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# New perspective on DNA response pathway (DDR) in glioblastoma, focus on classic biomarkers and emerging roles of ncRNAs

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## Abstract

**Background.** Glioblastoma (GBM) is the most frequent type of primary brain cancer, having a median survival of only 15 months. The current standard of care includes a combination of surgery, radiotherapy (RT) and chemotherapy with temozolomide, but with limited results. Moreover, multiple studies have shown that tumour relapse and resistance to classic therapeutic approaches are common events that occur in the majority of patients, and eventually leading to death. New approaches to better understand the intricated tumour biology involved in GBM are needed in order to develop personalised treatment approaches. Advances in cancer biology have widen our understanding over the GBM genome and allowing a better classification of these tumours based on their molecular profile.
**Methods.** A new targeted therapeutic approach that is currently investigated in multiple clinical trials in GBM is represented by molecules that target various defects in the DNA damage repair (DDR) pathway, a mechanism activated by endogenous and exogenous factors that induce alteration of DNA, and is involved for the development of chemotherapy and RT resistance. This intricate pathway is regulated by p53, two important kinases ATR and ATM and non-coding RNAs including microRNAs, long-non-coding RNAs and circular RNAs that regulate the expression of all the proteins involved in the pathway.
**Results.** Currently, the most studied DDR inhibitors are represented by PARP inhibitors (PARPi) with important results in ovarian and breast cancer. PARPi are a class of tumour agnostic drugs that showed their efficacy also in other localisations such as colon and prostate tumours that have a molecular signature associated with genomic instability. These inhibitors induce the accumulation of intracellular DNA damage, cell cycle arrest, mitotic catastrophe and apoptosis.
**Conclusions.** This study aims to provide an integrated image of the DDR pathway in glioblastoma under physiological and treatment pressure with a focus of the regulatory roles of ncRNAs. The DDR inhibitors are emerging as an important new therapeutic approach for tumours with genomic instability and alterations in DDR pathways. The first clinical trials with PARPi in GBM are currently ongoing and will be presented in the article. Moreover, we consider that by incorporating the regulatory network in the DDR pathway in GBM we can fill the missing gaps that limited previous attempts to effectively target it in brain tumours. An overview of the importance of ncRNAs in GBM and DDR physiology and how they are interconnected is presented.

## Introduction

Glioblastoma (GBM) is the most common primary brain tumour that accounts for almost half of all primary brain tumours with an survival rate of 4% at 5 years (Refs 1, 2). The worldwide incidence of GBM varies across studies between 0.59 and 5 per 100.000 (Refs 1, 3). The incidence is higher in men with a median age at diagnosis of 64 years old (Ref. 4). GBM is classified as a grade IV aggressive primary brain glioma, and due to advancements in understanding its genomic biology the current WHO classification includes molecular features in defining a GBM, such as the IDH and TERT mutation and EGFR amplification (Ref. 5). Several markers such as TP53 mutation, MGMT, TERT and ATRX play an important role in understanding the pathophysiology of GBM, being as well potential targets for future targeted therapies (Refs 1, 4).

While the next-generation sequencing technologies became more accessible in both research and clinical setups, a more in-depth characterisation of the tumour genomic profile became available (Ref. 6). This allowed the identification of specific genetic anomalies, present

in both the coding and non-coding regions of the genome. Therefore, the repository of therapeutic targets expended, supporting the development of new targeted therapies against GBM that are looking at specific genetic event inhibition or are targeting complex molecular pathways such as DDR (Refs 7–9).

The current therapeutic interventions for GBM include maximal surgical resection, radiotherapy (RT) and concomitant or maintenance temozolomide (TMZ) chemotherapy. However, none of them are curative and most of patients will experience multiple recurrences. In fact, regardless of the numerous ongoing clinical trials, the development of effective therapies is deficient due to high intratumor heterogeneity, lack of adequate control arms, selection bias and small sample size (Refs 10, 11).

Recently, the DNA damage response (DDR) pathway became subject of interest in GBM research after its implications in chemotherapy and RT resistance were better understood given the advanced in understanding of the underling mechanisms (Ref. 12). DDR is involved in detecting the DNA damage, signalling its presence and favouring its repair (Refs 12, 13).

Since both, RT and chemotherapy induce DNA damage and activate DDR, molecules that target DDR inhibition are investigated for their therapeutic role in overcoming treatment resistance (Ref. 14). Surprisingly, even though the DDR pathway activates post-irradiation in both parent cells and recurrent cells, Kaur *et al.* demonstrated on GBM cell line models (U87MG and SF268) that there are differences in the DDR pathway activation in primary *versus* recurrent post-treatment GBM cells. Post-irradiation, recurrent cells prefer a different DDR pathway in comparison to their parents, highlighting the adaptability of GBM cells. As a result, more studies on which DDR enzyme is acting in recurrent GBM should be conducted since the DDR enzymes ATR and ATM have different mechanisms of action (Ref. 15).

DDR are induced as an adaptive reaction to either single or double-strand breaks (SSB/DSB) of the genomic DNA, which occur as a consequence of the endogenous genomic instability associated with GBM development or as a post-conventional treatment side-effect. The restoration of SSB, which are the most common DNA lesions, is mediated by base excision repair (BER), nucleotide excision repair (NER) and mismatch repair (MMR) mechanisms, while the DSB restoration is mediated by either homologous recombination (HR) or nonhomologous end-joining (NHEJ) mechanisms (Refs 16, 17) One clear example for the use of DDR pathway in GBM therapy are the poly ADP-ribose polymerase (PARP) inhibitors, especially those that target PARP-1. Normally, this enzyme binds to the injured DNA site catalysing the formation of ADP-ribose polymers using $NAD^+$ as a substrate and activates the necessary enzymes for BER to be conducted. PARP inhibitors (PARPi) bind themselves to this enzyme, thus blocking the formation of ADP-ribose polymers from $NAD^+$. Therefore, DNA repair is ceased which leads to an increase in genomic instability, growth arrest and apoptosis (Refs 18, 19). These molecules have started to being investigated in GBM, but with limited results which might be due to increased intratumor heterogeneity that allows for rapid development of treatment resistance subclones or to lack of proper cancer biomarkers 25709118 and 34584069. A possible new source of biomarkers that will allow real-time evaluation of the treatment efficacy and development of resistance is represented by non-coding (ncRNAs) (Refs 20–22). Multiple research directions have emerged intending to investigate the expression levels and the regulatory impact of these ncRNAs, especially microRNAs (miRNAs), long non-coding RNAs (lncRNAs) and circular RNAs (circRNA) on the DDR pathways in the context of GBM pathogenesis, chemoresistance and radiation sensitivity. (Refs 23–27). NcRNAs have been intensively studied in multiple

cancers due to their wide tissue distribution, ease of access and potential to be used as biomarkers and treatment targets (Refs 22, 28). In GBM dysregulated ncRNAs can be used both to target altered tumour mechanisms using substation strategies or to down-regulate specific miRNAs (Ref. 29). Hence, due to their versatile activity as GBM promoting or suppressing agents, ncRNAs started to be investigated as valuable therapeutic targets or as biomarkers to evaluate treatment response.

The subject of DDR inhibitors in the management of GBM patients is a highly studied field with an international effort being in place to advance the available treatment options of GBM using DDR inhibitors. The National Brain Tumour Society is the institution driving these efforts, through establishment of a DDR consortium. The DDR consortium has the mission to move forward to the clinic DDR inhibitors through supporting research, encourage exchange of data and evaluation of clinical trials (Ref. 30).

The aim of this review is to decipher the intricated machinery involved in the regulation of DDR pathway in GBM. Our approach will focus on the functional mechanisms activated by both endogenous and exogenous DNA damage-inducible agents. We will use an innovative approach towards DDR by looking at the regulatory roles of ncRNA molecules and how we can use these molecules both as biomarkers and potential therapeutic targets.

## DDR pathway statut in GBM

DNA damage can be induced endogenously, by mutations inducing genomic instability or exogenously by exposure to various harmful factors such as ultraviolet, RT, chemotherapy, reactive oxygen species (ROS) and deregulated metabolism respectively (Refs 31, 32). Alterations in DNA as SSB or DSB are a common event that led to the development of a complex regulatory network to protect and restore DNA integrity represented by the DDR pathway (Ref. 33). DDR pathway prevents damaged DNA to be copied (G1 checkpoint) or transferred to the future generation of cells (G2 checkpoint) during cellular cycle (Ref. 34). The Phosphatidylinositol-3 kinase-like protein (PIKK), includes ataxia–telangiectasia mutated (ATM) and ataxia-telangiectasia Rad3 related (ATR), DNA-Dependent Protein Kinase Catalytic Subunit (DNA-PKs), mammalian target of rapamycin, suppressor of morphogenesis in genitalia and transcription-associated protein (Refs 31, 35). ATM, ATR and DNA-PK are the most known kinases involved in DDR, their integrity being instrumental to the pathway regulation and cell survival (Refs 31, 34).

GBM is a highly treatment-resistant brain malignancy and during its treatment with TMZ and RT is supposed to high DNA damage stress including SSB and DSB (Refs 36–38). ATM is activated in response to DSB and plays a role in repairing the DNA damage by recruiting proteins, signalling the cell checkpoint and inducing apoptosis (Ref. 39). The role of ATM in phosphorylating various compounds (p53, CHK2, H2AX), thus inducing the cell cycle arrest and apoptosis is well-known (Refs 31, 34). The phosphorylation of CHK2, which is the most important ATM transducer, induces the G1 checkpoint arrest and apoptosis (Refs 34, 40, 41). Activated CHK2 activation favours the phosphorylation of CDC25A (one of the most crucial cell cycle regulators) (Ref. 42) which leads to its reduction and blockade of the entrance to the G1 phase (Refs 39, 41, 43). However, G1 checkpoint arrest is mainly regulated through the p53 pathway (Ref. 44). Activated p53 induces the transcription of CDKN1A which encodes p21 (Ref. 45); p21 mediates the p53-dependent G1 cycle arrest through inhibition of the cyclin-dependent kinases (CDK1 and CDK2) (Refs 34, 46). *TP53* mutations are very frequent in GBM (up to 70%) and are associated with increased

tumour progression and the inactivation of p53 is associated with an aggressive phenotype and sustained cell viability (Ref. 47).

ATR is activated by various types of DNA damage, including DSB, cross-links and DNA replication stress (Ref. 48). Compared to ATM, ATR is an essential component of the cell for its viability and replication (Ref. 49). Studies showed that at the N-terminal, ATR presents a binding protein (ATRIP); the complex ATR/ATRIP is mediated through RPA (Replication protein A) to initiate the DDR by phosphorylating various targets, including CHK1 (Ref. 50). Further, CHK1 phosphorylates CDC25 proteins (A, B, C) to arrest the G2 checkpoint (Refs 34, 40, 51). Moreover, it was shown that ATR/CHK1 plays a role in the intra-S-phase cell cycle checkpoint by phosphorylating a Treslin protein that blocks the accumulation of CDC45, an important protein for initiation of DNA replication (Refs 31, 40, 51, 52). Wee1 is a kinase activated by CHK1 and is 'the gatekeeper' of the G2 checkpoint and S phase, arresting mitosis (Fig. 1). It favours the phosphorylation of CDK1, thus its inactivation during interphase. Studies have shown that the downregulation of Wee1 was associated with an increased entry in the mitotic phase (Ref. 53).

The above-mentioned DDR pathways represent specific targets that could be used for future therapy development (Fig. 1) (Ref. 9).

## DNA damage response induced by Temozolomide and Radiotherapy

Activation of the DDR pathway is a common mechanism for cancer induced chemotherapy or RT resistance which limit current therapeutic approaches (Ref. 31). TMZ is an alkylated agent, part of the triazene group compounds (Ref. 54). In 2005, it became the standard of care, together with surgery and RT in GBM, by showing prolongation of survival by 2.5 months when compared to RT alone. TMZ by alkylating the DNA, forms two compounds: N3-methyladenine and N7-methylguanine (∼90%) and O6-methylguanine (5–10%) (Ref. 55). O6-methylguanine (O6MeG) represents an important signal for generating DDR (Ref. 56). O6-methylguanine-DNA methyltransferase (MGMT) is an endogenous enzyme that contributes to DDR by removing the methyl group in O6-methylguanine thereby neutralising the drug-induced DNA damage and reducing the overall efficacy of TMZ (Ref. 57). MGMT is a well-known factor that contributes to TMZ resistance in GBM, yet not the only factor responsible for TMZ resistance (Refs 58, 59). For example, it was shown that GBM with a low level of MGMT had increased rho-associated kinase 2 (ROCK2), a cytoskeleton regulator, that was associated with a low survival rate, making it a potential target for future treatments (Ref. 60). O6MeG plays a role also in the MMR pathway damaging the DNA replication (Ref. 9).

Besides the afore-mention mechanism, MMR might influence the resistance to TMZ and also thorough development of de novo mutations or though MMR deficiency (Refs 57, 61). This pathways includes mainly 4 proteins (MLH-1, MSH-2, MSH-6 and PMS-2) which act as endonucleases (MSH-2 and MSH 6) or are signalling the initiation of repairment (MSH −1/PMS-2) (Ref. 62). The expression of these proteins can be routinely detected through immunohistochemistry (IHC). Their lack of expression is associated with a MMR deficient mechanism, microsatellite instable (Ref. 63).

RT is used as a standard treatment for GBM in shrinking the mass or post-surgery to eliminate the residuals (Refs 64, 65). RT induces a various types of DNA damage, directly by inducing DSB and indirectly by promoting accumulation of ROS which favours SSB (Ref. 64). In the SSB, PARP1 plays a central role in the detection and repair of the pathway (Ref. 9). SSB and base modification

form multiple DNA lesions, favouring a change in base-pairing properties and causing spontaneous mutations (Ref. 17). PARP 1 bounds SSB and PAR. The X-ray repair cross-complementing protein 1 (XRCC1), acts as a scaffold for SSB break proteins, thereby stimulating the repair process (Ref. 66). By targeting PARP and ATR pathway, the response to RT increase (Ref. 67).

DSBs are repaired through two mechanisms, the NHEJ and HR DNA repair pathways (Ref. 9). The HR mechanisms takes place in late G1 and G2 phases and is using the sister chromatid as a DNA replication template to correct the damage (Refs 9, 68).

RAD51 is a specific protein that has a role in DNA damage repair (DDR) through HR (Refs 69, 70). It is overexpressed in multiple cancers, including glioblastoma, and is associated with resistance to treatment. Furthermore, in various studies, its overexpression was associated with genetic mutations that favoured the evolution of the tumour and even metastasis (Ref. 71). In a study by Morrison et al., they showed that RAD51 in the HR pathway was overexpressed in patients with GBM versus normal brain, and it was associated with a reduced survival rate, making RAD51 a potential prognostic biomarker (Ref. 72). In NHEJ, Ku70–Ku80 hetero dimer (Ku) plays a central role in detecting the DSB and behaves as a loading protein on which other NHEJ proteins can be recruited as needed to promote the joining of DNA ends. The NHEJ pathway utilises proteins that have various roles from recognising to ligating the DNA ends in a flexible manner (Ref. 73). Both, HR and NHEJ are regulated by CDK activity (Ref. 9).

The Fanconi anaemia pathway is activated by both ATR and ATM kinases and has a role in facilitating lesion repair and restarting the replication via NER, HR- and MMR system (Fig. 2) (Ref. 9).

## Biomarkers of DDR pathway status

Assessing the level of activity of the DDR pathway is crucial for the identification of specific biomarkers that can predict the response to specific classes of DDR inhibitors (Ref. 74). Among the first proposed biomarkers is the status of the *TP53* gene which is altered in up to 20–30% of primary GBM and more than 60% of secondary GBM (Ref. 75). Intact p53 activity is required for efficient cell cycle progression through the G1/S checkpoint, its mutational loss of function is allowing cancer cells to pass the checkpoints with replicating errors and to accumulate DNA damage (Ref. 16). For example, treatment with RT and DNA-PK inhibitor M3814 on p53 mutated cell lines lead to mitotic catastrophe and apoptotic cell death, as the replication mechanism was unable to arrest the cell cycle and repair the induced DNA damage (Ref. 76). Also, dysfunction of p53 is influencing the cell response to the G2/M checkpoint, therefore increasing cellular susceptibility to ATR, CHK2 and Wee1 inhibitors (Ref. 16).

Mutated IDH1/2 enzymes will impact the citric acid cycle leading to the generation of increased quantities of 2-hydroxyglutarate, an oncometabolite that induces aberrant hypermethylation of histone 3 lysine 9 (H3K9) which impacts the efficacy of DDR proteins, such as p53 binding protein 1 (53BP1) (Ref. 77). Therefore, mutations in IDH1/2 genes are associated with a dysregulation of the DDR pathway to DSB which sensitises tumours harbouring these mutations to ATR and PARPi (Ref. 78).

*PTEN* is a tumour suppressor gene that is mutated or deleted in various cancers, including in 40% of GBM cases. *PTEN* is a negative regulator of the PI3 K/AKT signalling pathway, therefore *PTEN* dysregulation is associated with PI3 K/AKT activation which leads to cancer cell proliferation, failure to arrest the cell cycle at, G2/M, and resistance to classic GBM therapy (Refs 79–81). Loss of *PTEN* leads to sensitivity to genotoxic stress,
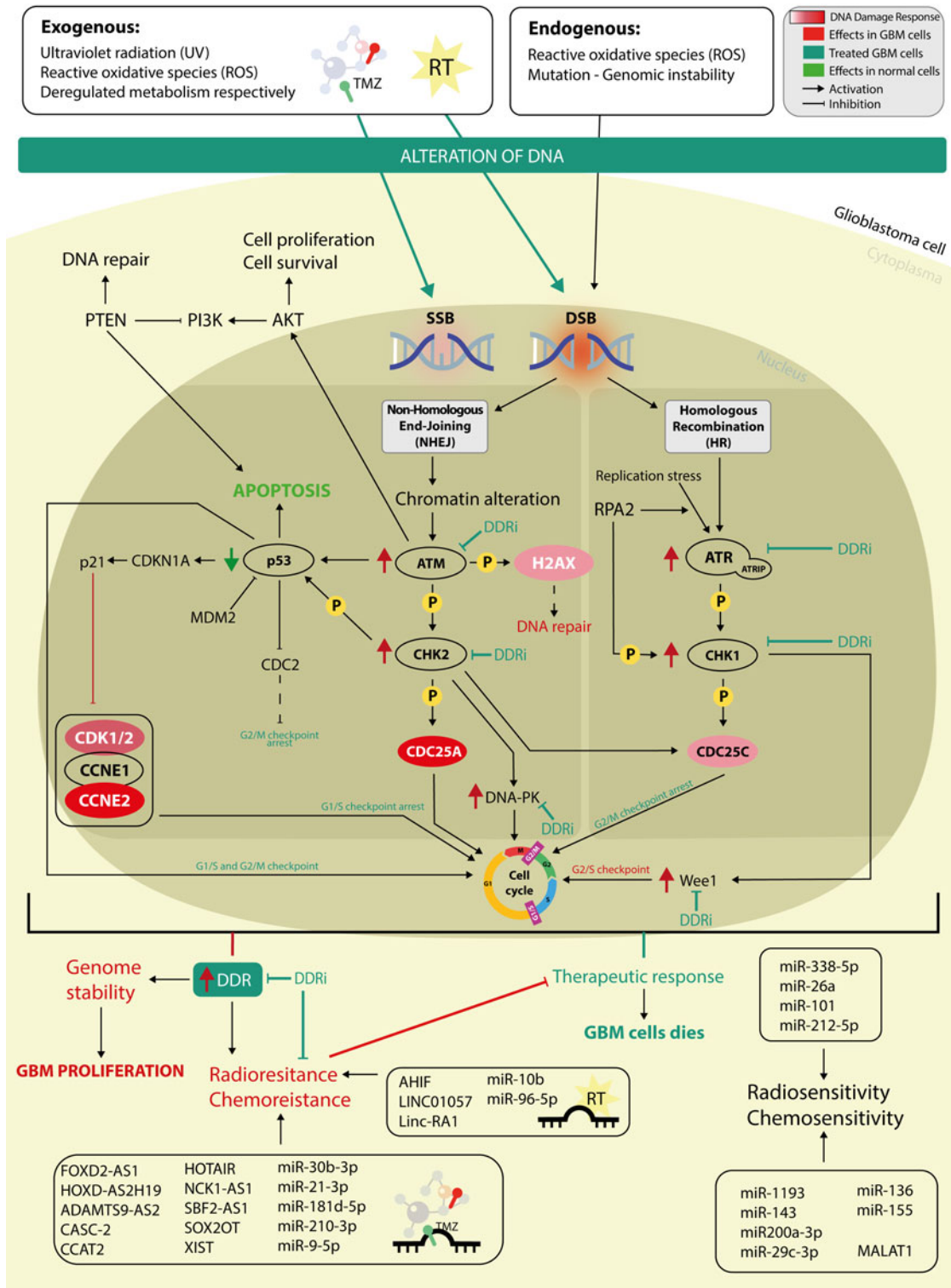
**Fig. 1.** The intricate mechanism of DDR pathway in glioblastoma with a focus on miRNAs and lncRNAs involved in the regulation of radioresistance and chemoresistance. DNA is damaged by exogenous and enogenous factors and is repaired by two principal repatory pathways, non-homologous end-joining (NHEJ) and homologous recombination, both which can be altered in the development of glioblastoma at different key points. Targeting an altered DDR pathway using DDR inhibitors (DDRi) represents an attractive treatment approach. The main molecules targeted by DDRi are represented by: ATM, ATR, Wee1, CHK1 and CHK2.

accumulation of DNA damage and hyperactivation of ATM. In a cell line model in which *PTEN* was inhibited using siRNA, treatment with ATM inhibitor KU-60019 caused increased catastrophic DNA damage, mitotic cell cycle arrest and apoptosis when compared with *PTEN* wild-type cells (Ref. 82). Therefore, *PTEN* gene loss of function mutation can be investigated as a possible biomarker for response to ATM inhibitors in GBM.

Additional strategies focus on targeting RAD51 protein in *PTEN* deficient GBM models (Ref. 83).

*PTEN* deficient tumour cells have increased replication stress and rely on the activation of PTEN-RAD51 signalling axis to ensure efficient DNA replication (Ref. 84). RAD51 is a key protein that is activated in conditions of increased DNA damage that acts on correcting errors in the replication forks through HR (Ref. 69).

**Fig. 2.** *A graphical representation of the main DDR mechanisms activated by RT (right) and TMZ (left) in GBM.* Right: RT induce direct damage, causing SSB and DSB; they are repaired by BER and DSB repair pathways (HR and NHEJ). Replication stress activates the ATR/ATM kinases, thus the cell cycle arrest (see Fig. 1). Stalled replication forks results from replication stress and are repaired via activation of the FA pathway. The detection of SSB and its repair is made by PARP1, together with XRCC1. ROS induced by RT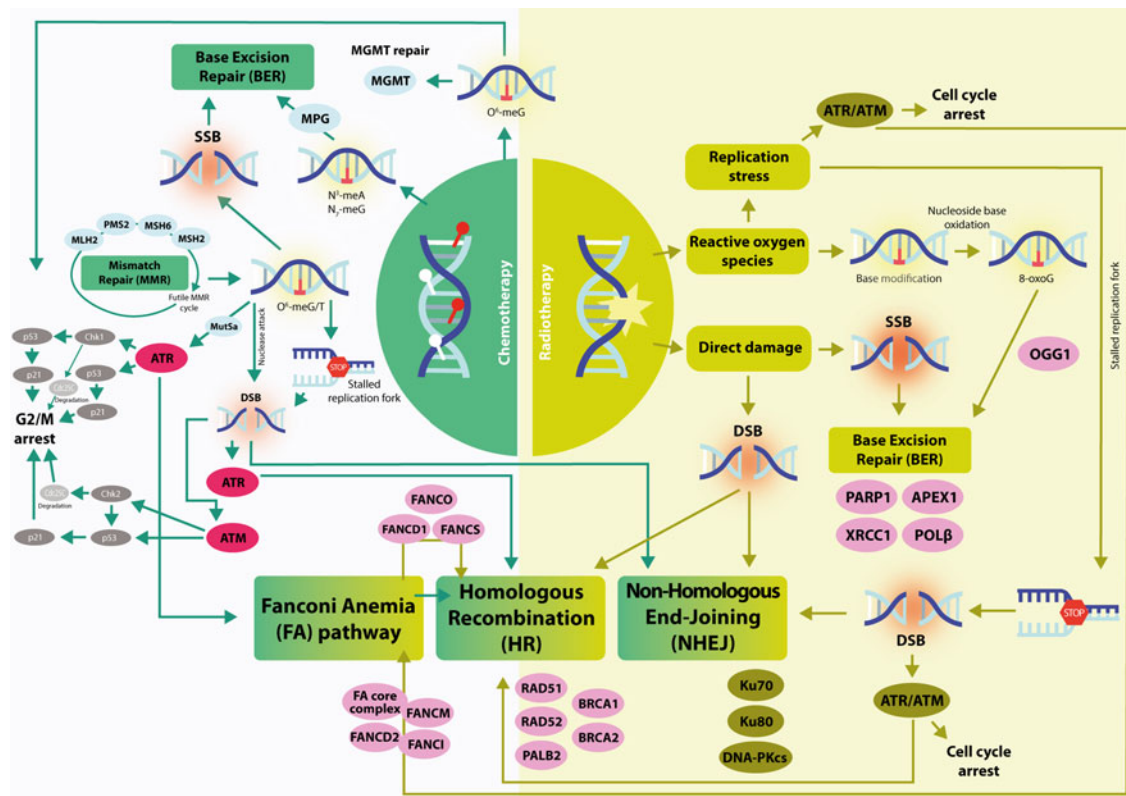 cause base modification which is repair by BER. Left: TMZ induces O6-MeG, which is repaired by MGMT or it can undergo the MMR cycle if an aberrant mutation is present (O6MeG/T). The O6-meG/T lesion can either be recognised by ATR and lead to cell cycle arrest and DNA repair, resulting in a stalled replication fork or form a DSB via nuclease attack, thereby activating G2/M arrest through ATM. It can be repaired through HR and NHEJ. N3- and N7-meA lesions are recognised and repaired through BER pathway via PARP1.

Intact function of the RAD51 protein and its paralogs is essential for efficiently bypassing blockades in the replication forks by allowing switching of the replication fork on the sister DNA chromatide (Ref. 85). RAD51 IHC can be used as a surrogate marker for the activation of the DDR pathway (Table 1) (Ref. 86). Blocking RAD51 in *PTEN* deficient cells using an efficient cell-penetrating autoantibody that inhibits RAD51, 3E10, is inducing mitotic catastrophe and subsequent apoptosis (Ref. 83). Furthermore, in various studies, its overexpression was associated with genetic mutations that favoured the evolution of the tumour and even metastasis (Ref. 71).

Recent advances in the understanding of genome biology and mechanism of the DDR pathways have led to the identification of possible surrogate biomarkers of the DDR pathway activation that can be more easily detected in clinical settings using classic detection methods widely available in pathology laboratories (Table 1) (Refs 87, 88). An example is the phospho-H2AX or γ-H2AX that functions as a sensitive marker for the presence of DNA DSB (Ref. 89). Accumulation of γ-H2AX, can be easily detected by both WB and IHC, is a marker of accumulating DNA damage and has been widely investigated for its role as a surrogate for response to agents targeting the DDR pathway (Ref. 90).

## DDR and non-coding RNAs in GBM targeted therapy

Despite all the efforts made in optimising the standard regiment of care for GBM, patient outcome has not significantly changed. Emerging experimental findings started to highlight the potential therapeutic benefit of targeting the DDR pathway and its related non-coding components (Refs 9, 12). As such, a growing body of

evidence emerged supporting the role of ncRNAs expression in the modulation of DDR genes during the pathogenesis and relapse of GBM, but also their potential utility as reliable biomarkers for targeted therapy (Refs 26, 98–100). Today, ncRNAs are recognised as a diverse group of transcripts that are not translated into proteins following transcription, but rather play a significant role in modulating the expression of several genes involved in different pathological processes, including GBM carcinogenesis, tumour development, metastasis and DDR (Refs 101–103). In particular, miRNAs, lncRNAs and circRNAs that have a dysregulated expression profile started to be investigated for their role of DDR mediators in GBM, Moreover, the strong regulatory influence of miRNAs, lncRNAs and circRNA upon the pathogenic properties of glioma cells, including treatment resistance and sensitivity, are making them attractive targets for therapy (Refs 104, 105).

Several research studies confirmed the active involvement of dysregulate ncRNAs expression profiles in the pathogenesis and development of GBM (Refs 106–108). Hence, their utility as therapeutic interventions against GBM is based on the concept that the malignant phenotype can be restored by targeting different ncRNAs. As such, two primary ncRNA-based approaches are currently investigated for the development of anti-GBM therapies: Gene-silencing therapy which uses specific single-stranded oligonucleotides with complementary sequences to inhibit the function of a targeted ncRNA, and replacement therapy which aims to restore the expression of silenced ncRNAs with ncRNA mimics. Therefore, both strategies could aid the development of better ncRNA-based GBM targeted therapeutics (Refs 109–112).

**Table 1.** Potential protein biomarkers for the evaluation of the DDR pathway status

| Biomaker | Regulation status (up/down) | Role in DDR | Detection method | Cancer type | Ref |
|---|---|---|---|---|---|
| pRAD50 | ↑ | Marker of DDR pathway activation in cancer. | IHC | GBM | (Ref. 91) |
| RAD51 | ↑ | Marker of DDR pathway activation in cancer. | IHC | GBM | (Ref. 86) |
| $\gamma$-H2AX | ↑ | Marker of DNA damage and genomic instability | IHC/WB | | (Ref. 90) |
| XPO1 | ↑ | Protein transporter; exports proteins from nucleus to cytoplasm | WB | GBM | (Ref. 92) |
| P53 | ↓ | Tumour gene suppressor, G1/S, G2/M checkpoints | IHC | GBM | (Ref. 93) |
| ATM | ↑ | Induces cell cycle arrest or apoptosis | IHC | GBM | (Ref. 94) |
| WEE1 | ↑ | Activated by ATR-Chk1 pathway; prolongs the G2 phase | IHC | GBM | (Refs 95, 96) |
| MMR proteins | ↓ | Repair of O6MeG C:T mismatched caused by TMZ | IHC | GBM | (Ref. 97) |

The repository of dysregulated ncRNAs that are currently explored as potential therapeutic targets against GBM is growing. However, extensive research and validation is required to safely translate such experimental knowledge into efficient clinical applications.

## DDR and miRNAs in GBM

In an experimental study conducted by Costa *et al.*, it was demonstrated that intravenously administered chlorotoxin-targeted stable nucleic acid lipid particle-formulated anti-miR-21 oligonucleotides, efficiently promoted miR-21 silencing. Moreover, increased mRNA and protein levels of RhoB, a direct target of miR-21, with no signs of systemic immunogenicity was clearly observed, while decreased GBM cell proliferation and tumour size, enhanced apoptosis and improvement of animal survival (Ref. 113). In a similar work by Lee *et al.*, anti-miR-21 was delivered using a multi-valent folate-conjugated three-way-junction-based RNA nanoparticle platform. As a result, anti-miR-21 specifically targeted and knocked down miR-21 expression in GBM cells *in vitro* and *in vivo* while also upregulating the expression of *PTEN* and *PDCD3* genes which increased GBM apoptosis and induced tumour regression (Ref. 114).

In a distinctive research study, Huang *et al.* investigated the regulatory effects of miR-93 on the autophagic activity of GSCs revealing that IR and TMZ, two first-line treatments for GBM, decreased miR-93 expression which resulted in enhanced autophagic processes. However, the researchers showed that ectopic miR-93 expression inhibited autophagy and enhanced the activity of IR and TMZ against GSCs (Ref. 29).

Replacement therapy has also been validated in the experimental setup. As such, Li *et al.* reported that overexpression of miR-519a, targeted with miR-519a mimic, enhanced TMZ chemosensitivity and promoted autophagy in GBM by regulating STAT3/Bcl2 signalling pathway (Refs 88, 115). In 2021, Nan *et al.* enhanced the expression of miRNA-451, a tumour suppressor that is usually suppressed in high grades GBM, using a transfected lentivirus expressing miR-451. This supports the utility of miR-451-targeted therapy for GBM, as its overexpression regulates NF-$\kappa$B signalling pathway by targeting IKK$\beta$, thus inhibiting tumour cells growth *in vitro* and *in vivo* (Ref. 116).

The role of miR-490 upon the activation of p53, a well-known master regulator of DDR, was observed by Vinchure *et al.* while conducting an *in vitro* telomerase fragility study on GMB cell lines U87MG (wild-type p53) and T98 G (p53 mutant c.711G > T). They have reported that miR-490 overexpression-induced DNA damage and DDR signalling in U87MG cells, with the upregulation of p53 due to an accumulation of p-$\gamma$H2AX (Refs 117–119). Thus, by upregulation of p53, miR-490 overexpression could indirectly orchestrate a variety of DDR mechanisms in GBM cells.

In another study, designed to evaluate the miR-338-5p effect upon radiation response in GBM cells, Besse *et al.* found that overexpression of miR-338-5p, gained by transient transfection of IR-treated GBM cell lines (A172, T98G, U87MG), lead to downregulation of NDFIP1, RHEB and PPP2R5a. These genes have been previously described as key components of the DDR pathway; thus, this study provides evidence supporting the regulatory effect of miR-338-5p on the IR response phenotype of GBM cells through direct upregulation of DDR genes (Ref. 120).

Previous studies reported the miR-181b pathway being activated as a reaction to several DNA lesions, including GBM response to TMZ-induced methylation and IR-induced DSB (Refs 121, 122). In a distinct study, Xu *et al.* reported that overexpression of miR-181b increases IR-induced NF-$\kappa$B activity by downregulating SENP2 in IR-treated GBM cell lines (T98G, U87MG). Taken together, these observations support the role of miR-181b as a positive regulator on the feedback loop of NF-$\kappa$B activation via targeting SENP2 in GBM cells exposed to DNA damaging agents, such as chemo and radiotherapies (Ref. 123).

Finally, two independent studies focused on exploring the implication of miR-221/miR-222 in the molecular process associated with GBM pathogenesis found different links between these genomic modulators and DDR-mediated treatment response. Li *et al.* found that radiation-induced c-jun transcription of miR-221/miR-222 modulated DNA-PK expression to affect DDR by activating Akt independent of PTEN status, contributing to a radio-resistance phenotype. Thus, miR-221/222 could serve as a therapeutic target for increasing radiosensitivity in GBM cells (Ref. 124). Separately, Quintavalle *et al.* found that miR-221/miR-222 are overexpressed in GBM cells and directly downregulate MGMT in GBM TMZ-resistant cell lines, inducing greater TMZ-mediated cell death. However, as MGMT is a key component of the GBM-associated DDR pathways, miR-221/222-mediated MGMT downregulation may render cells unable to overhaul genetic damage (Ref. 125). Taken together, these studies highlight two distinct mechanisms behind the regulation of DRR in GBM and GBM-resistant cell lines.

So far, over 250 miRNAs are known to be upregulated in GBM-associated pathways, including DDR, contributing to the development of either treatment-resistant or treatment-sensitive phenotypes (Refs 99, 126–128). Table 2 presents a summary of the principal miRNAs that influence the DDR pathway upon GBM treatment.

**Table 2.** Overexpressed miRNAs in GBM-associated DDR and the effect upon treatment

| miRNA | Study sample | Target | Effect | Ref |
|---|---|---|---|---|
| miR-30b-3p | Cell lines: Glioma stem-like cells (GSC) and GBM primary tumour cells derived from patients | ↑ HIF1$\alpha$<br>↑ STAT3 | Chemoresistance | (Ref. 129) |
| miR-21-3p | Cell lines: D54MG cells | ↓PDCD4<br>↓TPM1<br>↓PTEN | Chemoresistance | (Refs 130–132) |
| miR-181d-5p | FFPE samples: 114 GBM patients who had received postoperative TMZ chemotherapy and RT | ↓MGMT | Chemoresistance | (Ref. 133) |
| miR-210-3p | Cell lines: U87MG and primary tumour cells derived from GBM patients | ↑HIF1$\alpha$<br>↓HIF2$\alpha$ | Chemoresistance | (Ref. 134) |
| miR-9-5p | Cell lines: U87 and T98G | ↑PTCH1 | Chemoresistance | (Ref. 135) |
| miR-1193 | Cell lines: U118MG, M059J, M059K, U251, A549, HepG2, Huh7, RPE-1, 293T cells and fibroblasts | ↓FEN1 | Chemosensitivity | (Ref. 136) |
| miR-143 | Cell lines: U87, U251 | ↓N-RAS | Chemosensitivity | (Ref. 137) |
| miR200a-3p | Cell lines: U87, U373, T98G, LN18, U138 | ↓MGMT | Chemosensitivity | (Ref. 138) |
| miR-29c-3p | Fresh-frozen tissues: 21 GBM patients<br>Cell lines: U251, U251/TR | ↓Sp1/ MGMT | Chemosensitivity | (Ref. 139) |
| miR-136 | Cell lines: U251 cells | ↓AEG-1 | Chemosensitivity | (Ref. 140) |
| miR-155 | Cell lines: U251, U87, A172, SF767, SF126, SHG-44 | ↓p38 | Chemosensitivity | (Ref. 141) |
| miR-10b | Cell lines: A172, LN229 | ↑p-AKT | Radioresistance | (Ref. 142) |
| miR-96-5p | Cell lines: U87-MG, U251-MG, A172 | ↓PDCD4 | Radioresistance | (Ref. 143) |
| miR-338-5p | Cell lines: A172, T98G, U87MG | ↓Ndfip1<br>↓The<br>↓ppp2R5a | Radiosensitivity | (Ref. 120) |
| miR-26a | Cell lines: U87 | ↓ATM | Radiosensitivity | (Ref. 144) |
| miR-101 | Cell lines: U87MGD | ↓DNA-PKcs<br>↓ATM | Radiosensitivity | (Ref. 145) |
| miR-212-5p | Cell lines: U251, U-118MG, SHG-44 | ↓BRCA1 | Radiosensitivity | (Ref. 146) |

## DDR and lncRNAs in GBM

Generally, lncRNAs can function as molecular decoys, scaffolds, enhancers or repressors. Moreover, these genomic regulators can serve as phenotypic switches for GBM cells, as they can affect stemness, proliferation, invasion and DDR. Thus, aberrant expression of such transcripts may facilitate therapy resistance and responsiveness, leading to tumour recurrence (Refs 26, 108, 128, 147).

Gene-silencing technique was employed by Li *et al.* to demonstrate that silencing lncRNA SNHG15 had a beneficial outcome leading to suppression of GBM tumorigenesis, while also restoring TMZ sensitivity *in vitro* (Ref. 148). In 2022, Xu *et al.* conducted an innovative approach on the lncRNA PRADX. PRADX overexpression activates STAT3 phosphorylation and enhances ACSL1 expression, being associated with accelerated cellular metabolism and tumour growth. Combined ACSL1 and CPT1 inhibitors could reverse this malignant phenotype, which provides the means to further explore lncRNA PRADX as a potential therapeutic target (Ref. 149).

HMMR-AS1 was found upregulated following radiation therapy along with the increased expression of DDR proteins ATM, RAD51 and BMI1. Collectively, these findings confirm that chemo- and radiation-induced DDR could activate lncRNAs in GBM, making them attractive as potential therapeutic targets (Refs 26, 150, 151).

Zhang *et al.* reported that the overexpression of SBF2-AS1 backs the chemoresistant phenotype behind the TMZ-resistant GBM cells. Their study reported that SBF2-AS1 functions as a ceRNA for miR-151a-3p, upregulating its endogenous target,

XRCC4, which enhances DSB repair in GBM cells. These results showed that lncSBF2-AS1/miR-151a-3p/XRCC4 axis is involved in the DDR-regulation of TMZ resistance in GBM cells (Ref. 152).

MALAT1 is a well-studied lncRNA that is linked with the activation of DDR pathway. Activation of DDR pathway by TMZ induces overexpression of MALAT1 which is linked via NF-κB to p53. Down-regulation of MALAT1 using nanoparticle-encapsulated anti-MALAT1 siRNA were able to restore the chemosensibility to TMZ, making it an attractive target for the chemosensitization of GBM (Ref. 153).

A summary of additional overexpressed lncRNAs in GBM, their regulatory effect on DDR effector genes, and the treatment-associated phenotype are presented in Table 3.

## DDR and circRNA in GBM

CircRNAs are a particular group of ncRNAs produced mainly via back-splicing of pre-mRNA (Ref. 167). They are most abundant in brain tissues and found to be highly dysregulated in GBM, where they play significant roles in tumour growth, metastasis, epithelial-to-mesenchymal transition and therapy resistance (Refs 168, 169).

In a recent study conducted on GBM cells, Wang *et al.* found that low-dose RI could trigger the production of exosomes carrying cargoes abundant in circ-METRN, which in turn led to increased levels of γH2AX. Thus, circ-METRN was reported to exhibit oncogenic functions, such as GBM progression and radio-resistance, by deregulation of DDR-associated γH2AX via miR-4709-3p/GRB14/PDGFRα pathway (Ref. 170).

**Table 3.** Overexpressed lncRNAs in GBM-associated DDR and the effect upon treatment

| lncRNA | Study sample | Target | | Effect | Ref. |
|---|---|---|---|---|---|
| | | Gene | miRNA | | |
| FOXD2-AS1 | Cell lines: U251, A172 | ↓MGMT | | Chemoresistance | (Ref. 154) |
| HOXD-AS2 H19 | Fresh-frozen tissues: 41 brain tumour samples, 5 non-tumour brain samples from GBM patients<br>Cell lines: A172, U87, U251, LN229, U138, T98, DBTRG-05MG, D54 and normal human astrocytes (NHA) | ↑MGMT | ↓miR-198 | Chemoresistance | (Refs 155, 156) |
| ADAMTS9-AS2 | Fresh-frozen tissues: 140 samples from GBM patients<br>Cell lines: T98G-R, U118-R | ↑FUS | | Chemoresistance | (Ref. 157) |
| CASC-2 | Fresh-frozen tissues: 57 paired glioma tissues and peritumoral brain oedema tissues from GBM patients<br>Cell lines: U251, U373, SNB19, U118, LN229, NHA | ↑PTEN | ↓miR-181a | Chemoresistance | (Ref. 158) |
| CCAT2 | Fresh-frozen tissues: neoplastic tissues and corresponding adjacent non-tumour tissues<br>Cell lines: U251, U87, A172, SHG44, NHA | ↑CHK1 | ↓miR-424 | Chemoresistance | (Ref. 159) |
| HOTAIR | Serum: 51 samples from GBM patients<br>Cell lines: A172, LN229, NHA | ↑RRM1 | ↓miR-519a-3p | Chemoresistance | (Ref. 160) |
| NCK1-AS1 | Fresh-frozen tissues: 36 samples<br>Cell lines: U251, A172, HEK-293 | ↑TRIM24 | ↓miR-137 | Chemoresistance | (Ref. 161) |
| SBF2-AS1 | Fresh-frozen tissues: 20 primary tumour samples and their corresponding recurrent GBM specimens<br>Serum: 20 samples from GBM patients<br>Cell lines: U87, LN229, A172, T98, U251 | ↑XRCC4 | ↓miR-151a-3p | Chemoresistance | (Ref. 152) |
| SOX2OT | Fresh-frozen tissues: 118 glioma samples and 10 normal tissues<br>Cell lines: U87, U251 | ↑ALKBH5<br>↑SOX2<br>↑Wnt5a/$\beta$-catenin | | Chemoresistance | (Ref. 162) |
| XIST | Fresh-frozen tissues: 69 paired glioma and the PTBE tissues<br>Cell lines: U251, U373, LN229, U118, LN229, NHA | ↑SP1<br>↑MGMT | ↓miR-29c | Chemoresistance | (Ref. 163) |
| MALAT1 | Cell lines: U87, A172, U251 and patients-derived cell lines GSCs, GBM34, GBM44 | ↑NF-$\kappa$B<br>↑p53 | | Chemosensitivity | (Ref. 153) |
| AHIF | Fresh-frozen tissues: 31 tumour tissues and 7 adjacent normal brain tissues were collected from patients with GBM<br>Cell lines: U87, U251, A172, T98G | ↓HIF1a<br>↓p53 | | Radioresistance | (Ref. 164) |
| LINC01057 | Fresh-frozen tissues: 12 tumour tissue paired with their adjacent normal tissues<br>Cell-lines: LN229, T98G, HEK293 T | ↑IKK$\alpha$ | | Radioresistance | (Ref. 165) |
| Linc-RA1 | Fresh-frozen tissues: 120 tumour tissues<br>Cell lines: M059J, M059 K, U251, U87 | ↑H2Bub1 | | Radioresistance | (Ref. 166) |

Lou et al. found CDR1as is a particularly interesting circRNA as its expression decreases with the increase of glioma grade, which promotes it as a reliable predictor for overall survival, especially in GBM. The researchers reported that CDR1 interacts with the p53 DBD domain, thus disrupting the p53/MDM2 complex formation. This interaction with the p53 protein is essential for maintaining function and protect from additional DNA damage (Ref. 171).

Nonetheless, in a circRNAs expression profiling study conducted by Wang et al. on GBM patients, it was found that compared with the adjacent normal brain tissues, 254 circRNAs were upregulated and 361 circRNAs were downregulated in IDH-wt GBM. In fact, a comprehensive Gene Ontology analysis conducted by the same research group indicated that these differentially expressed circRNAs could be involved in different GBM-associated processes, including DDR and repair (Refs 12, 172).

The expression level of circHEATR5B is generally low in tissues and cells, being involved in aerobic glycolysis, a metabolic hallmark of GBM. However, Song et al. reported that circHEATR5B transfection-based overexpression contributed to suppressing the aerobic glycolysis process and GBM cells proliferation in vitro. Moreover, circHEATR5B overexpression proved to play a role in the inhibition of GBM xenograft growth, while also prolonging the survival rate of nude mice. This highlights the potential use of circHEATR5B for the advance of anti-GBM targeted therapies (Ref. 173). Similarly, Jiang et al. found that circLRFN5 is downregulated in GBM and associated with poor patient prognosis (Ref. 174).

## Current therapeutic approaches targeting DDR in GBM

As previously mentioned, DDR pathway is activated by SSB or DSB which are induced by RT or TMZ. Using this pathway, GBM cells acquired resistance to genotoxic anti-tumoral agents. PARP-1 plays a central role in both SSB and DSB, being highly sensitive to detect the DNA damage and favours its repair (Refs 17, 175, 176). PARP-1 inhibitors sensitise GBM to RT and chemotherapy (Refs 177–179). The OLA-TMZ-RTE-01 trial (Ref. 180) included 79 participants: 30 in phase I and 49 in phase IIa, participants with unresectable or partially resecable GBM tumours, aged between 18 and 70 years old. The study highlighted the benefits of PARP-1 inhibitor Olaparib, alongside RT and TMZ, at improving the 18 months' overall survival in patients with unresectable or partially resecable GBM, without harming the non-

**Table 4.** Current ongoing clinical trials with various DDR targets

| Agents | Targets | Tumour type | Phase | Status | Trial No. |
|---|---|---|---|---|---|
| Nedisertib (M3814) + RT → TMZ | DNA-PK | MGMT unmethylated GBM | I | Ongoing, expected 2023 | NCT04555577 |
| CC-122 | DNA-PK | GBM | I | Ongoing, Expected 2022 | NCT01421524 |
| AZD1390 + RT (GBM & AATMs) | ATM | GBM or other Brain Neoplasms | I | Ongoing, Expected 2024 | NCT03423628 |
| Selinexor (KPT-330) (GBM & XPO1i) | XPO1 | Recurrent childhood GBM, recurrent/ refractory solid and CNS tumours, recurrent/ refractory lymphoma | I | Ongoing, expected 2022 | NCT02323880 |
| NMS-03305293 + TMZ | PARP1 | diffuse gliomas, IDH wild-type recurrent glioblastoma | I | Ongoing, expected 2025 | NCT04910022 |
| Lomustine | PARP1 | IDH wild-type recurrent glioblastoma | II | | |
| Fluzoparil + TMZ | PARP1 | Recurrent GBM | II | Ongoing, expected 2022 | NCT04552977 |
| Veliparib (ABT-888) + RT → Veliparib + TMZ | PARP1 | New High-Grade Glioma (HGG) Without H3 K27 M or BRAFV600 Mutations | II | Ongoing, expected 2024 | NCT03581292 |
| BGB-290 + TMZ | PARP1 | Recurrent GBM, recurrent grade II, III glioma, IDH 1 or 2 mutation | I/II | Ongoing, expected 2023 | NCT03914742 |
| Niraparib | PARP1 | Recurrent GBM, glioma | II | Ongoing, expected 2026 | NCT05297864 |
| BGB-290 + TMZ | PARP1 | adolescents and young adults with IDH1/ 2-mutant grade I-IV glioma | I | Ongoing, expected 2029 | NCT03749187 |
| Single fraction, low dose (2 Gy) whole-brain RT → Talazoparib + Carboplatin | PARP1 | Recurrent High-grade Glioma with DDR deficiency (TAC-GReD) | II | Ongoing, expected 2023 | NCT04740190 |
| Veliparib + TMZ | PARP1 | New GBM with MGMT promoter hypermethylation | II/III | Active, not recruiting | NCT02152982 |
| Olaparib (AZD2281) | PARP1 | glioma, cholangiocarcinoma or solid tumours with IDH1 or IDH2 mutations | II | Ongoing, expected 2022 | NCT03212274 |
| Niraparib + TTF | PARP1 | recurrent glioblastoma | II | Ongoing, expected 2025 | NCT04221503 |
| Veliparib + RT + TMZ | PARP1 | Newly diagnosed diffuse pontine gliomas | I/II | Completed | NCT01514201 |
| olaparib + cediranib (AZD2171) | PARP1 | Recurrent GBM | II | Ongoing, expected 2022 | NCT02974621 |
| Niraparib + RT | PARP1 | Newly-diagnosed GBM and Recurrent IDH1/2 (+) ATRX Mutant Glioma | I | Ongoing, expected 2024 | NCT05076513 |
| AZD1775/adavosertib + TMZ + RT → adavosertib + TMZ | Wee1 | GBM | I | Active, not recruiting | NCT01849146 |

cancerous brain tissue and without affecting patients' cognition. The measurement of GBM penetration of Olaparib, as well as its safety and efficacy associated with TMZ was tested in a study that included 48 patients with recurrent GBM. Olaparib was well represented at the core of GBM as well as the margins and the patients receiving this treatment tolerated it well (Ref. 181).

The VERTU study (Ref. 182) included 125 patients (84 in the experiment group and 41 in the standard group) newly diagnosed with MGMT-unmethylated GBM. The experiment group received veliparib 200 mg twice a day and radiation for 6 weeks and veliparib and 40 mg BD and TMZ, while the standard group received just TMZ and RT. Veliparib was well tolerated but the study did not reach statistical significance (Ref. 182).

ATR plays an essential role in most replicating cells' survival (Ref. 183). Thus, there are some limitations regarding the treatment with ATR inhibitors, as they could harm both cancerous and noncancerous cells. At the moment, there are no specific ATR inhibitors clinical trials for GBM, due to their increased toxicity in preclinical studies (Refs 16, 95). Yet, there are ongoing clinical trials that test ATR inhibitors in combination with RT or other chemotherapy: Elimusertib (BAY1895344) with Pembrolizumab for advanced solid tumours (NCT04095273) or Elimusertib with Pembrolizumab and Stereotactic Body Radiation Therapy for recurrent head and neck cancer (NCT04576091) or AZD6738 with Olaparib (AZD2281) for

Clear Cell Renal Cell Carcinoma and Advanced Pancreatic Cancer (NCT03682289).

ATM inhibitors are a practical solution to resistant GBM since they enhance the toxic effects of RT and chemotherapy (Ref. 184). AZD1390 is one of the latest, highly effective ATM inhibitors, being able to effectively cross the blood-brain barrier, in comparison with older-generation ATM inhibitors such as KU-60019 (Refs 185, 186). Now, a phase 1 clinical trial is conducted in which AZD1390 in combination with RT being tested on 120 patients with primary/recurrent GBM (NCT03423628).

XPO1 is a protein transporter that facilitates the exports of proteins from the nucleus. XPO1 is upregulated in GBM and other cancers, thus being a potential effective antitumoral target (Ref. 187). The only XPO1 inhibitors available are Selinexor and Eltanexor. The first is currently given only in haematological malignancies, such as relapsed or refractory multiple myeloma or diffuse large B-cell lymphoma, while the latter is still undergoing clinical trials. (Ref. 92). In vivo and in vitro studies showed that Selinexor has radiosensitizing effects against GBM; in addition, it also affects gene translation, since XPO1 also facilitates the transport of ribosomal RNA across the nuclear membrane (Ref. 188). The KING trial (NCT01986348) was a phase II study using Selinexor conducted with 76 participants divided into 4 arms with various treatment regimens. Only at 80 mg/week Selinexor induced responses and had a relevant 6-month

progression-free survival rate. Although there were some haematological adverse effects (thrombocytopenia, neutropenia and anaemia) they were reversible with the adjustment of dose (Ref. 189). At the moment, Selinexor is tested in phase 1 clinical trial with 68 participants with recurrent and refractory paediatric solid tumours, including CNS tumours and GBM (NCT02323880).

Wee1 is a protein kinase of the ATR-CHK1 pathway. Its key role is to lengthen the G2 cellular phase, thus making it possible for DDR mechanisms to repair the injured DNA. One Wee1 activator is phosphatidylinositol 3-kinase (PI3 K) inhibition, as an adaptive mechanism of GBM cells (Ref. 190). Moreover, it was demonstrated a beneficial association between WEE1 inhibitors and PI3 K inhibitors in GBM therapy (Ref. 190). A recent phase 0 clinical trial with 20 participants (NCT02207010) highlighted only that WEE1 inhibitor Adavosertib (AZD1775) passes through the blood-brain barrier and reaches the, and not its efficiency in fighting the tumour (Ref. 191). The 20 participants were grouped into three cohorts and received a single dose of 100, 200 or 400 mg before tumour resection. Part 1 of the study planned the tumoral resection 8 hours post-AZD1775 administration in each cohort; in contrast, in part 2 the resection was planned for 8 hours or 24 hours. In the case of Adavosertib resistance, the biomarker Myt1 should be investigated, because it demonstrated an upregulation of Myt1 following WEE1 inhibitors treatment (Ref. 192).

Currently, there are no clinical trials that involve ncRNAs that target DDR pathway in GBM or are being used for disease monitoring. However, in the future these molecules could become of interest as we showed that they are important key regulators of DDR pathway and can be either used as single targets or as adjuvant therapy to current approaches Table 4.

## Conclusion

In conclusion, we consider that an in-depth characterisation of the molecular mechanisms involved in DDR can provide important insights into this particular field of GBM biology that can be exploited by the upcoming new DDR inhibitors. An integrated approach needs to consider the underlying genomic background of each individual GBM patient, to check for DDR pathway status in the tumour using both ncRNAs and protein biomarkers and to identify the genomic vulnerability that can be targeted in the particular genomic context of the tumour. By targeting specific vulnerable targets of the DDR pathway using above-mentioned, inhibitors we can try to overcome the current challenges in chemotherapy and RT resistance.

The novelty of this review resides in including in the regulatory loop of DDR in glioblastoma the roles of ncRNAs with a special focus on miRNAs and lncRNAs. We consider that an integrative view over the DDR pathway in glioblastoma which considers ncRNAs can fill the gaps in understanding that limited more consistent progression in this field. NcRNAs can be used assess the functionality of the DDR mechanism and to assess in dynamic treatment response with DDR inhibitors such as PARPi. The widespread distribution of ncRNAs, stability and sensibility are important characteristics that make them attractive biomarker for identifying and monitoring GBM patients in further clinical trials.

**Conflicts of interest.**  The authors declare no conflict of interest.

## References

1. **Grochans S** *et al.* (2022) Epidemiology of glioblastoma multiforme–literature review. *Cancers* **14**, 2412.
2. **Poon MTC** *et al.* (2020) Longer-term (≥2 years) survival in patients with glioblastoma in population-based studies pre- and post-2005: a systematic review and meta-analysis. *Scientific Reports* **10**, 11622.
3. **Grech N** *et al.* (2020) Rising incidence of glioblastoma multiforme in a well-defined population. *Cureus* **12**, e8195.
4. **Tamimi AF and Juweid M** (2017) Epidemiology and outcome of glioblastoma. In De Vleeschouwer S (ed.), *Glioblastoma* [Internet]. Brisbane, AU: Codon Publications, pp. 143–153, http://www.ncbi.nlm.nih.gov/books/NBK470003/.
5. **Louis DN** *et al.* (2021) The 2021 WHO classification of tumors of the central nervous system: a summary. *Neuro-Oncology* **23**, 1231–1251.
6. **Mardis ER** (2019) The impact of next-generation sequencing on cancer genomics: from discovery to clinic. *Cold Spring Harbor Perspectives in Medicine* **9**, a036269.
7. **Wang WT** *et al.* (2019) Noncoding RNAs in cancer therapy resistance and targeted drug development. *Journal of Hematology Oncology* **12**, 55.
8. **Shahzad U** *et al.* (2021) Noncoding RNAs in glioblastoma: emerging biological concepts and potential therapeutic implications. *Cancers* **13**, 1555.
9. **Rominiyi O and Collis SJ** (2022) DDRUgging glioblastoma: understanding and targeting the DNA damage response to improve future therapies. *Molecular Oncology* **16**, 11–41.
10. **Fernandes C** *et al.* (2017) Current standards of care in glioblastoma therapy. In De Vleeschouwer S (ed.), *Glioblastoma*. Brisbane, AU: Codon Publications, pp. 197–241, http://www.ncbi.nlm.nih.gov/books/NBK469987/.
11. **Weller M** *et al.* (2013) Standards of care for treatment of recurrent glioblastoma--are we there yet? *Neuro-Oncology* **15**, 4–27.
12. **Ferri A** *et al.* (2020) Targeting the DNA damage response to overcome cancer drug resistance in glioblastoma. *International Journal of Molecular Sciences* **21**, 4910.
13. **Jackson SP and Bartek J** (2009) The DNA-damage response in human biology and disease. *Nature* **461**, 1071–1078.
14. **Everix L** *et al.* (2022) Perspective on the use of DNA repair inhibitors as a tool for imaging and radionuclide therapy of glioblastoma. *Cancers* **14**, 1821.
15. **Kaur E** *et al.* (2022) Glioblastoma recurrent cells switch between ATM and ATR pathway as an alternative strategy to survive radiation stress. *Medical Oncology* **39**, 50.
16. **Bonm A and Kesari S** (2021) DNA damage response in glioblastoma: mechanism for treatment resistance and emerging therapeutic strategies. *Cancer Journal* **27**, 379–385.
17. **Caldecott KW** (2008) Single-strand break repair and genetic disease. *Nature Reviews Genetics* **9**, 619–631.
18. **Malyuchenko NV** *et al.* (2015) PARP1 inhibitors: antitumor drug design. *Acta Naturae* **7**, 27–37.
19. **Leonetti C** *et al.* (2012) Targeted therapy for brain tumours: role of PARP inhibitors. *Current Cancer Drug Targets* **12**, 218–236.
20. **Sakthikumar S** *et al.* (2020) Whole-genome sequencing of glioblastoma reveals enrichment of non-coding constraint mutations in known and novel genes. *Genome Biology* **21**, 127.
21. **Cardon T** *et al.* (2021) Unveiling a ghost proteome in the glioblastoma non-coding RNAs. *Frontiers in Cell and Developmental Biology* **9**, 703583.
22. **Zhang Y** *et al.* (2017) Noncoding RNAs in glioblastoma. In De Vleeschouwer S (ed.), *Glioblastoma*. Brisbane, AU: Codon Publications, pp. 95–130, http://www.ncbi.nlm.nih.gov/books/NBK469994/.
23. **Zeng Z** *et al.* (2022) NcRNAs: multi-angle participation in the regulation of glioma chemotherapy resistance (review). *International Journal of Oncology* **60**, 76.
24. **Wan G** *et al.* (2014) Noncoding RNAs in DNA repair and genome integrity. *Antioxidants & Redox Signaling* **20**, 655–677.

25. **Ahmed SP *et al.*** (2021) Glioblastoma and MiRNAs. *Cancers* **13**, 1581.

26. **Stackhouse CT *et al.*** (2020) Exploring the roles of lncRNAs in GBM pathophysiology and their therapeutic potential. *Cells* **9**, E2369.

27. **Parashar TR, Ravindran F and Choudhary B** (2021) DNA Damage Repair Genes and Noncoding RNA in High-Grade Gliomas and Its Clinical Relevance [Internet]. Central Nervous System Tumors. IntechOpen; [cited 2022 Jul 12]. Available at https://www.intechopen.com/chapters/undefined/state.item.id.

28. **Mousavi SM *et al.*** (2022) Non-coding RNAs and glioblastoma: insight into their roles in metastasis. *Molecular Therapy Oncolytics* **24**, 262–287.

29. **Huang T *et al.*** (2019) MIR93 (microRNA −93) regulates tumorigenicity and therapy response of glioblastoma by targeting autophagy. *Autophagy* **15**, 1100–1111.

30. **DNA Damage Response (DDR) Consortium [Internet]**. National Brain Tumor Society. [cited 2022 Jul 24]. Available at https://braintumor.org/our-research/ddr-consortium/.

31. **Weber AM and Ryan AJ** (2015) ATM and ATR as therapeutic targets in cancer. *Pharmacology Therapeutics* **149**, 124–138.

32. **Repair of Endogenous DNA Damage**. [cited 2022 Jul 10]. Available at http://symposium.cshlp.org/content/65/127.extract.

33. **Ciccia A and Elledge SJ** (2010) The DNA damage response: making it safe to play with knives. *Molecular Cell* **40**, 179–204.

34. **Smith HL *et al.*** (2020) DNA Damage checkpoint kinases in cancer. *Expert Reviews in Molecular Medicine* **22**, e2.

35. **Woods D and Turchi JJ** (2013) Chemotherapy induced DNA damage response. *Cancer Biology and Therapy* **14**, 379–389.

36. **Kaina B and Christmann M** (2019) DNA repair in personalized brain cancer therapy with temozolomide and nitrosoureas. *DNA Repair* **78**, 128–141.

37. **Ou A *et al.*** (2020) Molecular mechanisms of treatment resistance in glioblastoma. *International Journal of Molecular Sciences* **22**, E351.

38. **Aldea MD *et al.*** (2014) Metformin plus sorafenib highly impacts temozolomide resistant glioblastoma stem-like cells. *Journal of the Balkan Union of Oncology* **19**, 502–511.

39. **Estiar MA and Mehdipour P** (2018) ATM in breast and brain tumors: a comprehensive review. *Cancer Biology & Medicine* **15**, 210–227.

40. **Zannini L *et al.*** (2014) CHK2 kinase in the DNA damage response and beyond. *Journal of Molecular Cell Biology* **6**, 442–457.

41. **van Jaarsveld MTM *et al.*** (2020) Cell-type-specific role of CHK2 in mediating DNA damage-induced G2 cell cycle arrest. *Oncogenesis* **9**, 1–7.

42. **Shen T and Huang S** (2012) The role of Cdc25A in the regulation of cell proliferation and apoptosis. *Anti-Cancer Agents in Medicinal Chemistry* **12**, 631–639.

43. **Falck J *et al.*** (2001) The ATM–Chk2–Cdc25A checkpoint pathway guards against radioresistant DNA synthesis. *Nature* **410**, 842–847.

44. **Jeggo PA *et al.*** (2016) DNA repair, genome stability and cancer: a historical perspective. *Nature Reviews Cancer* **16**, 35–42.

45. **Fridman JS and Lowe SW** (2003) Control of apoptosis by p53. *Oncogene* **22**, 9030–9040.

46. **Abbas T and Dutta A** (2009) p21 in cancer: intricate networks and multiple activities. *Nature Reviews Cancer* **9**, 400–414.

47. **Lee YJ *et al.*** (2020) Gene expression profiling of glioblastoma cell lines depending on TP53 status after tumor-treating fields (TTFields) treatment. *Scientific Reports* **10**, 12272.

48. **Nam EA and Cortez D** (2011) ATR signaling: more than meeting at the fork. *Biochemical Journal* **436**, 527–536.

49. **Sirbu BM and Cortez D** (2013) DNA damage response: three levels of DNA repair regulation. *Cold Spring Harbor Perspectives in Biology* **5**, a012724.

50. **Rao Q *et al.*** (2018) Cryo-EM structure of human ATR-ATRIP complex. *Cell Research* **28**, 143–156.

51. **Saldivar JC *et al.*** (2017) The essential kinase ATR: ensuring faithful duplication of a challenging genome. *Nature Reviews Molecular Cell Biology* **18**, 622–636.

52. **Guo C *et al.*** (2015) Interaction of Chk1 with Treslin negatively regulates the initiation of chromosomal DNA replication. *Molecular Cell* **57**, 492–505.

53. **Esposito F *et al.*** (2021) Wee1 kinase: a potential target to overcome tumor resistance to therapy. *International Journal of Molecular Sciences* **22**, 10689.

54. **Strobel H *et al.*** (2019) Temozolomide and other alkylating agents in glioblastoma therapy. *Biomedicines* **7**, 69.

55. **Lee SY** (2016) Temozolomide resistance in glioblastoma multiforme. *Genes & Diseases* **3**, 198–210.

56. **Cui B *et al.*** (2010) Decoupling of DNA damage response signaling from DNA damages underlies temozolomide resistance in glioblastoma cells. *The Journal of Biomedical Research* **24**, 424–435.

57. **Singh N *et al.*** (2021) Mechanisms of temozolomide resistance in glioblastoma - a comprehensive review. *Cancer Drug Resistance* **4**, 17–43.

58. **Kitange GJ *et al.*** (2009) Induction of MGMT expression is associated with temozolomide resistance in glioblastoma xenografts. *Neuro-Oncology* **11**, 281–291.

59. **Chien CH *et al.*** (2021) Dissecting the mechanism of temozolomide resistance and its association with the regulatory roles of intracellular reactive oxygen species in glioblastoma. *Journal of Biomedical Sciences* **28**, 18.

60. **Zhang X *et al.*** (2022) Acquired temozolomide resistance in $MGMT^{low}$ gliomas is associated with regulation of homologous recombination repair by ROCK2. *Cell Death Disease* **13**, 1–15.

61. **Touat M *et al.*** (2020) Mechanisms and therapeutic implications of hypermutation in gliomas. *Nature* **580**, 517–523.

62. **Bateman AC** (2021) DNA mismatch repair proteins: scientific update and practical guide. *Journal of Clinical Pathology* **74**, 264–268.

63. **McCarthy AJ *et al.*** (2018) Heterogenous loss of mismatch repair (MMR) protein expression: a challenge for immunohistochemical interpretation and microsatellite instability (MSI) evaluation. *The Journal of Pathology: Clinical Research* **5**, 115–129.

64. **Borrego-Soto G *et al.*** (2015) Ionizing radiation-induced DNA injury and damage detection in patients with breast cancer. *Genetics and Molecular Biology* **38**, 420–432.

65. **Gzell C *et al.*** (2017) Radiotherapy in glioblastoma: the past, the present and the future. *Clinical Oncology* **29**, 15–25.

66. **Ray Chaudhuri A and Nussenzweig A** (2017) The multifaceted roles of PARP1 in DNA repair and chromatin remodelling. *Nature Reviews Molecular Cellular Biology* **18**, 610–621.

67. **Carruthers RD *et al.*** (2018) Replication stress drives constitutive activation of the DNA damage response and radioresistance in glioblastoma stem-like cells. *Cancer Research* **78**, 5060–5071.

68. **Krajewska M *et al.*** (2015) Regulators of homologous recombination repair as novel targets for cancer treatment. *Frontiers Genetic* **6**, 96.

69. **Hine CM *et al.*** (2008) Use of the Rad51 promoter for targeted anti-cancer therapy. *Proceedings of the National Academy of Sciences of the United States of America* **105**, 20810–20815.

70. **Balacescu O *et al.*** (2014) Gene expression profiling reveals activation of the FA/BRCA pathway in advanced squamous cervical cancer with intrinsic resistance and therapy failure. *BMC Cancer* **14**, 246.

71. **Gachechiladze M *et al.*** (2017) RAD51 as a potential surrogate marker for DNA repair capacity in solid malignancies. *International Journal of Cancer* **141**, 1286–1294.

72. **Morrison C *et al.*** (2021) Expression levels of RAD51 inversely correlate with survival of glioblastoma patients. *Cancers* **13**, 5358.

73. **Chang HHY *et al.*** (2017) Non-homologous DNA end joining and alternative pathways to double-strand break repair. *Nature Reviews Molecular Cell Biology* **18**, 495–506.

74. **Chen M *et al.*** (2022) DNA Damage response evaluation provides novel insights for personalized immunotherapy in glioma. *Frontiers in Immunology* **13**, 875648.

75. **England B *et al.*** (2013) Current understanding of the role and targeting of tumor suppressor p53 in glioblastoma multiforme. *Tumour Biology Journal* **34**, 2063–2074.

76. **Sun Q *et al.*** (2019) Therapeutic implications of p53 status on cancer cell fate following exposure to ionizing radiation and the DNA-PK inhibitor M3814. *Molecular Cancer Research* **17**, 2457–2468.

77. **Young LC *et al.*** (2013) Kdm4b histone demethylase is a DNA damage response protein and confers a survival advantage following $\gamma$-irradiation. *Journal of Biological Chemistry* **288**, 21376–21388.

78. **Sule A *et al.*** (2021) Targeting IDH1/2 mutant cancers with combinations of ATR and PARP inhibitors. *NAR Cancer* **3**, zcab018.

79. **Eich M *et al.*** (2013) Contribution of ATM and ATR to the resistance of glioblastoma and malignant melanoma cells to the methylating anticancer drug temozolomide. *Molecular Cancer Therapy* **12**, 2529–2540.

80. **Behrooz AB *et al.*** (2022) Wnt and PI3K/Akt/mTOR survival pathways as therapeutic targets in glioblastoma. *International Journal of Molecular Sciences* **23**, 353.

81. **Benitez JA** *et al.* (2017) PTEN regulates glioblastoma oncogenesis through chromatin-associated complexes of DAXX and histone H3.3. *Nature Communication* **8**, 15223.

82. **McCabe N** *et al.* (2015) Mechanistic rationale to target PTEN-deficient tumor cells with inhibitors of the DNA damage response kinase ATM. *Cancer Research* **75**, 2159–2165.

83. **Turchick A** *et al.* (2019) Synthetic lethality of a cell-penetrating anti-RAD51 antibody in PTEN-deficient melanoma and glioma cells. *Oncotarget* **10**, 1272–1283.

84. **He J** *et al.* (2015) PTEN regulates DNA replication progression and stalled fork recovery. *Nature Communication* **6**, 7620.

85. **Rein HL** *et al.* (2021) RAD51 paralog function in replicative DNA damage and tolerance. *Current Opinion in Genetics & Development* **71**, 86–91.

86. **Welsh JW** *et al.* (2009) Rad51 protein expression and survival in patients with glioblastoma multiforme. *International Journal of Radiation Oncology - Biology - Physics* **74**, 1251–1255.

87. **Pirlog R** *et al.* (2019) Proteomic advances in glial tumors through mass spectrometry approaches. *Medicina* **55**, 412.

88. **Susman S** *et al.* (2019) The role of p-Stat3 Y705 immunohistochemistry in glioblastoma prognosis. *Diagnostic Pathology* **14**, 124.

89. **Palla VV** *et al.* (2017) gamma-H2AX: can it be established as a classical cancer prognostic factor? *Tumor Biology* **39**, 1010428317695931.

90. **Nagelkerke A and Span PN** (2016) Staining against phospho-H2AX (γ-H2AX) as a marker for DNA damage and genomic instability in cancer tissues and cells. *Advances in Experimental Medicine and Biology* **899**, 1–10.

91. **Jones GN** *et al.* (2018) pRAD50: a novel and clinically applicable pharmacodynamic biomarker of both ATM and ATR inhibition identified using mass spectrometry and immunohistochemistry. *British Journal of Cancer* **119**, 1233–1243.

92. **Martin JG** *et al.* (2021) Chemoproteomic profiling of covalent XPO1 inhibitors to assess target engagement and selectivity. *Chembiochem* **22**, 2116–2123.

93. **Birner P** *et al.* (2002) Prognostic relevance of p53 protein expression in glioblastoma. *Oncology Reports* **9**, 703–707.

94. **Seol HJ** *et al.* (2011) Prognostic implications of the DNA damage response pathway in glioblastoma. *Oncology Reports* **26**, 423–430.

95. **Majd NK** *et al.* (2021) The promise of DNA damage response inhibitors for the treatment of glioblastoma. *Neuro-Oncol Advances* **3**, vdab015.

96. **Ghelli Luserna di Rorà A** *et al.* (2020) A WEE1 family business: regulation of mitosis, cancer progression, and therapeutic target. *Journal of Hematology Oncology* **13**, 126.

97. **Śledzińska P** *et al.* (2021) Prognostic and predictive biomarkers in gliomas. *International Journal of Molecular Sciences* **22**, 10373.

98. **DeOcesano-Pereira C** *et al.* (2020) Emerging roles and potential applications of non-coding RNAs in glioblastoma. *International Journal of Molecular Sciences* **21**, E2611.

99. **Banelli B** *et al.* (2017) MicroRNA in glioblastoma: an overview. *International Journal of Genomics* **2017**, 7639084.

100. **Wu P** *et al.* (2019) Lnc-TALC promotes O6-methylguanine-DNA methyltransferase expression via regulating the c-Met pathway by competitively binding with miR-20b-3p. *Nature Communications* **10**, 2045.

101. **Beylerli O** *et al.* (2022) Long noncoding RNAs as promising biomarkers in cancer. *Non-Coding RNA Research* **7**, 66–70.

102. **Van Roosbroeck K and Calin GA** (2017) Cancer hallmarks and microRNAs: the therapeutic connection. *Advances in Cancer Research* **135**, 119–149.

103. **Paulmurugan R** *et al.* (2019) The protean world of non-coding RNAs in glioblastoma. *The Journal of Molecular Medicine* **97**, 909–925.

104. **Lu E** *et al.* (2022) The mechanisms of current platinum anticancer drug resistance in the glioma. *Current Pharmaceutical Design* **28**, 1863–1869.

105. **Visser H and Thomas AD** (2021) MicroRNAs and the DNA damage response: how is cell fate determined? *DNA Repair* **108**, 103245.

106. **Chen M** *et al.* (2021) Role of microRNAs in glioblastoma. *Oncotarget* **12**, 1707–1723.

107. **Costa PM** *et al.* (2015) MicroRNAs in glioblastoma: role in pathogenesis and opportunities for targeted therapies. *CNS & Neurological Disorders - Drug Targets* **14**, 222–238.

108. **Yadav B** *et al.* (2021) LncRNAs associated with glioblastoma: from transcriptional noise to novel regulators with a promising role in therapeutics. *Molecular Therapy Nucleic Acids* **24**, 728–742.

109. **Winkle M** *et al.* (2021) Noncoding RNA therapeutics - challenges and potential solutions. *Nature Reviews Drug Discovery* **20**, 629–651.

110. **Lei Q** *et al.* (2023) MicroRNA-based therapy for glioblastoma: opportunities and challenges. *European Journal of Pharmacology* **938**, 175388.

111. **Reda El Sayed S** *et al.* (2021) MicroRNA therapeutics in cancer: current advances and challenges. *Cancers* **13**, 2680.

112. **Shetty K** *et al.* (2022) Multifunctional nanocarriers for delivering siRNA and miRNA in glioblastoma therapy: advances in nanobiotechnology-based cancer therapy. *3 Biotech* **12**, 301.

113. **Costa PM** *et al.* (2015) MiRNA-21 silencing mediated by tumor-targeted nanoparticles combined with sunitinib: a new multimodal gene therapy approach for glioblastoma. *Journal of Controlled Release* **207**, 31–39.

114. **Lee TJ** *et al.* (2017) RNA nanoparticle-based targeted therapy for glioblastoma through inhibition of oncogenic miR-21. *Molecular Therapy* **25**, 1544–1555.

115. **Li H** *et al.* (2018) miR-519a enhances chemosensitivity and promotes autophagy in glioblastoma by targeting STAT3/Bcl2 signaling pathway. *Journal of Hematology Oncology* **11**, 70.

116. **Nan Y** *et al.* (2021) miRNA-451 regulates the NF-κB signaling pathway by targeting IKKβ to inhibit glioma cell growth. *Cell Cycle* **20**, 1967–1177.

117. **Mah LJ** *et al.* (2010) γH2AX: a sensitive molecular marker of DNA damage and repair. *Leukemia* **24**, 679–686.

118. **Vinchure OS** *et al.* (2021) miR-490 suppresses telomere maintenance program and associated hallmarks in glioblastoma. *Cellular and Molecular Life Sciences* **78**, 2299–2314.

119. **Williams AB and Schumacher B** (2016) P53 in the DNA-damage-repair process. *Cold Spring Harbor Perspectives in Medicine* **6**, a026070.

120. **Besse A** *et al.* (2016) MiR-338-5p sensitizes glioblastoma cells to radiation through regulation of genes involved in DNA damage response. *Tumour Biology* **37**, 7719–7727.

121. **Wang W** *et al.* (2017) DNA damage-induced nuclear factor-kappa B activation and its roles in cancer progression. *Journal of Cancer Metastasis and Treatment* **3**, 45–59.

122. **Soubannier V and Stifani S** (2017) NF-κB Signalling in glioblastoma. *Biomedicines* **5**, E29.

123. **Xu RX** *et al.* (2015) DNA damage-induced NF-κB activation in human glioblastoma cells promotes miR-181b expression and cell proliferation. *Cellular Physiology and Biochemistry* **35**, 913–925.

124. **Li W** *et al.* (2014) miR-221/222 confers radioresistance in glioblastoma cells through activating Akt independent of PTEN status. *Current Molecular Medicine* **14**, 185–195.

125. **Quintavalle C** *et al.* (2013) MiR-221/222 target the DNA methyltransferase MGMT in glioma cells. *PLoS ONE* **8**, e74466.

126. **O'Brien J** *et al.* (2018) Overview of microRNA biogenesis, mechanisms of actions, and circulation. *Frontiers in Endocrinology* **9**, 402.

127. **Sati ISEE and Parhar I** (2021) MicroRNAs regulate cell cycle and cell death pathways in glioblastoma. *International Journal of Molecular Sciences* **22**, 13550.

128. **Peng Y and Croce CM** (2016) The role of microRNAs in human cancer. *Signal Transduction and Targeted Therapy* **1**, 15004.

129. **Yin J** *et al.* (2021) Extracellular vesicles derived from hypoxic glioma stem-like cells confer temozolomide resistance on glioblastoma by delivering miR-30b-3p. *Theranostics* **11**, 1763–1779.

130. **Wong STS** *et al.* (2012) MicroRNA-21 inhibition enhances in vitro chemosensitivity of temozolomide-resistant glioblastoma cells. *Anticancer Research* **32**, 2835–2841.

131. **Wang G** *et al.* (2015) Targeting strategies on miRNA-21 and PDCD4 for glioblastoma. *Archives of Biochemistry and Biophysics* **580**, 64–74.

132. **Aloizou AM** *et al.* (2020) The role of MiRNA-21 in gliomas: hope for a novel therapeutic intervention? *Toxicology Reports* **7**, 1514–1530.

133. **Chen YY** *et al.* (2018) Upregulation of miR-125b, miR-181d, and miR-221 predicts poor prognosis in MGMT promoter-unmethylated glioblastoma patients. *American Journal of Clinical Pathology* **149**, 412–417.

134. **Wang P** *et al.* (2020) The HIF1α/HIF2α-miR210-3p network regulates glioblastoma cell proliferation, dedifferentiation and chemoresistance through EGF under hypoxic conditions. *Cell Death Disease* **11**, 992.

135. **Munoz JL** *et al.* (2015) Temozolomide resistance in glioblastoma occurs by miRNA-9-targeted PTCH1, independent of sonic hedgehog level. *Oncotarget* **6**, 1190–1201.

136. **Zhang J** *et al.* (2020) Inhibition of miR-1193 leads to synthetic lethality in glioblastoma multiforme cells deficient of DNA-PKcs. *Cell Death Disease* **11**, 602.

137. **Wang L** *et al.* (2014) MiR-143 acts as a tumor suppressor by targeting N-RAS and enhances temozolomide-induced apoptosis in glioma. *Oncotarget* 5, 5416–5427.

138. **Berthois Y** *et al.* (2014) Differential expression of miR200a-3p and miR21 in grade II-III and grade IV gliomas: evidence that miR200a-3p is regulated by O$^6$-methylguanine methyltransferase and promotes temozolomide responsiveness. *Cancer Biology Therapy* 15, 938–950.

139. **Xiao S** *et al.* (2016) miR-29c contribute to glioma cells temozolomide sensitivity by targeting O6-methylguanine-DNA methyltransferases indirectly. *Oncotarget* 7, 50229–50238.

140. **Wu H** *et al.* (2014) MiR-136 modulates glioma cell sensitivity to temozolomide by targeting astrocyte elevated gene-1. *Diagnostic Pathology* 9, 173.

141. **Liu Q** *et al.* (2015) miR-155 regulates glioma cells invasion and chemosensitivity by p38 isforms in vitro. *Journal of Cellular Biochemistry* 116, 1213–1221.

142. **Zhen L** *et al.* (2016) MiR-10b decreases sensitivity of glioblastoma cells to radiation by targeting AKT. *Journal of Biological Research* 23, 14.

143. **Guo P** *et al.* (2018) Upregulation of miR-96 promotes radioresistance in glioblastoma cells via targeting PDCD4. *International Journal of Oncology* 53, 1591–1600.

144. **Guo P** *et al.* (2014) MiR-26a enhances the radiosensitivity of glioblastoma multiforme cells through targeting of ataxia-telangiectasia mutated. *Experimental Cell Research* 320, 200–208.

145. **Yan D** *et al.* (2010) Targeting DNA-PKcs and ATM with miR-101 sensitizes tumors to radiation. *PLoS ONE* 5, e11397.

146. **He X and Fan S** (2018) hsa-miR-212 modulates the radiosensitivity of glioma cells by targeting BRCA1. *Oncology Reports* 39, 977–984.

147. **DeSouza PA** *et al.* (2021) Long, noncoding RNA dysregulation in glioblastoma. *Cancers* 13, 1604.

148. **Li Z** *et al.* (2019) Modulating lncRNA SNHG15/CDK6/miR-627 circuit by palbociclib, overcomes temozolomide resistance and reduces M2-polarization of glioma associated microglia in glioblastoma multiforme. *Journal of Experimental & Clinical Cancer Research* 38, 380.

149. **Xu C** *et al.* (2022) lncRNA PRADX is a mesenchymal glioblastoma biomarker for cellular metabolism targeted therapy. *Frontiers in Oncology* 12, 888922.

150. **Li J** *et al.* (2018) Targeting long noncoding RNA HMMR-AS1 suppresses and radiosensitizes glioblastoma. *Neoplasia* 20, 456–466.

151. **Yuan E** *et al.* (2022) Modulating glioblastoma chemotherapy response: evaluating long non-coding RNA effects on DNA damage response, glioma stem cell function, and hypoxic processes. *Neuro-Oncology Advances* 4, vdac119.

152. **Zhang Z** *et al.* (2019) Exosomal transfer of long non-coding RNA SBF2-AS1 enhances chemoresistance to temozolomide in glioblastoma. *Journal of Experimental & Clinical Cancer Research* 38, 166.

153. **Voce DJ** *et al.* (2019) Temozolomide treatment induces lncRNA MALAT1 in an NF-κB and p53 codependent manner in glioblastoma. *Cancer Research* 79, 2536–2548.

154. **Shangguan W** *et al.* (2019) FoxD2-AS1 is a prognostic factor in glioma and promotes temozolomide resistance in a O6-methylguanine-DNA methyltransferase-dependent manner. *Korean Journal of Physiology and Pharmacology* 23, 475–482.

155. **Nie E** *et al.* (2021) TGF-β1 modulates temozolomide resistance in glioblastoma via altered microRNA processing and elevated MGMT. *Neuro-Oncology* 23, 435–446.

156. **Gong R** *et al.* (2021) Long noncoding RNA PVT1 promotes stemness and temozolomide resistance through miR-365/ELF4/SOX2 axis in glioma. *Experimental Neurobiology* 30, 244–255.

157. **Yan Y** *et al.* (2019) Novel function of lncRNA ADAMTS9-AS2 in promoting temozolomide resistance in glioblastoma via upregulating the FUS/MDM2 ubiquitination axis. *Frontiers in Cell and Developmental Biology* 7, 217.

158. **Liao Y** *et al.* (2017) LncRNA CASC2 interacts with miR-181a to modulate glioma growth and resistance to TMZ through PTEN pathway. *Journal of Cellular Biochemistry* 118, 1889–1899.

159. **Ding J** *et al.* (2020) lncRNA CCAT2 enhanced resistance of glioma cells against chemodrugs by disturbing the normal function of miR-424. *OncoTargets Therapy* 13, 1431–1445.

160. **Yuan Z** *et al.* (2020) Exosome-mediated transfer of long noncoding RNA HOTAIR regulates temozolomide resistance by miR-519a-3p/RRM1 axis in glioblastoma. *Cancer Biotherapy and Radiopharmaceuticals*. doi: 10.1089/cbr.2019.3499.

161. **Chen M** *et al.* (2020) NCK1-AS1 Increases drug resistance of glioma cells to temozolomide by modulating miR-137/TRIM24. *Cancer Biotherapy and Radiopharmaceuticals* 35, 101–108.

162. **Liu B** *et al.* (2020) LncRNA SOX2OT promotes temozolomide resistance by elevating SOX2 expression via ALKBH5-mediated epigenetic regulation in glioblastoma. *Cell Death Disease* 11, 384.

163. **Du P** *et al.* (2017) LncRNA-XIST interacts with miR-29c to modulate the chemoresistance of glioma cell to TMZ through DNA mismatch repair pathway. *Bioscience Reports* 37, BSR20170696.

164. **Dai X** *et al.* (2019) AHIF promotes glioblastoma progression and radioresistance via exosomes. *International Journal of Oncology* 54, 261–270.

165. **Tang G** *et al.* (2021) lncRNA LINC01057 promotes mesenchymal differentiation by activating NF-κB signaling in glioblastoma. *Cancer Letters* 498, 152–164.

166. **Zheng J** *et al.* (2020) Linc-RA1 inhibits autophagy and promotes radioresistance by preventing H2Bub1/USP44 combination in glioma cells. *Cell Death Disease* 11, 758.

167. **Hao Z** *et al.* (2019) Circular RNAs: functions and prospects in glioma. *Journal of Molecular Neurosciences* 67, 72–81.

168. **Rybak-Wolf A** *et al.* (2015) Circular RNAs in the mammalian brain are highly abundant, conserved, and dynamically expressed. *Molecular Cell* 58, 870–885.

169. **Sun J** *et al.* (2020) Functions and clinical significance of circular RNAs in glioma. *Molecular Cancer* 19, 34.

170. **Wang X** *et al.* (2021) Identification of low-dose radiation-induced exosomal circ-METRN and miR-4709-3p/GRB14/PDGFRα pathway as a key regulatory mechanism in glioblastoma progression and radioresistance: functional validation and clinical theranostic significance. *International Journal of Biological Sciences* 17, 1061–1078.

171. **Lou J** *et al.* (2020) Circular RNA CDR1as disrupts the p53/MDM2 complex to inhibit Gliomagenesis. *Molecular Cancer* 19, 138.

172. **Wang HX** *et al.* (2018) Expression profile of circular RNAs in IDH-wild type glioblastoma tissues. *Clinical Neurology and Neurosurgery* 171, 168–173.

173. **Song J** *et al.* (2022) A novel protein encoded by ZCRB1-induced circHEATR5B suppresses aerobic glycolysis of GBM through phosphorylation of JMJD5. *Journal of Experimental & Clinical Cancer Research* 41, 171.

174. **Jiang Y** *et al.* (2022) CircLRFN5 inhibits the progression of glioblastoma via PRRX2/GCH1 mediated ferroptosis. *Journal of Experimental & Clinical Cancer Research* 41, 307.

175. **Caron MC** *et al.* (2019) Poly(ADP-ribose) polymerase-1 antagonizes DNA resection at double-strand breaks. *Nature Communications* 10, 2954.

176. **Pascal JM** (2018) The comings and goings of PARP-1 in response to DNA damage. *DNA Repair* 71, 177–182.

177. **Sim HW** *et al.* (2022) PARP Inhibitors in glioma: a review of therapeutic opportunities. *Cancers* 14, 1003.

178. **Murnyák B** *et al.* (2017) PARP1 Expression and its correlation with survival is tumour molecular subtype dependent in glioblastoma. *Oncotarget* 8, 46348–46362.

179. **Gourley C** *et al.* (2019) Moving from poly (ADP-ribose) polymerase inhibition to targeting DNA repair and DNA damage response in cancer therapy. *Journal of Clinical Oncology* 37, 2257–2269.

180. **Kazlauskas A** *et al.* (2019) Isocytosine deaminase Vcz as a novel tool for the prodrug cancer therapy. *BMC Cancer* 19, 197.

181. **Hanna C** *et al.* (2020) Pharmacokinetics, safety, and tolerability of olaparib and temozolomide for recurrent glioblastoma: results of the phase I OPARATIC trial. *Neuro-Oncology* 22, 1840–1850.

182. **Sim HW** *et al.* (2021) A randomized phase II trial of veliparib, radiotherapy, and temozolomide in patients with unmethylated MGMT glioblastoma: the VERTU study. *Neuro-Oncology* 23, 1736–1749.

183. **Cimprich KA and Cortez D** (2008) ATR: an essential regulator of genome integrity. *Nature Review Molecular Cellular Biology* 9, 616–627.

184. **Frosina G** *et al.* (2019) The efficacy and toxicity of ATM inhibition in glioblastoma initiating cells-driven tumor models. *Critical Reviews in Oncology and Hematology* 138, 214–222.

185. **Jucaite A** *et al.* (2020) Brain exposure of the ATM inhibitor AZD1390 in humans—a positron emission tomography study. *Neuro-Oncology* 23, 687–696.

186. **Vecchio D** *et al.* (2014) Predictability, efficacy and safety of radiosensitization of glioblastoma-initiating cells by the ATM inhibitor KU-60019. *International Journal of Cancer* 135, 479–491.

187. **Green AL** *et al.* (2015) Preclinical antitumor efficacy of selective exportin 1 inhibitors in glioblastoma. *Neuro-Oncology* **17**, 697–707.

188. **Degorre C** *et al.* (2021) Bench to bedside radiosensitizer development strategy for newly diagnosed glioblastoma. *Radiation Oncology* **16**, 191.

189. **Lassman AB** *et al.* (2022) A phase II study of the efficacy and safety of oral selinexor in recurrent glioblastoma. *Clinical Cancer Research* **28**, 452–460.

190. **Wu S** *et al.* (2018) Activation of WEE1 confers resistance to PI3 K inhibition in glioblastoma. *Neuro-Oncology* **20**, 78–91.

191. **Sanai N** *et al.* (2018) Phase 0 trial of AZD1775 in first-recurrence glioblastoma patients. *Clinical Cancer Research* **24**, 3820–3828.

192. **Lewis CW** *et al.* (2019) Upregulation of Myt1 promotes acquired resistance of cancer cells to Wee1 inhibition. *Cancer Research* **79**, 5971–5985.