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REVIEW ARTICLE

PARP INHIBITION: ADVANCING THROUGH THE ONCOLYTIC ARENA

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SUMMARY

Poly(ADP-ribose)polymerase 1 (PARP-1) is an essential enzyme in the repair of single-strand breaks in DNA via the base excision repair pathway and has become an important novel target in cancer therapy. PARP inhibitors represent a major breakthrough for patients with hereditary BRCA-associated cancers as a promising new class of anticancer agents. This review will summarize PARP inhibition, including the mechanism of action, its role in the treatment of BRCA1- and 2-associated cancers, the promise of combination therapy with cytotoxic agents and future directions for PARP inhibition in the clinical setting.

INTRODUCTION

Targeting DNA repair mechanisms in cancer cells has long been an active area of anticancer research. Interest in this approach as a potential therapeutic strategy has recently become intense, with promising data emerging from studies using poly(ADP-ribose)polymerase (PARP) inhibitors. This review will discuss the emergence of these exciting new agents, review the current data on clinical efficacy (focusing on their activity in breast and ovarian cancer) and consider future developments in this field.

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POLY(ADP-RIBOSE)POLYMERASE 1 (PARP-1)

PARP is a 17-member superfamily of enzymes involved in cell signaling (1). PARP-1 is the most widely studied and was originally purified over 30 years ago. In 1980, PARP-1 was shown to be involved in DNA repair (2). It is a 113-kDa nuclear protein expressed in all human cells (except neutrophils). It is comprised of three functional domains: an amino-terminal DNA binding domain, an automodification domain (which allows the enzyme to poly[ADP-ribosyl]ate itself) and a *C*-terminal catalytic domain (Fig. 1).

PARP-1 has been implicated in multiple DNA-related processes, playing a crucial role in the repair of metabolic, chemical or radiation-induced DNA single-strand breaks (SSBs) via the base excision repair (BER) pathway (3, 4). PARP-1 detects and signals the presence of an SSB by binding to the altered DNA. Once bound, its catalytic activity increases 10-500-fold. Subsequently, PARP-1 catalyzes the cleavage of the coenzyme nicotinamide adenine dinucleotide (NAD^+) into nicotinamide and ADP-ribose, to produce highly negatively charged branched chains of poly(ADP-ribose) (PAR), thus interfering with the functions of DNA-modifying proteins such as histones and topoisomerases (3) and attracting the formation of a multiprotein DNA repair complex. Directly and indirectly recruited proteins include the repair enzymes DNA ligase III, DNA polymerase B and scaffolding proteins such as XRCC1 (X-ray repair cross-complementing protein 1), which assemble and activate the BER machinery. At the same time, ADP ribosylation reduces the affinity of histones and PARP-1 for DNA, allowing release of PARP-1, modulation of local chromatin structure and access to other repair complex proteins. After repair, the PAR polymers are degraded via poly(ADP-ribose) glycohydrolase (PARG) (5-7) (Fig. 2).

The role of PARP-1 may not be limited to just BER. Rapid recruitment of the components of the homologous recombination (HR) pathway is dependent on poly(ADP-ribose) synthesis, suggesting that PARP-1 also plays a role during this process (8). In addition, in vitro studies suggest its involvement in nonhomologous end-joining (9-11). Furthermore, PARP-1 may also regulate transcription by modulating chromatin structure, altering patterns of DNA methylation and regulating transcription factors (12, 13).

PARP INHIBITION

The role of PARP in DNA repair suggested the potential use of PARP inhibition as a means of enhancing the effects of alkylation and

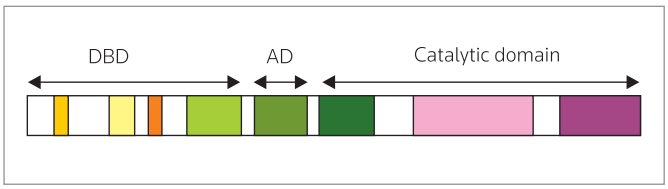

Figure 1. Functional domains of PARP-1. DBD, DNA-binding domain; AD, automodification domain.

other DNA damage. After initial PARP-1 inhibition studies demonstrated enhanced sensitivity to radiation (14), a new generation of PARP inhibitors was developed. These drugs inhibit PARP-1 by competing with NAD^+ at the catalytic domain. Although these agents inhibit PARP-1, they may also inhibit other members of the PARP family that use NAD^+. Clinical development of the PARP inhibitors is following two complementary trajectories: in combination with DNA-damaging agents, including cytotoxic chemotherapy and radiation, to enhance their effect; and secondly, in cancers which have "lost" DNA repair capacity either through genetic or epigenetic mechanisms and are therefore predisposed to die if PARP inhibition occurs. This followed the observation that PARP inhibition is particularly potent in patients who have defects in DNA repair, such as those with mutations in the *BRCA1* and *BRCA2* genes (15, 16).

PARP INHIBITION IN *BRCA1* AND *BRCA2* CARRIERS

BRCA1 or *BRCA2* mutations occur in 0.1-0.8% of the general population and are inherited in an autosomal dominant manner (17). They are well recognized to have a higher incidence in certain ethnic groups, such as individuals of Ashkenazi Jewish descent (18). Women carrying a mutation in *BRCA1* or *BRCA2* have an increased lifetime risk of developing a number of cancers, including epithelial ovarian cancer and breast cancer (19).

The *BRCA1* gene is located on chromosome 17q21, while *BRCA2* is located on chromosome 13q12 (20, 21). *BRCA1* and *BRCA2* play major roles in the repair of DNA double-strand breaks (DSB) by HR. *BRCA1* signals the presence of DSBs, while *BRCA2* is directly involved in the mechanism of HR. In the absence of *BRCA1* or *BRCA2*, alternative DNA repair pathways are used, which results in chromosomal instability and cell death. Normal cells of carriers are usually heterozygotic and loss of the second allele occurs during tumorigenesis in the tumor cells of these individuals (20).

In a normal cell, PARP-1 inhibition leads to a failure of SSB repair, resulting in the formation of a DSB in the DNA when a replication fork encounters the SSB. Thus, the DSB can be repaired by HR and the fidelity of the genome maintained. However, in cells carrying defects in *BRCA1/2*, HR is defective and results in an attempted repair of the DSB by the more error-prone nonhomologous end-joining pathway (4). As a result, the cell acquires lethal levels of damage and cellular viability is lost. This concept is termed "synthetic lethality", with the malignant cell able to function with the loss of one DNA repair mechanism (HR), but ceasing to be viable with the loss of a second (BER) (22, 23). As most *BRCA1/2* carriers have one normal allele, the hope was that inhibition of PARP would be selectively lethal for tumor cells.

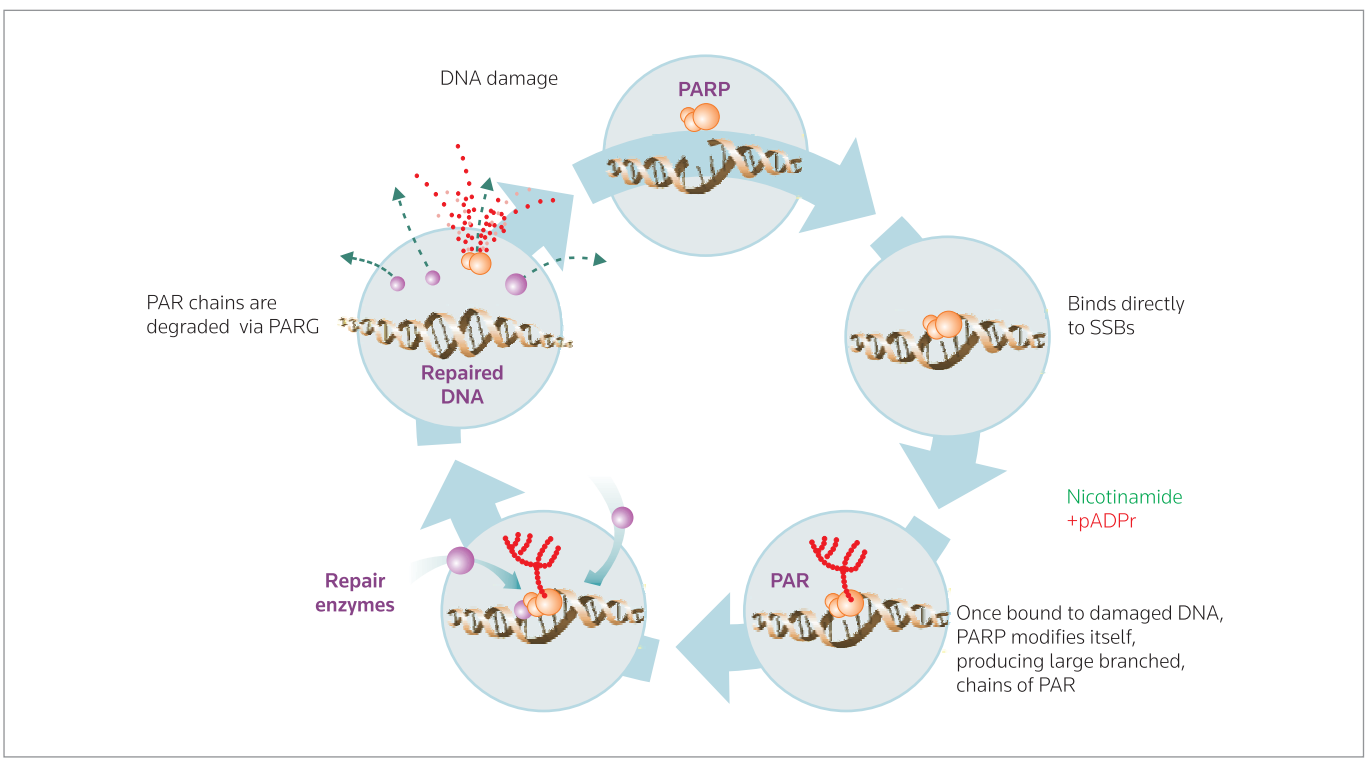

Figure 2. The role of PARP in the repair of single-strand DNA breaks via the base excision repair pathway. PARG, poly(ADP-ribose)glycohydrolase; PAR, poly(ADP-ribose); PARP, poly(ADP-ribose)polymerase; SSBs, single-strand breaks.

In 2005, two preclinical papers demonstrated the sensitivity of *BRCA1*- and *BRCA2*-deficient cell lines to PARP inhibition (15, 16). The first paper by Bryant and colleagues demonstrated reduced survival of *BRCA2*-deficient cell lines with four PARP inhibitors (15). In the same year, Farmer and colleagues demonstrated how both *BRCA1*- and *BRCA2*-deficient cell lines were sensitive to inhibition by PARP-1 (16). Both of these papers demonstrated how homozygotes (tumor cells) are sensitive to the mechanism of PARP inhibition, whereas heterozygotes (the rest of the patient's cells) are insensitive to this mechanism and therefore there should be minimal toxicity to normal tissue.

PHASE I AND II TRIALS IN *BRCA*-ASSOCIATED CANCERS

A number of PARP inhibitors have entered the clinic as both intravenous (i.v.) and oral formulations (Table I). Olaparib (AZD-2281, KU-0059436; AstraZeneca) is an oral small-molecule PARP inhibitor. The first clinical evidence demonstrating the sensitivity of *BRCA*-mutated cancers to PARP inhibitor monotherapy was presented in a study by Yap et al. in 2007 (24). This phase I trial included 44 patients, of whom 11 had a *BRCA* mutation-associated cancer. Based on the encouraging antitumor activity, including patients with *BRCA1/2* mutations, in the initial dose-escalation phase they investigated an expanded cohort of patients with *BRCA* mutations at the recommended phase II dose. The drug was well tolerated in both *BRCA*-mutated and normal populations. Most toxicities were grade 1-2 (≥ 95%), consisting of fatigue (28%), nausea (28%), vomiting (18%), loss of taste (13%) and anorexia (12%). Grade 3-4 toxicities were rare (< 5%) (25). Nineteen patients with *BRCA* mutations were evaluated, with 12 of these patients (63%) demonstrating a clinical benefit for olaparib (25). No response was seen in patients who did not have a *BRCA* mutation. Reassuringly, there was no increase in toxicity reported for patients with *BRCA* mutations, supporting the theory that PARP inhibitors should not result in increased toxicity to heterozygote cells (15, 16).

A phase II study of olaparib in women with *BRCA1/2*-associated epithelial ovarian cancer was conducted using two schedules of olaparib (26). The objective response rate (27) in women receiving 400 mg b.i.d. was 33% compared to 12.5% in those receiving 100 mg b.i.d., suggesting that there may be a dose–response effect. Once more, toxicity was low, consisting of grade 1/2 nausea (44%) and fatigue (35%). Interestingly, although numbers were low, the response rate in platinum-resistant patients (38% vs. 14%) was higher in this study than in the earlier phase I study (28) (Table II). Laboratory studies have previously suggested that platinum-resistant patients may reacquire *BRCA* function (29), thus potentially making them resistant to the effects of PARP inhibition. Whether this is clinically relevant remains to be seen in larger-scale studies. Furthermore, early data suggest that response rates may be higher in those patients with *BRCA2* mutations (30), and this too requires further study.

A phase II study of 54 women with previously treated advanced *BRCA1/2*-associated breast cancer demonstrated a response rate of 41% (11/27 patients) for the 400 mg b.i.d. regimen of olaparib, with a lower rate seen in those receiving 100 mg b.i.d. (31). A median progression-free survival of 5.7 months (4.6-7.4 months) suggested

Table I. *PARP inhibitors in clinical trials (data from ref. 59 and 60).*

Agent	Company	Route	Clinical trials	Phase	References
Olaparib (AZD-2281)	AstraZeneca/KuDOS (London, UK)	Oral	Platinum-sensitive ovarian cancer *BRCA1/2*-associated cancers (with carboplatin) Triple-negative breast cancer (single agent or with carboplatin) Other solid tumors	II II II I/II	25, 61-63
BSI-201	BiPar (Brisbane, CA, USA) (sanofi-aventis)	I.v.	Triple-negative breast cancer (with gemcitabine and carboplatin) Ovarian cancer, glioblastoma multiforme and uterine cancer (various combinations) *BRCA2*-associated pancreatic cancer (various combinations) Other solid tumors	II II Ib I/II	46
AG-014699	Pfizer (La Jolla, CA, USA)	I.v.	*BRCA1/2*-associated cancers Melanoma (complete)	II I/II complete	44, 64, 65
ABT-888	Abbott Laboratories (North Chicago, IL, USA)	Oral	Glioblastoma multiforme (with temozolomide) Solid tumors and leukemia (various combinations) *BRCA1/2*-associated cancers	II I I	52, 66-71
INO-1001	Inotek/Genentech (Beverly, MA, USA)	I.v.	Melanoma	I	72
MK-4827	Merck & Co. (NJ)	Oral	Solid tumors, BRCA ovarian	I	73
GPI-21016	MGI Pharma (Bloomington, MN, USA)	Oral	Solid tumors	I planned	
CEP-8983/ CEP-9722	Cephalon (PA)	Oral	Solid tumors (with temozolomide)	I	41

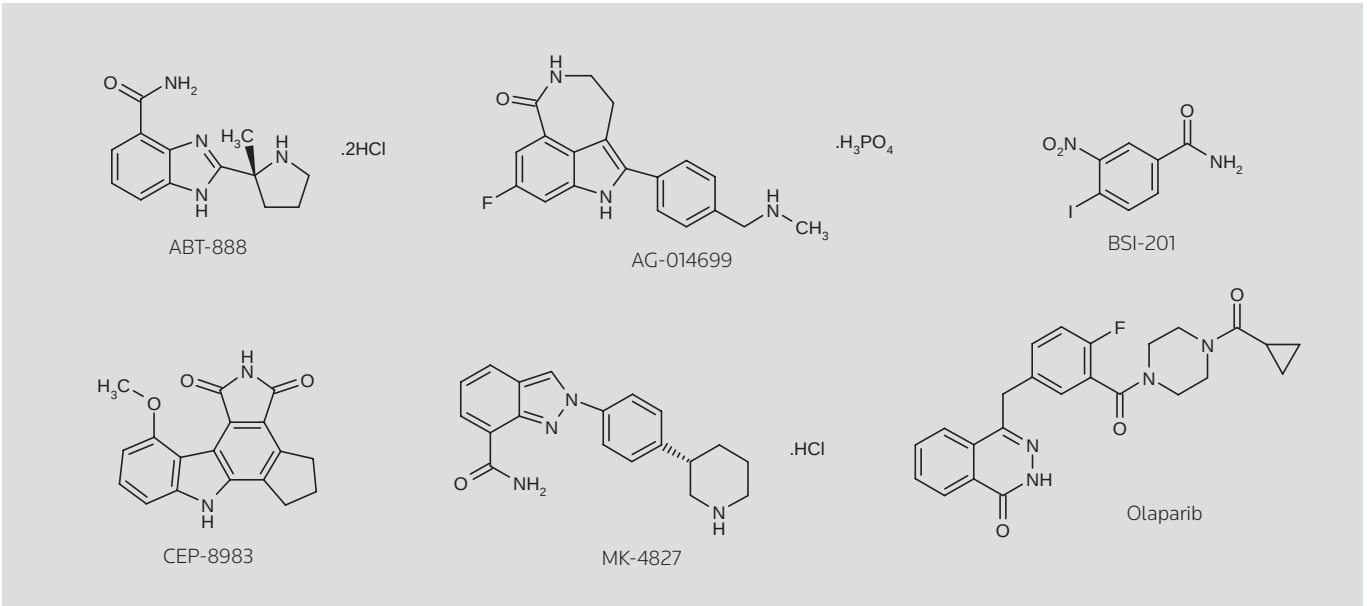

Table II. *Response rates of women with epithelial ovarian cancer to olaparib (AZD-2281) by platinum sensitivity in phase I and phase II trials (data from ref. 28 and 26, respectively).*

	No. of patients evaluated		Responders by RECIST (%)		Responders by RECIST or GCIG (%)	
	Phase I (28)	Phase II (26)	Phase I (28)	Phase II (26)	Phase I (28)	Phase II (26)
Total	46	33	13 (28%)	11 (33%)	21 (46%)	20 (61%)
Platinum-sensitive (> 6 months)	10	7	5 (50%)	1 (14%)	8 (80%)	–
Platinum-resistant (≤ 6 months)	25	26	8 (32%)	10 (38%)	11 (44%)	–
Platinum-refractory	11	–	0 (0%)	–	2 (18%)	–

RECIST, Response Evaluation Criteria In Solid Tumors; GCIG, Gynecologic Cancer InterGroup.

promising efficacy. The toxicity profile was similar to that observed in the patients with epithelial ovarian cancer.

PARP INHIBITION IN NON-*BRCA1/2*-RELATED CANCERS

BRCA1/2-related tumors represent a minority of cancers. However, observations of defects in HR from either loss of functional *BRCA1/2* or from defects in other proteins suggest that single-agent PARP inhibitors may have broader applications. Triple-negative (estrogen receptor, progesterone receptor and *ERBB2*-negative) breast cancers and sporadic high-grade serous epithelial ovarian cancer, exhibit some properties of *BRCA1*- and *BRCA2*-deficient cells. Observations in these tumor subtypes suggest that PARP inhibition alone may be relevant for sporadic tumors. Epigenetic gene inactivation is a well-recognized phenomenon, with 13% of sporadic breast cancers and 15% of sporadic epithelial ovarian cancers exhibiting aberrant methylation of the *BRCA1* promoter, resulting in loss of transcription (32, 33). In addition, overexpression of the *EMSY* gene, which represses *BRCA2* function, has been observed in 13% of sporadic breast cancers and 17% of sporadic high grade ovarian cancers (34). These tumors appear to be similar to *BRCA1*- or *BRCA2*-mutated tumors, although they do not have mutations in any of these genes, a concept called "BRCAness" (35, 36). One molecular characterization study suggested that over 50% of patients with high-grade ovarian cancer had loss of *BRCA* function, either due to genetic or epigenetic events (37). Recent data suggest that the loss of the tumor suppressor *PTEN* gene may be important for the expression of DNA repair proteins. *PTEN*-negative tumors appear to be very sensitive to PARP inhibition. *PTEN* expression is lost in many different tumor types; for example, 53% of metastatic endometrial cancers have lost *PTEN* expression (38), and further studies are thereby warranted in these patient populations. It remains to be seen whether patients who have lost other mechanisms of DNA repair will benefit from PARP inhibition. Studies have shown that the loss of functional proteins in the HR pathway may lead these cells to be sensitive to PARP inhibition (39). Identification of "*BRCA*-like" and other patient populations who have lost functional DNA repair and who may benefit from this approach remains an active area of ongoing research.

COMBINING PARP INHIBITORS WITH OTHER TREATMENT MODALITIES

As one might expect, preclinical studies have shown that PARP inhibitors enhance the cytotoxicity of a diverse range of DNA-damaging agents, including topoisomerase I inhibitors such as topotecan, platinum agents and alkylating agents such as temozolomide (40-43). Furthermore, in preclinical models, they enhance the effects of ionizing radiation.

Initial clinical studies of PARP inhibitors in combination with cytotoxic agents have produced intriguing results. A phase I study of AG-014699 (Pfizer) in combination with the methylating agent temozolomide in solid tumors (44) demonstrated that these two agents could safely be combined with myelosuppression, with dose-limiting toxicity when > 90% PARP inhibition occurred. This combination in a phase II study in patients with malignant melanoma produced a response rate of 18% and a longer median time to progression compared to temozolomide alone (45).

A randomized phase II study in women with triple-negative breast cancer showed a significantly increased response rate when the PARP inhibitor BSI-201 (Bipar Sciences) was added to the combination of gemcitabine (1000 mg/m^2) and carboplatin (AUC2) (days 1 and 8) (46). Patients receiving the PARP inhibitor had an objective response rate of 48% compared to 16% in the women who received chemotherapy alone ($P = 0.002$), albeit the response rate in the control arm was low compared to other published studies using platinum agents in triple-negative breast cancer (47). Furthermore, this translated into a significantly improved progression-free (6.9 months vs. 3.3 months; hazard ratio [HR]: 0.342; $P < 0.002$) and overall survival (9.2 months vs. 5.7 months; HR: 0.348; $P < 0.0001$). Interestingly, there was no increased toxicity in the BSI-201-containing arm, which is in contrast to other phase I studies combining PARP inhibitors with cytotoxic regimens, such as the myelosuppression seen with olaparib in combination with cisplatin and gemcitabine (48, 49), and that for ABT-888 with topotecan (50). A phase III study of BSI-201 with gemcitabine and carboplatin is ongoing (51).

Finally, there is also preclinical evidence that PARP inhibitors potentiate the cytotoxic effects of radiotherapy (14). In vivo studies of AG-14361 and ABT-888 have shown significant delay in tumor growth when combined with radiation in colorectal, non-small cell lung cancer and glioblastoma multiforme models (14, 52). A clinical trial combining radiation and ABT-888 in patients with brain metastases is ongoing (53).

FUTURE DIRECTIONS

At least eight PARP inhibitors, including AG-0146999 (Pfizer) and MK-4827 (Merck & Co.) are under investigation either as single agents and/or in combination with other agents or treatment modalities (Table I). The role of PARP inhibitors as maintenance therapy is being explored (54). Furthermore, some suggest that PARP inhibitors could be used to prevent cancers in patients who are *BRCA* mutation carriers (55). While these are very exciting developments, there are many questions which remain unanswered with regard to the use of PARP inhibition. Further elucidation of the levels and function of PARP-1 in normal cells is required, as little is known about "normal" levels and whether they have implications for the "therapeutic index" when PARP inhibitors are used. The long-term effects of PARP inhibition are unknown, specifically as PARP plays a role in the development of memory and in cardiovascular protection, and therefore longer-term follow-up is required. At the beginning of this review we discussed that PARP appears to play a role in many pathways; whether long-term inhibition results in an increased risk of secondary malignancy remains to be seen. An increased incidence of tumors in knockout mice indicates that there is reason for concern (56-58). Development of PARP inhibitor resistance in *BRCA1*- and *BRCA2*-deficient cancer cells suggests that we need to further determine the finer details of the DNA repair pathway in order to devise a rational therapeutic strategy to overcome this. Further elucidation of the pathways surrounding DNA repair will also allow rational design of combination studies using other biological agents.

CONCLUSIONS

The evolution of PARP inhibition as a therapeutic strategy has evolved at a very rapid pace. Its potential for patients with hereditary defects in DNA repair is coming to fruition. Furthermore, its role in sporadic tumors is leading to further exploration of the mechanisms of DNA repair and how this can be exploited to further benefit our patients. Studies in combination with existing drugs and radiation are producing encouraging results. Over the next few years, we expect to see an explosion in the publication of studies exploring the use and role of PARP inhibitors in the clinic. This, in addition to the use of diagnostic tests in order to select patients likely to be sensitive to PARP inhibitors, will continue to be developed. However, careful clinical trial design incorporating quality translational research together with further evaluation in the laboratory is essential if we are to make the most of this opportunity and avoid long-term negative consequences for our patients.

DISCLOSURES

The authors state no conflicts of interest.

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