ADP-Ribose) Polymerase Inhibitors: Recent Advances and Future Development

The following represents disclosure information provided by authors of this manuscript. All relationships are considered compensated. Relationships are self-held unless noted. I = Immediate Family Member, Inst = My Institution. Relationships may not relate to the subject matter of this manuscript. For more information about ASCO's conflict of interest policy, please refer to www.asco.org/rwc or jco.ascopubs.org/site/ifc.

Clare L. Scott
Honoraria: Roche, Pfizer
Consulting or Advisory Role: AstraZeneca, Clovis Oncology
Speakers' Bureau: Prime Oncology
Expert Testimony: AstraZeneca
Travel, Accommodations, Expenses: Pfizer, Roche, AstraZeneca, Clovis Oncology
Other Relationship: Clovis Oncology

Elizabeth M. Swisher
Research Funding: Clovis Oncology (Inst), AbbVie (Inst)

Scott H. Kaufmann
Research Funding: Eli Lilly (Inst)
Patents, Royalties, Other Intellectual Property: Methods and materials for assessing responsiveness to PARP inhibitors and platinating agents US 8, 729, 048132 (Inst)