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Poly(ADP-Ribose) Polymerase Inhibition as a Model for Synthetic Lethality in Developing Radiation Oncology Targets

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DNA double-strand breaks (DSBs) induced during clinical radiotherapy are potent inducers of cell death. Poly(ADP-ribose) polymerase (PARP)-1 is a 113-kD nuclear protein that binds to both single- and double-strand DNA breaks and is actively involved in DNA single-strand break repair and base excision repair. Recently, potent and specific chemical inhibitors of PARP activity have been developed that are effective tumor cell radiosensitizers in vitro and in vivo. Because of synthetic lethality, PARP inhibitors may be highly effective as a single agent in patients whose tumors have germline or somatic defects in DNA damage and repair genes (eg, ATM, BRCA1, BRCA2, and NBS1) or defects in genes involved in phosphatase and tensin homolog gene (PTEN) signaling. Defects in specific DNA repair pathways also appear to enhance the radiosensitizing effects of PARP inhibition. In addition to inherent genetics, tumor cells may also be preferentially sensitized to radiotherapy by diverse mechanisms, including proliferation-dependent radiosensitization, targeting of the endothelium and tumor vasculature, and increased sensitivity to PARP inhibitors within repair-deficient hypoxic cells. Because biologically active doses of PARP inhibitors caused minimal toxicity in phase I to II clinical trials, careful scheduling of these agents in combination with radiotherapy may maintain the therapeutic ratio and increase tumor radiocurability.
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Proteins with poly(ADP-ribose) polymerase (PARP) activity have been studied for decades and are known to play important roles in a variety of cellular functions.[1] This research heritage has revealed the existence of a number of different PARP enzymes with diverse but partly overlapping functions.[2] Equally valuably, it has allowed the development of a range of potent and specific chemical inhibitors that have been well characterized in cellular and preclinical models.[3] The observation that biologically active doses of PARP inhibitors cause minimal toxicity in animal models has contributed to the enthusiasm with which therapeutic roles for these compounds have been sought. PARP-1 and its close relative PARP-2 are known to play a role in the cellular response to DNA damage, and a potential role for PARP inhibitors as sensitizers to cytotoxic DNA damaging agents has emerged.[4] The revelation in 2005 that PARP inhibitors have potent, single-agent activity against tumor cells deficient in the DNA repair proteins BRCA1 or BRCA2 confirmed that "synthetic lethality" is a genuine and highly valuable therapeutic approach.[5,6] Furthermore, it supported the idea that the DNA repair defects that are a cardinal feature of carcinogenesis can provide tumor-specific therapeutic possibilities. This review addresses the radiosensitizing properties of PARP inhibitors, describing the mechanisms responsible and the potential clinical applications. It focuses particularly on whether radiosensitization is likely to be tumor specific and, if so, which tumors are likely to benefit.

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Biological Functions of PARP

The PARP family of proteins is defined by the catalytic capacity to modify target proteins by the covalent addition of chains of poly(ADP-ribose) polymers. Of the PARP family, PARP-1 is by far the most abundant, accounting for at least 80% of cellular PARP activity. PARP-1 and its closest relative PARP-2 possess DNA-binding domains, and their catalytic function is activated when they bind to sites of DNA damage. By this mechanism, they play an important role in detecting the presence of damaged DNA and activating signaling pathways that promote appropriate cellular responses.[7]

PARP-1 is a 113-kD nuclear protein with 4 major functional domains: an N-terminal DNA-binding domain that mediates binding to breaks or nicks in DNA; a nuclear localization signal domain; a central domain that facilitates binding of PARP-1 to itself and is also a target for poly(ADP-ribosylation); and a C-terminal catalytic domain that binds NAD^+ and uses it to modify target proteins by the addition of long, branching chains of poly(ADP-ribose) (pADPr). PARP-1 is relatively abundant in the nucleus. In response to DNA damage, it binds rapidly to DNA breaks, a process that activates its catalytic function leading to the modification of histones and proteins involved in single-strand break (SSB) repair, double-strand break (DSB) repair, and DNA replication. Such modifications are transient because pADPr polymers are rapidly degraded by the action of poly(ADP-ribose) glycohydrolase (Fig 1).[1]

DSBs in DNA, if unrepaired, are potent inducers of cell death. In mammalian cells, DSBs may be repaired by 2 different pathways.[8] Most radiation-induced DSBs are repaired by nonhomologous end joining (NHEJ). This process is rapid and may occur at any stage of the cell cycle, but because it lacks the capacity to restore any DNA that is lost during the breakage event or subsequent processing, it is error prone. Homologous recombination repair (HR) uses the sister chromatid as a template and faithfully restores DNA sequence, but as a result can only take place in cells that have 2 copies of the relevant region of DNA. Therefore, HR operates only during the S and G2 phases of the cell cycle and indeed plays a particular role in resolving DSB that arise during DNA replication.

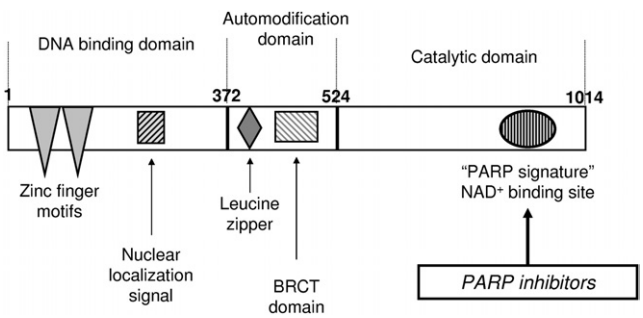

Figure 1 A schematic diagram of the PARP-1 protein showing the key functional domains. The vast majority of PARP inhibitors compete with NAD^+ at the "PARP signature" site in the catalytic domain.

PARP-1 binds to both single- and double-strand DNA breaks. Although its role in SSB repair is well established, the functional relevance of its DSB-binding activity remains uncertain. With respect to SSB repair, binding of PARP-1 to SSBs that are induced either directly or as intermediate products of base excision repair (BER) appears to protect the damaged site from inappropriate recombination events. PARP-1 also enhances the recruitment and activation of multiple components of the BER repair complex, most notably the scaffold protein XRCC1, by both direct interaction and poly(ADP-ribosylation).[9] PARP-1–deficient (or inhibited) cells show reduced rates of BER[10] and hypersensitivity to agents that induce SSB.[11] Because repair of SSBs is delayed but not abolished by PARP inhibition and SSBs are not thought to be associated directly with cell death, additional mechanisms by which PARPi promote cytotoxicity after radiation have been investigated.

Mechanisms Underlying Radiosensitizing Effects of PARP Inhibitors: Dependence on DNA Replication

Ionizing radiation used in the clinical treatment of cancer generates SSBs and DSBs in an approximate ratio of 25:1. As described previously, the major effect of PARP inhibition is to delay, but not abolish, the repair of SSBs. Because DSBs are the most important cytotoxic lesions, the effect on SSB repair has a minimal impact on the survival of nonreplicating cells. In contrast, PARP inhibition does increase radiosensitivity of rapidly proliferating cells, and the magnitude of the radiosensitizing effect in cell culture systems has been shown to correlate with the proportion of cells that are replicating.[12,13] PARP inhibition increases the level of unrepaired DSB in replicating cells by at least 2 mechanisms. (1) The delayed repair of radiation-induced SSB increases the probability of unrepaired lesions colliding with the DNA replication machinery, generating excess DSBs. (2) The inhibition of the catalytic activity of PARP does not impede binding to DNA breaks but prevents PARP from modifying itself by the addition of pADPr. Unmodified PARP remains bound to sites of DNA damage so PARP inhibitors also interfere with downstream repair processes, increasing the toxicity of DSB that are generated in their presence.[14]

The fact that PARP inhibitors increase radiosensitivity only in replicating cells is of great interest to radiation oncologists. In general terms, tumors contain a higher proportion of replicating cells than normal tissues, and the cell-cycle checkpoint responses of these cells are often defective.[15] Furthermore, some of the critical normal tissues that limit radiation doses in the clinic (eg, the brain and spinal cord) are composed almost entirely of nonreplicating G0 or G1 phase cells. Thus, PARP inhibitors have the potential to increase the therapeutic index of radiation therapy in a variety of tumor sites by increasing damage in highly replicating tumor cells, but sparing noncycling normal tissues, which are often responsible for dose-limiting late damage after radiotherapy. This theory has been substantiated by in vitro and in vivo studies showing that PARP inhibitors enhance radiosensitivity by

Table 1 Selected New-Generation PARP Inhibitors in Clinical Trials and Sensitivity to RT In Vitro

Treatment	Model	Assays	Conclusions
GPI-15427: 7 μmol/L, 15 min before radiation: 2 Gy; 24, 48, 72, 96 h (Khan et al, 2009)	JHU012 head and neck cancer cells	Neutral comet assay, apoptosis (annexin V/PI)	Compared with RT alone, GPI-15427 + RT decreased DNA DSB repair and increased apoptosis
KU-0059436 (AZD2281, Olaparib): 1 μmol/L 1 h before + 3 or 24 h after radiation: 0-5 Gy (Dungey et al, 2009)	Human glioblastoma cell lines: T98G and U87-MG	Clongenic survival, γ-H2AX foci	KU-0059346 + RT reduced clonogenic survival compared with RT alone Decreased DNA repair; DNA replication-dependent Fractionation sensitive
ABT 888: 2.5 μmol/L; radiation: 5 Gy (Liu et al, 2008)	Human lung cancer cells H1299, prostate cancer cell lines DU145 and 22RV1	Clonogenic survival, repair foci assay	Combination ABT888 + RT reduced clonogenic survival compared with RT alone Effect in both oxic and acutely hypoxic cells
ABT888: 5 μmol/L as neoadjuvant; radiation: 0-6 Gy (Albert et al, 2007)	H460 lung carcinoma cells	Clonogenic survival, apoptosis (annexin V/PI), endothelial damage assay	Decreased clonogen survival with ABT888 + RT compared with RT alone Increased apoptosis Inhibition of endothelial tubule formation
E7016: 3-5 μmol/L treated 6 h before radiation: 0-8 Gy (Russo et al, 2007)	Human U251 glioblastoma cells, MiaPaCa pancreatic cancer cells, DU145 prostate cancer cells	Clonogenic survival, γ-H2AX foci, mitotic catastrophe, apoptosis (annexin V)	Increased clonogen kill Increased mitotic catastrophe No change in apoptosis
AG14361: 0.4 μM adjuvant to radiation: 8 Gy (Calabrese et al, 2004)	Human colon carcinoma cells: LoVo and SW620	Clonogenic survival	Combined with RT, PARPi decreased survival by inhibiting recovery from potentially lethal damage
KU-0059456 (AZD2281, Olaparib): 500 nmol/L 1 h before IR and left on for 22 h post-radiation: 0-8 Gy (Loser et al, 2010)	Human and murine primary cells defective in Artemis, ATM, DNA ligase IV	Clonogenic survival, alkaline comet assays, γ-H2AX foci	Sensitization to KU-0059346 + RT was enhanced in cells defective for ATM, Artemis and DNA ligase IV (SER increased from ~1.3 to ~1.6) Overall, clonogenic survival after KU-0059456 + RT was decreased in rapidly dividing and DNA repair-deficient cells

SER, sensitizer enhancement ratio.

factors ranging from 1.3 to more than 2.[13,16-18] In vitro and in vivo fractionation of the radiation regimen was seen to preserve or even enhance the radiosensitizing effect of the PARP inhibitor. A summary of radiosensitization data pertaining to new generation PARP inhibitors that are being tested in phase I to III trials is shown in Tables 1 and 2.

PARP Inhibition and HR: Synthetic Lethality

Under normal conditions, PARP proteins do not contribute directly to DSB repair,[19] but defects in HR can be augmented by PARP inhibition to cause cell death. In the absence of exogenous DNA damage, the continuous exposure of replicating cells to a PARP inhibitor causes a significant increase in HR activity that is thought to represent the repair of DNA replication forks that have stalled or collapsed after encountering unrepaired SSB.[20] PARP inhibitors probably play a dual role in this process by (1) reducing the rate of repair of endogenously arising single-stranded lesions and (2) obstructing the efficient resolution of stalled or collapsed replication forks by impeding the release of PARP molecules from damaged sites. The absolute requirement of HR for repair of these lesions is shown by the extreme sensitivity to PARP inhibitors of HR-defective cells, such as breast or ovarian cancer cells that lack the crucial HR proteins BRCA1 or BRCA2.[5,6]

Targeting cells with a specific DNA repair defect by inhibiting a second DNA repair pathway is an example of "synthetic lethality," and the promising data derived from cellular systems has been rapidly translated into effective treatments for patients with hereditary BRCA1 and BRCA2 deficient can-

Table 2 Selected New-Generation PARP Inhibitors in Clinical Trials and Sensitivity to RT In Vivo

Treatment	Model	Assays	Conclusions
GPI-15427: 10, 30, 100, 300 mg/kg orally Neoadjuvant (1 h before) Radiation: 2 Gy for 2 d (Khan et al, 2009)	JHU012 and JHU006 head and neck cancer xenografts	Tumor growth delay apoptosis (TUNEL)	GPI - 15427 + RT inhibits tumor regrowth when compared with RT alone; increased apoptosis
ABT888: 7.5 mg/kg twice a day, p.o. Monday-Saturday 1 h before TMZ TMZ: 33 mg/kg/d Monday to Friday for 2 wk radiation: 2 Gy Monday to Friday for 2 wk (total 20 Gy for 11 d) (Clarke et al, 2009)	Intracranial xenograft model of glioblastoma using (MGMT-hypermethylated glioblastoma models: GBM12 and GBM22)	Animal survival body weight	Combination therapy of ABT888 and TMZ + RT increased animal survival compared with RT alone; minimal weight loss
ABT888: 25 mg/kg/d via osmotic pumps 2 d before RT radiation: 2 Gy/d for 10 consecutive days (Donawho et al, 2007)	Human colon carcinoma HCT116 xenograft	Animal survival	ABT888 + RT increased mean survival time from 23 to 36 d compared with RT alone
ABT888: 25 mg/kg ip for 5 consecutive days, 1 h before RT radiation: 2 Gy/d for 5 consecutive days (Albert et al, 2007)	Human Lung carcinoma H460 xenograft	Tumor growth delay Ki-67 staining Apoptosis (TUNEL) CD34 staining for blood vessel density	ABT 888 delayed tumor regrowth by 6.5 d compared with radiation alone Associated with decreased tumor vasculature, decreased proliferation and increased apoptosis No increased toxicity with combination treatment
AG14361: 5 or 15 mg/kg/d ip daily for 5 d, 30 min before radiation: 2 Gy locally to the tumor daily for 5 d (Calabrese et al, 2004)	Subcutaneous implants of human colon carcinoma, SW620	Tumor growth delay	Combination AG14361 + RT delayed tumor regrowth (from 19 to 37 d) when compared with RT alone Increased toxicity for combination treatment
E7016: 30 mg/kg oral TMZ: 3 mg/kg oral radiation: 4 Gy single dose (Russo et al, 2009)	Human U251 glioblastoma xenograft	Tumor growth delay	TMZ + E7016 + RT delayed tumor regrowth compared with RT alone

ip, intra-peritoneal.

cers.[21] Another biomarker that may select for PARP inhibitor synthetic lethality is the status of the phosphatase and tensin homolog gene (PTEN) that is located on chromosome 10 and is frequently deleted in human cancer. Preclinical studies have suggested that PTEN loss is associated with PARP inhibitor sensitivity in vitro and in vivo although the mechanism by which this occurs is controversial.[22,23] Furthermore, in prostate cancer, we have documented functional defects in the HR and NHEJ DNA repair pathways[24] along with allelic losses in PARP-1, p53, and ATM[25] and methylation of BRCA1 and BRCA2 that may also affect PARP inhibitor sensitivity.[26] In addition to the obvious benefits for this subset of patients, the accumulating clinical evidence supports earlier predictions that the short-term use of PARP inhibitors would be extremely well tolerated, even in patients who have undergone multiple previous cytotoxic therapies.

From the therapeutic perspective, the simultaneous inhibition of PARP and HR should give rise to marked radiosensitization of replicating cells but would also carry a risk of inducing synthetic lethality in nonirradiated but proliferating tissues. To date, no specific inhibitor of HR has been developed. However, the heat shock protein 90 inhibitor 17-(allylamino)-17-demethoxygeldanamycin selectively accumulates in tumor cells and inhibits HR by downregulating Rad51 and BRCA2. Consistent with this hypothesis, 17-(allylamino)-17-demethoxygeldanamycin and the PARP inhibitor olaparib synergistically increased the radiosensitivity of proliferating glioma cell populations without affecting nonreplicating cells.[27]

Effects of PARP Inhibition on Cells Defective in NHEJ, BER, or ATM-p53 Signaling

Many researchers have sought a direct role for PARP-1 in DSB repair. In cells that are deficient in core components of NHEJ (such as Ku70/80 and DNA ligase IV), an alternative (or

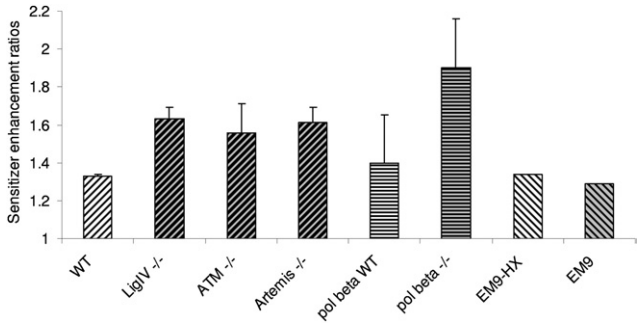

Figure 2 Sensitizer enhancement ratios (±standard error of the mean or standard deviation where indicated) for the PARP inhibitor olaparib derived from clonogenic survival assays in a range of DNA repair proficient and deficient cell lines. In repair proficient cells (2 different parental mouse embryo fibroblast (MEF) cell lines and Chinese hamster ovary (CHO) EM9 cells with XRCC1 expression restored by stable transfection with the HX plasmid), enhancement ratios of 1.3 to 1.4 were observed. In ligase IV, ATM, and Artemis-deficient MEFs, enhancement ratios for olaparib were significantly higher (SER = c.1.6). XRCC1-deficient (EM9) and proficient (EM9-HX) CHO cells were sensitized to the same extent (SER = c.1.3), whereas DNA polymerase β–defective MEFs were sensitized to a greater degree than the parental controls (SER = 1.9 vs 1.4), indicating that the radiosensitizing effects of PARP inhibition are maintained or enhanced in cells with defective BER. (Reprinted with permisiion.[31])

"backup") end-joining pathway exists that partly compensates for the NHEJ defect,[28] and the inhibition of PARP activity suppresses this pathway.[29] It has been proposed that the absence of key NHEJ components allows PARP-1 to bind and activate alternative end joining; this process is obstructed by PARP inhibition. Cells that are deficient in core NHEJ and therefore highly radiosensitive can be further sensitized by treatment with a PARP inhibitor, and the degree of sensitization is greater than in wild type cells (Fig 2).[30] Many cancers exhibit some degree of impairment of DNA repair,[31,32] but it is uncommon for tumors to be profoundly deficient in one or more of the core NHEJ proteins. However, the fact that PARP inhibitors increase radiosensitivity of nonreplicating cells only if they are deficient in NHEJ predicts that many normal tissues will be relatively unaffected by such agents. Figure 3 depicts a simplified overview of interactions between PARP inhibition and DNA repair pathways.

Because PARP-1 has an established role in the BER pathway, it might be expected that PARP inhibitors would have little effect on the radiosensitivity of cells deficient in other BER proteins. This does not appear to be the case. In collaboration with Conchita Vens, we have shown that the radiosensitivity of cells deficient in XRCC1 or another BER protein DNA polymerase β is increased by PARP inhibition to an equal or greater degree than parental controls (Fig 2). Because defects in BER have been reported in a range of human tumors and appear to be relatively common,[33,34] the treatment of these patients with PARP inhibitors may have merit.

Many tumors exhibit defective cell-cycle checkpoints, and mutations in checkpoint signaling proteins, such as p53, ATM, NBS1, MRE11, and BRCA1, are associated with a predisposition to cancer.[16] In the absence of exogenous DNA damage, PARP inhibitors promote the activation of ATM, probably in response to collapsed replication forks.[35] Because ATM is required to stimulate the pathways that repair this damage, ATM-deficient cancer cells, such as mantle cell lymphomas, are sensitive to PARP inhibition.[36] Furthermore, ionizing radiation causes a G2/M checkpoint response that is stimulated by ATM. Because radiation-induced accumulation of cells in G2 phase is enhanced in the presence of PARP inhibitors, the downregulation of ATM or its downstream signaling proteins might exacerbate the radiosensitizing effects of these PARP inhibitors.[30,37]

Taken together, these observations indicate that the genetic or functional status of DNA repair in human cancers should be characterized before considering the use of PARP inhibitors as single-agent or radiosensitizing therapies. A number of functional assays have been proposed;[38] one approach is to quantify DNA repair foci in situ in tumor tissues and in surrogate normal tissues, such as lymphocytes or skin fibroblasts.[21,39,40]

PARP Inhibition In Vivo; Effects on Blood Vasculature and Hypoxia

Preclinical efficacy of PARP inhibitors has now been observed in murine tumor models with DNA repair defects involving mismatch repair, BRCA-1, and ATM deficiency. Chemosensitization of p53-deficient breast cancer has also been documented.[36,41-44] These models have not yet been used to test for radiosensitization by PARP inhibition. However, several combination studies of PARP inhibition and experimental

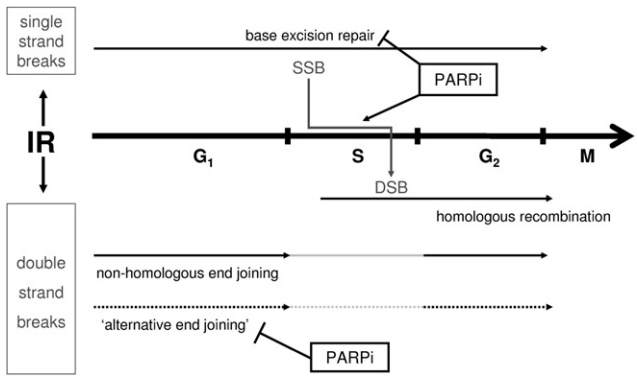

Figure 3 A simplified diagram showing the major pathways that contribute to the repair of radiation-induced DNA damage and their relationship to cell-cycle phase. The key mechanisms of action of PARP inhibitors are indicated. IR is a potent inducer of SSB that are predominantly repaired by BER, which functions throughout the cell cycle. PARP inhibitors delay BER, increasing the burden of unrepaired SSB, and promote replication-dependent conversion of SSB to DSB during S-phase. DSB arising in this manner are repaired by the homologous recombination repair pathway. Radiation also induces DSB directly. These are repaired mainly by NHEJ, which functions during G1 and G2 phases of the cell cycle. In the absence of core NHEJ, an alternative end joining process can repair radiation induced DSB. This pathway is impeded by PARP inhibition.

radiotherapy have been performed in human xenografts, and the promising results of these preclinical studies are summarized in Table 1. AG14361 enhanced tumor growth delay in a colorectal cancer xenograft model, GPI-15427 caused sustained tumor control in head and neck cancer xenografts, ABT-888 has shown radiosensitization in colorectal and lung cancer models, and E7016 (in combination with temozolomide) significantly improved tumor growth delay in glioblastoma xenografts.[18,19,45-48]

In many of these in vivo models, the sensitizing effect of the inhibitor was greater than might be predicted by the more modest effects observed in cell lines. In some cases, this might be attributable to superior effects with fractionated radiation schedules, but in some studies only large single doses were used. None of these studies used ex vivo clonogenic assays or measured the dose required to control 50% of tumors so they were unable to show direct sensitization of tumor clonogens or whether this was achieved in cycling versus noncycling tumor cells. This is an important issue because mechanistic studies using solely the growth delay assay could be biased by effects of radiotherapy and novel drugs on the tumor vasculature. Indeed, it is possible that some PARP inhibitors could induce short-term, vasodilatory effects by virtue of their structural similarities to nicotinamide. This could enhance tumor growth delay after radiotherapy by increasing tumor blood flow, enhancing drug penetration, and increasing oxygen concentrations to offset hypoxic cell radioresistance. Vasoactive properties and/or antiendothelial effects have been documented for AG14361 and ABT888,[45,46,49] as outlined in Tables 1 and 2, and PARP inhibition appears to be prolonged in tumors compared with normal tissues. This pharmacokinetic property might favor an enhanced in vivo response in tumor cells during radiotherapy and further improve the therapeutic ratio.

All solid tumors contain hypoxic cells. Hypoxia generates aggressive tumor cell phenotypes in part because of ongoing genetic instability and a "mutator" phenotype. The latter may be caused by the suppression of Rad51, BRCA1, BRCA2, and other HR protein expression via decreased transcription or translation.[50-53] We have shown that these hypoxia-mediated HR repair defects can be specifically targeted by DNA-damaging agents and/or "contextual synthetic lethality" to kill repair-deficient cells and preserve the therapeutic ratio. Specifically, HR defects render hypoxic cells more sensitive to ionizing radiation (IR), mitomycin C, and cisplatin.[52] More recent data suggest that HR-deficient hypoxic cells that have adapted and are proliferating are also more sensitive to PARP inhibition.[5,6]

Finally, to explore the possibility that effects of PARP inhibition could be altered by differential NAD^+ and ATP metabolism in hypoxic tumor cells, we tested the role of ABT888 as a radiosensitizer under hypoxic conditions in human prostate (DU-145 and 22RV1) and non–small-cell lung (H1299) cancer cell lines.[54] These studies showed that when ABT-888 was combined with IR, clonogenic radiation survival was decreased by 40% to 50% under oxic or hypoxic conditions. This sensitization of hypoxic cancer cells further supports the clinical use of PARP inhibitors to improve the therapeutic ratio achieved by radiotherapy.

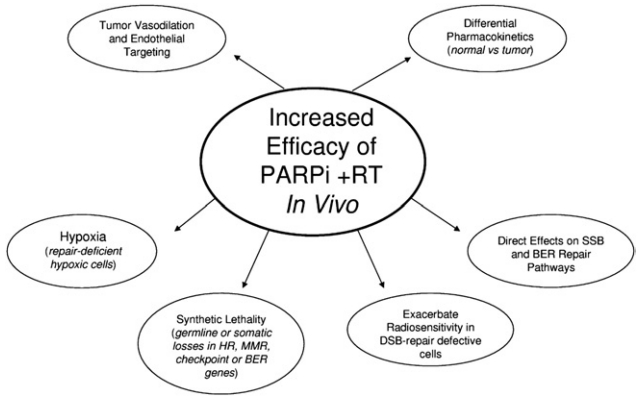

Figure 4 Mechanisms by which PARP inhibitors may increase clinical radiocurability (see text for details).

In summary, the contributing mechanism(s) in vivo remain largely unaddressed and may be model specific (see summary in Fig 4). At present, careful studies in human xenografts to directly determine the therapeutic ratio in vivo by comparing tumor efficacy with normal tissue toxicity in combined PARP inhibitor plus radiotherapy studies have not been conducted.[26,55] As such, it is not possible to conclude from the preclinical data that PARP inhibition always enhances the therapeutic ratio in combination with radiotherapy.

Clinical Trials of PARP Inhibitors Plus Radiation

Despite the encouraging preclinical studies, very few clinical studies of PARP inhibitors in combination with radiation are underway. In general, it is the late, irreversible effects of radiation that limit total radiotherapy dose, and these effects are not observed until months or years after treatment. Conventional drug dose-escalation studies combined with radical radiotherapy regimens are therefore difficult to design, and current studies have instead focused on combining PARP inhibition with palliative radiotherapy. At the time of writing, only 2 ongoing studies were identified. Both involve the Abbott compound ABT-888, and both are targeting brain tumors. The first is a phase I study combining escalating doses of oral ABT-888 with whole-brain radiation therapy (37.5 Gy in 15 fractions or 30 Gy in 10 fractions) in patients with cerebral metastases. The second is a phase I/II study of the same drug in combination with radical radiation therapy with concomitant and adjuvant temozolomide in the first-line treatment of patients with glioblastoma multiforme. The phase I component seeks to establish the maximum tolerated dose of the PARP inhibitor in this setting, and the subsequent phase II study will estimate the efficacy of this combination. Because PARP inhibition sensitizes glioma cells to both radiation and temozolomide, this is an ambitious study that has the potential to significantly improve outcomes for this difficult patient group. However, the risks of hematologic and

neurologic toxicity are also high, and the determination of the therapeutic ratio of the treatment will be important.

Conclusions and Outstanding Questions

In this review, we have discussed a variety of mechanisms that may underlay the radiosensitizing effects of PARP inhibitors in vitro and in vivo. Provocative data suggest that these sensitizing effects are likely to be more pronounced in tumors than in normal tissues during fractionated treatments, but careful preclinical and clinical studies are needed to prove that the therapeutic ratio is enhanced. A number of questions are still outstanding with respect to the use of this combination: (1) Are all PARP inhibitors equally efficacious in terms of maintaining the therapeutic ratio? (2) How should PARP inhibitors and fractionated radiotherapy be scheduled? (3) What are the best predictors for sensitization to PARP inhibition: proliferation rates, genetic status of tumor cells (ATM, BRCA1/2, or PTEN deficiency), and/or hypoxic fraction? (4) Do PARP inhibitors alter normal tissue genetic instability, and could they increase secondary malignancies when used in combination with radical radiotherapy? Even with these remaining questions, early data are supportive of a unique role for PARP inhibitors to augment tumor radiocurability, and the results of defined palliative and radical radiotherapy combination trials are eagerly awaited.

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