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DNA damage response in brain tumors: A Society for Neuro-Oncology consensus review on mechanisms and translational efforts in neuro-oncology

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Abstract

DNA damage response (DDR) mechanisms are critical to maintenance of overall genomic stability, and their dysfunction can contribute to oncogenesis. Significant advances in our understanding of DDR pathways have raised the possibility of developing therapies that exploit these processes. In this expert-driven consensus review, we examine mechanisms of response to DNA damage, progress in development of DDR inhibitors in IDH-wild-type glioblastoma and IDH-mutant gliomas, and other important considerations such as biomarker development, preclinical models, combination therapies, mechanisms of resistance and clinical trial design considerations.

Keywords

DDR inhibitors | DNA damage response | DNA repair | glioma | radiation therapy

DNA damage response (DDR) is a collective term for a suite of intra- and inter-cellular signaling events that play critical roles in maintaining genomic stability. Loss of these mechanisms can lead to the accumulation of deleterious mutations that contribute to oncogenesis. With better understanding of how DDR pathways function in cancer, our ability to exploit these processes for therapeutic benefit will increase. In this review, we provide a broad overview of DDR pathways, discuss how these processes are altered in primary brain tumors, and highlight translational efforts to leverage this knowledge for developing new therapies to treat patients with brain tumors.

Therapeutically Relevant DNA Damage Repair Mechanisms in Glioma

Before discussing therapeutic efforts to target DDR deficits in glioma, we provide an overview of DDR pathways that are relevant to translational opportunities in neuro-oncology (Figure 1).

Base Excision Repair and Methylguanine-DNA Methyltransferase

DNA damage limited to a single base is generally repaired by base excision repair (BER).[1] Alterations affecting single bases, such as oxidation, oxidative deamination, and/or alkylation/methylation (including alkylating chemotherapies) trigger BER.[2] Repair of this damage goes through the steps of excising the damaged base, the sugar backbone, and the deoxyribose-phosphate site, which effectively creates a strand break. A replacement base with the sugar and phosphate moiety is ligated in place. Alkylating agents such as temozolomide (TMZ) induce damage of DNA bases and trigger BER in glioma tumors. Importantly, and relevant to glioma treatment, the function of the methylguanine methyltransferase (MGMT) protein counteracts DNA damage caused by alkylating agents. MGMT acts as a "suicide enzyme" that repairs the DNA damage caused by TMZ by removing alkyl groups from guanine residues (Figure 2A) , hence reversing the effect ofTMZ.[2] Silencing of MGMT gene expression via methylation of the MGMT gene

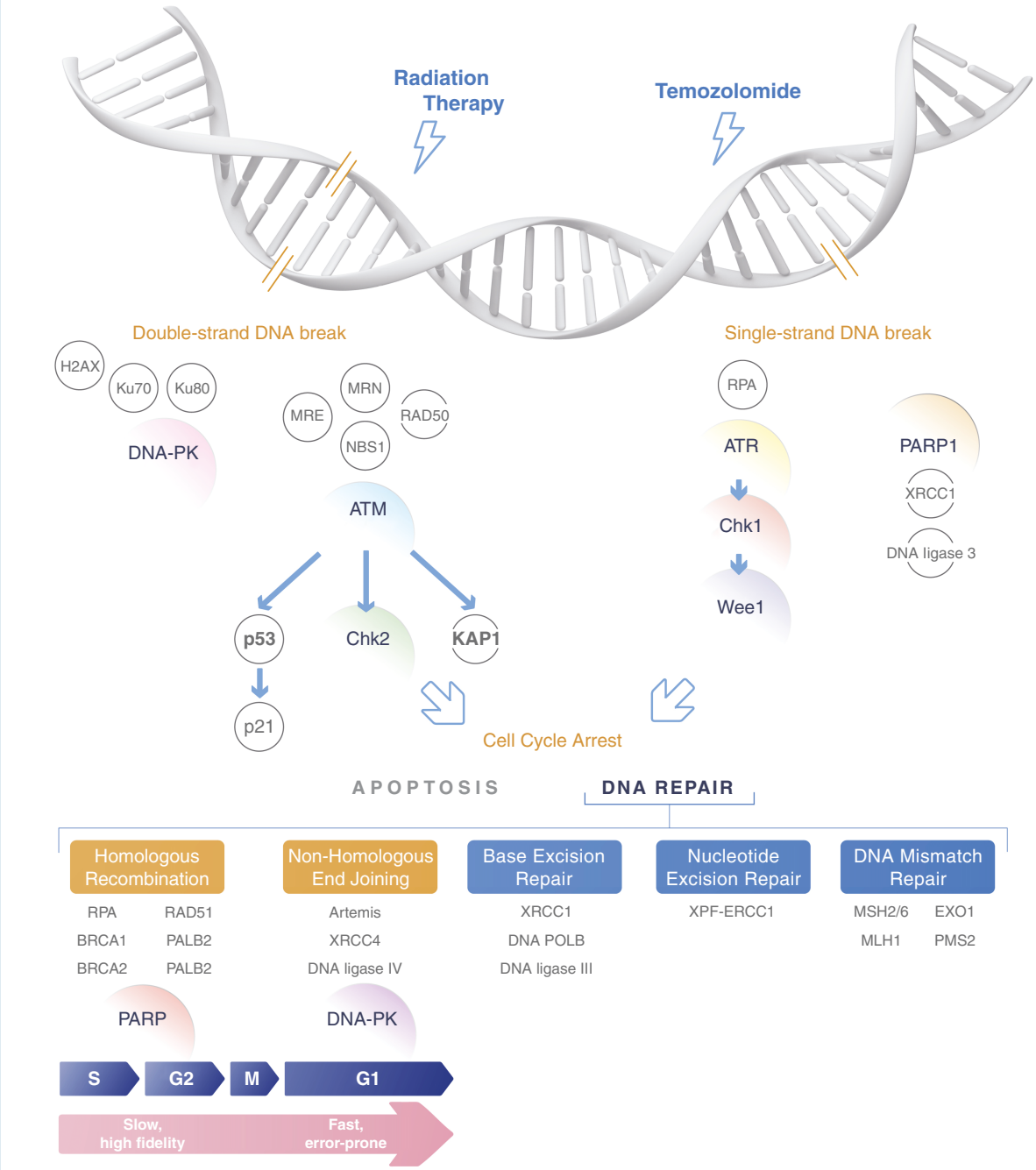

Figure 1. Overview of relevant DNA DNA damage response (DDR) pathways in response to standard of care therapies of radiation therapy and alkylating chemotherapy (temozolomide). Antitumor therapy can cause DNA damage via double-strand breaks (DSBs) or single-strand breaks (SSBs). In the setting of double-strand breaks, DNA-PK is a multi-enzyme complex consistent with DNA binding domains and catalytic subunit, and it regulates non-homologous end-joining (NHEJ), which can occur in the absence of sister chromatids (eg, G1 arrest). DSBs can also activate the ATM pathway, which includes downstream phosphorylation of proteins including CHK2 and p53. ATR can be activated by several genotoxic stresses, including SSBs, and it phosphorylates several targets. DNA DDR pathways lead to cell cycle arrest, and possible outcomes include apoptosis or successful DNA repair. Relevant DNA repair mechanisms and relevant molecular factors are listed at the bottom of the figure. Cell-cycle specific timing and kinetics of double-strand break repair mechanisms, homologous recombination (HR), and NHEJ, are highlighted. The balance of HR versus NHEJ repair mechanisms reflects factors such as whether a template strand exists for HR-mediated repair, chromatin accessibility, the presence of relevant co-occurring mutations such as BRCA1/2 deficiency, and the burden of DNA damage. Targets of therapies that are under active testing in neuro-oncology are shaded with color and bolded.

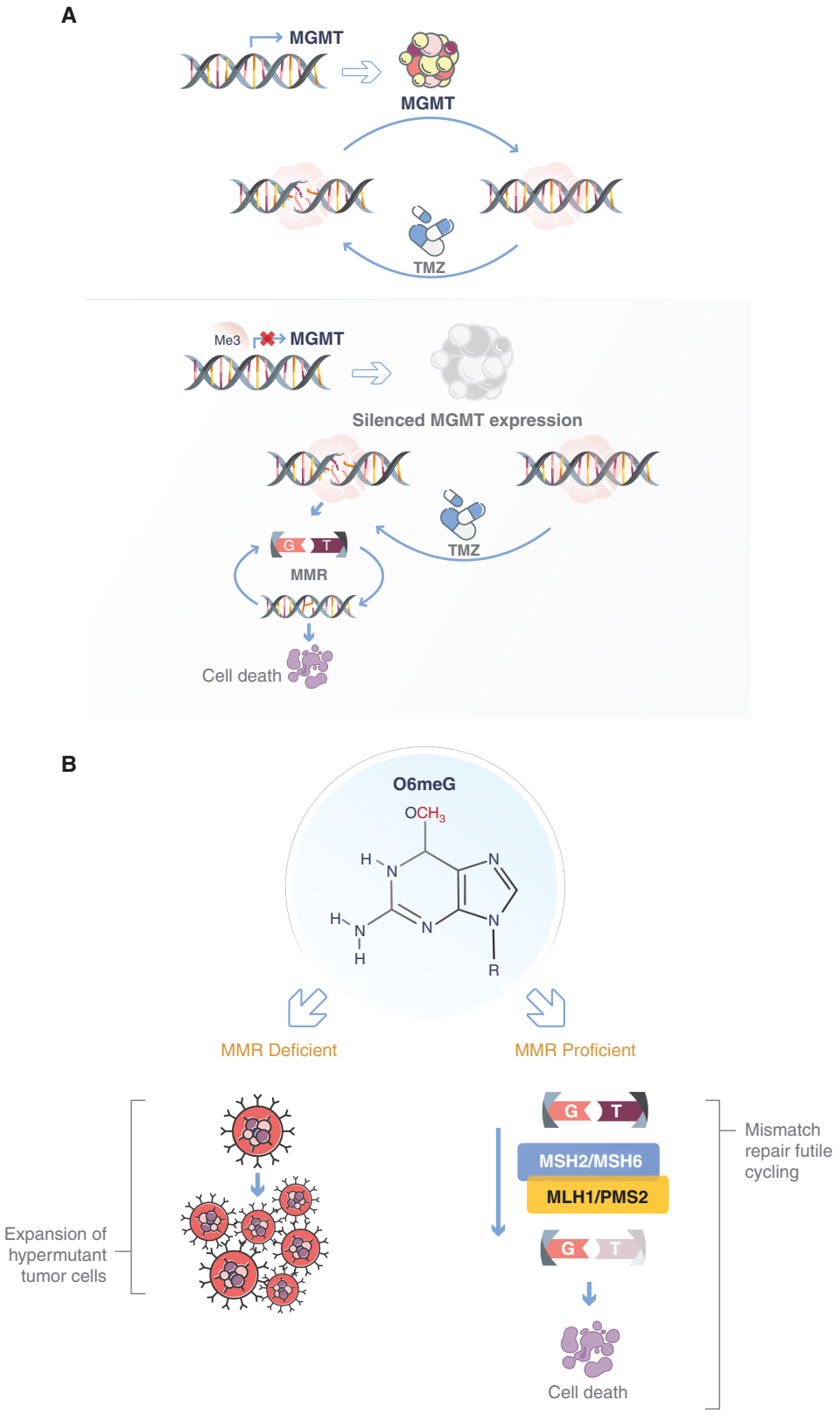

Figure 2. (A, top) MGMT, when unmethylated and expressed, repairs TMZ-induced DNA damage by removing alkyl groups from guanine residues. (A, bottom) Methylation of the MGMT gene promoter silences the expression of MGMT. In this scenario, TMZ-induced DNA alkylation is less able to be removed by MGMT. In MMR-proficient tumor cells, this may trigger a futile cycle of MMR. (B) The active form of temozolomide, methyl diazonium ion, acts as a methyl donor at O^6-methylguanine (O^6MeG) adducts. If these alkylated lesions are not repaired by MGMT, MMR-proficient cells can undergo futile cycling of mismatch repair and subsequent cell death. In MMR-deficient cells, cells may proliferate and develop hypermutated phenotypes that may confer therapy resistance. Abbreviations: MGMT, O^6-methylguanine-DNA methyltransferase; TMZ, temozolomide; Me3, methyl group; O^6meG, O^6-methylguanine; G, guanine; T, thymine; MMR, mismatch repair.

promoter, a tumor-specific epigenetic event and rarely a germline event, is estimated to occur in 30%–40% of glioblastoma (GBM) patients.[3,4] MGMT promoter methylation, which inactivates this repair gene, has been associated with improved prognosis.[3]

Mismatch repair.—Mismatch repair (MMR), as its name suggests, repairs DNA base pairing errors. MMR machinery, comprised of multiple MMR proteins, follows the replisome as a "check" on replication.[5,6] In mismatch repair, the MSH2/6 complex recognizes small mismatches, such as a single-base mismatch or 1 or 2 unpaired bases. In contrast, the MSH2/3 complex recognizes relatively larger insertion–deletion loops of ≤15 nucleotides, although it can also recognize small mismatch sites of 1 or 2 unpaired bases. These complexes then recruit MLH1 and PMS2 to the site of repair, resulting in cleavage of mismatched DNA, excision of the mismatched region, and finally ligation. Defects in MMR tend to increase mutational burden through increases in point mutations and mutational load (as opposed to large chromosomal losses). The phenotype of microsatellite instability has been described in MMR-deficient cancers such as colon cancer,[7] ovarian cancer,[8] gastric cancer,[9] and others.[10] Owing to their high mutational burden characteristic of MMR-deficient cancers, immune checkpoint inhibitors are often effective in such tumors and represent a recent paradigm shift in drug approval from tumor-specific indications to mutation-burden-driven indications.[11] Of note, while MMR germline deficiency is rare in gliomas, a hypermutated phenotype following chronic exposure to alkylating therapy is more commonly seen in glioblastoma (see Additional Considerations in Targeting DNA Damage Pathways, "Combination with immunotherapy").[12,13]

Non-homologous end joining.—The most common mechanism for double-stranded break (DSB) repair is non-homologous end joining (NHEJ). NHEJ entails repair of DSBs in the absence of a sister chromatid template (Figure 3). As such, it is a lower-fidelity mechanism that is more prone to error.[14,15] Given that a sister chromatid template is not required, NHEJ can occur at any phase of the cell cycle and often occurs during G1. NHEJ begins with localization of the KU dimer (KU70 and KU80) to the double-strand DNA break (DSB) and recruitment of DNA-PK. DNA-PK, a multi-enzyme complex consisting of DNA binding domains (Ku70, Ku80) with a catalytic subunit (DNA-PKcs), regulates NHEJ while also functioning to detect and modulate signaling of DSBs. After Ku proteins bind to exposed chromosome ends, they interact with DNA-PKcs to create the repair complex. Once bound, DNA-PKcs is activated and can facilitate downstream activation of XRCC4, XLF, and ARTEMIS to facilitate NHEJ repair. The complex can recruit endonucleases to resect DNA and recruit polymerases to complete repair and join the resected ends of DSBs. DNA-PKcs has been implicated as a drug target in ATM-deficient tumors (see "Targeting DDR kinases and PARP in GBM").[16]

Homologous recombination.—In contrast to NHEJ, homologous recombination (HR) utilizes a sister chromatid template for DNA repair and as such is less error-prone compared to NHEJ.[17,18] The first step entails nuclease-mediated resection of portions of each of the strands to generate DNA "overhangs." Replication protein A (RPA) binds to these overhangs and recruits single-stranded DNA to "fill the gap." RPA is then exchanged for the Rad51 protein, which promotes strand invasion into the sister chromatid that is used as the template for DNA synthesis. Following strand invasion, a new DNA strand is synthesized using the sister chromatid as a template, and this high-fidelity strand joins the 2 ends of the strand break. Cancer patients with defects in HR (usually germline) may benefit from PARP inhibitors since these agents cause persistent, unrepaired single-strand breaks (SSBs). Persistence of these SSBs through DNA replication in the S-phase yields a DSB in the daughter cell, which cannot be adequately repaired in the presence of germline HR defects. Two important and overlapping strategies have emerged for inhibiting PARP: Blocking the catalytic enzyme activity and preventing release of PARP from DNA (ie, PARP trapping), the latter of which refers to PARP complexes at damaged DNA, preventing their catalytic function and stalling replication.[19] PARP inhibitors can trap PARP1 in its bound form to DNA, causing replication fork stalling. BRCA1/2 proteins play a role in protecting stalled replication forks,[20,21] and a deficiency in BRCA1/2 in setting of PARP inhibition can result in synthetic lethality.[22,23] Various PARP inhibitors are felt to have different potencies as a direct inhibitor versus PARP trapper, which may have implications for clinical efficacy though further study is needed to explore these nuances.[24] There is increasing interest in using PARP inhibitors in tumors with other HR defects beyond BRCA1/2 mutations, a state sometimes referred to as "BRCAness," which is of particular interest in isocitrate dehydrogenase-mutant gliomas (see "IDH-mutant gliomas").

Ataxia-Telangiectasia Mutated Kinase and Ataxia-Telangiectasia and Rad3-Related Kinase

The DDR kinases ataxia-telangiectasia mutated kinase (ATM) and ataxia-telangiectasia and rad3-related kinase (ATR) are particularly relevant DDR mechanisms in glioma given ongoing clinical testing of inhibitors of these proteins. ATM is an apical serine/threonine kinase that is the master orchestrator of the cellular response to DSBs. ATM activity leads to downstream phosphorylation of many substrates, including key DNA damage pathway proteins such as p53, CHK2, MDM2, and BRCA1. Several of these targets (CHK2, p53) are direct regulators of cell cycle progression, allowing cells to repair DNA before progressing through G1/S, G2/M, and S-phase. Furthermore, ATM phosphorylates targets that are more directly involved in DSB repair, including NBS1, a component of the MRN complex.[25–28] Patients with ataxia-telangiectasia (A-T) who harbor germline loss-of-function mutations in the ATM gene are profoundly sensitive to ionizing radiation as they are inefficient at repairing DSBs. This raises the possibility that ATM inhibition could be used to enhance the efficacy of radiation therapy, and possibly other DNA-damaging therapies. ATM preferentially triggers HR (as opposed to NHEJ) by phosphorylating numerous downstream targets.

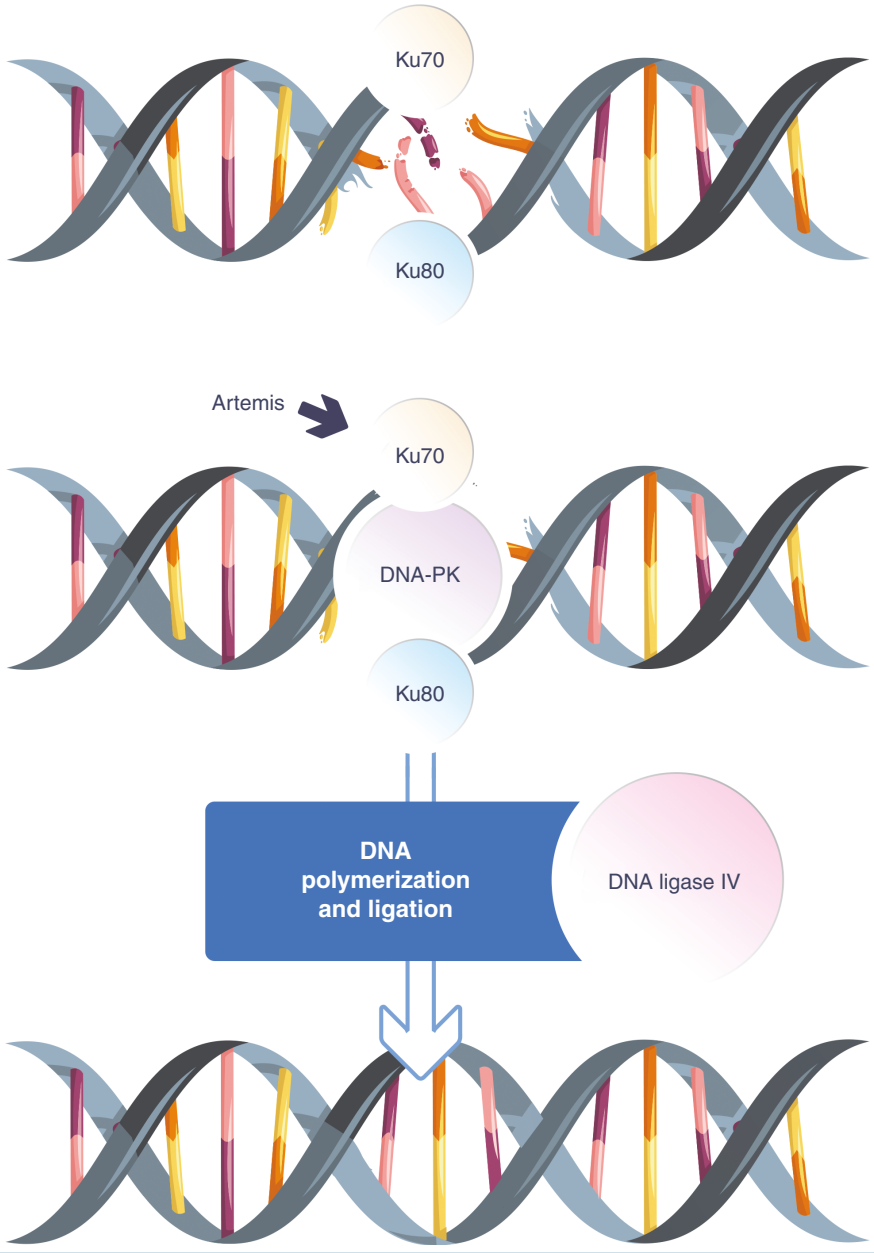

Figure 3. Overview of non-homologous end joining repair (NHEJ). NHEJ repair occurs in the absence of a sister chromatid template. First, there is localization of the KU dimer to the double-strand break. KU recruits DNA-PK and there is subsequent recruitment of ARTEMIS to facilitate processing of DNA ends. There is subsequent activation of XRCC4, XLF, and ARTEMIS. The complex recruits endonucleases and polymerases to complete repair and join resected ends.

ATM loss dramatically increases radiosensitivity in a variety of gliomas in experimental models, and this effect is even more profound in models harboring nonfunctional p53 in several reports.[29,30] As many GBMs harbor genetic alterations inactivating p53, these results imply that ATM inhibition may be particularly effective in a large subset of GBMs.

While inhibiting ATM might present a therapeutic vulnerability for brain tumors, there is evidence suggesting that such inhibition could also offer protection to the normal brain from radiation. ATM plays a crucial role in radiation-induced apoptosis in the developing mouse brain, and its deletion appears to safeguard certain neuronal cell populations.[31] Recent research in mouse genetics has further implicated ATM in DDR-induced apoptosis specifically in immature brain progenitor cells, while other DDR kinases seem to be more vital in other brain cell types. This discovery hints at the possibility that some normal tissues could be shielded from radiation-induced toxicity when ATM inhibitors are administered alongside radiation therapy.

ATR, the other master regulator of the DDR, is activated by replication stress and generally promotes NHEJ

(see "Targeting DDR kinases and PARP in GBM"). Unlike ATM, ATR deletion is embryonically lethal in mice. ATR can be activated by a variety of genotoxic stresses such as SSBs. ATR is recruited to sites of DNA damage flagged by coating of single-strand DNA with RPA. Similar to ATM, ATR orchestrates the phosphorylation of numerous downstream targets. One of the most notable downstream targets is CHK1. ATM and ATR share numerous downstream targets, and ATM deficiency confers sensitivity to ATR inhibition,[32] which raises the possibility of synthetic lethality by targeting ATR in tumors lacking functional ATM. Given the embryonic lethality in mice, ATR inhibition initially raised major off-target toxicity concerns. However, evidence has accumulated that there may be a therapeutic window for ATR inhibition to exploit genomic instability in cancer cells, especially in combination with other DNA-damaging therapies. Downstream of ATR-CHK1, Wee1 acts as a regulator and phosphorylates CDK1 leading to its inhibition and block of the G2-M transition.[33] Wee1 phosphorylates and inhibits CDK2 activity which can also delay the G1/S transition.[34] Prior preclinical evidence suggests that Wee1 inhibition can sensitize tumors to radiation, and this represents another possible avenue for therapeutic development.[35]

Glioblastoma, IDH Wild Type

GBM, wild-type for IDH (IDH-WT) by definition, represents the most common primary malignant intracranial neoplasm in adults.[36] Here we first review standard-of-care treatment, including mechanisms of response and resistance[37], emphasizing links to DDR. We then describe how this underpins the rationale for investigating specific DDR-directed therapies in GBM. Selected clinical trials of DDR agents for GBM are summarized in Table 1.

Standard of Care: Surgery, Radiation/ Temozolomide, and Tumor-Treatment Fields

Adjuvant radiation (RT) following maximal safe resection showed a benefit in early trials, and dose escalation to 60 Gy in 30 daily fractions was shown to confer superior survival compared to lower doses.[53–55]

In 2005, the European Organization for Research and Treatment of Cancer and the National Cancer Institute of Canada (EORTC trial 26981/22981 and NCIC trial CE.3) showed a significant increase in median survival from 12 to 15 months with the addition of concurrent and adjuvant temozolomide (TMZ) to RT.[56] In addition to surgery and chemoradiation, the use of tumor treating fields (TTF) has also demonstrated a survival benefit with an improvement of median survival from 16.0 to 20.9 months with the use of TTF in the adjuvant phase of therapy.[57]

Radiation therapy induces lethal DSBs, which trigger HR and/or NHEJ repair mechanisms. The factors that dictate specific mechanisms of repair include the burden and complexity of DNA damage, the phase of the cell cycle in which the damage occurs, and chromatin accessibility. The type of radiation delivered may invoke varying DNA damage repair mechanisms with evidence suggesting that high linear energy transfer sources of radiation such as carbon ions may induce a greater density of breaks and utilize HR-mediated repair, whereas low linear energy transfer radiation sources utilize less HR-mediated repair.[58,59] In addition to the direct DSB and SSBs caused by radiation, it should be noted that radiation also induces indirect DNA damage through mechanisms such as reactive oxygen species (ROS) generation that may also result in lethal DNA damage.

Mechanism of TMZ Efficacy and Resistance

TMZ is an oral pro-drug, and the active moiety produces widespread base alkylation that is mostly readily repaired by BER. However, alkylation at the N^7 and O^6 positions of guanine residues is poorly repaired, especially in the context of a specific deficiency of the repair enzyme MGMT. MGMT is inactive in a subset of newly diagnosed GBMs in which the MGMT promoter is methylated, silencing MGMT expression. Base alkylation by TMZ sets in motion a cascade of events that exert cytotoxicity in a DDR-dependent manner. The O^6-methylguanine lesions generated by TMZ frequently cause a guanine-thymine mismatch, exacerbated in the context of unavailable "exchange methyl" groups from a deficiency of MGMT, and this mismatch activates MMR. This leads to cleavage and reinsertion of thymine, ultimately leading to futile cycling of the MMR pathway that causes DNA breaks, triggers response to DNA damage, and leads to apoptosis (Figure 2A).[60,61] As such, response to TMZ and mechanisms of resistance to TMZ can vary based on co-occurring mutations. For example, p53 mutations can alter the duration of G2-M arrest induced by TMZ and trigger different mechanisms of cytotoxicity in glioma.[62] Additionally, downregulation of the MMR pathway, including mutations in MLH1, MSH2, MSH6, and PMS2, have been associated with resistance to TMZ.[63–65] Suppression of the HR machinery has also been linked to increased sensitivity to TMZ,[66] as has inhibition of Fanconi Anemia pathway genes,[67] and inhibition of ATM or ATR kinases.[68] Of note, TMZ has been posited to drive a hypermutation phenotype by inducing MMR deficiency as a resistance mechanism that then leads to genome-wide hypermutation, and there is some evidence suggesting that this phenotype may not be as responsive to immunotherapy as in other tumors (Figure 2B).[69]

MGMT promoter methylation.—In a setting with decreased expression of MGMT when the promoter is methylated, there is greater accumulation of O^6-methylguanine lesions generated by TMZ.[70] While there was preliminary evidence suggesting that the use of dose-dense TMZ may rapidly and persistently deplete intracellular MGMT, this dosing strategy did not yield a survival benefit in a phase III randomized trial.[71] Suppression of MGMT activity to sensitize tumor cells to TMZ has been investigated both preclinically and in early-phase clinical trials, but these efforts have largely been stymied by excessive myeloid toxicity.[72,73] Attempts to circumvent these toxicities include use of a monoamine oxidase B-specific prodrug that is converted to

Table 1. Clinical Trials of DNA Damage Response Inhibitors in Adult Glioblastoma

Trial	Agent	Pathway/ target	Disease setting	Design/combination	Comments/results
ATM inhibitor					
NCT03423628	AZD1390[38]	ATM	ndGBM uMGMT, rGBM	Phase 1 study, 3 arms (ndGBM uMGMT, rGBM, brain metastases)	- Initial results demonstrate pharmacologically relevant concentration in non-enhancing tissue
Wee1 inhibitor					
NCT01849146	AZD1775[39]	WEE1	ndGBM, rGBM	Phase 0: AZD1775 -> surgery	- 20 patients - Good brain tumor penetration and evidence of Wee1 pathway suppression
NCT01849146	AZD1775	WEE1	rGBM	Phase 1 nonrandomized: Arm I: RT +TMZ + AZD1775 ->TMZ Arm II: RT/TMZ ->TMZ.+ AZD1775	- 3 + 3 design - Initial results: MTD 200 mg for concurrent dosing with RT; MTD 425 mg for adjuvant dosing
DNA-PK inhibitor					
NCT04555577	M3814 (Nedisertib)[40]	DNA-PK	ndGBM uMGMT	Phase I, 2 stages: Stage I: RT/M3814->TMZ Stage II RT/M3814->resection->TMZ	- Ongoing recruitment
NCT02977780	CC-115[41]	DNA-PK and mTOR	ndGBM uMGMT	Phase II randomized with safety lead-in: Randomized: RT/CC-115 -> CC-115 vs. RT/TMZ->TMZ	- 12 patients received CC-115 - No evidence of PFS or OS benefit - Arm terminated due to concerns of toxicity and absence of efficacy signal
PARP inhibitor					
NCT00770471 (ABTC-0801)	Veliparib (ABT-888)[42]	PARP	ndGBM	Phase I: RT +TMZ + veliparib	- With concurrent RT, DLT occurred in 4/12 patients; 3/6 patients at a lower dose - Veliparib is not tolerable with standard RT/TMZ at tested doses
VERTU[43]	Veliparib	PARP	ndGBM, uMGMT	Phase II: RT +TMZ +/– veliparib (2:1 randomization to experimental arm)	- 125 participants, 84 receiving veliparib - OS 12.7 vs. 12.8 months (ns)
NCT01026493 (NRG/RTOG 0929)[44]	Veliparib	PARP	rGBM	Phase I/II randomized in BEV-naïve and BEV refractory rGBM Arm 1: Veliparib +TMZ (75 mg/m2) Arm 2: Veliparib +TMZ (150mg/m2)	- OS similar in both arms - PFS-6 17% in BEV-naïve and PFS-6 4% in BEV-refractory
NCT02152982 (Alliance A071102)[45]	Veliparib	PARP	ndGBM, mMGMT	Phase II/III randomized, placebo-controlled: RT/TMZ ->TMZ +/– veliparib	- 421 patients - Median OS 28.1 months (veliparib) vs. 24.8 months (placebo) - Median PFS 13.2 vs. 12.1 months - Subset exploratory analysis suggests benefit for TMZ + veliparib with TMZ at first recurrence
NCT01390571 (OPARATIC)[46]	Olaparib	PARP	rGBM	Phase 0/1 nonrandomized Stage I: Olaparib -> surgery Stage II: Olaparib -> surgery -> TMZ + Olaparib	- Olaparib reliably penetrates rGBM at radiosensitizing concentrations - Olaparib was detected in 71/75 tumor specimens with 36 evaluable patients (PFS6 39%)
CRUK/13/034 (PARADIGM)[47]	Olaparib	PARP	ndGBM	Phase I: Hypofractionated RT + olaparib	- 16 patients (median age 72 years) with RP2D 200 mg BID daily - mOS 10.3 months
ISRCTN51253312 (PARADIGM-2)[47,48]	Olaparib	PARP	ndGBM	Phase I mMGMT: RT +TMZ + Olaparib uMGMT: RT + Olaparib	- Ongoing, early results showing treatment is well-tolerated
NCT03212742[49]	Olaparib	PARP	ndHGG unresectable	Phase I/IIa nonrandomized: RT +TMZ + olaparib	- Ongoing recruitment
NCT02974621[50]	Olaparib + cediranib	PARP	rGBM	Phase II randomized: Olaparib + cediranib vs. BEV	- 70 total patients - No benefit for Olaparib + cediranib - Median PFS 118 vs. 92 days (ns) - Median OS 269.5 vs. 192 days (ns)

Table 1. Continued

Trial	Agent	Pathway/ target	Disease setting	Design/combination	Comments/results
NCT05188508	Olaparib + pembrolizumb + TMZ	PARP	rGBM[#]	Phase II nonrandomized Arm 1: recurrent IDH mutant Arm 2: Recurrent IDH-wt gliomas and homologous recombination deficient	- Ongoing recruitment
NCT05463848	Pembro + Olaparib + TMZ	PARP	rGBM	Phase II randomized; surgical: Arm A: Pembrolizumab +TMZ + Olaparib Arm B: Pembrolizumab	- Ongoing recruitment
NCT05076513[51]	Pamiparib	PARP	ndGBM, rGBM	Phase 0 trigger trial Arm A (ndGBM): pamiparib -> surgery Arm B (rGBM): pamiparib -> surgery Arm C (rGBM): pamiparib	- Generally well-tolerated with meaningful concentration in nonenhancing tissue - PK threshold for expansion phase by all patients Arm A + B - Median PFS 5.4 mo (Arm A), 5.0 mo (Arm B), 2.7 mo (Arm C)
NCT03150862[52]	Pamiparib	PARP	ndGBM uMGMT, rGBM	Phase 1b/2 ndGBM (uMGMT): Arm 1: RT + pamiparib Arm 2: RT +TMZ + pamiparib rGBM: RT + pamiparib	- Manageable safety profile for pamiparib +/– RT +/–TMZ - Median PFS 4.4 months and median OS 12.7mo in Arms A/B - Median PFS 1.9 months and median OS 7.3 months in Arm C
NCT04221503	Niraparib	PARP	rGBM	Phase II nonrandomized: TTFields + niraparib	- Active, not recruiting

ndGBM, newly diagnosed glioblastoma; rGBM, recurrent glioblastoma, uMGMT, unmethylated MGMT promoter; mMGMT, methylated MGMT promoter; RT, radiation therapy; TMZ, temozolomide; PFS, progression-free survival, OS, overall survival; PFS-6, PFS at 6 months; BEV, bevacizumab.
[#]NCT05188508 also has separate cohort for recurrent enhancing grade 2–3 IDH-mutated gliomas.

an MGMT inhibitor in glioma, which has been shown to potentiate the effects of TMZ.[74] Finding effective ways to inhibit MGMT could perhaps improve the efficacy of this agent, but so far this approach remains challenging.[75]

Genetic drivers.—Genetic drivers of glioma may also themselves affect response to DNA damage. For example, p53 and MDM2 mutations are commonly observed in GBM and are direct regulators of DDR, in addition to mutations in cell cycle regulators such as CDK4 and CDKN2A/B.[37] While mutations in these presumed driver mutations may directly affect response to DNA damage, the interactions between driver mutations and DDR are likely more complex. GBM is heterogeneous, and data from studies of glioma stem cells suggests that this subpopulation may exhibit an *increase* in DDR activation, which may drive clinically aggressive phenotypes such as radioresistance.[76,77] There has been increased understanding of interplay of genetic drivers of GBM and DDR, including work showing increased sensitivity to PARP in patients with EGFR amplification[78] and interplay between PTEN phosphorylation and attenuated DNA repair.[79] Likewise, there is work evaluating the role of the PI3K pathway, often activated in GBM, in DNA replication and genomic stability.[80]

Targeting DDR Kinases and PARP in GBM

ATM.—Several ATM inhibitors have been developed for clinical applications. KU-55933 was one of the first identified ATM small molecules and was noted to have potent radiosensitizer properties in vitro.[81] This led to improved compounds such as the non-CNS-penetrant KU60019,[82] CP466722,[83] AZ31, AZ32,[30] WSD-0628,[84] and SJ573017/SJ573226.[85] Recently, AZD0156, an ATM inhibitor optimized from AZ31, showed potentiation of irradiation and PARP inhibition in preclinical models of extracranial tumors.[86] Importantly, a brain-penetrant, orally-available inhibitor of ATM, AZD1390, has been developed and shown to significantly potentiate radiation response across a number of brain tumor models, including adult and pediatric GBM, without obvious CNS toxicity, consistent with findings in mice selectively lacking ATM in the brain.[87,88] PET imaging in humans suggests that AZD1390 crosses the intact blood-brain barrier (BBB).[89]

Several ATM inhibitors have entered clinical trials, and almost all combine ATM inhibition with either RT or DNA-damaging chemotherapy. In addition to clinical trials evaluating AZD0156 and M3541 (combined with olaparib, NCT03225105) in extracranial tumors,[40,90] a phase 0/Ib trial of AZD1390 administered concurrently with RT for adults with brain metastases and GBM is nearing completion (NCT03423628), with initial results suggesting that it is well-tolerated and achieves meaningful concentration in non-enhancing tissue and suppresses induction of pRAD50 ex vivo after RT.[91]

ATR.—ATR inhibition enhances the effects of TMZ-induced cell death in GBM cell lines by inducing apoptosis.[68] However, TMZ can also trigger survival mechanisms such as senescence, which are characterized by activation of

DDR and cell cycle arrest.[92] This senescence induction contributes to recurrence and depends on the ATR-CHK1 pathway. It has been shown that TMZ activates ATR in an MGMT-dependent fashion, and MGMT-deficient cells treated with TMZ demonstrate an increased sensitivity to ATR inhibitors in in vitro and in vivo GBM models.[93] Additionally, ATR and CHK1 pathways are activated in glioma stem cells after radiation, and inhibiting these pathways can increase radiation sensitivity and induce mitotic catastrophe.[41]

Several clinical investigations of ATR inhibition have been pursued. Berzosertib (formerly M6620, VX-970) is an ATR inhibitor evaluated in GBM and lung cancer brain metastasis, with conflicting preclinical data regarding CNS penetration and in vivo intracranial efficacy.[94] Another ATR inhibitor AZD6738, which has shown manageable toxicity and efficacy in patients with gastric cancer,[95] has entered phase I/II trials; however, AZD6738 did not appear to have a radiosensitizing effect in preclinical GBM models.[96] Other ATR inhibitors such as BAY1895344 have shown blood-brain barrier penetrance[97] and promising antitumor efficacy in combinations with DNA-damaging therapies in preclinical studies.[98]

There are several ongoing clinical trials that involve treating advanced solid cancers with RT or chemoradiation in combination with ATR inhibitors, such as BAY1895344, which has shown an acceptable safety profile as monotherapy.[99] An ongoing neuro-oncology-focused trial is evaluating the ATR inhibitor M6220 in combination with whole-brain RT to treat non-small cell lung cancer patients with brain metastases (NCT02589522). Taken together, these findings suggest that the addition of an ATR inhibitor to chemoradiation may increase tumor sensitivity to these standard-of-care adjuvant therapies. BBB penetration and acceptable toxicity profiles, particularly in combinatorial regimens, remain ongoing areas of investigation.

DNA-PK.—Significant work evaluating the role of DNA-PK in gliomas is ongoing. DNA-PK inhibitors may be combined with DNA-damaging therapies (chemotherapy and/or RT), or may have monotherapy potential in tumors with aberrant DNA repair mechanisms.[100] Earlier studies have suggested that hyperactivation of DNA-PKcs was associated with glioma development and survival of glioma cells.[101] Inhibition of DNA-PKcs sensitized glioma cells to TMZ, primarily through regulation of AKT signaling. Furthermore, in studies of glioma cancer stem cells, there is evidence that DNA-PK serves an important role in regulating cellular overgrowth, radioresistance, and glioma progression.[102] Initial studies with the DNA-PK inhibitor KU0060648 showed reduction in tumor proliferation in vitro and in vivo.[101] Treatment with VX-984 (also known as M9831), a DNA-PKcs inhibitor, has been shown to enhance radiosensitivity of GBM cells in a concentration-dependent fashion, both in vitro and in vivo.[103] Additionally, absence of DNA-PKcs appears to correlate with radiosensitivity of glioma cell lines, an effect that is rescued with DNA-PKcs re-expression.[46,100]

There are several DNA-PK inhibitors that have progressed into clinical trials. VX-984 with and without pegylated liposomal doxorubicin has been tested in a phase I study for advanced solid tumors (NCT02644278), as well as AZD7648 with or without other cancer agents (NCT03907969). DNA-PK inhibition has also been combined with other chemotherapies to potentiate effects in GBM cells.[47] Further along in development, M3814 (nedisertib), an orally available agent, was first shown to have antitumor activity in mouse models in combination with RT.[48,49] It is currently being tested in a phase I window-of-opportunity trial in newly diagnosed MGMT unmethylated GBM combined with RT (NCT04555577).

CC-115 is a CNS-penetrant, oral dual inhibitor of mTOR and DNA-PK.[104] While initial phase I testing demonstrated good tolerability and ability to cross the BBB,[105] further testing through the Individualized Screening Trial of Innovative Glioblastoma Therapy (INSIGhT) phase II trial (NCT02977780)[106] did not demonstrate significant clinical benefit and was discontinued due to unfavorable toxicity.[42] Despite these initial findings, there remains interest in exploiting these pathways for therapeutic advances.

PARP.—PARP inhibition is a proven therapeutic strategy in several malignancies and is now approved by the US Food and Drug Administration for use in breast and ovarian cancers with BRCA1/2 mutations.[20] While only a minority of gliomas have a conventional BRCA defect, PARP1 inhibition has been shown to increase radiosensitivity and enhance the therapeutic ratio of RT in human glioma lines in vitro.[107] Likewise, the use of PARP inhibitors has shown chemo-potentiating effects with TMZ in both in vitro and in vivo GBM models.[108] Of note, MGMT methylation appears to also predict benefit from the combination of TMZ and veliparib in orthotopic xenograft models.[44]

Given the established safety profiles of several PARP inhibitors in non-glioma populations and the preclinical data demonstrating potential efficacy in primary brain tumor patients, multiple clinical trials have tested PARP inhibitors in GBM patients. For example, rucaparib and talazoparib likely do not have sufficient BBB penetration to have meaningful activity in the brain based on animal models of GBM,[45,109] and clinical testing in glioma patients has focused primarily on alternative PARP inhibitors, including olaparib, veliparib, and niraparib.

The PARP inhibitor olaparib has been shown to have radiosensitizing effects in multiple glioma cell lines, as well as pediatric brain tumor cell lines.[107,110] A phase I trial evaluated pharmacokinetics and tolerability of olaparib combined with TMZ (OPARATIC, NCT01390571) and showed that olaparib can reliably reach recurrent GBM tumors at clinically meaningful concentrations.[51] Of note, these results contrasted with initial preclinical work that suggested olaparib had poor BBB penetration. PARADIGM and PARADIGM-2 are currently evaluating olaparib in newly diagnosed GBM patients receiving concurrent radiation therapy, with or without TMZ based on MGMT methylation status.[111,112] The OLA-TMZ-RTE-01 trial is testing the use of olaparib with chemoradiation in unresectable high-grade gliomas (NCT03212742).[113] A randomized phase II study compared olaparib combined with cediranib relative to bevacizumab in recurrent GBM patients and did not show a significant survival benefit (NCT02974621).[114]

Veliparib is another PARP inhibitor known to cross the BBB,[115,116] and it has been shown to have better brain penetration relative to talazoparib and rucaparib though initial clinical trial results have not been favorable in GBM.[116] Clinical testing has demonstrated toxicity concerns with veliparib and TMZ (NCT00770471)[117] and lack of significant clinical benefit in combination with radiotherapy (eg, ACTRN12615000407594, VERTU).[118–120] Given prior evidence that veliparib-mediated TMZ sensitization preferentially occurs in MGMT promoter methylated patients,[44] veliparib was tested in a randomized phase II/III trial in patients with MGMT-methylated GBM (NCT02152982, Alliance A071102), but it again did not demonstrate evidence of progression-free or survival benefit in the overall population.[121]

A preclinical study evaluating the pharmacokinetics of PARP inhibitors showed that niraparib has more favorable tumor and brain distribution compared to olaparib.[122] Ongoing trials are evaluating the use of niraparib in newly diagnosed and recurrent GBM (NCT05076513), as well as with tumor-treatment fields (NCT04221503). Pamiparib, a potent PARP inhibitor and trapper,[123] was generally well-tolerated in a phase 0 study with meaningful accumulation in nonenhancing GBM tissue (NCT03150862).[124] A phase Ib/II study assessing the use of pamiparib combined with RT or chemoradiation in newly diagnosed unmethylated GBM and methylated or unmethylated recurrent GBM has completed accrual (NCT03150862).

Most of the first-generation PARP inhibitors are dual PARP1 and PARP2 inhibitors/trappers. Since only PARP1 trapping is required for synthetic lethality in HR repair, while PARP2 inhibition is linked with hematologic toxicity, there is interest in developing selective PARP1 inhibitors and DNA trappers such as AZD5305 or AZD9574 (NCT05417594) alone and in combination with temozolomide.[125]

Moving forward, there will need to be continued emphasis on rigorous preclinical and clinical assessment of BBB penetration, pharmacokinetic/pharmacodynamic endpoints, and monitoring of toxicities when combined with radiation therapy and TMZ in the clinical development of PARP inhibitors for the treatment of GBM. The interplay of DDR inhibitors with TTF also remains an area of interest given possible synergistic benefits in preclinical studies that should be further explored.[126]

IDH-Mutant Gliomas

IDH mutant gliomas exhibit distinct biology from their IDH-WT counterparts. The most common IDH mutation observed in glioma patients is IDH1-R132H, representing an arginine-to-histidine substitution. The IDH1-R132H mutant protein is a neomorph and synthesizes the oncometabolite (*R*)-2-hydroxyglutarate (2HG), which competitively inhibits 2-oxoglutarate (2OG)-dependent enzymes and contributes to glioma formation.[127] Many of the elucidated cellular changes conferred by mutant IDH1 converge on mechanisms related to DNA damage, and we highlight efforts to translate these efforts into clinical practice in this section. Selected clinical trials of DDR agents for IDH-mutant gliomas are summarized in Table 2.

Mutant IDH and Homologous Recombination

Given that many 2OG-dependent enzymes function as histone and DNA demethylases, it is perhaps unsurprising that multiple reported mechanisms of DNA damage alterations in IDH-mutant gliomas invoke epigenetic changes. One of the more mature lines of work in this regard

Table 2. Select Completed and Ongoing Clinical Trials in IDH-Mutant Gliomas

Trial	Patient population/ disease setting	Target	Experimental therapy	Phase of testing	Status
NCT03561870[128]	Recurrent IDH-mutant gliomas -35 patients	PARP inhibitor	Olaparib	II	Completed - Olaparib was well tolerated - Median PFS 2.3 months - Median OS 15.9 months
NCT05417594	Recurrent IDH-mutant glioma	PARP1 inhibitor	AZD9574 and TMZ	I	Accruing
NCT05188508	Recurrent grade 2–3 IDH-mutant	PARP inhibitor	Olaparib, TMZ, and pembrolizumab	II	Accruing
NCT05076513	Recurrent grade 2–4 IDH-mutant astrocytoma	PARP inhibitor	Niraparib	0	Accruing
NCT03914742	Recurrent grade 2–4 IDH-mutant glioma	PARP inhibitor	Pamiparib and metronomic TMZ	I/II	Not accruing
NCT03991832	Recurrent IDH-mutant glioma	PARP inhibitor	Olaparib and durvalumab	II	Accruing
NCT03528642	Grade 2–3 IDH-mutant gliomas	Glutaminase inhibitor	Telaglenastat	I	Not accruing
N/A	Grade 4, recurrent IDH- mutant	de novo pyrimidine synthesis inhibitor	Orludodstat	0	In development

TMZ, Temozolomide; PFS, progression-free survival; OS, overall survival.

includes a series of preclinical studies and ongoing clinical trials testing the hypothesis that IDH-mutant gliomas display a "BRCAness" phenotype with defects in HR.[129] In this model, (*R*)-2HG inhibits the 2OG-dependent KDM4B dioxygenase, resulting in hypermethylated H3K9 and a defect in DNA damage recognition. Therefore, DSB repair is compromised and renders IDH-mutant gliomas sensitive to PARP inhibitor treatment. In addition to this mechanism based on H3K9 hypermethylation, hypersensitivity to PARP inhibitor and TMZ treatment may also be related to impaired NAD + metabolism in IDH-mutant gliomas.[130] Of note, recent data challenge this model and suggest that mutant IDH increases replication stress due to an increase in heterochromatin protein formation and slowing of cell cycle progression, in a HR-independent manner.[131]

Clinical trials testing PARP inhibitors in IDH-mutant gliomas are now ongoing (NCT03561870, NCT05076513, and NCT03914742). The OLAGLI trial was a single-arm phase II study that enrolled 35 recurrent IDH-mutant high-grade glioma patients, who were treated with olaparib monotherapy. At a median follow-up of 11 months, 30 of the 35 patients had developed tumor progression. Median progression-free survival and overall survival were 2.3 and 15.9 months, respectively.[132] Results from a phase II trial testing olaparib and durvalumab combination therapy in IDH-mutant gliomas have also been reported, with 1 out of 9 patients achieving objective tumor response after 8 cycles. Ongoing efforts are testing olaparib in combination with TMZ (ABTC1801, NCT03991832), and another trial is testing this combination in newly diagnosed and recurrent pediatric/adolescent and young adult IDH-mutant gliomas (PNOC017; NCT03749187). While most PARP inhibitors target PARP1 and PARP2, AZD9574 is a brain-penetrant selective inhibitor and optimized trapper of PARP1 that is being tested with TMZ in IDH-mutant tumors (NCT05417594). Combining PARP inhibitors with additional systemic agents and/or radiation may yield enhanced efficacy in IDH-mutant gliomas.[133,134]

It should be noted that the effect of IDH mutation on PARP inhibitor sensitivity may be context-dependent and could partially explain the lack of strong clinical benefit in the prospective trial data reported thus far. Mutant IDH was also reported to increase RAD51-mediated HR and confer resistance to TMZ-induced DNA damage.[135] This is also consistent with other data[136] suggesting that IDH mutations increase RAD51 expression as compared to IDH-WT controls.

Sensitivity to DNA-Damaging Therapies

In addition to potential defects in HR in IDH-mutant gliomas, multiple other mechanisms linking IDH mutations to DNA damage have been explored (Figure 4). Many of these mechanisms rely on therapeutically exploiting vulnerabilities related to DNA damage repair conferred by mutant IDH through epigenetic, transcriptional, and/or metabolic reprogramming.

Standard of care treatment for IDH-mutant glioma involves DNA-damaging therapies, namely radiation therapy, TMZ, and/or other alkylating agent-based regimens such as procarbazine, lomustine, and vincristine. As such, there have been efforts to better understand how mutant IDH affects response to these agents in gliomas. The effect of IDH mutations on radiation sensitivity has been investigated, though with conflicting results. While some studies in engineered glioma cells or hematopoietic stem cells report that mutant IDH may synergistically increase sensitivity to radiation,[133,137,138] other preclinical data suggest that IDH mutations increase DNA repair mechanisms[135] and may confer radioresistance.[136] The direct role of IDH mutations in TMZ response has also been explored in previous studies.[139,140] These studies delineate the function of the AlkB family of proteins, which are 2OG-dependent enzymes that directly repair alkylated DNA.[141,142] These AlkB proteins are thought to be inhibited by mutant IDH, sensitizing IDH-mutant glioma cells to the alkylating agents CCNU and procarbazine. Importantly, this effect was dependent on the catalytic activity of mutant IDH1, as catalytically dead double-mutant IDH1 protein rescued sensitization to alkylating agents.

Many preclinical studies have introduced promising novel therapeutic strategies exploiting DNA damage deficits in IDH-mutant gliomas, leveraging a synthetic lethality approach. Consistent with the known epigenetic alterations caused by IDH mutations in gliomas, recent work demonstrated that mutant IDH increases expression of NRF2 pathway genes involved in antioxidant gene expression, the kinetics of which mirror that of an epigenetic mechanism. This results in reliance on these genes to maintain ROS homeostasis,[143] suggesting that mutant IDH may alter response to ROS-induced DNA damage through changes in the epigenome. In this regard, others have reported multiple mechanisms of disrupted ROS homeostasis conferred by mutant IDH in glioma. Mutant IDH1 generates (*R*)-2HG through an NADPH-dependent reaction, which can then affect the ability to generate reduced glutathione, necessary for protecting DNA from ROS-induced DNA damage.[144] Glutathione pools are also affected by (*R*)-2HG-dependent inhibition of 2OG-dependent transaminases (BCAT1 and BCAT2), resulting in an increased reliance on glutaminase for glutathione synthesis.[145] Pharmacologic inhibition of glutaminase has been shown to deplete glutathione and sensitize IDH-mutant gliomas to radiation[145] with acceptable early safety reported with the glutaminase inhibitor telaglenastat.[146]

More recently, the susceptibility to DNA damage has been shown to render IDH-mutant gliomas sensitive to drugs that inhibit pyrimidine nucleotide synthesis. IDH mutations predict sensitivity to the drug orludodstat (BAY 2402234), an inhibitor of the de novo pyrimidine synthesis enzyme dihydroorotate dehydrogenase. Orludodstat treatment induces nucleotide pool imbalance, increased DNA damage, and cell death in the presence of mutant IDH, and this drug will now undergo clinical testing in an upcoming early-phase clinical trial. Additional metabolic vulnerabilities conferred by mutant IDH have been described. In this work, mutant IDH1 decreased pools of NAD+, an important substrate for PARP-mediated repair of DNA damage, which can be exploited by the use of NAMPT inhibitors that further decrease NAD + pools and limit the PARP-mediated repair of DNA damage caused by alkylating agents. NAD + depletion through inhibitors of poly(ADP-ribose) glycohydrolase (PARG), which leads to NAD + sequestration, has also been shown to be a promising strategy for IDH-mutant gliomas based on preclinical data.[147–149]

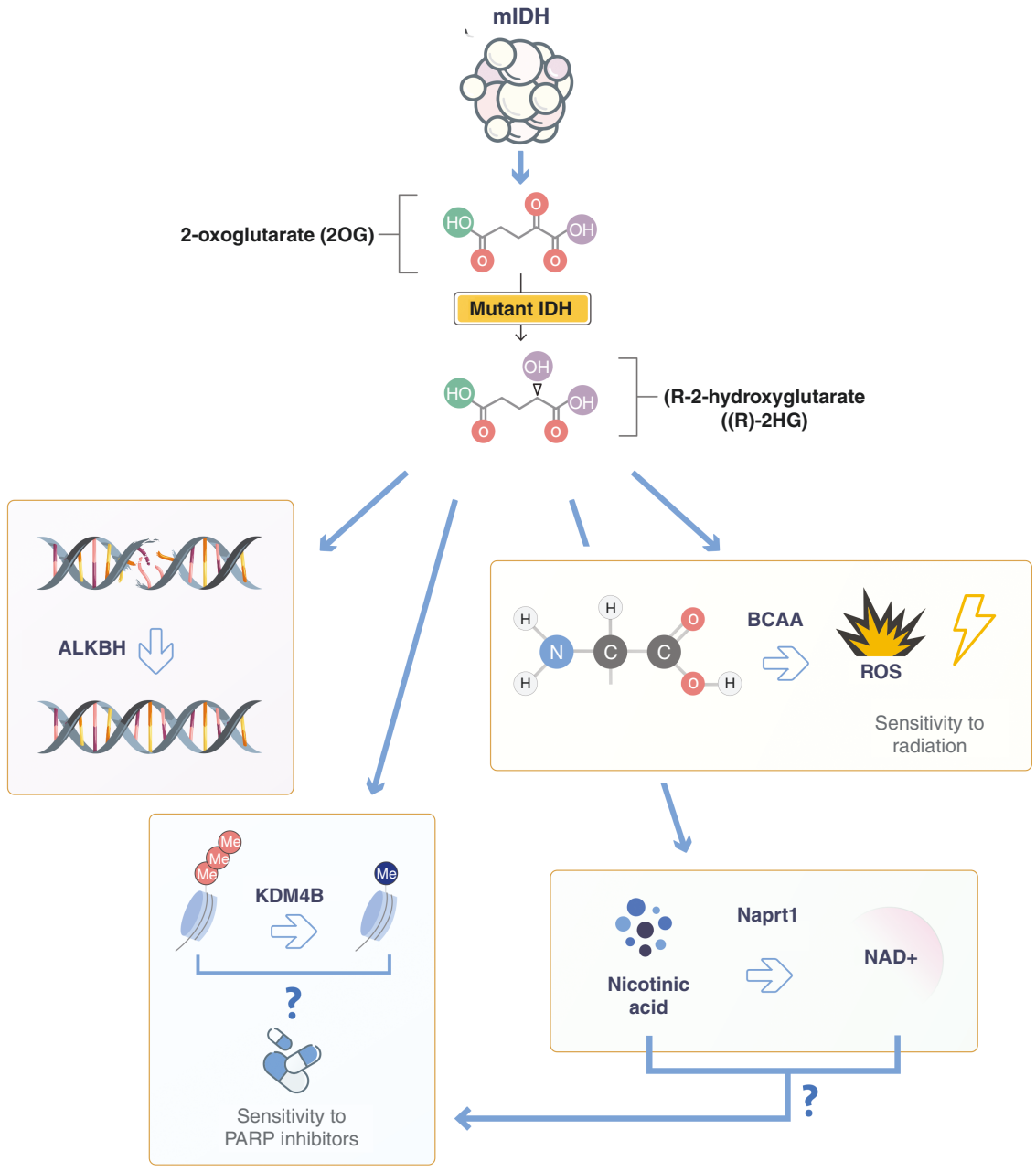

Figure 4. Overview of select altered DNA damage repair pathways that have been reported in IDH-mutated gliomas. (*R*)-2HG-mediated inhibition of ALKBH is thought to sensitize IDH-mutant gliomas to alkylating agents (far left). Inhibition of the histone demethylase KDM4B by (*R*)-2HG sensitizes IDH-mutant gliomas to PARP inhibition (middle left) and depletes NAD + pools (middle right), rendering IDH-mutant gliomas vulnerable to DNA repaired by NAD+-dependent enzymes such as PARP. (*R*)-2HG inhibits BCAAs including BCAT1, which depletes glutathione pools and sensitizes IDH-mutant gliomas to oxidative stress (far right). Abbreviations: mIDH, mutant IDH; BCAA, branched-chain amino acids.

Ongoing clinical trials will provide important insights into how IDH mutations may predict response to some of the novel targeted therapies discussed above, as well as standard-of-care treatments including radiation and TMZ. Clinically, the timing and use of TMZ as concurrent, adjuvant, or monotherapy treatment has been the subject of large clinical trials. The benefit of adjuvant TMZ in IDH-mutant, 1p/19q non-codeleted tumors has been supported by recently updated results from the CATNON trial,[150] where 12 cycles of adjuvant TMZ conferred an overall survival benefit specifically within the IDH-mutant subset of patients. Of note, the addition of concurrent TMZ with radiation did not improve survival in this cohort, suggesting that TMZ may be more effective as an adjuvant monotherapy rather than delivered concurrently with radiation. However, among IDH-mutant, 1p/19q codeleted patients,

the CODEL trial reported worse progression-free survival outcomes in the TMZ alone arm (no radiation) as compared to the radiation-containing arms (radiation alone and radiation with concurrent and adjuvant temozolomide).[151] Taken together, these data suggest that TMZ could be most effective when used sequentially following radiation; alternatively, it is possible that the most effective use and timing of TMZ are different in IDH-mutant 1p/19q codeleted versus non-codeleted tumors. The relative benefit of TMZ in IDH-mutated 1p/19q codeleted versus non-codeleted tumors has not been compared in a prospective randomized clinical trial. The potential disease control benefits of TMZ monotherapy should be contextualized within the risk of possible malignant transformation. Low-grade astrocytomas have been reported to possibly undergo TMZ-induced malignant transformation to more hypermutated and aggressive tumors,[152,153] which should be a consideration when using TMZ monotherapy in low-grade IDH-mutant gliomas.

Additional Considerations in Targeting DNA Damage Pathways

Molecular Predictors of Response

The discovery of molecular markers in glioma over the past several decades has revealed important insights into the biological heterogeneity of this disease. While some molecular markers (such as IDH and H3K27M alterations) comprise formal diagnostic criteria in the WHO classification,[154] the impact of these markers on predicting response to specific therapies is still not fully understood. As a representative example, the OLAGLI trial presented modest clinical outcomes in a heavily pretreated IDH-mutant glioma population, but 2 patients had a durable benefit and remained on treatment 16–18 months after treatment started, suggesting that a subset of patients may benefit.[132] Given the known intertumoral heterogeneity of therapeutic response to such therapies, the discovery of predictive biomarkers will be an important component of the development of DDR agents.

Perhaps the prognostic and predictive biomarker with the most mature data is the use of alkylating agent TMZ in MGMT-methylated gliomas. As discussed above, MGMT-methylated tumors have been shown to respond better to TMZ,[3] and MGMT methylation status may now guide use of TMZ in the adjuvant setting. Preclinical data also suggest that IDH mutations may confer specific DNA damage deficits that sensitize these tumors to various DNA-damaging agents (see "IDH-mutant gliomas") and may be a useful biomarker, pending ongoing clinical studies.

Aside from these established molecular biomarkers, other molecular biomarkers have been proposed as ways to predict response to DNA-damaging therapies. For example, preclinical data may support the use of therapies based on p53 mutation status, with one preclinical study demonstrating that p53 mutant gliomas display increased sensitivity to the ATM inhibitor KU60019.[29] This is further supported by separate work demonstrating that p53-deficient, but not p53 wild-type, DIPG in genetically engineered mice display an increase in radiosensitivity upon genetic deletion of *Atm*, which supports the use of ATM inhibitors as radiosensitizers in p53 mutant gliomas.[155] In contrast, treatment with the clinically advanced ATM inhibitor AZD1390 significantly improved the efficacy of radiation in both p53 wild-type and p53 mutant isogenic cell lines and distinct orthotopic xenograft models of pediatric HGG, including H3K27M-mutant diffuse midline glioma,[87] suggesting that in the context of genetically diverse human tumors, the use of p53 mutation status as a singular predictive biomarker for combination ATM inhibition therapy may be limiting. Furthermore, when tested in a colorectal cancer cell line, the effect of ATM inhibition on radiosensitivity was independent of p53 status.[156] These differences may be due to cancer context-dependent effects of p53, impact of other mutations concurrent with p53 loss, and/or differential effect of p53 mutations on the use of ATM inhibitors as a monotherapy versus as a radiosensitizer. Indeed, the effect of ATM inhibition appears to have tissue-specific effects, as evidenced by the fact that *Atm* deletion appears to induce differing degrees of radiosensitivity depending on tissue type.[157–159]

While ATM inhibitors have been tested in synthetic lethal strategies with p53 loss as described above and are undergoing clinical testing in brain tumor patients, ATM loss may also serve as a predictive biomarker in and of itself, with a prior study suggesting that it impairs HR, raising the possibility that ATM loss may predict sensitivity to PARP inhibitors.[160] The effect of ATM loss on HR was partly compensated for by ATR in this study, in line with separately reported data suggesting that ATM deficiency may be sensitized to ATR inhibitors in leukemia[161] and pediatric high-grade-glioma.[87]

With regards to PARP inhibitors, genomic alterations beyond BRCA1/2 mutation, such as genomic signatures associated with HR deficiency, represent important possible biomarkers that will need to be further evaluated across different cancers to potentially identify patients most likely to benefit from these therapies.[162]

As data within glioma and other cancer types mature, insights into the mechanisms and cell biology of DNA damage pathways in glioma remain critical to inform rational design of therapeutic strategies and identification of relevant biomarkers.

Combination With Immunotherapy

Immunotherapy approaches have largely failed to improve outcomes in gliomas.[163–165] While there is extensive ongoing work to better understand the biology behind this lack of efficacy, GBMs are generally thought to be immunologically "cold" or "exhausted." There has been substantial interest in using DDR inhibitors to increase DNA damage, stimulate neoantigen production, and alter the inflammatory tumor microenvironment to increase the likelihood of improving the activity of immunotherapy (Figure 5).[166] A prior window of opportunity study in patients with recurrent GBM receiving pembrolizumab showed an absence of an effector immunologic response in most patients, potentially due to scarcity of T cells within the tumor microenvironment.[167]

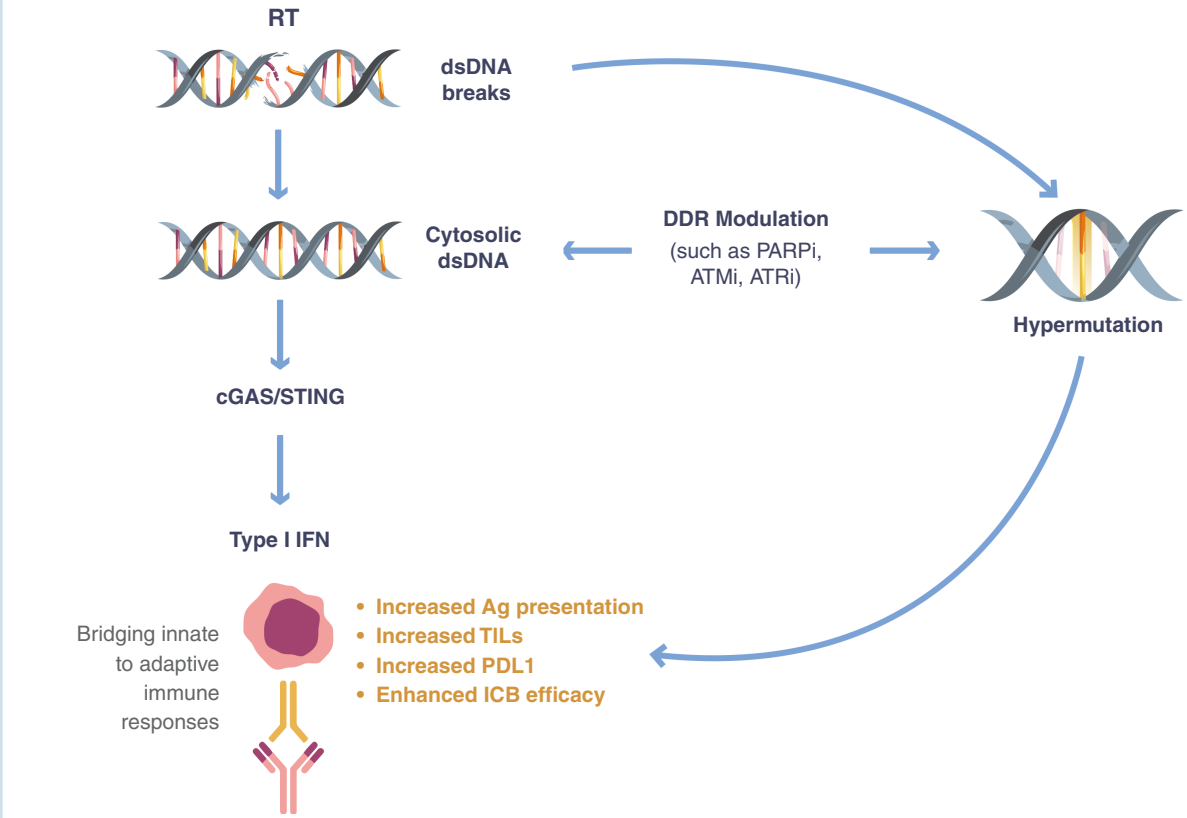

Figure 5. Molecular pathways by which modulation of the DNA damage response (DDR) could potentiate immune processes. DDR modulating agents such as PARP inhibitors, ATM inhibitors, and ATR inhibitors may increase production of extrachromosomal double-stranded DNA (dsDNA) in the cytosol, especially in the setting of cytotoxic therapies such as radiation therapy that can cause double strange DNA breaks. Such cytosolic dsDNA can be detected by cGAS/STING to potentiate innate immune responses by stimulating type I interferon expression, antigen presentation, tumor-infiltrating lymphocyte recruitment, PDL1 expression, and improved responses to immune checkpoint blockade therapy. Alternatively, DDR modulation could drive tumor hypermutation, which may also have immune modulatory effects. Abbreviations: Ag, antigen; TILs, tumor-infiltrating lymphocytes; ICB, immune checkpoint blockade; DDR, DNA damage response.

There is growing evidence that DNA damage and DDR deregulation can enhance immune responses and increase PD-L1 expression.[168,169] Indeed, emerging data suggest that pediatric gliomas with germline MMR deficiency may be uniquely sensitive to immune checkpoint blockade.[170] Given evidence of increased likelihood of benefit with checkpoint inhibitor therapy in other solid tumors with high tumor mutation burden,[171] an active area of study is to evaluate whether DDR inhibitor-induced hypermutation could augment responses to checkpoint inhibitors. This may be of particular interest in light of recent work describing hypermutated states of GBM following treatment with TMZ.[12] Preliminary data, however, suggest that checkpoint inhibitor therapy may have less activity in hypermutated GBM following temozolomide therapy relative to other malignancies.[12] Strategies to increase tumor mutational burden in glioma include the use of PARP inhibitors. It has been increasingly appreciated that PARP inhibitors may act at least in part through the innate immune system and activate the cGAS-STING pathway and potentially other DDR pathways (ATM-TR-CHK1).[172,173] Activation of the cGAS-STING pathway may also stimulate type 1 interferon, increase tumor-infiltrating lymphocytes, and induce antitumor activity independent of BRCA status.[174] As such, there is an ongoing basket trial evaluating the use of olaparib with durvalumab for IDH-mutated solid tumors (NCT 03991832),[175] and trials testing olaparib, pembrolizumab, and TMZ in recurrent IDH mutant gliomas (NCT05188508) and recurrent IDH-WT GBM (NCT05463848). Further trials are expected given the rationale for combining DDR inhibitors with immunotherapy.

Non-canonical Targets for DNA-Damaging Therapies

While direct inhibition of key functional enzymes in DNA damage repair may be a promising therapeutic strategy and is currently undergoing testing (see "Molecular Predictors of Response"), many promising targets exist that are not canonical DNA damage enzymes. For example, multiple synthetic lethal drug targets that exploit DNA damage deficits that are not directly involved in DNA damage repair (see "IDH-mutant gliomas"). Additionally, preclinical and early clinical data suggest roles for druggable targets that may potentiate DNA-damaging therapies such as radiation.

For example, cell cycle inhibitors such as CDK4/6 inhibitors have been shown to act as radiosensitizers in in vitro models across cancer types, including medulloblastoma,[176] atypical teratoid rhabdoid tumors,[177] and GBM.[178] More recent work has also demonstrated the efficacy of combining CDK4/6 inhibitors with cytotoxic chemotherapy.[179] However, the mechanisms underlying these interactions are not fully understood. Furthermore, while CDK4/6 inhibitors are incorporated into standard of care in subsets of breast cancer, use of CDK4/6 inhibitors as a radiosensitizer may pose challenges with regard to toxicity and tolerability, although initial studies recently completed have not identified any undue toxicities.[180]

Additional potential radiosensitizers include drugs blocking vascular endothelial growth factor (VEGF), which have been shown experimentally to increase radiosensitivity through suppression of autophagy in nasopharyngeal carcinoma[181] and enhance radiation response in preclinical schwannoma models.[182] While some conflicting data exist,[38] other groups have reported radiosensitization with VEGF inhibitors across multiple cancer types, including GBM.[39,43] Similar to the radiosensitization effects of CDK4/6 inhibitors, the mechanism of potential synergy between VEGF inhibitors and radiation is not fully understood. Importantly, the VEGF-A inhibitor bevacizumab was not shown to improve survival when added to chemoradiation in GBM patients,[50] suggesting that if such a radiosensitization effect does exist in glioma, further work to identify subsets of patients that benefit is needed to better translate these findings to clinical practice.

Cell cycle inhibitors and VEGF inhibitors are 2 examples of therapeutic targets outside of classical DNA damage enzymes that may directly potentiate DNA damage or prevent DNA damage repair. Emerging data continue to shed light on additional enzymes that may play important roles in DNA damage and serve as additional therapeutic targets. For example, some evidence suggests that the MAPK/ERK/Akt pathway harbors direct DNA damage repair function.[52,128,183,184] As such, these "non-canonical" DNA damage enzymes may become additional viable therapeutic targets that leverage alterations in the DDR for antitumor efficacy.

Clinical Trial Considerations

While there are many clinical trials evaluating DDR inhibitors across neuro-oncology, several considerations have become apparent in designing clinical trials for these therapies. Some of the challenges with the development of DDR inhibitors include poor correlation between preclinical studies and clinical outcomes, poor BBB penetration associated with limited drug exposure, poorly defined patient-selection criteria and endpoints, difficulty ascertaining response (particularly in the setting of chemoradiation)[185] or obtaining pathological correlates via biopsy, challenging toxicity in combining with existing standard-of-care therapies of radiation therapy and chemotherapy, and/or insufficient financial investment.[186]

Given the distinct challenges with drug development for the treatment of CNS diseases, there have been many failures. Thus, more efficient testing of therapies early in development is critical to better identify therapies most likely to be successful.[186]

Prior studies have indicated significant variability of clinically tested DDR inhibitors with respect to BBB penetration. Furthermore, while BBB penetration is commonly cited as a primary concern, it is just as important to ensure that biologically active concentrations and target engagement can be achieved in tumor tissue. Because BBB is often disrupted in malignant gliomas, potent drugs can still achieve sufficient exposure in the tumor core, but only drugs specifically designed to cross the BBB will be able to target glioma cells that have invaded surrounding "normal" brain parenchyma with intact BBB.

Evaluation of treated tumor tissue in patients has long been identified as a need in neuro-oncology,[187] though implementation has been slow. Surgical window of opportunity trial designs represent an important consideration that can be used to identify intratumoral concentrations of experimental therapies and generate pharmacodynamic data to understand the effects of drugs on tumor tissue by assessing surgical specimens after therapy administration.[188,189]

A representative example of a window of opportunity study tests the DNA-PK inhibitor M3814 in combination with radiation therapy in MGMT unmethylated newly diagnosed GBM (NCT0455577).[190] In the first stage, patients receive M3814 concurrently with RT; in the second stage, patients receive standard-of-care RT with M3814 followed by surgical resection within 1–14 days of completing RT. Window of opportunity trials provides an opportunity to understand target engagement as well as the mechanism of response. Use of this trial design could allow for a more efficient and reliable selection of therapies more likely to be successful in later phase testing. Pharmacokinetics/pharmacodynamics (PK/PD) and window of opportunity studies to confirm target inhibition will also continue to be important in this regard.

Beyond the window of opportunity trials, testing of DDR inhibitors could be more efficiently conducted in later-phase trials that also utilize novel trial designs.[186,191] DDR inhibitors have been tested on adaptive platform trials, including CC-115 on INSIGhT.[192] Regardless of trial design, there should be careful monitoring of toxicities, particularly when combining them with standard-of-care therapies. Assessment of toxicity for new therapeutic strategies that combine a new therapy with radiation can be challenging, and causality can be difficult to determine against the expected toxicity of treatment. Furthermore, there may be higher rates of pseudoprogression with DDR agents, which raises the need for confirmation scans and implementation of response assessment criteria that allow for confirmation scans to increase fidelity of response assessments.[185]

Future Directions

The future of therapies targeting the DDR in gliomas requires focused investigations in several key areas. First, there is a crucial need for improved preclinical models that faithfully recapitulate the complex biology and genetic heterogeneity of gliomas, enabling better prediction

of therapeutic responses. Furthermore, exploring the potential of combination therapies is essential, as targeting multiple DDR pathways simultaneously may enhance treatment efficacy. In parallel, correlative studies aimed at elucidating the molecular determinants of response and resistance to DDR-targeted therapies are imperative. Understanding the mechanisms underlying resistance will guide the development of strategies to overcome treatment obstacles. Biomarker discovery and validation are pivotal for identifying patients most likely to benefit from DDR-targeted therapies, enabling personalized treatment approaches. As described above, the rational combination of DDR-targeted therapies with immunotherapies holds immense potential, as it can exploit the interplay between DNA damage and the immune system, enhancing the overall therapeutic response. Finally, comprehensive studies exploring the timing, sequencing, and dosage of radiation in combination with DDR-targeted agents will be vital to determine the most effective methods to rationally combine investigational therapies with radiation. Future investigations in these areas will propel the development of effective and personalized treatments for gliomas, improving patient outcomes.

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