The potential of PARP inhibitors in neuro-oncology

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Practice Points

- Tumor cells may differ in DNA-damage response and repair in comparison to normal cells and effects of cancer therapies could be maximized by interfering with tumor cell DNA-damage responses with agents such as PARP inhibitors.
- PARP inhibitors have effects on the base excision repair pathway and delay repair of ssDNA breaks produced by ionizing radiation or chemotherapy.
- PARP inhibitors have shown promising results as monotherapies in BRCA-deficient cancers; however, combination with cytotoxic chemotherapy has been challenging due to problems with hematological toxicity. The investigation of PARP inhibitors as radiosensitizers may have important therapeutic potential.
- Several trials have either commenced recruitment or are underway and are investigating the following areas:
 - Penetration of olaparib beyond the blood–brain barrier into tumor tissue.
 - Toxicity of PARP inhibitor therapy concurrent with temozolomide.
 - Whole-brain radiotherapy and PARP inhibitor therapy.
- The identification of appropriate biomarkers is essential if trials of PARP inhibitors are to be successful and functional analysis of whole-DNA repair pathway integrities and DNA-damage levels in general may provide more relevant information than assays of expression of individual DNA repair genes.
- PARP inhibitors are potent DNA-damage response-modulating agents with promising anti-tumor effects.
- Glioblastoma is an obvious target for application of PARP inhibitors.

SUMMARY DNA damaging agents have an integral role in the treatment of brain tumors. Recent advances in our understanding of how cancer cells repair DNA damage have made it possible to consider modification of the DNA damage response as a way in which resistance to radiotherapy and chemotherapy might be overcome. PARP inhibitors are potent but nontoxic drugs that inhibit repair of DNA single-strand breaks and increase the cytotoxic

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effects of radiotherapy and alkylating chemotherapy agents, including temozolomide. PARP inhibitors have potential applications in neuro-oncology because there is increasing evidence that their radio- and chemo-sensitizing effects are tumor specific. This review explores the mechanisms of action of PARP inhibitors and describes their putative mechanisms of radio- and chemo-sensitization in the context of CNS oncology. The authors go on to review their development in recent clinical trials, with a focus on glioblastoma.

DNA-damaging agents, whether in the form of chemotherapy or radiotherapy, play an important role in the treatment of cancer. Until recently, treatment efforts have focused on maximizing the DNA damage that can be inflicted upon tumors, with little consideration of how malignant and normal cells repair DNA damage. Such dose escalation strategies are inherently limited by the toxic effects of DNA-damaging agents on normal tissues, so for many types of cancer it will be necessary to adopt a more tumor-specific approach in order to increase cure rates. It is becoming increasingly clear that tumor cells repair and respond to DNA damage in different ways from normal tissues, and this creates a possibility of maximizing the anticancer effects of DNA-damaging therapies by interfering with the DNA-damage response (DDR) of malignant cells. A number of small molecule inhibitors of the DDR are available and some of these are undergoing preclinical assessment in combination with cytotoxic agents. PARP inhibitors are the most developed DDR modifiers and the purpose of this review is to explore the potential applications of these agents within CNS oncology. The review will focus on the treatment of glioblastoma (GBM), but wider applications of PARP inhibitors in neuro-oncology will also be considered.

The DDR

Because they are continuously exposed to genotoxic agents from without and within, mammalian cells have developed a network of pathways that enables them to mount an efficient response to DNA damage and maintain genomic integrity. Consistent with the fundamental nature of this process, the pathways are highly conserved and feature crossover and redundancy between the different repair pathways. There are two main components of the DDR. Cell cycle checkpoints prevent DNA replication and/or mitosis from proceeding in the presence of DNA damage by arresting cell cycle progression at the G1/S and G2/M checkpoints. Activation of these checkpoints increases the time available for repair of DNA damage and reduces the risk of nonlethal

damage evolving into more complex, double-stranded lesions that might threaten cell viability. Defects in the checkpoint signaling pathways are associated with genomic instability and an increased risk of cancer [1–3]. Acting in concert with these checkpoints is a broad range of repair pathways, which recognize and repair sites of damaged DNA. Many of the components of DNA repair pathways appear to be essential for life, but a number of human syndromes have been associated with mutations in DNA repair genes, and these are generally characterized by hypersensitivity to DNA-damaging agents and increased cancer susceptibility [4].

Full descriptions of the known checkpoint and repair pathways are beyond the scope of this article. The main pathways relevant to the discussion of PARP inhibitors in neuro-oncology are base excision repair (BER) [5], which overlaps extensively with single-strand break (SSB) repair [6]; and nonhomologous end joining (NHEJ) [7] and homologous recombination repair (HRR) [8], which execute double-strand break (DSB) repair (Figure 1). For a concise review of DNA repair see Fleck and Nielsen [9].

DDR & PARP

The PARPs are a family of proteins which have many functions, for example, DNA repair, transcription, mitotic segregation, telomere homeostasis and cell death, but share the common catalytic function of poly(ADP-ribosylation) [10]. There are up to 18 members of the PARP family, however only PARP-1, -2 and -3 have a well characterized role in the DDR [10–13]. Of these, PARP-1 is by far the most abundant and its role in DNA repair is the best understood. For the remainder of this review, the term ‘PARP’ will be used to refer primarily to PARP-1, but since many PARP inhibitors have activity against all three enzymes, effects on PARP-2 and -3 may also be encompassed. Several investigators have reported the identification of selective inhibitors of PARP isoforms, which may be attractive candidates for future clinical development [14–17]. The specificity of current PARP inhibitors for various family members of the PARP family was

Figure 1. A simplified diagram of the major pathways and checkpoints involved in DNA damage response.

BER: Base excision repair; NHEJ: Nonhomologous end joining.

investigated by Wahlberg *et al.* [18], who demonstrated that the major PARP inhibitors in current clinical development (olaparib, ABT-888 and rucaparib) exhibit activity against PARP-1, -2 and -3. However, rucaparib was also observed to exert stabilizing effects on PARP-5a and -5b. PARP-5a and -b are tankyrases known to have important roles in telomere homeostasis [19]; however, McCabe *et al.* have also demonstrated a synthetic lethal effect of PARP-5a and -5b inhibition in BRCA-deficient cell lines via centrosome amplification and spindle abnormalities [20]. Although not related to DDR, these effects could be of therapeutic importance.

PARP has an integral role in ssDNA repair: it detects and binds to nicks and breaks in DNA, protecting the damaged sites from inappropriate recombination events and acting as a signaler to downstream components of the repair pathway, recruiting them to the site of damage.

Upon binding to DNA, PARP-1 undergoes dimerization, leading to activation of its catalytic function. Activated PARP then modifies its nuclear protein targets by the sequential, covalent addition of ADP-ribose molecules leading to the

formation of branching chains of poly(ADP-ribose) (PAR) polymers. The substrate from which PAR is synthesized is NAD⁺, and changes in cellular levels of NAD⁺ can influence PARP activity (and *vice versa*). Poly(ADP-ribosylation) alters both the catalytic activity and protein–protein interactions of its substrates, thus modifying the function of various cellular pathways. The targets of PARP are multiple and include proteins involved in DNA metabolism, such as DNA ligases and DNA polymerases [10]. With regard to DNA repair, PARP interacts directly with and modifies XRCC1, recruiting it to sites of ssDNA damage where it acts as a scaffold protein on which additional BER proteins are assembled and activated. PARP also undergoes automodification, which is important when considering its role in DNA repair and the impact of chemical inhibitors. Automodification generates long chains of PAR polymers on the DNA-bound PARP molecule. This creates a negative charge that causes PARP to dissociate from the DNA strand, allowing access of downstream components of the DNA repair machinery to the damaged site [21–24]. These processes are summarized

Figure 2. The role of PARP in single-strand DNA break repair.

BER: Base excision repair; SSB: Single-strand break.

in **Figure 2**. In the context of cancer treatments, BER has a critical role in the repair of damaged bases and SSBs induced by alkylating agents, such as temozolomide, and by ionizing radiation [25].

The role of PARP in the repair of dsDNA breaks is much less well defined. In mammalian cells, DSBs are usually repaired via NHEJ, or by HRR if they arise during DNA replication. PARP has no direct involvement in the HRR pathway, and PARP inhibitors have been shown to increase the cellular activity of HRR, probably by increasing the burden of replication-associated damage [26]. By contrast, PARP does interact with components of the NHEJ pathway but does not appear to be vital to this process. In some tumors an alternative end-joining pathway appears to be upregulated,

possibly in response to defects in NHEJ. This pathway is not well defined but has been termed 'microhomology-associated end joining' (MMEJ) [27]. There is some evidence that PARP is involved in MMEJ, and in cellular systems that are deficient in core NHEJ, inhibition of PARP has been shown to impede the backup repair pathway and exacerbate the repair defect [28].

PARP inhibitors

PARP inhibitors compete with NAD⁺ at the catalytic site of PARPs, inhibiting their enzymatic function and preventing synthesis of PAR. The catalytic sites of PARP-1 and -2 are very similar and most PARP inhibitors have similar activity against these two enzymes. A recent study has shown that effects of PARP inhibitors in current clinical use are generally restricted to PARPs 1 to 4 [18]. As yet, the clinical consequences of inhibition of the different PARP isoforms are unknown. Several PARP inhibitors are currently undergoing evaluation in early-phase clinical trials. These include AZD2281 (olaparib), ABT888 (veliparib), AG014699 (rucaparib), ABT767, BMN673, INO-1001, GPI21016, CEP-9722 and MK4827.

PARP inhibitors as radiosensitizers

PARP inhibitors are known to be modest radiosensitizers *in vitro* with sensitivity enhancement ratios of 1.2–1.7 in cell lines [29,30]. The PARP inhibitor olaparib has recently been shown to enhance the radiosensitivity of non-small-cell lung cancer both *in vitro* and *in vivo* [31]. The mechanism of radiosensitization is related to inhibition of BER, which executes repair of the vast majority of radiation-induced SSBs, but an additional mechanism involves inhibition of automodification of PARP at the damaged site. This has been shown to delay its dissociation from DNA, a phenomenon that is likely to further interfere with damage resolution by blocking access to downstream DNA repair components. Experimentally, this is observed as an increase in the burden of unrepaired SSBs. In proliferating cells, these unresolved SSBs can be converted into DSBs during DNA replication via the generation of collapsed replication forks (**Figure 3**). Thus, in replicating cells, SSBs produced by ionizing radiation (which are normally repaired efficiently) can be converted into potentially lethal DSBs, enhancing the cytotoxic effects of radiation. This theory has been supported by work showing that the radiosensitizing

effect of PARP inhibitors is abolished if DNA replication is inhibited and increased if cells are synchronized in S phase [29,32].

The glial tissues of the brain have been demonstrated to replicate extremely slowly, with an estimated time for cellular turnover of 200 days [33]. Neuronal tissue in the brain is essentially nondividing so it is reasonable to predict that the addition of a PARP inhibitor to radiation would have a relatively greater effect on GBM cells, which are highly proliferative, than on the tissues of the normal brain. PARP inhibitors have also been observed to accumulate in tumor tissue, perhaps because of increased levels of DNA damage in tumor cells. This may enhance the tumor specificity of their radiosensitizing activity.

GBM are characterized by extensive hypoxia and angiogenesis. Liu and colleagues have shown that the PARP inhibitor ABT-888 (now veliparib)

radiosensitizes oxic and hypoxic cell lines to a similar degree [34], which is an important consideration because GBM have large hypoxic cell populations. Furthermore, PARP inhibitors may have additional effects on hypoxic tumour tissue. As they have been designed to compete with NAD⁺, many of the agents in current use have structural similarities to nicotinamide, which is a potent vasodilator. Consistent with this, PARP inhibitors have been shown to exert vasodilatory effects in preclinical tumor models, reducing tumor hypoxia and enhancing radiation sensitivity [35]. Antiangiogenic effects have also been observed [36–38] and it has been suggested that these effects might explain why *in vivo* studies have shown more pronounced radiosensitization effects than studies conducted in cell culture.

PARP inhibitors may also have protective effects in normal tissues. Activated PARP

Figure 3. Putative mechanism of radiosensitization by PARP inhibition.
DSB: Double-strand break. SSB: Single-strand break.

depletes the cell of NAD⁺, which may inhibit energy-dependent cellular pathways, such as apoptosis. Cells subject to massive DNA damage may then die by necrosis, a process that can exacerbate damage to adjacent normal tissues by promoting inflammation. If PARP is inhibited, depletion of NAD⁺ is less likely to occur and cells are more likely to execute apoptosis, thus limiting damage to surrounding tissues. Preclinical studies have demonstrated significant protective effects of PARP inhibitors in myocardial infarction, cerebral ischemia, spinal cord trauma, renal injury, hemorrhagic shock and acute lung injury [39–47], but these have yet to be replicated in patients. In the context of radiotherapy it is possible to speculate how concomitant treatment with a PARP inhibitor would affect acute and long-term toxicities: the replication dependence of radiosensitization predicts that acute toxicities in rapidly proliferating tissues would be exacerbated whereas late effects in slowly proliferating tissues would be unaffected. Indeed, Beller *et al.* have demonstrated in an *ex vivo* model that PARP inhibition can ameliorate vascular function following irradiation [48]. The radiosensitizing effects of PARP inhibitors are summarized in [Figure 3](#).

PARP & synthetic lethality

A situation whereby the effects of two individually innocuous genetic mutations combine to result in cell death is termed ‘synthetic lethality’. In cancers associated with hereditary mutations in the *BRCA1* or *BRCA2* genes, a form of synthetic lethality has been achieved by continuous exposure to PARP inhibitors. In this setting, monotherapy with a variety of PARP inhibitors has generated sustained tumor responses with minimal toxicity [49–51]. Synthetic lethality represents an ideal therapeutic mechanism whereby exploitation of a cancer-specific defect induces cancer cell kill whilst almost entirely sparing normal tissues. In this scenario, cancer cells accumulate DNA SSBs due to replicative and oxidative stress. These breaks would normally be repaired by the BER pathway but in the presence of PARP inhibition they accumulate and, in replicating cells, generate DSBs via collapsed replication forks. These lesions would usually be repaired by HRR (as is the case in the normal tissues of the patient) but cancer cells bearing a homozygous loss of function of *BRCA1* or *2* are deficient in this repair pathway. *BRCA* mutated cancer cells are thus unable to repair DSBs

arising in S phase and are extremely sensitive to PARP inhibition.

BRCA mutations are infrequent in GBM so an exact replication of this scenario is unlikely. However, some GBM tumor specimens have HRR pathway mutations which may produce a synthetic lethal effect. *PTEN* mutations have been observed in up to one-third of GBMs, and this genetic defect has been associated with HRR deficiency in some experimental systems [52]. To date, however, there is no convincing evidence that *PTEN*-deficient GBMs are sensitive to single-agent treatment with PARP inhibitors.

A related phenomenon, which has been termed ‘synthetic sickness’, involves defects in DNA repair rendering tumor cells more sensitive to the combined effects of radiation and PARP inhibition. *In vitro* studies have shown that PARP inhibitors increase the radiosensitivity of cells with deficiencies in the BER, HRR and NHEJ pathways to a greater extent than cells in which these pathways are intact. The underlying mechanisms vary according to the precise nature of the repair defect, but in general it appears that the combination of PARP inhibition and radiation-induced DNA damage renders cells particularly vulnerable to DNA repair defects that might otherwise be compensated for by alternative pathways [53,54]. Although it is well established that defects in DNA damage signaling and repair are a fundamental component of the malignant phenotype, it is not yet clear whether these defects will be sufficient to enhance the radiosensitizing effects of PARP inhibitors in the clinic.

PARP inhibitors & chemotherapy

Concurrent treatment with radiotherapy and temozolomide is now standard of care for GBM. While a proportion of patients derive significant benefit from the addition of temozolomide to standard radiotherapy, the majority of tumors appear to be resistant to the drug and these patients have a dismal prognosis. Temozolomide is a DNA-methylating agent that reacts with DNA bases to generate a variety of potentially cytotoxic lesions. The major products are *O*⁶-methylguanine (5%), *N*³-methyladenine (9%) and *N*⁷-methylguanine (70%). *O*⁶-methylguanine is the most important lesion, since it causes inappropriate base pairing with thymine during DNA replication. This triggers repeated, unsuccessful attempts at repair by the mismatch repair pathway that eventually result in the formation of DSBs.

Methylguanine DNA methyltransferase (MGMT) is responsible for reversal of O^6 -methylguanine lesions, and high levels of this enzyme correlate with resistance to temozolomide both *in vitro* and *in vivo*. Approximately 60% of GBMs exhibit lack of methylation of the *MGMT* gene promoter; this genetic marker is associated with high tumor levels of MGMT and appears to confer a poor overall prognosis as well as predicting lack of benefit from temozolomide treatment [55]. Cells that are defective in mismatch repair are also insensitive to temozolomide, but this resistance mechanism is uncommon in GBM [56] (although mismatch repair defects have been observed in recurrent tumors that were previously treated with temozolomide [57]). The other DNA lesions induced by temozolomide, N^3 -methyladenine and N^7 -methylguanine, are efficiently reversed via the BER pathway and under normal conditions do not contribute to temozolomide cytotoxicity.

However, in the presence of a PARP inhibitor, resolution of these lesions is impaired and they become cytotoxic.

In the context of MGMT-mediated resistance, therefore, the addition of a PARP inhibitor might increase tumor sensitivity to temozolomide by facilitating alternative cytotoxic mechanisms. In fact, there is relatively little experimental or clinical data to support this specific hypothesis. By contrast, there is a wealth of cellular and preclinical data showing that PARP inhibitors increase temozolomide sensitivity in general and overcome mismatch repair-mediated resistance in particular [58]. The ability of PARP inhibitors to overcome temozolomide resistance in patients with a range of solid tumor types was investigated in a Phase I trial that showed some evidence of potentiation of temozolomide activity by the intravenous compound AGO14699 (rucaparib) [59]. A subsequent Phase II study of this agent in metastatic melanoma supported the concept

Figure 4. Putative mechanism of action of PARP inhibitors in potentiating DNA damage produced by temozolomide.
MGMT: Methylguanine DNA methyltransferase.

that PARP inhibitors enhance temozolomide sensitivity, but this potentiation was accompanied by an exacerbation of hematological toxicity that necessitated temozolomide dose reductions and offset any clinical advantage. It is possible that alternative, less myelotoxic temozolomide schedules might be safely combined with PARP inhibitors and ongoing clinical trials are testing this hypothesis in GBM patients. The putative actions of PARP inhibitors when combined with temozolomide are summarized in [Figure 4](#).

PARP inhibition & the glioma stem cell theory

Evidence is accumulating for the existence of a subpopulation of tumor cells in GBM known as ‘tumor stem-like cells’ or ‘glioma stem cells’ [60]. These are malignant cells that possess some of the properties of neural stem cells and are thought to be responsible for initiation, maintenance and propagation of GBM. There is some evidence that these cells are also resistant to radiotherapy and chemotherapy and may be responsible for treatment failure and subsequent tumor recurrence. The mechanism of radioresistance has been attributed to upregulation of DDR pathways, such that glioma stem cells are able to repair DNA damage more effectively than

other tumor cells [61]. If this is the case, targeting the DDR of glioma stem cells may provide a means of overcoming resistance and reducing recurrence rates. It is not yet known whether inhibition of PARP will sensitize glioma stem cells to DNA-damaging agents, but preclinical investigation of this possibility is warranted.

PARP inhibitors in clinical trials

Fong *et al.* investigated the safety and toxicity profile of single agent AZD2281 (now named olaparib) in a landmark study of 60 patients with solid tumors. This study identified tumor responses in nine patients, all of whom were either known *BRCA1* or *2* mutation carriers or had highly suggestive family histories. This important trial supported the BRCA/PARP synthetic lethality model and confirmed the expectation that single-agent PARP inhibitors would be well tolerated [49].

Since then there have been several trials combining PARP inhibitors with cytotoxic chemotherapy agents. A Phase II trial in triple-negative breast cancer showed improved progression-free survival when the putative PARP inhibitor BSI201 (iniparib) was combined with carboplatin and gemcitabine with minimal additional toxicity [62]. However, a Phase III trial

Figure 5. Potential applications of PARP inhibitors in CNS oncology.
PARPi: PARP inhibitor.

of the same combination was ended when the BSI201 arm failed to reach primary end points. It is now thought that BSI201 does not inhibit PARP at the doses achieved in patients in the trial. Samol and colleagues investigated the combination of olaparib and topotecan in a Phase I trial in patients with advanced solid tumors and observed severe myelosuppression [63]. Combined with the AG014699 plus temozolomide data described above, these findings indicate a lack of tumor specificity when combining systemic chemotherapy with systemic PARP inhibition.

Despite these early setbacks, a number of Phase I trials of PARP inhibitors in combination with systemic agents are in development. It is hoped that revised dosage schedules will ameliorate dose limiting hematological toxicity. Of interest are several trials exploring combinations of targeted agents, such as gefitinib (an EGFR tyrosine kinase inhibitor) with PARP inhibition. These combinations would not be expected to cause hematological toxicity. In parallel with these novel combination strategies, ongoing clinical trials are attempting to optimize and extend the role of PARP inhibitor monotherapy in patients with solid tumors that are defective in DDR pathways such as homologous recombination. In particular there is growing interest in the potential role of single agent PARP inhibitors as maintenance treatment for patients who have responded to conventional cytotoxic chemotherapy [64].

Despite the encouraging preclinical data, clinical trials combining PARP inhibitors with radiotherapy have yet to commence. A number of studies are in development, in tumor sites including esophagus, lung, head and neck, and locally advanced breast cancer. Various approaches have been taken, with some studies cautiously combining escalating doses of PARP inhibitor with palliative radiation regimes and others combining inhibitors with radical chemoradiation schedules. These studies are still in the early stages of recruitment so published data are not available. However, up-to-date information can be obtained on the NIH clinical trials website [101].

PARP inhibitors in CNS oncology

There is a strong rationale for the use of PARP inhibitors in neuro-oncology (Figure 5). The radio- and chemo-sensitizing effects observed in cellular systems are supported by *in vivo* data

showing enhanced tumor growth delay in animal models of GBM when PARP inhibitors are added to radiotherapy and/or temozolomide treatment regimes [65,66].

Testing PARP inhibitors in neuro-oncological disease states poses unique difficulties. The brain lays behind the ‘blood–brain barrier (BBB)’, a physiological structure that excludes harmful agents from the CNS but also prevents potentially therapeutic drugs from entering the brain. It is important to note, however, that the BBB is grossly abnormal in many brain tumors, particularly GBM and cerebral metastases, as demonstrated by the florid contrast enhancement that is a characteristic feature of these lesions. That olaparib exhibits therapeutically relevant levels of BBB penetration is indicated by radiological responses in brain metastases of patients treated with single-agent olaparib [49]. Furthermore, radiation treatment of the brain causes an increase in BBB permeability that is particularly marked in tumors, so concomitant treatment with radiotherapy is likely to enhance tumor drug levels [67].

The OPARATIC study will investigate penetration of GBM by olaparib as its primary end point. This Phase I trial of olaparib in combination with temozolomide for recurrent GBM is being conducted in two stages. In stage 1, patients undergoing surgical resection of recurrent GBM will be dosed with olaparib for 3 days prior to their operation. Tissue removed at surgery will then be analyzed for drug levels. In stage 2, olaparib will be combined with daily low-dose temozolomide and a dose-escalation strategy will determine the maximum tolerated doses of these agents in combination. The dosing schedule of temozolomide has been based on the daily low-dose regimen used with concurrent radiotherapy, which is generally associated with minimal bone marrow toxicity. Furthermore the biologically effective dose of PARP inhibitor when given concomitantly with DNA-damaging agents may be much lower than when the agent is used as monotherapy. Hence dose escalation of olaparib in the OPARATIC study will begin at lower doses than have been used in previous studies.

If olaparib is detected in GBM tissue, subsequent trials will investigate the safety and efficacy of combining it with radiotherapy and chemo-radiation. Various populations of patients might benefit from this approach: patients undergoing radical chemoradiation whose tumors have either methylated or unmethylated

MGMT genotype, with the benefit of increased sensitivity to both radiation and temozolomide; and elderly patients who are currently treated with short-course radiotherapy alone.

With regards to other compounds, a Phase I trial of ABT-888 (veliparib) in combination with concurrent radiotherapy and temozolomide in the treatment of pediatric diffuse pontine glioma is under development, and the combination of veliparib and temozolomide in recurrent adult GBM multiforme is also being tested in a Phase I study.

In pediatric neuro-oncology the PARP inhibitor AG104699 (rucaparib) has been shown to have CNS penetration and to enhance temozolomide activity in *in vivo* models of childhood medulloblastoma [68]. Furthermore it has been demonstrated that the effects of topotecan and temozolomide are enhanced by AG104699, with the authors concluding that there is a strong rationale for investigation of PARP inhibitors in pediatric early clinical studies [69].

Patients with brain metastases represent a large and increasing proportion of patients with cancer, and their therapeutic options are limited. Investigating PARP inhibitors in the treatment of brain metastases would allow the radiosensitizing effects of PARP inhibitors to be explored without the complications of concurrent chemotherapy. Also, interpretation of toxicity data might be more straightforward in the setting of whole-brain radiotherapy, where the volume of irradiated normal tissue is more consistent than in the primary brain tumor setting [70]. A Phase I study of veliparib in brain metastases is currently recruiting patients, with the primary aim of determining the maximum tolerated dose of veliparib when delivered in combination with whole-brain radiotherapy at a dose of 37.5 Gy in 15 daily fractions.

Biomarkers

It is highly unlikely that all patients will benefit from the addition of a PARP inhibitor to their treatment regimen, so it is important to develop effective biomarkers in order to identify those patients who are most likely to benefit. However, the multiple pathways involved in DDR and the extensive overlap and/or redundancy between the different pathways make this a difficult task. It is likely that successful biomarker strategies will rely upon identifying the integrity or otherwise of entire DDR pathways, rather than examining one particular gene or protein. The use of

DNA microarray technology may facilitate this approach.

Nevertheless, it appears that markers for potential sensitivity to PARP inhibitors can be identified. According to the synthetic lethality paradigm, defects in HRR predict sensitivity to PARP inhibition. Mutations in *BRCA1* or *BRCA2* are the most obvious examples of this situation, but such mutations are rare in brain tumors. Other defects in HRR might yield sensitivity to PARP inhibition, and mutations in *PTEN* have been associated with homologous recombination (HR) deficiency [52]. Even in HR-deficient tumors, however, resistance to PARP inhibition can occur [49], either by acquisition of secondary mutations restoring HR function, upregulation of other DDR pathways, expression of drug efflux pumps or by other, as yet unknown, mechanisms.

Defective NHEJ may also enhance radiosensitization by PARP inhibitors, as it may be accompanied by upregulation of MMEJ, which is a PARP-dependent process [27]. Examination of the functionality of NHEJ by measuring individual effectors such as DNA-PKcs, Ku70/80 and DNA ligase IV may therefore be informative, but again the integrity of the overall pathway is probably the key factor.

With this in mind, it may be of value to assess more general markers of DNA damage and repair. A simplistic viewpoint is that defective DDR will result in increased levels of unrepaired DNA damage in tumor cells. This can be detected by immunocytochemical staining for γ -H2AX and/or 53BP1, both of which are established markers of dsDNA damage, or for markers of base damage, such as 8-oxoguanine.

In terms of monitoring biological activity of PARP inhibitors in tumor tissue, there are a number of established assays that detect PAR, the product of PARP activity. Because baseline levels of PARP activity vary enormously, and because evidence of PARP inhibition is provided by a reduction in PARP activity, tissue specimens are required before and after (or during) treatment. When replicating cells are exposed to PARP inhibitors an acute increase in γ -H2AX staining is frequently observed, but pre- and post-treatment tissue samples would be required for a reliable demonstration of PARP inhibition. Multiple tumor sampling is not usually possible in patients with brain tumors so surrogate markers are urgently required. In most cases, peripheral blood is used for practical reasons,

but this does not address the important issue of CNS tumor bioavailability. Further work in this important area is required if PARP inhibitors are to become part of routine clinical care.

Clinical trial design for PARP inhibitor radiosensitization

Designing appropriate trials for small-molecule radiosensitizers poses difficulties unique from those usually encountered in Phase I trials. The interruption of a radiotherapy schedule due to dose-limiting toxicity is likely to be detrimental to tumor control and patient outcome, so ethical and safety issues must be addressed in the study design. Both acute and late toxicity are often dependent on the volume of tumor irradiated, so for GBM in particular there will be significant heterogeneity in any analysis of toxicity. Potential late effects of cranial radiotherapy on cognitive function are of particular concern, whereas conventional Phase I studies are primarily concerned with assessment of acute toxicity. However, waiting years between dose escalation cohorts to look for late toxicity would not be feasible [70].

One solution to these problems would be to study potential radiosensitizers, such as PARP inhibitors, in patients who are undergoing palliative radiotherapy. In these patients the total radiation dose is well below the maximum tolerated level so the likelihood of severe toxicity is low, the consequences of interrupting therapy due to toxicity are less severe and the probability of patients surviving long enough to experience late toxicity is small. Other investigators have argued that such studies in the palliative setting will not usefully predict safety and toxicity in the radical setting, and have advocated a more ambitious approach introducing novel agents into first-line, radical treatment regimens. Until the studies have been conducted it will not be possible to judge which of these strategies is the most appropriate.

Conclusion

PARP inhibitors are novel agents that have promising anti-tumor effects. These have been described most strikingly in *BRCA1*- and 2-deficient cancers, where single agent PARP inhibitors have been shown to produce sustained tumor responses with minimal toxicity utilizing a synthetic lethal effect. However, PARP inhibitors are also known to be potentiators of chemotherapy and radiotherapy via their effects on DDR.

In CNS oncology GBM is the most obvious target for initial application of a PARP inhibitor strategy. Targeting radio- and chemo-resistant tumors with a DDR-inhibiting strategy, such as PARP inhibition, may lead to useful clinical benefit for patients. There are inherent challenges when incorporating PARP inhibitors into radical chemoradiotherapy protocols and it is important to design appropriate clinical trials with associated biomarker studies in order to fully realize the clinical potential of these exciting novel therapies.

Future perspective

In the near future, the results of the first Phase I trials of PARP inhibitors in combination with radiotherapy in a variety of tumor sites will become available. If toxicity is acceptable, Phase II and III trials will be conducted to evaluate benefits from PARP inhibitors and radiotherapy.

Early attempts to combine PARP inhibitors with cytotoxic chemotherapy drugs have been limited by bone marrow toxicity. Modification of dosage and scheduling may make combination therapy with PARP inhibitors achievable in some tumor sites.

Combinations of PARP inhibitors with other classes of targeted agents (e.g., tyrosine kinase inhibitors) are likely to be tolerated, but it is not yet clear which, if any, tumor types will benefit.

An important goal of current research is developing clinically applicable assays that can identify which patients will benefit from treatment with PARP inhibitors. Relevant parameters are likely to include proliferation rates and markers of DNA repair integrity. In the neuro-oncology setting, such assays would ideally be noninvasive.

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