The potential role and application of PARP inhibitors in cancer treatment

Anthony J. Chalmers*
Genome Damage and Stability Centre, Brighton and Sussex Medical School, Brighton, UK

Background: Since many anti-cancer agents act by inflicting DNA damage on tumour cells, there is increasing interest in the use of inhibitors of DNA repair to increase the cytotoxicity of these agents. Poly(ADP-ribose) polymerase (PARP) is an abundant nuclear enzyme that binds to sites of DNA damage and promotes repair by modifying a number of key proteins. Potent and specific inhibitors of PARP are available; these have been shown to increase the cytotoxicity of a range of anti-cancer agents including temozolomide, irinotecan and radiation.

Sources of data: Data from laboratory studies on human tumour cell lines, pre-clinical studies including tumour xenograft models and early phase clinical testing in human subjects are discussed.

Areas of agreement: Pre-clinical and early clinical testing indicates that PARP inhibitors are extremely well tolerated. As single agents they have activity against BRCA1- and BRCA2-deficient cancers, and in combination they increase the cytotoxic effects of certain chemotherapy agents.

Areas of controversy: In order for PARP inhibitors to improve outcomes for patients, their sensitizing effects must be tumour specific. Early clinical data indicate that systemic toxicity may be exacerbated, so future trials must address this issue. The mechanism of action of PARP inhibitors in combination with cytotoxic agents is also uncertain.

Growing points: Among BRCA-deficient cancers, mechanisms of inherent and acquired resistance to PARP inhibitors are under investigation. Combining these agents with radiotherapy appears promising but designing clinical trials to test the efficacy and toxicity of this combination is problematic.

Areas timely for developing research: A particularly promising role for PARP inhibitors in the treatment of malignant brain tumours is outlined.

Keywords: poly (ADP-ribose) polymerase/chemotherapy/radiotherapy/DNA repair/glioblastoma multiforme/PARP inhibitors/temozolomide/irinotecan
Rationale for the use of DNA repair inhibitors in cancer therapy

Many of the agents used in the treatment of cancer exert their cytotoxic effects by damaging the chromosomes of the target tumour cells. Since the integrity of normal tissues depends on the maintenance of intact chromosomes, such agents necessarily have unwanted effects. Indeed, effects on normal tissues generally determine the maximum dose of an agent that may be safely delivered and are therefore responsible in many cases for the failure of cytotoxic agents to achieve cure. However, treatment with conventional cytotoxic therapies, such as ionizing radiation and/or chemotherapy, does deliver clinical benefit, so tumours in general must deal with DNA damage less efficiently than normal tissues. By understanding how cellular responses to DNA damage differ between malignant and healthy cells, it may be possible to accentuate these differences and enhance the therapeutic ratio achieved by existing treatments.

Different cytotoxic agents induce different spectra of DNA lesions, which in turn elicit characteristic DNA damage responses in the cell (reviewed in Fleck and Nielsen1). The details of these responses are beyond the scope of this review, but the pathways share a number of similar features, as shown in Figure 1. Essentially, the chemical nature of the DNA lesion determines which protein(s) are able to sense it. In some cases, damage sensing proteins also repair the lesion, but more commonly binding of sensor proteins to damaged sites initiates a variety of signalling cascades that alert the cell to the presence of damaged DNA, minimize the consequences of the damage and activate appropriate networks of proteins to repair it. Inhibiting any of these pathways increases the likelihood that DNA damage will cause cell death—the challenge for translational researchers is to identify targets that are tumour specific.

An important consequence of DNA damage signalling is the activation of cell cycle checkpoints (reviewed in Houtgraaf et al.2). The function of these checkpoints is to protect cells from the adverse consequences of replicating DNA damage (during S phase) or attempting to execute mitosis in the presence of damaged DNA (Fig. 2). Defective cell cycle checkpoint responses have been widely documented in many tumour types and are likely to contribute to the relative tumour specificity of DNA damaging agents.3 Partly as a result of blunted cell cycle checkpoint activation, many tumour cells exhibit rapid proliferation rates that may further increase their susceptibility to DNA damaging agents. Furthermore, specific repair mechanisms are required for processing chromosomal damage associated with
DNA replication. These represent potential therapeutic targets and are discussed later.

Although less extensive, there is also evidence to suggest that the actual DNA repair machinery may be compromised in some human tumours. The breast cancer susceptibility proteins BRCA1 and BRCA2 are components of the homologous recombination (HR) repair pathway and loss of function of either protein is associated with a profound increase in cancer susceptibility. Interestingly, BRCA1 and BRCA2 also have putative cell cycle checkpoint roles, as do several of the other DNA repair proteins that are associated with cancer susceptibility (e.g. ATM and p53). Since one of the features of the DNA repair network is redundancy—the capacity for alternative proteins or pathways to compensate for specific repair deficiencies—there is increasing interest in the concept of ‘synthetic lethality’. In this scenario, deficiency of one DNA repair component renders tumour cells highly sensitive to specific inhibition of a backup pathway that would otherwise be non-essential.

Fig. 1 Simplified overview of events involved in detection and repair of DNA damage. The left column indicates the generic sequence of events, which is applicable to many DNA repair pathways. These are illustrated in the central column with reference to the key events that occur during base excision repair of single-stranded DNA breaks induced by ionizing radiation. These events are described in the right column.
Hence, there is a convincing and increasing body of evidence to indicate that inhibition of DNA damage signalling and/or repair might enhance the cytotoxicity of DNA damaging agents in a tumour-specific manner. This review focuses entirely on the therapeutic potential of inhibiting PARP.

**PARP functions in DNA repair**

The PARP family of proteins, of which there may be as many as 18 members, is characterized by the enzymatic property of poly(ADP-ribosylation). This reaction uses NAD$^+$ as a substrate and catalyses the addition of long, branching chains of poly(ADP-ribose) polymers to target proteins. Such poly(ADP-ribosylation) modulates the catalytic activity and protein–protein interactions of these targets and thus influences a range of cellular processes (reviewed in...
D’Amours et al.\textsuperscript{5}). Poly(ADP-ribose) polymerase-1 (PARP-1) is responsible for at least 80% of total cellular PARP activity, and together with its nearest relative PARP-2, constitutes the DNA damage response arm of the PARP family.\textsuperscript{6} Tankyrase 1 and its close homologue tankyrase 2 are PARP proteins that play regulatory roles in telomere maintenance and mitosis and may therefore influence genomic stability (reviewed in Hsiao and Smith\textsuperscript{7}), while the remaining members of the PARP family are not thought to be involved in DNA repair. In this article, the term ‘PARP’ will be used to indicate the combined effects of PARP-1 and PARP-2, since in many cases their relative contributions have not been fully elucidated.

PARP-1 and PARP-2 are DNA damage sensors, in that they bind rapidly to sites of DNA damage, and also DNA damage signallers, in that this binding activates their catalytic function which in turn modulates a wide range of proteins involved in the DNA damage response (reviewed in Malanga and Althaus\textsuperscript{8}). The major target is PARP itself: poly(ADP-ribosylation) of the automodification domain (Fig. 3) creates a negative charge that brings about dissociation of PARP from DNA. This is important because it renders damaged DNA accessible to the proteins that are recruited to execute its repair. Dissociated PARP is recycled by the action of poly(ADP-ribose) glycohydrolase (PARG), which enzymatically removes poly(ADP-ribose) polymers.\textsuperscript{9} PARP inhibitors disrupt the catalytic function of PARP but do not interfere with its ability to bind to DNA.\textsuperscript{10} In the presence of a PARP inhibitor, therefore, it is probable that PARP binds to sites of damage but fails to automodify, remaining bound to the lesion, preventing access of repair factors and exacerbating the impact of PARP inhibitors on DNA repair.

Although PARP-1 binds to both single- and double-stranded DNA breaks, its role in single-strand break (SSB) repair via the base excision repair (BER) pathway has been most clearly defined.\textsuperscript{11} Even here, however, complete inhibition of PARP activity by molecular or chemical means does not abolish SSB repair entirely. Rather, lesions are repaired with delayed kinetics. The importance of PARP activity in repair of double-strand breaks (DSB) is even more uncertain and appears to vary according to the DNA damaging agent involved. Nevertheless, inhibition or downregulation of PARP has a significant effect on cellular sensitivity to a range of DNA damaging agents.

BER plays an important role in repairing damaged bases and SSB induced by alkylating agents and ionizing radiation (reviewed in Caldecott\textsuperscript{12}). PARP binds both to directly induced SSB and to SSB that arise during repair of base damage. Such binding appears to protect these lesions from deleterious interactions with adjacent DNA. Activated PARP also interacts with BER proteins such as...
The role of poly(ADP-ribose) polymerase (PARP) in detecting and signalling single-stranded DNA breaks (left column), and the effect of chemical inhibition of PARP on this process (right column). Briefly, the presence of a DNA single-strand break (A) stimulates attachment of the DNA-binding domain of PARP, which activates the catalytic domain (B). Poly(ADP-ribosylation) of nuclear proteins occurs, along with poly(ADP-ribosylation) of the automodification domain of PARP (C). Automodification stimulates release of PARP from the damaged site, leaving it accessible to DNA repair proteins (D), some of which (e.g. X) have been recruited and activated by PARP. Others (e.g. Y) are recruited via other pathways. Concerted activity of these proteins repairs the damaged site (E). Chemical inhibitors of PARP compete with NAD\(^{+}\) and bind to the catalytic domain (A). The DNA-binding function of PARP is not affected (B) but catalytic activity is inhibited (C). This prevents automodification of PARP, which remains bound to DNA (D). Recruitment of PARP-dependent repair proteins (e.g. X) is inhibited, and access of PARP-independent repair proteins (e.g. Y) to the damaged site is impeded. The DNA break is unrepaired, and inhibited PARP may remain bound to the damaged site (E). The kinetics of this interaction are unclear.
XRCC1, promoting recruitment and activity of these factors and facilitating repair. Hence PARP-1-deficient mice are hypersensitive to alkylating agents, as are organisms or cells subjected to PARP inhibition.

DSB induced by ionizing radiation or cytotoxic agents are repaired primarily by the non-homologous end-joining (NHEJ) pathway, although lesions arising during DNA replication are preferentially repaired by the HR pathway (reviewed in O’Driscol and Jeggo13). PARP-1 has been shown to interact with components of NHEJ but does not appear to be a vital component of the pathway. PARP-1 does not appear to participate in HR,14 indeed inhibition of PARP increases HR activity in replicating cells, a phenomenon that will be discussed later. PARP-1-deficient organisms show a modest increase in radiosensitivity; whether this reflects defects in SSB and/or DSB repair has been a matter of some debate.15

PARP inhibitors

Chemical inhibitors of PARP have been in the laboratory for decades. The earliest compounds were analogues of the nicotinamide component of NAD+, which is the substrate for PARP’s catalytic function. These inhibitors competed with NAD+ for PARP’s catalytic site and prevented synthesis of poly(ADP-ribose). Since then, a huge array of more potent inhibitors has been developed, all of which act in essentially the same way. Since the catalytic sites of PARP-1 and PARP-2 are virtually identical, it has not been possible to develop inhibitors with differential activity, so the compounds referred to in this article should be considered as effective inhibitors of both PARP-1 and PARP-2. While these inhibitors exhibit relative specificity for PARP-1 and PARP-2, their potential to exert ‘off-target’ effects via other PARPs should always be considered. For a more detailed review of PARP inhibitors, see Woon and Threadgill.10

The cellular effects of PARP inhibitors vary according to the cellular environment. In particular, the presence and nature of DNA damage and/or metabolic stress have an important impact on the consequences of PARP inhibition.16 In the presence of damaged DNA, PARP functions to enhance repair and suppress potentially deleterious interactions between damaged sites. Because PARP is abundant and rapidly activated by DNA damage, however, this protective effect often occurs at the expense of NAD+ depletion, since the reaction catalysed by PARP consumes NAD+. This compound is essential for the synthesis of ATP, which is required as a source of energy for a vast array of metabolic processes. High doses of DNA
damaging agents have been shown to reduce cellular NAD\(^+\) levels by 80\% within 5–15 min, and the resulting lack of ATP renders cells unable to execute cell death by apoptosis, which is an energy-consuming pathway. Consequently cell death occurs by necrosis, which is associated with uncontrolled cell lysis and release of inflammatory mediators. Pre-treatment with a PARP inhibitor prevents NAD\(^+\) depletion and appears to enable cells to execute apoptosis. So although DNA repair is impaired, the overall damage to tissues or organs exposed to massive toxic insults may in fact be reduced by PARP inhibition, as reviewed in Horvath and Szabo.\(^17\)

This property has been explored in pathological conditions such as reperfusion after myocardial infarction\(^18\) or ischaemic stroke.\(^19\) In preclinical models, PARP inhibition appeared to suppress inflammatory and necrotic tissue responses. Clinical evaluation remains at an early stage: a recent randomized phase I study of the PARP inhibitor INO-1001 given immediately prior to coronary artery stenting as primary treatment for acute myocardial infarction indicated that the drug was well tolerated and showed a trend towards blunting of the reperfusion-associated peak in inflammatory markers (C-reactive protein, interleukin-6) 24 h post-procedure.\(^20\) In the context of cancer treatment, however, the impact of PARP inhibition on DNA repair pathways appears to be of greater significance than effects on NAD\(^+\) metabolism. This may reflect the fact that tumour cells are generally characterized by rapid replication rates, so efficient repair of DNA damage is critical to survival. In non-replicating tissues such as the brain, DNA damage is likely to have less immediate impact than depletion of NAD\(^+\).

**Therapeutic effects of PARP inhibitors as single agents**

*Background*

In most cell lines, treatment with PARP inhibitors at doses that successfully inhibit PARP activity does not cause cell death, consistent with the concept that PARP is only activated in the presence of DNA damage. There was great interest, therefore, in the discovery in 2005 that breast cancer cells bearing homozygous mutations in either the BRCA1 or BRCA2 cancer susceptibility genes were extremely sensitive to PARP inhibition.\(^21,22\) Investigation of the underlying mechanisms revealed that both BRCA1 and BRCA2 play important roles in the HR DNA repair pathway, and that continuous exposure of cycling cells to a PARP inhibitor resulted in accumulation of DNA DSB that could not be repaired. The explanation centres on the long-established
observation that our chromosomes are continuously subjected to high levels of damage by reactive chemicals generated within the cell as by-products of metabolism. PARP is involved in detection and repair of this endogenous damage, and inhibition of PARP increases the prevalence of unrepaired lesions that can cause stalling and collapse of the DNA replication machinery. As stated earlier, DNA breaks arising during replication can only be repaired by HR. In BRCA1- or BRCA2-deficient cells, which lack HR, these lesions go unrepaired and evolve into cytotoxic DSB.

Based on these observations, PARP inhibitors were proposed as the ultimate tumour-specific therapy for tumours with BRCA mutations. Patients generally inherit a single mutant copy of BRCA1 or BRCA2 and develop cancer only when a cell acquires an additional mutation that inactivates the normal allele. Tumour cells are therefore BRCA1 or BRCA2 deficient, whereas the cells of the normal tissues retain one intact copy of the gene, express apparently normal levels of functional protein and are resistant to PARP inhibition.

Clinical trial data

An initial dose escalation study of the Astra Zeneca compound AZD2281 confirmed that PARP activity could be successfully inhibited in tumour and white blood cells at non-toxic doses of the drug, and a number of clinical responses in BRCA-deficient ovarian cancer were observed. A subsequent study confined to patients with metastatic BRCA-deficient ovarian cancer yielded an overall response rate of 44%, with median response duration of 30 weeks. Interestingly, responses were much more frequent in patients whose tumours were cisplatin sensitive than in cisplatin refractory cases. Resistance of BRCA2-deficient cell lines to PARP inhibition has been shown to be associated with restoration of function of the HR pathway, and the observation of a similar mechanism underlying resistance to platinum-based cytotoxic agents may explain this cross-resistance.

Similar studies in patients with BRCA-mutated breast or ovarian cancer are underway in various centres. It will be interesting to see whether the different PARP inhibitors, which share a very similar mode of action, will demonstrate different levels of clinical effectiveness. Other unresolved questions include whether BRCA1-and BRCA2-deficient cancers are equally sensitive and whether response rates will be superior in ovarian cancer compared with breast cancer as was the case in the AZD2281 study.
PARP inhibitors and chemotherapy

Data from tumour cell lines

Evidence that PARP inhibition enhances the potency of a range of cytotoxic agents derives from studies on tumour cell lines in vitro and from pre-clinical animal models in vivo (Table 1). This evidence has been comprehensively reviewed elsewhere, but to summarize, several different PARP inhibitors have been shown to increase the cytotoxic effects of alkylating agents such as temozolomide and topoisomerase I poisons such as irinotecan. Less substantial evidence exists to support the concept that PARP inhibition enhances tumour

Table 1 In vivo effects of PARP inhibitors in combination with cytotoxic agents.

<table>
<thead>
<tr>
<th>PARP inhibitor</th>
<th>Cytotoxic agent(s)</th>
<th>Model organism</th>
<th>Tumour type (human origin unless stated)</th>
<th>Effect</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>AG14699</td>
<td>Temozolomide</td>
<td>Human</td>
<td>Malignant melanoma</td>
<td>Encouraging response rates, exacerbation of haematological toxicity</td>
<td>Plummer et al.(^{38}), Plummer et al.(^{40})</td>
</tr>
<tr>
<td>AG14361</td>
<td>Temozolomide, irinotecan, radiation</td>
<td>Mouse xenograft</td>
<td>Colorectal (LoVo, SW620)</td>
<td>Enhanced tumour growth delay</td>
<td>Calabrese et al.(^{32})</td>
</tr>
<tr>
<td>ABT-888</td>
<td>Temozolomide</td>
<td>Mouse syngeneic, rat orthotopic</td>
<td>Mouse melanoma (B16F10), rat glioma (9L)</td>
<td>Enhanced tumour growth delay, increased survival</td>
<td>Donawho et al.(^{28})</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Cisplatin, carboplatin, cyclophosphamide, Radiation</td>
<td>Breast (MX-1)</td>
<td>Enhanced tumour growth delay</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mouse xenograft</td>
<td>Colorectal (HCT-116)</td>
<td>Enhanced tumour growth delay</td>
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</tr>
<tr>
<td>GPI 15427</td>
<td>Temozolomide</td>
<td>Mouse syngeneic, mouse xenograft (all intracranial)</td>
<td>Mouse melanoma (B16), mouse lymphoma (LS178Y), glioblastoma (SJGBM2) (all intracranial)</td>
<td>Increased survival</td>
<td>Tentori et al.(^{35})</td>
</tr>
<tr>
<td>CEP-6800</td>
<td>Temozolomide, irinotecan, cisplatin</td>
<td>Mouse xenograft</td>
<td>Glioblastoma (U251), colorectal (HT29), non-small cell lung (Calu-6)</td>
<td>Increased complete regression rates, enhanced tumour growth delay</td>
<td>Miknyoczki et al.(^{36})</td>
</tr>
<tr>
<td>CEP-8983</td>
<td>Temozolomide, irinotecan</td>
<td>Mouse xenograft</td>
<td>Colorectal (HT29), rat glioma (RG2)</td>
<td>Enhanced tumour growth delay</td>
<td>Miknyoczki et al.(^{33})</td>
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<tr>
<td>AZD2281</td>
<td>Temozolomide</td>
<td>Mouse xenograft</td>
<td>Colorectal (SW620)</td>
<td>Enhanced tumour growth delay</td>
<td>Meneer et al.(^{34})</td>
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responses to DNA cross-linking agents such as cisplatin. The mechanisms underlying these effects have not been fully elucidated, and probably vary according to the properties of the cytotoxic agent. Data relating to temozolomide and irinotecan will be discussed here.

Temozolomide acts by methylating the DNA bases guanine and adenine at specific sites. Methylation changes the structure of these bases so that they pair inappropriately during DNA replication. Mispaired bases are recognized by the mismatch repair (MMR) pathway, which attempts to insert the correct base. However, the methylated base persists, so this cycle is repeated with each round of DNA replication, eventually resulting in DNA strand breaks and cell death.

The most cytotoxic lesion is O6-methylguanine, which is repaired primarily by O6-methylguanine methyltransferase (MGMT). Tumours that express this protein are generally resistant to temozolomide. Indeed, expression of MGMT by high-grade primary brain tumours known as glioblastoma multiforme (GBM) is associated with poor prognosis and resistance to temozolomide. In other tumour types, defects in the MMR pathway are associated with temozolomide resistance, because mispaired bases are not detected, no attempts at repair are made and no DNA breaks are generated.

Whatever the mechanism of resistance, PARP inhibition has the potential to reverse it. In the case of MGMT-mediated resistance, the other methylation products of temozolomide (N7-methylguanine and N3-methyladenine) are usually non-toxic because they are efficiently repaired by BER. Inhibition of PARP should therefore render these lesions cytotoxic and overcome MGMT-mediated resistance. There is also good evidence that PARP inhibitors have a greater impact on the temozolomide sensitivity of MMR-deficient than MMR-proficient tumour cells, suggesting that impeding BER-mediated repair of the N7-methylguanine and N3-methyladenine lesions also overcomes MMR-related resistance.

Topoisomerase I (topo I) poisons such as irinotecan and topotecan exert their cytotoxic effects by binding to and stabilizing the DNA helicase enzyme topo I. This enzyme plays a vital role in facilitating unwinding of the DNA double helix during DNA replication to relieve torsional strain. The mechanism involves temporary insertion of DNA breaks to allow unwinding, and an intermediate DNA/protein structure termed the ‘cleavable complex’ is generated. Topo I poisons stabilize the cleavable complex and extend the lifetime of the associated DNA strand breaks. PARP-1 is involved in the resolution of these breaks, so inhibition of PARP activity increases the yield of unrepaired DNA damage and consequent cell death.
In vivo data from animal models

Several studies have shown potent sensitization of temozolomide by PARP inhibitors in a range of human tumour cell lines transplanted as xenografts into animals.\textsuperscript{32–34} In particular, sensitization of intracranial tumours has been demonstrated for some compounds.\textsuperscript{35} Likewise, significant improvements in tumour growth delay have been observed when PARP inhibitors have been added to irinotecan\textsuperscript{32,33} and, in two reports, cisplatin.\textsuperscript{28,36} In most cases, PARP inhibition did not significantly worsen normal tissue toxicity, although it is often difficult to detect clinically relevant effects on normal tissues in these animal models. In one study, the intestinal toxicity associated with irinotecan was found to be reduced by concomitant PARP inhibition\textsuperscript{37} and another study used a granulocyte-macrophage colony-forming unit assay to show that PARP inhibition would be unlikely to exacerbate chemotherapy-related bone marrow toxicity.\textsuperscript{33} Despite these results, clinical studies in patients are required to demonstrate whether the sensitizing effects of PARP inhibitors are truly tumour specific.

Data from clinical trials

The first clinical trials of a PARP inhibitor in combination with chemotherapy were performed in patients with metastatic melanoma. After promising safety and toxicity outcomes from a phase I study of temozolomide in combination with the Pfizer compound AG014699, a phase II study was undertaken. Combination therapy yielded encouraging response rates, but the haematological toxicity of temozolomide was exacerbated.\textsuperscript{38} There was one toxic death, three neutropenic hospitalizations and a temozolomide dose reduction was required in 12 of the 40 patients. This study illustrates the perennial problem associated with modifying the cellular response to cytotoxic agents—that of parallel sensitization of the critical normal tissues.

The results of this trial do not necessarily sound the death knell for the temozolomide/PARP inhibitor combination. Alternative schedules of temozolomide are available, and daily low-dose regimes cause much less neutropenia and thrombocytopenia than the 5-day regime that was used in the trial. It may be possible to enhance the anti-tumour effects of such alternative regimes while keeping toxicity profiles within acceptable parameters.

At the time of writing, a number of clinical trials of PARP inhibitors combined with chemotherapy are either underway or in preparation, but data are not yet available.
PARP inhibitors and radiotherapy

Studies on tumour cell lines

Ionizing radiation induces a combination of SSB and DSB. Of these, DSB are the lethal lesions, but SSB are approximately 25-fold more numerous. As previously described, PARP plays a significant role in SSB repair but its role in DSB repair is disputed. The overall impact of PARP inhibition appears to be a modest increase in radiosensitivity; this has been demonstrated in a number of human tumour cell lines\(^39,40\) and in PARP-1-deficient mice.\(^15\) Some studies have reported minimal sensitization in certain cell lines;\(^41\) the likely explanation for this is that the radiosensitizing effects of PARP inhibitors are dependent on DNA replication. In non-dividing cells, PARP inhibition causes a delay in SSB repair which has no impact on DSB formation or cell survival, whereas in replicating cells the unrepaired SSB generate collapsed replication forks which give rise to potentially lethal DSB.\(^42\) It is not clear whether PARP inhibition simply increases the burden of unrepaired SSB or has an additional effect in impeding repair of the collapsed replication forks.

The net result is that PARP inhibition may preferentially sensitize tumour cells by virtue of their elevated replication rates. This may be particularly useful in the treatment of high-grade brain tumours where the critical normal tissue—the brain—is composed almost entirely of non-dividing cells. The potential role of PARP inhibitors in the treatment of brain tumours will be discussed in more detail later.

In vivo data from animal models

Pre-clinical studies have supported the potential role for PARP inhibitors as radiosensitizers. Tumour growth delay associated with radiation treatment was enhanced in colorectal cancer xenografts treated with AG14361\(^32\) and in colorectal and lung cancer xenografts treated with ABT-888.\(^28,43\) Combination therapy appeared to be well tolerated in all cases, although the radiation doses delivered in these experiments are not comparable with radical doses used in the clinic.

No data from clinical trials combining radiotherapy with PARP inhibitors were available at the time of writing.

The journey from laboratory to patient

Clinical testing of conventional anti-cancer therapies follows a well-established pathway. Phase I studies establish the safety and toxicity of
a compound, and the treatment of successive cohorts with escalating doses establishes the maximum tolerated dose (MTD). This dose is then tested for efficacy and additional toxicity information in phase II studies. If pre-determined response rates (or other endpoints if appropriate) are achieved, the compound may proceed to phase III testing in which the new agent is compared with current standard therapy in a randomized manner.

This pathway is not appropriate for evaluating targeted therapies such as PARP inhibitors for two reasons. First, if the compound can be shown to completely inhibit the activity of its target at a certain dose, there is no value in further dose escalation. Hence the first relevant endpoint is the optimum biological dose (OBD) rather than the MTD. Since the critical question is whether the agent achieves target inhibition in tumour cells, it is becoming increasingly common for agents to undergo ‘Phase 0’ testing. In this scenario, patients with a cancer diagnosis who are due to undergo definitive surgical therapy are treated with the novel agent immediately prior to surgery. Surgical resection specimens are then analysed for drug levels (pharmacokinetics) and for biological effects (pharmacodynamics) such as PARP inhibition. Dose escalation during phase 0 enables identification of the OBD.

Secondly, targeted agents are commonly used in combination with other agents, such as chemotherapy or radiotherapy, with the aim of enhancing efficacy. The next stage of testing therefore requires phase I assessment of the novel agent (given at OBD) in combination with the intended cytotoxic partner. With any sensitizer there is a risk that toxicity will be exacerbated, so it is necessary to undertake dose escalation of the conventional agent to establish its MTD in combination with the new agent. In the ideal scenario, sensitizing effects will be tumour specific and the conventional agent can be delivered at full dose. Combining novel therapies with radiation is particularly challenging, since there is only one opportunity to deliver radical dose radiotherapy, so dose escalation is not ethically acceptable. New strategies are required for effective testing of the many targeted therapies, including PARP inhibitors, that have clinical potential in combination with radiotherapy.

**GBM: the ideal tumour target for PARP inhibitors?**

GBM are the commonest and most aggressive primary brain tumours. Current therapy comprises surgical resection followed by radical radiotherapy with concomitant and adjuvant temozolomide chemotherapy. Despite such aggressive treatment, median survival is only 1 year. For elderly or poor performance status patients, average life expectancy is
3–6 months. Hence there is an urgent need for novel treatments for both groups of patients.

A unique feature of GBM is that they exhibit relatively high proliferation rates while the surrounding, dose limiting, normal tissue is essentially non-dividing. In terms of radiotherapy delivery, it is necessary to treat large volumes of apparently normal brain to encompass infiltrative tumour that cannot be removed surgically. Escalating radiation dose to the normal brain results in debilitating and irreversible neurotoxicity, so any radiosensitizer must be tumour specific. Since the radiosensitizing effects of PARP inhibitors are specific to cells undergoing DNA replication, such agents may be of therapeutic value.

PARP inhibitors also have the potential to increase the sensitivity of GBM to temozolomide. At least half of GBM are refractory to temozolomide because they express the repair protein MGMT. These tumours can be identified using methylation-specific polymerase chain reaction techniques because the promoter regions of their MGMT genes are unmethylated. Since PARP inhibition increases the sensitivity of MGMT expressing tumour cell lines to temozolomide, the combination of all three agents may be a therapeutic option for MGMT expressing tumours, for which current therapies are ineffective.

Summary

PARP activity plays an important role in the sensing and signalling of DNA damage arising both endogenously and as a result of cytotoxic treatments. Cancers with defects in the HR DNA repair pathway are sensitive to PARP inhibitors; clinical trials have shown encouraging response rates in BRCA1- and BRCA2-deficient ovarian cancers and have confirmed that PARP inhibitors can be used at effective doses with minimal toxicity. In a range of common cancer types, PARP inhibitors enhance the cytotoxicity of a variety of agents that are routinely used in cancer treatment, notably alkylating agents such as temozolomide, topoisomerase I poisons such as irinotecan, and ionizing radiation. The mechanisms responsible for this sensitization indicate that GBM are a particularly promising target for PARP inhibitors in combination with chemotherapy and radiation. Ongoing and future clinical trials will demonstrate whether the sensitizing effects of these agents are truly tumour specific and will identify the tumour types, patient groups and current therapies that benefit most from the addition of a PARP inhibitor.
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