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Review

# Repair mechanisms help glioblastoma resist treatment

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ABSTRACT

Glioblastoma multiforme (GBM) is a malignant and incurable glial brain tumour. The current best treatment for GBM includes maximal safe surgical resection followed by concomitant radiotherapy and adjuvant temozolomide. Despite this, median survival is still only 14–16 months. Mechanisms that lead to chemo- and radio-resistance underpin treatment failure. Insights into the DNA repair mechanisms that permit resistance to chemoradiotherapy in GBM may help improve patient responses to currently available therapies.



## 1. Introduction

Glioblastoma multiforme (GBM) is a highly malignant glial tumour (World Health Organization grade IV) that accounts for up to 78% of all malignant central nervous system (CNS) tumours [1]. Its diffuse and infiltrative nature makes complete resection impossible as tumour cells spread beyond the macroscopic margins [2,3]. Numerous innovative treatments have been proposed, including photodynamic therapy [4–6], but the current standard treatment with maximal safe surgery and concomitant radiotherapy (RT) and temozolomide (TMZ) chemotherapy (CTx) can provide a median overall survival (OS) of 14–16 months [7–13]. Stupp et al. reported improved OS rates at 2 years (27.2% *versus* 10.9%) and 5 years (9.8% *versus* 1.9%) for those receiving concomitant TMZ and RT *versus* RT alone [11,12]. Hegi et al. proposed that one of the predictors of this improved outcome was $O^6$-methylguanine DNA methyltransferase (MGMT) promoter methylation, which was present in up to 45% of the randomised cohort [11,12,14,15]. Within the MGMT methylated subgroup, those treated with RT and TMZ had a significantly improved median OS of 21.7 months (95% confidence interval [CI] 17.4–30.4) compared to 15.3 months (95% CI 13.0–20.9) for those treated with RT alone [11,12,14,15]. It is now likely that this is just one example of what might represent a genome wide epigenetic phenomenon, but how the methylation status of various genes influences tumour activity remains to be fully elucidated [16–18]. However, the correlation of MGMT promoter region methylation with improved treatment-related survival has helped emphasise the potential of personalised, targeted therapies. Some of these novel targets influence cell survival pathways, including phosphoinositide 3-kinase (PI3K)/mammalian target of rapamycin (mTOR), cyclic-nucleotide response element-binding protein, β-catenin/Wnt, Salvador–Warts–Hippo and yes-associated protein [19–22]. PI3K inhibitors (BKM120) and dual PI3K/mTOR inhibitors (BEZ235) have since entered phase I/II clinical testing and results are pending [23].

Other pathways of interest mediate the processes of cell proliferation (for example, epidermal growth factor receptor [EGFR] pathway) [24] and angiogenesis (vascular endothelial growth factor receptor [VEGFR] pathway) [25]. Phosphorylated signal transducer and activator of transcription (STAT) 3 has been shown to initiate the transcription of multiple cancer associated genes that influence these pathways and has been detected at high frequency in GBM [26]. Treatments targeting EGFR (such as erlotinib) and VEGF (such as bevacizumab) have undergone evaluation in clinical trials [27]. Although up to 40–60% of GBM tumours exhibit upregulation of EGFR signalling, there was no significant improvement in OS in response to anti-EGFR treatment in phase II clinical trials of erlotinib [28]. Furthermore, there was no evidence of EGFR signalling modulation in the post-surgical tissue specimens in response to anti-EGFR treatment, despite patient toxicity [29]. Similarly, there was no significant improvement in OS following treatment with bevacizumab (anti-VEGF anti-angiogenic treatment) in a phase III clinical trial [30]. Significantly, there are concerns that the strategy to starve tumours of their blood supply using anti-angiogenic therapies may need to be augmented with anti-invasive

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therapies [31]. This is extremely relevant as glioma cells have been shown to possess structures known as invadopodia that facilitate the invasion process [32], in addition to overexpressing a key protein involved in their formation known as Tks5 [33]. αvβ3-Integrin has been shown to be involved in invasion and angiogenesis and cilengitide is selective for $\alpha_v$ integrins, therefore acting as an angiogenic inhibitor [34,35]. However, the recently completed Cilengitide, Temozolomide, and Radiation Therapy in Treating Patients With Newly Diagnosed Glioblastoma and Methylated Gene Promoter Status (CENTRIC) phase III randomised clinical trial (clinicaltrials.gov identification [ID] NCT00689221) found no survival benefit in response to cilengitide in MGMT promoter methylated patients, with the median OS being 26.3 months in both arms [36]. The CORE phase II clinical trial (clinicaltrials.gov ID NCT00813943) investigating the clinical response of unmethylated patients to cilengitide has recently been completed and its results are pending.

While new (effective) targets continue to be identified it is important to optimise the treatments currently available. Advancements have been made to more precisely deliver ionising radiation such as stereotactic radiation, stereotactic radiosurgery, intensity-modulated radiation therapy, three-dimensional conformal radiation therapy and proton beam therapy [37–39]. However, given glial tumours are comprised of a diffuse and often large tumour bulk, advances in this modality alone may still have a limited impact. Therefore, GBM cells must be re-sensitised not only to RT, but other currently available cytotoxic therapies such as TMZ. To this point, there is mounting evidence suggesting that most cancer cells are deficient in at least one DNA repair pathway – a situation that leads to apoptosis in normal cells [40]. Loss of function in some DNA repair pathways may render malignant cells susceptible to specific targeting of other intact pathways that are normally redundant. Therapeutic targeting of these pathways leads to compounding genomic damage that ultimately causes cytotoxicity in susceptible cancer cells via a process referred to as synthetic lethality [41,42]. Synthetic lethality allows cancer cells that are deficient in DNA repair to be selectively targeted whilst sparing normal cells that have intact backup DNA repair systems [43,44].

## 2. Breaking the resistance

RT remains a keystone treatment for GBM despite the challenges posed by large and diffuse tumour volumes and its radioresistant properties [45]. RT induces cytotoxic DNA single-strand breaks (SSB) and double-strand breaks (DSB) which activate cell death programs (Fig. 1) [46]. The base excision repair (BER) pathway repairs SSB that occur following cleavage of a damaged residue from the genome. SSB are bound by poly (adenosine diphosphate ribose [ADP]) polymerase (PARP) which then recruits additional proteins (including BER components) to re-synthesise and re-join the damaged strands [47]. Although PARP participates in DSB repair, it is generally performed by the non-homologous end-joining (NHEJ) pathway [48–50].

PARP is an important component of the BER pathway as it recognises and binds to chemoradiotherapy (CRT)-induced DNA strand breaks (both SSB and DSB) and recruits X-ray repair cross-complementing protein 1 (XRCC1) [47]. XRCC1 then recruits DNA polymerases and ligases that resynthesise and ligate the damaged DNA [47]. Upon binding to DNA SSB or DSB, PARP-1 and PARP-2 (herein, collectively referred to as PARP), poly-ADP-ribosylate their own automodification domain, enabling the recruitment of DNA repair proteins. Inhibitors of the PARP catalytic site can prevent it from recruiting additional DNA repair proteins, but this only results in delayed DNA repair kinetics [51]. Nonetheless, PARP inhibition may help overcome MGMT-mediated resistance and re-sensitise tumours to CTx, particularly in patients with normal/

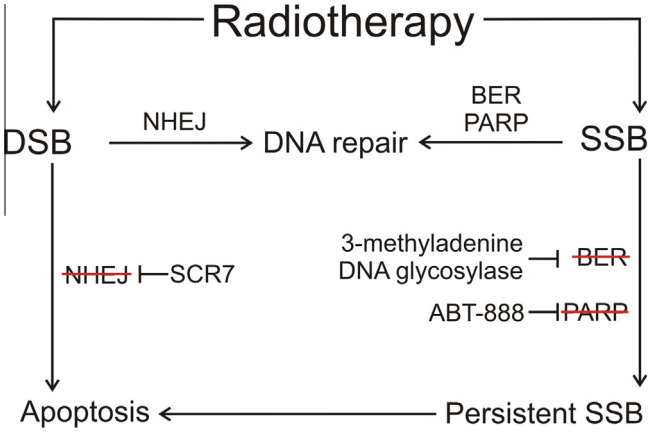


**Fig. 1.** Schematic representation of the cellular response to radiotherapy-induced damage. ABT-888 = veliparib, a PARP inhibitor, BER = base excision repair, DNA = deoxyribonucleic acid, DSB = double-strand DNA breaks, NHEJ = non-homologous end joining pathway, PARP = poly (adenosine diphosphate ribose) polymerase, SCR7 = ligase IV inhibitor, SSB = single-strand DNA breaks.

elevated MGMT levels [47]. *In vitro* studies of the PARP inhibitor ABT-888 (veliparib) have shown that it sensitises GBM cells to the cytotoxic effect of RT by 1.12–1.37 fold, and concomitant TMZ further increased tumour cell sensitivity by 1.30–1.44 fold [47]. Phase I–II clinical trials are currently underway to assess the efficacy of ABT-888 in CNS tumours (Table 1) [47].

Another PARP inhibitor (AZD-2281, olaparib), which is currently in phase I trials (Table 1), has shown some early clinical potential in patients with BRCA1/2 mutated recurrent ovarian and metastatic breast cancer [52–55]. According to the Response Evaluation Criteria in Solid Tumours (RECIST) criteria, the objective response rate to AZD-2281 (400 mg twice per day) in recurrent ovarian cancer was 31–33% resulting in a progression-free survival of 8.4–8.8 months compared to 7.1 months for pegylated liposomal doxorubicin and 4.8 months on placebo [53–57]. In another phase I trial of AZD-2281 in metastatic breast cancer patients, it was reported that 36% of patients had a partial clinical response, but the authors recommended modifications in the dose scheduling to address the frequency of adverse events (including neutropenia at a rate of 58%) [58]. Although PARP inhibitors have shown early promise further investigation is required.

Failure to reseal DSB (persistent DSB) ultimately culminates in cell death and can be induced by selective inhibition of ligase IV (a crucial NHEJ protein) with SCR7 [59]. Recently, it was shown that inhibition of ligase IV with SCR7 sensitises tumour cells to DSB induced by CRT [59]. Murine breast adenocarcinoma xenograft models treated with SCR7 survive up to four times longer than untreated animals (average survival of 52 days) [59]. Significant delays in tumour growth were also reported in SCR7-treated murine ovarian cancer xenograft models [59]. Treatment of Dalton's lymphoma murine models with SCR7 and RT has also demonstrated a synergistic reduction in tumour growth relative to those treated with RT alone [59]. After 7 days, the tumours in SCR7 and RT treated mice were approximately half the size of those in mice treated with RT alone ($p < 0.001$), suggesting that SCR7 potentiates the cytotoxic effects of RT [59]. The clinical effect of NHEJ inhibitors on GBM is yet to be elucidated, but the potential of them re-sensitising GBM to fractionated RT and alkylating CTx is an interesting prospect [59].

## 3. The role of "methylation"

TMZ has been an important part of the standard treatment regimen for GBM since the 2005 European Organisation for Research