Review

The Promise of Poly(ADP-Ribose) Polymerase (PARP) Inhibitors in Gliomas

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Abstract

Diffuse infiltrating gliomas are a clinically and molecularly heterogeneous group of tumors that are uniformly incurable. Despite our growing knowledge of genomic and epigenomic alterations in gliomas, standard treatments have not changed in the past 2 decades and remain limited to surgical resection, ionizing radiation, and alkylating chemotherapeutic agents. Development of novel therapeutics for diffuse gliomas has been challenging due to inter- and intra-tumoral heterogeneity, diffuse infiltrative nature of gliomas, inadequate tumor/drug concentration due to blood–brain barrier, and an immunosuppressive tumor microenvironment. Given the high frequency of DNA damage pathway alterations in gliomas, researchers have focused their efforts in targeting the DNA damage pathways for the treatment of gliomas. A growing body of data has shed light on the role of poly(ADP-ribose) polymerase (PARP) in combination with radiation and temozolomide in high-grade gliomas. Furthermore, a novel therapeutic strategy in low-grade glioma is the recent elucidation for a potential role of PARP inhibitors in gliomas with *IDH1/2* mutations. This review highlights the concepts behind targeting PARP in gliomas with a focus on putative predictive biomarkers of response. We further discuss the challenges involved in the successful development of PARP inhibitors in gliomas, including the intracranial location of the tumor and overlapping toxicities with current standards of care, and promising strategies to overcome these hurdles.

Keywords: glioma, PARP inhibitors, radiation, temozolomide, MGMT methylation

INTRODUCTION

Diffuse infiltrating gliomas are histologically and molecularly diverse malignancies that account for 25% to 30% of primary brain tumors.[1] The 2016 World Health Organization (WHO) classification system classifies diffuse infiltrating gliomas based on histologic grade, isocitrate dehydrogenase (*IDH*) mutation, and 1p19q co-

deletion status.[2] Low-grade diffuse infiltrating gliomas (WHO grade II) compromise tumors with low proliferation index and commonly harboring *IDH1/2* mutations. These include grade II *IDH* mutant or *IDH* wild-type diffuse astrocytoma and grade II *IDH* mutant and 1p19q-codeleted oligodendroglioma. High-grade gliomas consist of WHO grade III and IV tumors. Grade III or anaplastic diffuse infiltrating gliomas include *IDH* wild-type and *IDH* mutant anaplastic astrocytoma and *IDH* mutant, 1p19q-codeleted anaplastic oligodendroglioma. Grade III glioma are defined as tumors with focal or dispersed anaplasia and significant proliferative activity. Grade IV astrocytoma or glioblastoma is an infiltrating astrocytic tumor featuring nuclear atypica, cellular pleomorphism, mitotic activity, and microvascular proliferation and/or necrosis with or without *IDH1/2* mutations.[2] Recent advances in molecular pathogenesis of gliomas have led to efforts to integrate updated molecular information into clinical practice between WHO updates. The Consortium to Inform Molecular and Practical Approaches to CNS Tumor Taxonomy (cIMPACT-NOW) has addressed the current classification and grading questions and their recommendations are expected to be included in the upcoming WHO 2020 classification system.[3] Of relevance to this review is the recommendation for new classification of diffuse infiltrating gliomas, *IDH* wild-type with *EGFR* amplification, combined +7/−10 (gain of chromosome 7 or losses of chromosome 10) or *TERT* promoter mutation as diffuse astrocytic glioma, *IDH* wild-type, with molecular features of glioblastoma, WHO grade IV based on the observation that patients with WHO grade II or III tumors with these alterations have significantly shorter survival compared with patients with other WHO grade II or III tumors.[4] In addition, glioblastoma, *IDH* mutant, WHO grade IV are recommended to be classified as astrocytoma, *IDH* mutant, WHO grade 4 and the term "glioblastoma" will be reserved for *IDH* wild-type tumors, which are clinically and genetically distinct from *IDH* mutant tumors.[5] The use of Arabic instead of Roman numerals is recommended to decrease the possibility of typographic errors.[3] Furthermore, *CDKN2AB* homozygous deletion was found to be associated with worse outcome across *IDH* mutant astrocytoma[6] and was recommended as a criterion for WHO grade 4 classification for *IDH* mutant astrocytoma.[5] The WHO 2020 classification system of brain tumors integrating cIMPACT-NOW recommendations will allow for more unified patient populations with similar prognostic molecular characteristics to be enrolled into clinical trials.

Glioblastoma remains the most malignant primary brain tumor with dismal prognosis. The majority of de novo or primary glioblastomas are *IDH1/2* wild-type. Secondary glioblastoma, or *IDH1/2* mutant glioblastoma, accounts for 5% to 10% of all glioblastoma and arise from low-grade gliomas. Current standard-of-care treatment for high-risk low-grade gliomas and high-grade gliomas include surgery, radiation (RT) and alkylating agents, such as temozolomide (TMZ), or combination chemotherapy, such as procarbazine, lomustine, and vincristine. Despite multimodality treatments, all diffuse glioma grades are incurable and recur invariably leading to neurological disability and death. There is therefore an unmet need to explore novel therapeutic approaches in gliomas.

Diffuse infiltrating gliomas harbor a range of oncogenic mutations associated with resistance to both chemotherapy and RT. DNA repair pathways are among the most important key players of these genetic alterations. The four major DNA repair alterations in glioblastoma include downregulation of p53 and retinoblastoma signaling pathways in approximately 70% of all glioblastoma, and alteration of *PTEN* and upregulation of EGFR/PI3K and methylation of O^6-methylguanine-DNA methyltransferase (*MGMT*) promoter in approximately 30% of all glioblastoma.[7] The high frequency of alterations in DNA repair pathways in glioblastoma suggests that targeting these pathways may provide therapeutic benefit.

Ionizing RT, the cornerstone of treatment in glioblastoma, generates DNA single-strand breaks and double-strand breaks (DSBs).[8] DNA single-strand breaks are repaired through the base excision repair pathway through poly(ADP-ribose) polymerase (PARP)[9] and DNA DSBs are repaired through DNA damage response (DDR) kinases, DNA-dependent protein kinase (DNA-PK), ataxia telangiectasia mutated (ATM), and ataxia telangiectasia and Rad3 related (ATR).[10] The most commonly used chemotherapy for malignant astrocytoma is TMZ; TMZ is an alkylating agent that induces cell death by methylating guanine at the O^6 position. The major DNA adduct induced by TMZ is O6-methylguanine (O(6)-MeG). MGMT protein removes the alkyl group from the O^6 position and reverses the damage caused by TMZ. O(6)MeG, if unrepaired, causes O(6)MeG:T mismatches that are detected and processed by mismatch repair enzymes, which in turn signal to activate the DDR enzymes.[11,12] *MGMT* promoter methylated glioblastoma, with lack of MGMT expression, is more susceptible to DNA damage caused by TMZ than *MGMT* promoter unmethylated glioblastoma and confers improved survival.[13] There is strong rationale to leverage the mechanism of action of PARP and DDR inhibitors to augment the antitumor activity of RT and TMZ in gliomas as both these interventions kill tumor cells by inducing DNA damage. Here, we review our current knowledge of preclinical and clinical studies of PARP inhibitors in glioma, discuss challenges involved for their successful clinical implementation, and recommend strategies to overcome these challenges.

TARGETING POLY(ADP-RIBOSE) POLYMERASE (PARP)

PARP1 and PARP2 are enzymes that sense DNA damage and transduce signals by synthesizing negatively-

charged, branched poly(ADP-ribose) (PAR) chains (PARylation) on target proteins.[14] PARP1 binds single-strand breaks, which results in allosteric changes in PARP1 structure, activation of its catalytic function (PARylation), and recruitment of DNA repair proteins to the site of DNA damage.[15] AutoPARylation leads to release of PARP1 from the repaired DNA.[16] PARP inhibitors function by blocking PARP catalytic activity by preventing binding of PARP enzyme cofactor (β nicotinamide adenine dinucleotide) to PARP1 and PARP2. They also have varying ability to prevent release of PARP from DNA, which is referred to as "trapping," a phenomena that correlates directly with the inhibitors' cytotoxic potency.[17] There are currently six PARP inhibitors that are approved by the United States Food and Drug Administration (FDA) or in late stages of clinical development, including veliparib, olaparib, rucaparib, niraparib, pamiparib, and talazoparib.[18,19] These inhibitors demonstrate many similarities and yet have critical structural differences that lead to varying PARP trapping, antitumor activity, and tolerability.[20] Preclinically, talazoparib is the most potent PARP1 trapping PARP inhibitor, while veliparib is believed to be the least.

PARP inhibitors are the first class of drugs approved that exploit synthetic lethality in cancers with germline DNA repair deficiency. The antitumor effect of PARP inhibitors was first discovered in cancers with homologous recombination deficiency (HRD), such as those arising in carriers of *BRCA1/2* mutations.[21] BRCA1/2 are tumor suppressor proteins that repair DSBs via homologous recombination (HR) repair. Cancer cells with deleterious *BRCA1/2* mutations, such as breast cancer and ovarian cancer, rely on PARP function for effective DNA repair.[22] Therefore, PARP inhibitors induce tumor cell death and synthetic lethality in the absence of effective HR repair function. Several PARP inhibitors have been FDA-approved for ovarian cancer (olaparib, rucaparib, niraparib) and metastatic breast cancer (olaparib and talazoparib) with *BRCA* mutations and numerous clinical trials of PARP inhibitor combinations with cytotoxic chemotherapy, DDR inhibitors, and immunotherapy are ongoing.[19,23–25] HRD is not confined to *BRCA* mutant cancers and mutations in a variety of genes involved in HR repair leads to a HRD phenotype including, but not limited to, *ATM*, *PALB2*, *CHEK2*, *BAP1*, as germline or somatic mutations.[26]

PARP INHIBITORS FOR LOW-GRADE GLIOMAS

Aside from genes directly involved in DNA repair, genomic alterations leading to epigenetic changes can also result in an HRD phenotype. The best example of such genomic alterations in gliomas is *IDH* mutation. Mutations in *IDH1* have been found in approximately 70% of grade II and III gliomas and secondary glioblastoma.[27] Activating mutations of *IDH1/2* result in production of 2-hydroxyglutarate (2HG), which leads to histone and genome-wide hypermethylation termed the "CpG Island Methylator Phenotype," or "G-CIMP."[28,29] This genome-wide hypermethylation results in altered cellular metabolism, promotion of tumorigenesis via effect on chromatin structure, and blockade of cancer cell differentiation.[30] Selective mutant IDH1 and pan-IDH inhibitors have been shown to engage their target in the brain and shrink gliomas. Several IDH inhibitors are currently in early stage clinical trials for treatment of *IDH* mutant gliomas.[31]

A growing body of literature suggests that an alternative approach exploiting vulnerabilities imposed by *IDH* mutation in the cell maybe more effective. In a landmark article, Sulkowski et al[32] demonstrated that mutant *IDH* cell lines and patient-derived glioma cells are deficient in DNA DSB break repair and that *IDH1/2* mutation-dependent HRD confers synthetic lethality with PARP inhibition. They demonstrated that PARP sensitivity in *IDH1/2* mutant cells was reversed by addition of IDH inhibitors and that treatment with 2HG enantiomers in cells with intact IDH1/2 proteins conferred PARP sensitivity demonstrating the effect of 2HG in inducing synthetic lethality with PARP inhibition. In addition, they showed that olaparib selectively inhibits the growth of IDH1 R132H mutant tumor xenografts. Independent laboratories have since confirmed these findings.[33,34] Lu et al[35] demonstrated that targeting PARP DNA repair mechanisms remarkably potentiated the cytotoxic effects of temozolomide in *IDH* mutant glioma cells. *IDH1/2* mutations-induced "BRCAness" provided the basis for clinical investigation of PARP inhibitors in *IDH1/2* mutant solid tumors, including gliomas (NCT03212274).

PARP INHIBITORS FOR HIGH-GRADE GLIOMAS

High-grade gliomas have a high prevalence of genetic alterations affecting DNA repair pathways, therefore making PARP inhibition an attractive therapeutic intervention in these aggressive brain tumors.

MGMT promoter methylation is present in 30% of patients with glioblastoma and tumors with *MGMT* promoter methylation are more susceptible to DNA damage caused by TMZ.[13,36] O(6)MeG, the primary cytotoxic DNA adduct induced by TMZ, is removed by MGMT in tumors with MGMT expression (*MGMT* promoter unmethylated glioblastoma). Other TMZ adducts, N7-methylguanine and N3-methyladenine, are repaired through base excision repair.[37] PARP1 is a prominent enzyme in the base excision repair component and is highly expressed in glioblastoma in comparison with normal brain tissue.[38]

Early on, the synergy between TMZ and PARP inhibitors cytotoxicity was demonstrated in leukemic cells.[39,40] This was later confirmed in glioma cells, most pronounced in cells resistant to TMZ due to high MGMT levels or mismatch repair deficiency.[41] In addition, Glioma Stem Cells (GSCs), responsible for recurrence and

resistance to radiation and chemotherapy in glioblastoma, demonstrate upregulation of DNA response targets, including PARP1, ATM, ATR, CHK1.[42] PARP inhibitors and TMZ combinations exert synergistic antitumor effects in GSC lines.[43]

Perhaps the most studied PARP inhibitor in preclinical glioblastoma models is ABT-888 or veliparib and *MGMT* promoter methylation status has been delineated as a potential biomarker of veliparib-induced sensitization.[44,45] Barazzuol et al[45] demonstrated veliparib combined with RT yielded enhanced antitumor activity in 4 glioblastoma cell lines, which was augmented with addition of TMZ. Veliparib antitumor effects were enhanced in *MGMT* promoter methylated glioblastoma cell lines as compared with unmethylated lines.[45] Gupta et al[44] evaluated in vivo efficacy of veliparib combined with TMZ in a large panel of glioblastoma patient-derived xenografts and demonstrated improved survival of *MGMT* promoter hypermethylated lines, an effect that was lost when MGMT was overexpressed in these lines. This improvement in survival was associated with increased phosphorylation of damage-response proteins only in *MGMT* promoter hypermethylated lines. On the contrary, Erice et al[46] showed that melanoma and glioblastoma MGMT-positive cells responded strongly to the combination of PARP inhibitors and TMZ, whereas MGMT deficient cells did not. A novel mechanism of MGMT activity by PARP has been reported, which provides a possible explanation for the discordance between preclinical studies regarding the role of MGMT in TMZ sensitization by PARP inhibition. Wu et al[47] found that PARP physically interacts with MGMT and PARylation of MGMT by PARP is required for MGMT binding to DNA and to remove O(6)-MeG adducts in damaged DNA induced by TMZ. Of note, they showed 4 PARP inhibitors with varying PARP trapping activity (talazoparib, pamiparib, veliparib, olaparib) inhibited PARP-MGMT binding. They demonstrated that PARP inhibitors augmented TMZ toxicity in *MGMT* methylated and unmethylated GSCs, but more profoundly in unmethylated tumors both in vitro and in vivo. The discordance in the role of *MGMT* promoter methylation in PARP mediated sensitivity in the studies from Gupta et al[44] and Wu et al[47] may be due to different models used. The former group used orthotopic glioblastoma patient-derived xenografts models and Wu et al[47] used GSC murine models. Utilization of different preclinical models to discover biomarkers of response poses challenges in successful translation of this information from the laboratory to clinical trials. We advocate for inclusion of both *MGMT* promoter methylated and unmethylated glioblastoma patients in clinical trials of PARP inhibitors and to stratify patients based on *MGMT* status to enhance our understanding of the role of *MGMT* promoter status in PARP induced TMZ sensitization.

Another important factor to consider for clinical trial design is timing of combination of PARP with TMZ, in TMZ naïve or TMZ resistance glioblastoma. Clarke et al[48] demonstrated that veliparib increased the survival benefit of TMZ in TMZ-sensitive cell lines, however, after in vivo selection for TMZ resistance, this benefit was lost demonstrating that veliparib sensitization was limited to lines without prior exposure to TMZ. These data justified clinical development of veliparib in newly diagnosed glioblastoma. Consistent with this preclinical data, efficacy of veliparib in combination with TMZ was not impressive in recurrent TMZ refractory glioblastoma patients[49] and is now being explored in the newly diagnosed setting (Table 1).

Combination of veliparib with RT/TMZ in newly diagnosed glioblastoma in a phase I trial showed poor tolerability due to hematologic toxicities.[50] The benefit of TMZ in *MGMT* promoter unmethylated patients is questionable and TMZ can be eliminated from standard of care of these patients and replaced with investigational agents. Given the concern with added toxicity of veliparib and RT/TMZ, a phase II trial of veliparib in newly diagnosed *MGMT* promoter unmethylated glioblastoma was conducted in which TMZ was eliminated from the concurrent phase of therapy and replaced by veliparib. One hundred twenty-five patients were randomized between RT plus veliparib followed by TMZ plus veliparib in the adjuvant phase (treatment arm) and glioblastoma standard of care (RT plus TMZ followed by TMZ) (control arm).[51] The primary endpoint was PFS at 6 month and it was 53% (41–63) in the treatment arm and 37% (22–52) in the control arm; the prespecified primary endpoint was not met. The median PFS was 6.2 months (95% CI 4.9–7.1) for the treatment arm and 4.4 months (95% CI 4.0–6.0) for the control arm (HR = 0.81, 95% CI 0.54–1.21). Of all patients, 53% and 50% experienced grade 3 or greater adverse events (in the treatment arm and control arm, respectively), with the most common being thrombocytopenia (13% in treatment arm and 5% in control arm). Veliparib and TMZ combination is now being tested in a phase II/III clinical trial in patients with newly diagnosed *MGMT* promoter methylated glioblastoma in the adjuvant phase after concurrent RT/TMZ (NCT02152982).

New generation PARP inhibitors, olaparib and pamiparib, which cross the blood–brain barrier and have significant PARP trapping, may be more efficacious than valiparib.[52,53] Several phase I clinical trials of olaparib in combination with TMZ and RT in glioblastoma have been conducted or are ongoing (Table 1). The OPARATIC (Olaparib and Temozolomide in Treating Patients with Relapsed Glioblastoma) trial was a phase I study of olaparib in combination with TMZ in patients with recurrent glioblastoma, which showed the combination is well tolerated. Pharmacokinetics studies showed adequate tumor penetration of the drug, with tumor: plasma ratios ranging from 0.01 to 0.9 (mean 0.25).[54] PARADIGM (OlaPArib And RADiotherapy In newly diagnosed GlioblastoMa) trial was a phase I study of olaparib and RT in glioblastoma, which recommended a

Table 1.—Clinical trials of PARP inhibitors in gliomas

Investigational Agent	Combination Treatments	Disease Setting	Phase	Biomarker	Outcome or Status (if Results Pending)	ClinicalTrial.gov Identifier/ Reference
Olaparib						
OPARATIC	TMZ	Rec glioblastoma	I	None	MTD: Olaparib 150 mg (OD) days 1–3 weekly plus TMZ 75 mg/m^2 daily Olaparib penetrated the tumor	NCT01390571, [54]
PARADIGM-1	Hypo-RT	ND GBM—elderly	I	None	RP2D: Olaparib 200mg BID plus hypofractionated RT (40 Gy radiation in 15 fractions)	[55]
PARADIGM-2	RT or RT/TMZ	ND glioblastoma	I	*MGMT*	Ongoing Parallel 1: Olaparib/RT/TMZ → Olaparib → TMZ (MGMT-m) Parallel 2: Olaparib/RT → Olaparib (MGMT-un)	[56]
OLA-TMZ-RTE-01	RT/TMZ	Unresectable HGG	I	None	Ongoing Olaparib/RT/TMZ → Olaparib → Olaparib/TMZ	NCT03212742
Olaparib	none	Rec glioma	II	*IDH1/2* mutations	Ongoing	NCT03212274
Olaparib	Cediranib Maeate	Rec glioblastoma	II	None	Ongoing Arm A: Olaparib and Cediranib Maleate Arm B: bevacizumab	NCT02974621
Veliparib	RT and TMZ	ND glioblastoma	I	None	Veliparib in combination with concurrent RT/TMZ was not tolerable	NCT00770471, [50]
Veliparib	TMZ	Rec glioblastoma	I/II	None	MTD: Veliparib 40 mg BID + TMZ 75 mg/m^2 21/28 d PFS 6: BEV refractory: 4.4% BEV naive: 17%	[49]
Veliparib	RT and TMZ	ND glioblastoma	II	*MGMT*-unmethylated	PFS 6: Arm A: RT/TMZ → TMZ: 37% (22–52) Arm B: RT/Veliparib → TMZ + Veliparib: 53% (41–63) * did not meet the prespecified primary endpoint	[51]
Veliparib	RT and TMZ	ND glioblastoma	II/III	*MGMT*-methylated	Ongoing Arm I: RT/TMZ → TMZ + Veliparib Arm II: RT/TMZ → TMZ + placebo	NCT02152982
Niraparib (MK-4827)	TMZ	Advanced cancers to include gliomas	I	None	Ongoing	NCT01294735
Pamiparib	TMZ	Rec glioma	I/II	*IDH1/2* Mutations	Ongoing	NCT03914742
Pamiparib	RT and/or TMZ	ND and Rec glioblastoma	I/IIb	*MGMT*	Ongoing Arm A: Pamiparib/RT (ND MGMT-un) Arm B: Pamiparib/RT/TMZ (ND MGMT-un) Arm C: Pamiparib/TMZ (Rec any MGMT)	NCT03150862

BEV, bevacizumab; Hypo-RT, hypofractionated radiation; MGMT, O6-methylguanine DNA methyltransferase; MGMT-m, methylated MGMT; MGMT-un, unmethylated MGMT; MTD, maximum tolerated dose; ND: newly diagnosed; OD, once daily; PFS, progression free survival; Rec, recurrent; RP2D, recommended phase 2 dose; RT, radiation; TMZ, temozolomide

phase 2 dose (RP2D) of olaparib plus 40 Gy of radiation in the elderly.[55] PARADIGM-2 is a phase I multicenter, open-label, nonrandomized, dose-escalation clinical trial of olaparib in combination with RT, with or without TMZ, in patients with newly diagnosed *MGMT* promoter methylated (Parallel 1) and unmethylated status (Parallel 2).[56] Another phase I/IIa study of olaparib in combination TMZ and RT in newly diag-

nosed glioblastoma is planned after determination of the RP2D in phase I trials of olaparib in combination with RT and TMZ in the concurrent phase and then in combination with TMZ in the adjuvant phase.[57] Pamiparib has also been tested in phase I/II studies in combination with RT and/or TMZ in patients with newly diagnosed and recurrent glioblastoma and is generally well tolerated in combination with lower doses of TMZ than standard of care[58,59] (Table 1).

Discovering precise biomarkers of response is crucial for successful clinical development of PARP inhibitors in glioblastoma. PARP inhibitors are more effective in patients with HR-deficient tumors, such as those with mutation in *BRCA1/2*, *ATM*, *PALB2*, *CHEK2*, and *BAP1*[60]; however, these mutations are rare in glioblastoma and may not confer the same magnitude of benefit seen in other cancers. Wu et al[47] recently identified *EGFR* amplification (present in ~30% of glioblastoma) as a potential biomarker for response to talazoparib. In their study, *EGFR*-amplified GSCs showed enhanced DNA damage, increased PARP-DNA trapping and were remarkably sensitive to talazoparib.[47] Another common DNA repair pathway modification in glioblastoma is alteration of *PTEN* and upregulation of PI3K in 34% of glioblastoma.[7] PTEN is a lipid phosphatase and its loss results in upregulation of PI3K/AKT pathway. In addition, disruption of PTEN leads to genomic instability and PTEN null cells exhibit spontaneous DNA DSBs and defects in HR repair.[61] PTEN maintains chromosomal instability via regulation of Rad51, which reduces the incidence of spontaneous DSBs. In preclinical glioma cell lines, loss of PTEN has been shown to confer sensitivity to veliparib due to synthetic lethality.[62] Veliparib also enhances TMZ efficacy in PTEN deficient glioblastoma allografts and spontaneous tumors.[63] Aside from MGMT, other potential biomarkers of response, such as EGFR, PTEN, and IDH, need to be further explored in PARP inhibitor trials in glioblastoma.

CHALLENGES AND STRATEGIES FOR SUCCESSFUL CLINICAL DEVELOPMENT OF PARP INHIBITORS IN GLIOMA

Several clinical trials of PARP inhibitors have been developed for patients with newly diagnosed and recurrent glioma (Table 1). Clinical development of these inhibitors in glioblastoma is challenging because of multiple factors. The intracranial location of the tumor, limited blood–brain barrier penetration, difficulties with repeated sampling, and overlapping hematologic toxicities of PARP inhibitors when combined with glioblastoma standard of care (RT and TMZ) are all important challenges. One strategy to successfully develop these drugs is to first confirm adequate blood–brain barrier penetration and intratumoral pharmacodynamic endpoints via window-of-opportunity studies. Strategies to minimize cumulative hematological toxicities in ongoing clinical trials and limit central nervous system neurotoxicity are critical and can be achieved through TMZ dose reductions, and careful design of dose combinations in terms of dose intervals and sequencing of PARP inhibitors in relation to TMZ and RT. In addition, parallel correlative studies need to be carefully designed to lay the foundation for future rational combinatorial trials with novel therapies, such as DDR inhibitors and immunotherapy.

CONCLUSION

Clinical benefit with PARP inhibitors in patients with cancer with HRD phenotype has generated great interest in exploiting synthetic lethality in cancer for novel therapeutic development. There is now interest in the clinical development of PARP inhibitors in gliomas with HRD phenotype (eg, *IDH1/2* mutant glioblastoma) or in combination with DNA damaging agents, such as RT and TMZ. Key molecular prognostic and predictive biomarkers in gliomas, such as *IDH* mutation and *MGMT* methylation status will likely play important roles in determining response to PARP inhibitors. It is therefore crucial that patients are stratified based on *IDH* and *MGMT* status to isolate the prognostic value of these molecular features and their impact on the response to PARP inhibitors. Novel study designs with close attention to blood–brain barrier penetration of the drugs and pharmacodynamic endpoints in resected brain tissue as well as modified dosing regimens to reduce the risk of hematologic toxicities in combination with TMZ are needed for successful development of PARP inhibitors in gliomas.

References

1. Ostrom QT, Cioffi G, Gittleman H, et al. CBTRUS statistical report: primary brain and other central nervous system tumors diagnosed in the United States in 2012–2016. *NeuroOncology.* 2019;21:v1–v100.
2. Louis DN, Perry A, Reifenberger G, et al. The 2016 World Health Organization Classification of Tumors of the Central Nervous System: a summary. *Acta Neuropathol*. 2016;131:803–820.
3. Louis DN, Wesseling P, Aldape K, et al. cIMPACT-NOW update 6: new entity and diagnostic principle recommendations of the cIMPACT-Utrecht meeting on future CNS tumor classification and grading. *Brain Pathol*. 2020;30:844–856.
4. Brat DJ, Aldape K, Colman H, et al. cIMPACT-NOW update 3: recommended diagnostic criteria for "Diffuse astrocytic glioma, IDH-wildtype, with molecular features of glioblastoma, WHO grade IV". *Acta Neuropathol*. 2018;136:805–810.
5. Brat DJ, Aldape K, Colman H, et al. cIMPACT-NOW update 5: recommended grading criteria and terminologies for IDH-mutant astrocytomas. *Acta Neuropathol*. 2020;139:603–608.
6. Shirahata M, Ono T, Stichel D, et al. Novel, improved grading system(s) for IDH-mutant astrocytic gliomas. *Acta Neuropathol*. 2018;136:153–166.

7. Brennan CW, Verhaak RG, McKenna A, et al. The somatic genomic landscape of glioblastoma. *Cell*. 2013;155:462–477.
8. Rothkamm K, Lobrich M. Evidence for a lack of DNA double-strand break repair in human cells exposed to very low x-ray doses. *Proc Natl Acad Sci U S A*. 2003;100:5057–5062.
9. Caldecott KW. Protein ADP-ribosylation and the cellular response to DNA strand breaks. *DNA Repair (Amst)*. 2014;19:108–113.
10. Finzel A, Grybowski A, Strasen J, Cristiano E, Loewer A. Hyperactivation of ATM upon DNA-PKcs inhibition modulates p53 dynamics and cell fate in response to DNA damage. *Mol Biol Cell*. 2016;27:2360–2367.
11. Yoshioka K, Yoshioka Y, Hsieh P. ATR kinase activation mediated by MutSalpha and MutLalpha in response to cytotoxic O6-methylguanine adducts. *Mol cell*. 2006;22:501–510.
12. Caporali S, Falcinelli S, Starace G, et al. DNA damage induced by temozolomide signals to both ATM and ATR: role of the mismatch repair system. *Mol Pharmacol*. 2004;66:478–491.
13. Hegi ME, Diserens AC, Gorlia T, et al. MGMT gene silencing and benefit from temozolomide in glioblastoma. *N Engl J Med*. 2005;352:997–1003.
14. Satoh MS, Lindahl T. Role of poly(ADP-ribose) formation in DNA repair. *Nature*. 1992;356:356–358.
15. Eustermann S, Wu WF, Langelier MF, et al. Structural basis of detection and signaling of DNA single-strand breaks by human PARP-1. *Mol Cell*. 2015;60:742–754.
16. Dawicki-McKenna Jennine M, Langelier M-F, DeNizio Jamie E, et al. PARP-1 activation requires local unfolding of an autoinhibitory domain. *Mol Cell*. 2015;60:755–768.
17. Murai J, Huang S-yN, Das BB, et al. Trapping of PARP1 and PARP2 by clinical PARP inhibitors. *Cancer Res*. 2012;72:5588.
18. Pilie PG, Gay CM, Byers LA, O'Connor MJ, Yap TA. PARP inhibitors: extending benefit beyond BRCA-mutant cancers. *Clin Cancer Res*. 2019;25:3759–3771.
19. Yap TA, Plummer R, Azad NS, Helleday T. The DNA damaging revolution: PARP inhibitors and beyond. *Am Soc Clin Oncol Educ Book*. 2019;39:185–195.
20. Pommier Y, O'Connor MJ, de Bono J. Laying a trap to kill cancer cells: PARP inhibitors and their mechanisms of action. *Sci Transl Med*. 2016;8:362ps17.
21. Sonnenblick A, de Azambuja E, Azim HA, Jr., Piccart M. An update on PARP inhibitors–moving to the adjuvant setting. *Nat Rev Clin Oncol*. 2015;12:27–41.
22. Farmer H, McCabe N, Lord CJ, et al. Targeting the DNA repair defect in BRCA mutant cells as a therapeutic strategy. *Nature*. 2005;434:917–921.
23. Coleman RL, Oza AM, Lorusso D, et al. Rucaparib maintenance treatment for recurrent ovarian carcinoma after response to platinum therapy (ARIEL3): a randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet*. 2017;390:1949–1961.
24. Swisher EM, Lin KK, Oza AM, et al. Rucaparib in relapsed, platinum-sensitive high-grade ovarian carcinoma (ARIEL2 Part 1): an international, multicentre, open-label, phase 2 trial. *Lancet Oncol*. 2017;18:75–87.
25. Faraoni I, Graziani G. Role of BRCA mutations in cancer treatment with poly(ADP-ribose) polymerase (PARP) inhibitors. *Cancers*. 2018;10:487.
26. Bertelsen B, Tuxen IV, Yde CW, et al. High frequency of pathogenic germline variants within homologous recombination repair in patients with advanced cancer. *NPJ Genom Med*. 2019;4:13.
27. Yan H, Parsons DW, Jin G, et al. IDH1 and IDH2 mutations in gliomas. *N Engl J Med*. 2009;360:765–773.
28. Malta TM, de Souza CF, Sabedot TS, et al. Glioma CpG island methylator phenotype (G-CIMP): biological and clinical implications. *Neuro Oncol*. 2018;20:608–620.
29. Turcan S, Makarov V, Taranda J, et al. Mutant-IDH1-dependent chromatin state reprogramming, reversibility, and persistence. *Nat Genet*. 2018;50:62–72.
30. Lu C, Ward PS, Kapoor GS, et al. IDH mutation impairs histone demethylation and results in a block to cell differentiation. *Nature*. 2012;483:474–478.
31. Golub D, Iyengar N, Dogra S, et al. Mutant isocitrate dehydrogenase inhibitors as targeted cancer therapeutics. *Front Oncol*. 2019;9:417.
32. Sulkowski PL, Corso CD, Robinson ND, et al. 2-Hydroxyglutarate produced by neomorphic IDH mutations suppresses homologous recombination and induces PARP inhibitor sensitivity. *Sci Transl Med*. 2017;9:eaal2463.
33. Wang Y, Wild AT, Turcan S, et al. Targeting therapeutic vulnerabilities with PARP inhibition and radiation in IDH-mutant gliomas and cholangiocarcinomas. *Sci Adv*. 2020;6:eaaz3221.
34. Philip B, Yu DX, Silvis MR, et al. Mutant IDH1 promotes glioma formation in vivo. *Cell Rep*. 2018;23:1553–1564.
35. Lu Y, Kwintkiewicz J, Liu Y, et al. Chemosensitivity of IDH1-mutated gliomas due to an impairment in PARP1-mediated DNA repair. *Cancer Res*. 2017;77:1709.
36. Stupp R, Mason WP, van den Bent MJ, et al. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. *N Engl J Med*. 2005;352:987–996.
37. Jiapaer S, Furuta T, Tanaka S, Kitabayashi T, Nakada M. Potential strategies overcoming the temozolomide resistance for glioblastoma. *Neuro Med Chir (Toyko)*. 2018;58:405–421.
38. Galia A, Calogero AE, Condorelli R, et al. PARP-1 protein expression in glioblastoma multiforme. *Eur J Histochem*. 2012;56:e9.
39. Tentori L, Portarena I, Vernole P, et al. Effects of single or split exposure of leukemic cells to temozolomide, combined with poly(ADP-ribose) polymerase inhibitors on cell growth, chromosomal aberrations and base excision repair components. *Cancer Chemother Pharmacol*. 2001;47:361–369.
40. Tentori L, Turriziani M, Franco D, et al. Treatment with temozolomide and poly(ADP-ribose) polymerase inhibitors induces early apoptosis and increases base excision repair gene transcripts in leukemic cells resistant to triazene compounds. *Leukemia*. 1999;13:901–909.
41. Tentori L, Portarena I, Torino F, Scerrati M, Navarra P, Graziani G. Poly(ADP-ribose) polymerase inhibitor increases growth inhibition and reduces G(2)/M cell accumulation induced by temozolomide in malignant glioma cells. *Glia*. 2002;40:44–54.
42. Ahmed SU, Carruthers R, Gilmour L, Yildirim S, Watts C, Chalmers AJ. Selective inhibition of parallel DNA damage response pathways optimizes radiosensitization of glioblastoma stem-like cells. *Cancer Res*. 2015;75:4416–4428.
43. Tentori L, Ricci-Vitiani L, Muzi A, al. Pharmacological inhibition of poly (ADP-ribose) polymerase-1 modulates resistance of human glioblastoma stem cells to temozolomide. *BMC Cancer*. 2014;14:151.
44. Gupta SK, Kizilbash SH, Carlson BL, et al. Delineation of MGMT hypermethylation as a biomarker for veliparib-

mediated temozolomide-sensitizing therapy of glioblastoma. *J Natl Cancer Inst.* 2015;108:djv369.
45. Barazzuol L, Jena R, Burnet NG, et al. Evaluation of poly (ADP-ribose) polymerase inhibitor ABT-888 combined with radiotherapy and temozolomide in glioblastoma. *Radiat Oncol*. 2013;8:65.
46. Erice O, Smith MP, White R, Goicoechea I, Barriuso J, Jones C, et al. MGMT expression predicts PARP-mediated resistance to temozolomide. *Mol Cancer Ther.* 2015;14:1236–1246.
47. Wu S, Gao F, Koul D, Yung A. DRES-02. Parylation of mgmt by PARP is required for mgmt mediated temozolomide-induced o6-methylguanine repair: a novel mechanism of MGMT action. *NeuroOncology.* 2019;21:vi71–vi2.
48. Clarke MJ, Mulligan EA, Grogan PT, et al. Effective sensitization of temozolomide by ABT-888 is lost with development of temozolomide resistance in glioblastoma xenograft lines. *Mol Cancer Ther.* 2009;8:407–414.
49. Robins HI, Zhang P, Gilbert MR, et al. A randomized phase I/II study of ABT-888 in combination with temozolomide in recurrent temozolomide resistant glioblastoma: an NRG oncology RTOG group study. *J Neurooncol*. 2016;126:309–316.
50. Kleinberg L, Supko JG, Mikkelsen T, et al. Phase I adult brain tumor consortium (ABTC) trial of ABT-888 (veliparib), temozolomide (TMZ), and radiotherapy (RT) for newly diagnosed glioblastoma multiforme (GBM) including pharmacokinetic (PK) data. *J Clin Oncol.* 2013;31:2065.
51. Khasraw M, McDonald KL, Rosenthal M, et al. A randomized phase II trial of veliparib (V), radiotherapy (RT) and temozolomide (TMZ) in patients (pts) with unmethylated MGMT (uMGMT) glioblastoma (GBM). *J Clin Oncol.* 2019;37:2011.
52. Murai J, Zhang Y, Morris J, et al. Rationale for poly(ADP-ribose) polymerase (PARP) inhibitors in combination therapy with camptothecins or temozolomide based on PARP trapping versus catalytic inhibition. *J Pharmacol Exp Ther.* 2014;349:408–416.
53. Fong PC, Boss DS, Yap TA, et al. Inhibition of poly (ADP-ribose) polymerase in tumors from BRCA mutation carriers. *N Engl J Med.* 2009;361:123–134.
54. Halford SER, Cruickshank G, Dunn L, et al. Results of the OPARATIC trial: a phase I dose escalation study of olaparib in combination with temozolomide (TMZ) in patients with relapsed glioblastoma (GBM). *J Clin Oncol.* 2017;35:2022.
55. Chalmers A, Stobo J, Short SC, et al. ACTR-22. Results of phase i of the paradigm trial: a phase i dose escalation study of olaparib in combination with short course radiotherapy in elderly patients with newly diagnosed glioblastoma (GBM). *NeuroOncology.* 2017;19:vi5–vi.
56. Fulton B, Short SC, James A, et al. PARADIGM-2: Two parallel phase I studies of olaparib and radiotherapy or olaparib and radiotherapy plus temozolomide in patients with newly diagnosed glioblastoma, with treatment stratified by MGMT status. *Clin Transl Radiat Oncol*. 2017;8:12–16.
57. Lesueur P, Lequesne J, Grellard J-M, et al. Phase I/IIa study of concomitant radiotherapy with olaparib and temozolomide in unresectable or partially resectable glioblastoma: OLA-TMZ-RTE-01 trial protocol. *BMC Cancer.* 2019;19:198.
58. Piotrowski A, Puduvalli V, Wen P, et al. ACTR-39. Pamiparib in combination with radiation therapy (RT) and/or temozolomide (TMZ) in patients with newly diagnosed or recurrent/refractory (R/R) glioblastoma (GBM); phase 1B/2 study update. *NeuroOncology.* 2019;21:vi21–vi2.
59. Shih K, Schiff D, Kim L, et al. ACTR-30. Phase 1B/2 study to assess the clinical effects of pamiparib (bgb-290) in combination with radiation therapy (RT) and/or temozolomide (TMZ) in patients with newly diagnosed or recurrent/refractory glioblastoma (GBM). *NeuroOncology.* 2018;20:vi17–vi8.
60. Lord CJ, Ashworth A. PARP inhibitors: synthetic lethality in the clinic. *Science (New York, NY)*. 2017;355:1152–1158.
61. Shen WH, Balajee AS, Wang J, et al. Essential role for nuclear PTEN in maintaining chromosomal integrity. *Cell*. 2007;128:157–170.
62. McEllin B, Camacho CV, Mukherjee B, et al. PTEN loss compromises homologous recombination repair in astrocytes: implications for glioblastoma therapy with temozolomide or poly(ADP-ribose) polymerase inhibitors. *Cancer Res*. 2010;70:5457–5464.
63. Lin F, de Gooijer MC, Roig EM, et al. ABCB1, ABCG2, and PTEN determine the response of glioblastoma to temozolomide and ABT-888 therapy. *Clin Cancer Res*. 2014;20:2703.