Glioblastoma is the most frequently diagnosed type of primary brain tumour in adults. These aggressive tumours are characterised by inherent treatment resistance and disease progression, contributing to ~190,000 brain tumour-related deaths globally each year. Current therapeutic interventions consist of surgical resection followed by radiotherapy and temozolomide chemotherapy, but average survival is typically around 1 year, with <10% of patients surviving more than 5 years. Recently, a fourth treatment modality of intermediate-frequency low-intensity electric fields [called tumour-treating fields (TTFields)] was clinically approved for glioblastoma in some countries after it was found to increase median overall survival rates by ~5 months in a phase III randomised clinical trial. However, beyond these treatments, attempts to establish more effective therapies have yielded little improvement in survival for patients over the last 50 years. This is in contrast to many other types of cancer and highlights glioblastoma as a recognised tumour of unmet clinical need. Previous work has revealed that glioblastomas contain stem cell-like subpopulations that exhibit heightened expression of DNA damage response (DDR) factors, contributing to therapy resistance and disease relapse. Given that radiotherapy, chemotherapy and TTFields-based therapies all impact DDR mechanisms, this Review will focus on our current knowledge of the role of the DDR in glioblastoma biology and treatment. We also discuss the potential of effective multimodal targeting of the DDR combined with standard-of-care therapies, as well as emerging therapeutic targets, in providing much-needed improvements in survival rates for patients.
1. Introduction

1.1. Glioblastoma and current treatment regimes

Brain tumours are globally responsible for around 190,000 deaths per year (around 5000 of which are in the UK) and are responsible for the greatest reduction in life expectancy of any cancer (WHO data) [3]. Glioblastoma is ascribed the highest glioma grade designated by the World Health Organization (WHO grade IV glioma). It is the most frequently diagnosed primary brain tumour in adults and is associated with an exceptionally poor clinical course characterised by treatment resistance, rapid disease progression and dire patient survival rates of around 12–15 months following diagnosis in clinical studies [3]. However, average survival for unselected patients in the real-world setting is typically closer to 8 months [4]. In most cases, glioblastoma arises rapidly without previous clinical presentation or histological confirmation of a less malignant precursor lesion, although, in a minority of cases, signs of progression from a lower grade diffuse (WHO grade II) or anaplastic (WHO grade III) astrocytoma are evident [5].

Despite some recent advances in the genetic, epigenetic and molecular subtyping of gliomas [6], the current standard-of-care treatment remains maximal safe surgical resection followed by a course of radiotherapy with concomitant and adjuvant chemotherapy [7–9]. The mainstay chemotherapeutic agent is oral delivery of the DNA alkylating agent temozolomide following successful clinical trial data evidencing a 2.5-month increase in patient survival rates [7,8]. However, despite this aggressive course of therapy, median patient survival was estimated at 14.6 months, with < 10% of patients surviving more than 5 years. Importantly, however, in the subset of patients with promoter methylation of the DNA repair gene MGMT (discussed below), the addition of temozolomide was associated with overall survival of 21.7 months, representing an increase of 6.4 months [10]. More recent studies corroborate the benefit of temozolomide in the context of methylated MGMT status [11,12]. However, in contrast, efforts to improve the survival of patients with an unmethylated MGMT promoter have been less successful [13,14].

Since the approval of temozolomide in 2005, no new approved treatments for glioblastoma were forthcoming until the recent development and clinical approval of tumour-treating fields (TTFields) therapy, which uses locoregional delivery of alternating electrical fields within a narrow frequency range to specifically target rapidly dividing cancer cells within a defined brain region [15–17]. Akin to the introduction of temozolomide into existing radiotherapy regimes, the inclusion of TTFields to current temozolomide dosing schedules increased overall patient survival rates by approximately 5 months, leading to clinical approval for newly diagnosed glioblastoma by the Food and Drug Administration (FDA) in the United States [17] in 2015, following its initial approval for recurrent glioblastoma in 2011. However, the incorporation of TTFields into standard-of-care therapy for glioblastoma is not universally accepted for numerous reasons, including the lack of a placebo treatment, such as a sham TTFields device, in key phase 3 randomised trials [17,18]; incomplete understanding of the mechanistic basis for TTFields therapeutic effects [16]; the challenge of maintaining high treatment compliance; and the current high financial costs associated with this treatment regime. Consequently, opportunities for patients to receive TTFields are not geographically equitable and are largely dependent on the country’s healthcare system approval structure for cancer therapies [16]. Importantly, and relevant to this Review, the current landscape of multimodal therapy used to treat glioblastoma – that is, chemotherapy, radiotherapy and TTFields – all induces DNA damage, replication stress and mitotic-mediated cell death within tumour cells. As such, a deeper understanding of the innate DNA damage response (DDR) mechanisms and coordinated cell cycle regulation within these tumours offers opportunities to uncover exploitable tumourspecific genetic and phenotypic vulnerabilities which could lead to more efficient targeting and effective treatment strategies in the clinic to confer much-needed improvements in survival rates for patients.

1.2. Therapeutic challenges for glioblastoma

A cornerstone of glioblastoma treatment is effective surgical resection of the tumour bulk. However, clinical studies have suggested that neurosurgical removal of as much as 98% of the tumour volume may be required to provide an impact on median survival [19]. As such, a plethora of surgical innovations have been applied to help maximise surgical resection rates. Multimodal neuronavigation, intraoperative magnetic resonance imaging and ultrasound (MRI/US), and fluorescence-guided surgery with 5-aminolevulinic acid (5-ALA) have demonstrated the potential to augment surgical reduction of tumour burden [20–24]. Additionally, forthcoming innovations, including the application of iKnife,
which utilises rapid evaporative ionisation mass spectrometry (REIMS) to differentiate between tumour and healthy tissue [25,26] or Raman spectroscopy, which is used to achieve similar tumour-brain resolution in a nondestructive manner [27–30], may yield further improvements in resection rates in the near future. However, given the highly infiltrative nature of glioblastoma and limited ability to resect infiltrated regions of eloquent cortex without unacceptable morbidity, it is unfortunately hard to envisage advances in surgical technology impacting patient survival outcomes beyond incremental gains.

In a similar manner to surgical interventions, radiotherapy has also seen technological advancements, including intensity-modulated radiation therapy, stereotactic radiosurgery and proton beam therapy, which have the potential to lead to improved delivery of effective radiation doses to tumour sites whilst sparing the surrounding healthy brain tissue [31,32].

Beyond the challenges associated with effective and maximal surgical resection and targeted radiotherapy regimes, delivery of a therapeutically effective dose of chemotherapy to postsurgical residual tumour cells is hampered by the presence of the blood–brain barrier/blood–tumour barrier (BBB) [3,33], although there are several groups developing ways in which to disrupt or circumvent the BBB (discussed later).

The development of effective future therapies for glioblastoma will also need to tackle the biological mechanisms that help facilitate treatment resistance in these aggressive cancers. Unlike lower grade tumours, the majority of glioblastomas are classed as isocitrate dehydrogenase (IDH) wild-type but often exhibit mutations and/or deletions in phosphatase and tensin homolog (PTEN), cyclin-dependent kinase inhibitor 2A/B (CDKN2A/B), telomerase reverse transcriptase (TERT) promoter, tumour protein P53 (TP53), neurotrophicin 1 (NF1), phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (PIK3CA) and phosphoinositide-3-kinase regulatory subunit 1 (PIK3R1), as well as amplification of epidermal growth factor receptor (EGFR) and/or gain of chromosome 7, chromosome 10 monosomy and MGMT promoter methylation [5,34–36]. However, at present, no targeted therapy designed to exploit such genetic and/or phenotypic traits has proven successful within the clinic [37]. One of the main reasons for this is that glioblastomas exhibit profound intratumoural heterogeneity, with spatiotemporally divergent subpopulations within a tumour displaying varying profiles of vulnerability and resistance [38–41], which likely contributes to local disease recurrence. Additionally, increasing evidence supports the existence of a subpopulation of glioblastoma cells that possess an innate capacity for unlimited regeneration and self-renewal, which are often described as cancer stem cells (CSCs) or glioblastoma stem cells (GSCs) [3,42–47]. Importantly, GSCs are often deemed responsible for resistance to conventional chemoradiotherapy treatment regimens through enhanced activation of DNA damage checkpoints and DNA repair capacity [48–50]. This will be highlighted with specific examples later within this Review.

1.3. Clinically relevant models of glioblastoma

Over the last decade, there has been a disappointing lack of success in translating promising novel agents or drug repurposing from preclinical studies into clinical survival benefit for patients with glioblastoma [51]. To some degree, this failure is representative of a lack of clinically and postsurgically relevant preclinical models of glioblastoma. In particular, the reported and further emerging intratumoural heterogeneity that exists within gliomas is a vital consideration for the development of future therapies. Variation in the genetic aberrations, phenotypic characteristics and clinical progression of individual patient tumours presupposes the need for personalised therapeutic strategies informed by a range of molecular biomarkers [52–56]. Furthermore, accumulating evidence suggests that a phylogenetic hierarchy of spatiotemporally divergent subclones exists within individual glioblastomas, each possessing a distinct collection of putative driver mutations and a characteristic transcriptome [38,40,53,57,58]. Creating appropriate preclinical models that are capable of recapitulating such profound intratumoural heterogeneity as well as GSC states represents a key challenge for the development of new therapeutic agents that can impact patient survival [3,46,59,60]. A detailed discussion of the advantages and disadvantages of the various models is beyond the scope of this Review, but these currently consist of in vitro preclinical glioblastoma models that range from traditional commercially available immortalised cell lines or fresh patient-derived primary cell cultures (‘bulk’ or stem cell enriched) which can all be maintained in either two- or three-dimensional (2D or 3D) architectures, as well as more complex tumouroid and organoid cocultures and microfluidic ‘glioblastoma-on-a-chip’ cultures [61–73]. Likewise, there are a plethora of in vivo preclinical glioblastoma models available, including subcutaneous, syngeneic, orthotopic and patient-derived (PDX) xenografts as well as de novo genetically engineered rodent models [74–77].

What is clear from the historic lack of newly approved therapies and limited improvement in patient
survival rates over the last 50 years compared with other solid tumours is that glioblastoma represents a complex and difficult therapeutic challenge. However, recent work by numerous groups has started to reveal further genetic and molecular insights into glioblastoma biology, particularly within the area of DDR mechanisms that are triggered by current standard-of-care radiochemotherapy regimes used to treat these tumours. Furthermore, an evolving understanding of the GSC niche together with the development of novel preclinical models that better reflect postsurgical residual disease should hopefully begin to yield new therapeutic strategies over the next 5–10 years.

2. The DNA damage response and glioblastoma treatment

2.1. Cellular responses to DNA damage

The structural integrity of DNA is constantly threatened due to replication stress, telomere attrition and a multitude of endogenous and exogenous agents that generate high levels of varying DNA lesions. Such agents include metabolic by-products, such as reactive oxygen species (ROS) and aldehydes, UV light, ionising radiation (IR) and chemical toxins [78–80]. Failure to repair DNA lesions induced by these processes can lead to mutagenesis, tumorigenesis or cell attrition. Given the potential deleterious effects on genome integrity induced by such lesions, it is perhaps not surprising that an intricate network of signalling pathways and reparative mechanisms have evolved to deal with a plethora of DNA lesions, preserving genomic architecture and integrity (genome stability). This network is collectively referred to as the DNA damage response (DDR), which encompasses a coordinated and interconnected network of pathways that regulate cell cycle progression/checkpoints, DNA repair mechanisms, DNA replication and mitotic progression, as well as transcriptional and cell death processes, in order to preserve the integrity of the genome [79,81–85]. Importantly, given that radiation and chemotherapy treatments cause DNA lesions and affect cell cycle progression, heightened and/or dysregulated DDR mechanisms within tumour cells can often give rise to innate and/or acquired treatment resistance. However, such dysregulation to DDR mechanisms/processes during cancer development and progression can also offer vital cancer-selective vulnerabilities that can be exploited for an improved therapeutic index as part of either monotherapy- or combination therapy-targeted treatment strategies [82,85–89].

The mechanisms associated with physically repairing DNA damage can be categorised into four main types based on the type of DNA lesion present: (a) base modifications (including alkylation damage) and mis-paired bases; (b) intrastrand or interstrand DNA crosslinks; (c) single-strand breaks (SSBs); and (d) double-strand breaks (DSBs) [79,81,84,85,90]. Additionally, although DSBs are often considered amongst the most toxic of DNA lesions, it is worth considering that each type of lesion is not an enduringly separate entity. For example, radiation-induced damage often leads to so-called ‘complex lesions’ or ‘clustered damage’, in which multiple lesions are present within a few hundred base pairs of the DNA helix [91]. The collision of replication forks with SSBs or DNA interstrand crosslinks (ICLs), particularly as a consequence of oncogene activation, can result in the formation of DSBs and other deleterious lesions. Similarly, DNA : RNA hybrids known as R-loops can be generated from replication forks colliding with transcription bubbles, which can have a plethora of deleterious effects if not adequately resolved [92,93]. As such, the dynamic relationships that exist between various DNA lesions lead to functional redundancy within the reparative processes, which likely contributes to treatment resistance and forms the rationale for targeting multiple DDR pathways/processes to potentially overcome treatment resistance [78,82,83,86–89].

Maintenance of genome integrity relies not only on accurate DNA repair, but also on the processes that detect DNA damage and co-ordinate cell cycle checkpoints, allowing time for DNA to be repaired. The regulation of cell cycle checkpoints and subsequent DNA repair processes as part of the early DDR process is controlled by three main related protein kinases – ataxia telangiectasia mutated (ATM); ataxia telangiectasia and Rad3-related kinase (ATR); and DNA-dependent protein kinase (DNA-PK) – along with the various cyclin-dependent kinases (CDKs) and the central cell cycle regulator p53 and its associated factors [83,90] (Fig. 1). ATM represents an apical, multifunctional kinase within the DDR and typically plays a key role in cellular responses to DNA DSBs, where it helps co-ordinate all major cell cycle checkpoints via the ATM-CHK2 axis [90,94]. Through the ATR-CHK1 signalling axis, ATR helps preserve DNA integrity in response to replication stress (perturbation to ongoing replication forks) [95], although it is important to note that there is significant crosstalk and some functional redundancy amongst the activities of these related kinases [90,96]. However, due to the main differing functions of these kinases, they are considered as credible separate drug targets in cancer biology as...
part of either monotherapy or combinatorial (adjuvant) therapeutic approaches [88,90,97,98]. Beyond the initial DDR signalling and cell cycle checkpoint regulation, a key part of the cellular responses to DNA damage is obviously the physical repair of the vast array of DNA lesions that can be present within both heterochromatic and euchromatic regions of the genome [99–102]. An exhaustive discussion of the vast array of DDR pathways is not possible within the confines of this Review; however, brief summaries of some of the DNA repair pathways most relevant to the therapy-induced DNA damage within the context of glioblastoma treatment will be discussed in the following section.

Fig. 1. The role of ATM and ATR in cell cycle regulation following DNA damage. The processes of cell division (mitosis, M phase) and DNA synthesis (S phase) are separated by two important gap phases (G1 and G2). Progression of mitotic cells through the cell cycle is controlled by periodic accumulation and destruction of the aptly named cyclin-dependent kinases (CDKs) and cyclins. Inappropriate progression through phases of the cell cycle is prevented by three main checkpoints (G1/S, intra-S and G2/M checkpoints; dashed red lines). Following DNA damage, checkpoint activation is critical to provide ample time and recruit the necessary machinery required to maintain genomic integrity. Checkpoint activation: DNA double-strand breaks (DSBs) activate the apical DNA damage response (DDR) kinase ataxia telangiectasia mutated (ATM), which can influence all three major cell cycle checkpoints via the phosphorylation of checkpoint kinase 2 (CHK2) and subsequent downstream signalling. In contrast, ataxia telangiectasia and Rad3-related kinase (ATR) is activated by the presence of replication protein A (RPA)-coated single-stranded DNA (ssDNA) and contributes to maintenance of the intra-S phase and G2/M checkpoints via phosphorylation of checkpoint kinase 1 (CHK1) and subsequent downstream signalling as indicated. G1/S checkpoint: Phosphorylation of p53 by CHK2 and ATM directly (arrow not shown) results in a reduction in the binding of mouse double minute 2 homolog (MDM2) to p53 and p53 activation, promoting its nuclear accumulation and stabilisation. Subsequently, elevated p53 levels promote increased transcription of p21, which inhibits CDK2–cyclin-E activity, resulting in prevention of progression to S phase. Intra-S checkpoint: Within S phase, the activation of cell division cycle 25 (CDC25) phosphatases predominantly by prevention of cell division cycle 45 (CDC45) loading onto replication origins (preventing subsequent DNA replication) primarily via the ATR–CHK1 axis, but also via ATM-CHK2-mediated phosphorylation of CDC25A, can instigate an intra-S checkpoint in response to replication stress or other perturbations to optimal DNA synthesis, permitting a slowing of DNA replication. G2/M checkpoint: Both ATM- and ATR-mediated phosphorylation of CHK2 and CHK1, respectively, lead to the phosphorylation of CDC25C phosphatases, which influence the G2/M checkpoint via interaction with the cyclinB1–CDK1 complex. This figure is adapted, with permission, from Ref. [227].

2.2. Therapy-induced DNA lesions and associated repair mechanisms

Abrogation of the mechanisms that glioblastoma cells use to repair the DNA lesions induced by chemo- and radiotherapy may represent a key to effective multimodal treatment approaches [97] (Fig. 2). The most effective chemotherapeutic agent in current standard-of-care clinical use for glioblastoma is the alkylating agent temozolomide [8]. Of the array of methylation lesions induced on both nitrogen and oxygen molecules within DNA nucleotides, O6-methylguanine (O6MeG) is considered the most toxic lesion induced by temozolomide once metabolised from its prodrug
form to MTIC [3-methyl-(triazen-1-yl)imidazole-4-carboxamide] [103]. O6MeG can act as a miscoding base during DNA replication, leading to a corresponding C-to-T transversion within the complementary DNA strand. If O6MeG is not successfully excised by the mismatch repair (MMR) DNA repair machinery [104], it endures as a perpetually miscoding base, instigating ‘futile cycles’ of MMR with consequent stalling of DNA replication forks or double-strand breakage [104,105]. As such, MMR capacity within GSCs harbouring genetic or epigenetic defects that affect MMR gene expression can impact on temozolomide sensitivity as well as other phenotypic traits due to the well-established hypermutation phenotype conferred by MMR defects [106–108] (Fig. 2). Additionally, the de-alkylating enzyme methylguanine methyltransferase (MGMT) can sequester the methyl group from O6MeG to restore the guanine residue to its original state, but this leaves MGMT irreversibly inactivated and subject to ubiquitin-mediated proteasomal degradation [105,109]. Consequently, high expression levels of MGMT can contribute to temozolomide resistance in glioblastoma [109]. On the other hand, approximately 40% of IDH-wild-type glioblastomas exhibit
The effects of clinically approved therapies on the DNA damage response (DDR) and novel strategies to enhance efficacy of current standard-of-care treatments. Schematic representation of the main DNA damage lesions (in blue italic) induced by therapies approved for clinical use to treat glioblastoma and associated DDR mechanisms. For each approved treatment, putative strategies to enhance therapeutic efficacy through targeting relevant DDR mechanism(s) are indicated. (A) Radiotherapy: generates large amounts of DNA single-strand breaks (SSBs) and double-strand breaks (DSBs), which activate ATR and ATM, respectively. DSB repair is then predominantly undertaken by either nonhomologous end joining (NHEJ), which is available throughout the cell cycle but compromises fidelity, or homologous recombination (HR) DNA repair, which provides a high-fidelity repair mechanism, but is only available during S and G2 phases of the cell cycle due to the requirement for a sister chromatid. SSB repair relies on PARP1 to detect SSBs and facilitate the recruitment of XRCC1. However, the presence of strand breaks also leads to stalling of DNA replication forks, which depend on the functions of ATR and proteins within the Fanconi anaemia pathway (FAP) for stability and replication restart. Consequently, a strong scientific rationale exists supporting inhibition of either ATM (ATMi), ATR (ATRI), PARP1 (PARPi) or the FAP (FAPi) to enhance the efficacy of radiotherapy. (B) Temozolomide: produces an array of methylation lesions including N3-methyladenine (N3MeA) and N7-methylguanine (N7MeG), which are substrates for effective removal via DNA base excision repair (BER), and O6-methylguanine (O6MeG), which is removed directly by the enzyme MGMT in a suicide reaction. Hypermethylation of the MGMT gene promoter region leads to reduced MGMT expression, shifting the balance in favour of persistent O6MeG. O6MeG can act as a miscoding base during DNA replication, leading to a corresponding C-to-T transition within the complementary DNA strand. If O6MeG is not successfully excised by the mismatch repair (MMR) DNA repair machinery, it endures as a perpetually miscoding base, instigating ‘futile cycles’ of MMR with consequent stalling of DNA replication forks or DSBs. (C) Tumour-treating fields (TTFields): may negatively impact FAP and HR-mediated DNA repair processes. TTFields-induced ‘BRCAness’ (reflecting a relative HR deficiency) provides a compelling rationale to combine this therapeutic modality with PARPi, or potentially FAPI, ATRi or even ATMi. (D) Carmustine (BCNU) – Gliadel® wafers: provide local delivery of this bidirectional DNA alkylating agent, leading to the generation of DNA interstrand crosslinks that impede DNA replication during S phase. This leads to activation of the FAP, within which monoubiquitination of FANCD2 within the FANCD2-I complex is a key quantifiable step. Activated FANCD2-I coalesces as foci at sites of DNA damage and acts as a master regulator of downstream DNA repair, recruiting proteins involved in nucleotide excision repair (NER), translesion synthesis (TLS) and HR. Interplay with associated DDR mechanisms, for example ATM and ATR, leads to the phosphorylation of multiple FAP proteins (examples indicated), providing a rationale for the use of non-FAP DDR inhibitors (e.g. ATRi or ATMi) to sensitise to crosslinking chemotherapy, and for the concept of combining multiple DDR inhibitors (including FAPI) to potentially maximise therapeutic enhancement.

The mere notion that a DNA repair mechanism such as MGMT expression can profoundly influence survival for some patients provides further evidence of the critical nature of DNA repair in glioblastoma. However, it is important to note that, although both MMR activity and MGMT expression can impact temozolomide sensitivity of glioblastomas, longitudinal genomic profiling has revealed that such defects do not account for the majority of therapy resistance exhibited within these tumours [106]. In addition to O6MeG, the more prevalent (but less toxic) temozolomide-induced DNA lesions N3-methyladenine and N7-methylguanine are primarily removed via base excision repair (BER), leaving an intermediary abasic [or apurinic/apyrimidinic (AP)] site. The nucleotide gap is subsequently filled through triggered DNA synthesis processes and strand integrity restored through ligation of the DNA ends – a process involving an AP endonuclease (APEX1), DNA polymerase (pol-β) and DNA ligases I and III, respectively [110–112]. Additionally, nucleotide excision repair (NER) is able to remove a wide variety of structurally unrelated DNA lesions that can be generated by both chemo- and radiotherapy. The process can be activated by DNA helix distortions associated with structural changes to nucleotides or by stalling of RNA polymerase II due to the presence of a DNA lesion during transcript elongation, and involves the DNA unwinding before removal of a section of DNA containing the lesion followed by resynthesis using the template strand and subsequent ligation [113].

Radiotherapy exerts cellular damage by inducing a wide range of DNA lesions, but it is particularly associated with the generation of large amounts of SSBs and DSBs (Fig. 2). Poly (ADP-ribose) polymerase-1 (PARP1) plays a pivotal role in detection of SSBs, facilitating the colocalisation of the single-strand break repair (SSBR) polypeptide x-ray repair cross-complementing protein 1 (XRCC1) [114,115]. DNA DSBs are predominantly repaired by the nonhomologous end joining (NHEJ) and homologous recombination (HR) DNA repair pathways. The HR pathway represents a complex, high-fidelity mechanism of DNA DSB repair. However, given its reliance on a homologous DNA sequence (duplicate DNA strand on a sister chromatid) as a template for resynthesis of removed DNA sequences around the site of DNA damage, HR-mediated repair mechanisms are only possible during S and
G2 phases of the cell cycle, when such sister chromatids are available [116,117]. Interestingly, although TTFields therapy does not directly induce DNA breaks (unlike IR), recent work has suggested that, in addition to its effects on mitotic cells, TTFields may negatively impact on HR-mediated DNA repair processes [16,118], which could be important for future DDR-targeting strategies that combine with this recently approved glioblastoma therapy. In contrast to HR, the NHEJ DNA repair pathway provides rapid DSB repair capabilities throughout the cell cycle (since a homologous sister chromatid is not required), permitting repair of a range of DNA-end configurations (Fig. 2). However, the flexibility and rapidity of NHEJ is provided at the expense of fidelity [119,120]. As one might expect given the cell cycle regulated nature of these pathways, both HR and NHEJ are tightly regulated by CDK activity [120,121], a family of kinases often dysregulated in human cancers [122,123].

Finally, the Fanconi anaemia (FA or FA/BRCA) pathway is frequently activated in response to DNA strand breaks, alkylatation damage and other cytotoxic DNA lesions induced by both alkylating and DNA crosslinking chemotherapeutic agents that impede ongoing DNA replication [124–128]. The FA pathway is also activated during normal S-phase progression and is regulated by both ATM and ATR kinases [124–134]. The FA pathway consists of at least 22 proteins which can be broadly categorised into three distinct functional groups (core complex, ID complex and downstream effectors) that, through sequential interaction, facilitate lesion repair and the restart of replication forks via physical removal of lesions by NER and HR-mediated processes, as well as interactions with components of the MMR system [135].

3. Targeting functional interplay within the glioblastoma DDR network

3.1. Exploiting synthetic lethality and synthetic sensitivity strategies

As mentioned previously, there are a plethora of interconnected interactions and functional crosstalk between the various DDR, regulatory and DNA repair pathways that open up a multitude of potential therapeutic targeting strategies in heterogeneous tumours such as glioblastoma [88]. Perhaps most notable over the past 10 years or so is the concept of synthetic lethality: a concept that was originally described as a simultaneous genetic mutation in, or functional aberration of the product of, two genes which causes cell death; but in isolation, either change is survivable [136]. More recently, synthetic lethality in terms of oncology-based therapeutic strategies has expanded to include scenarios in which specific phenotypic traits such as defective HR-mediated DNA repair function, or aberration in the function of certain genes or pathways, result in impaired cell growth or proliferation, promoting lethal effects in the presence of additional insults such as IR or cytotoxic chemotherapy [137]. As such, the term ‘synthetic sensitivity or lethality’ (SSL) can be used to amalgamate these related concepts.

Although major advancements in the generation of RNAi and CRISPR/Cas9 libraries have undoubtedly advanced our ability to uncover potential SSL relationships at the genome scale, the most successful implementation of an SSL strategy to date in cancer treatment, the targeting of BRCA-deficient cells with PARP1 inhibitors, was derived from hypothesis-driven research rooted in a fundamental understanding of the specific molecular pathways concerned and relevant interplay between them, rather than broad screening approaches [138,139]. This seminal work has led to a wave of clinical approvals for PARP1 inhibitors that are delivering survival benefits to patients with a range of cancers globally [140–142]. In the context of gliomas, tumours with IDH1/2 mutations may exhibit a ‘BRCA-ness’ phenotype as a consequence of inhibition of HR DNA repair processes by the enhanced levels of oncometabolites, which could explain why a subset of IDH-mutant tumours respond well to conventional DNA damaging chemotherapies such as temozolomide as well as PARP1 and ATR inhibitors [141,143,144]. However, as highlighted earlier, the majority of high-grade aggressive gliomas do not exhibit such defects in IDH genes and associated metabolism but, as discussed below, disruption to HR and associated DDR pathways may represent a credible mechanism to produce a similar phenotype in these tumours. Furthermore, challenges in efficient delivery of such compounds across the BBB are a major clinical consideration; however, at least for PARP1 inhibitors, early indications are encouraging in that therapeutically active doses can be achieved at the tumour site [145].

3.2. Single DDR inhibitor strategies to enhance therapeutic response

As mentioned previously, glioblastoma cells within a single tumour can demonstrate remarkable heterogeneity in the expression of DDR factors and consequently exhibit varying resistance profiles [146,147], which likely plays a key role in treatment failure.
Given the interconnected nature of the DDR pathways [88], it is perhaps not surprising that, although promising in preclinical models, targeting any single DDR pathway might not yield an effective therapeutic response clinically. The D’Andrea group were the first to demonstrate that components of the FA pathway could confer resistance to temozolomide in glioma cells [148], which was further corroborated by Kondo and colleagues [149]. Building upon these findings, we showed that the FA pathway is re-expressed and active within high-grade gliomas compared with low-grade tumours as well as normal healthy tissue and that inhibition of the FA pathway in both established and primary glioma cells could confer an increased sensitivity to temozolomide [150]. These findings were recently confirmed by large-scale CRISPR-Cas9 screens that identified the FA and related HR pathways as key modulators of temozolomide resistance within glioma stem cells [108]. Importantly, we showed that disruption to FA pathway function was able to render glioma cells sensitive to temozolomide irrespective of MGMT status/expression levels [150], demonstrating a potential large scope for such an approach within the clinical setting. This is particularly important, as PARP1 inhibition (the most successful DDR-targeting drug to date) may only confer a similar increased temozolomide sensitivity within cells that have MGMT promoter methylation [151], which represents ~40% of all patients diagnosed with glioblastoma and whose tumours are already intrinsically more sensitive to temozolomide. This is consistent with previous work by Gupta and colleagues, who demonstrated that siRNA-mediated knockdown of either N-methylpurine DNA glycosylase (MPG) or XRCC1 (two indispensable components of a functional BER pathway) did not confer temozolomide sensitivity in resistant glioma cell lines [152], even though PARP1 can act as a scaffold to promote BER-mediated repair of alkylating damage [153].

Given that persistent bulky O6MeG lesions in the absence of MGMT result in elevated DNA replication stress [154,155] and that replication fork stability and recovery is influenced by a number of DDR factors, such as PARP1, as well as the HR and FA proteins RAD51/FANCN, FANCD2, BRCA1/FANCS and BRCA2/FANCD1, the integrity of some of these interconnected DDR processes may underpin the heterogeneous preclinical success of PARP1 inhibitor-mediated temozolomide sensitisation. At least theoretically, these data would support the use of adding an additional DDR inhibitor to maximise replication fork collapse and/or failure of resultant DNA DSB resolution, potentially resulting in more pronounced neoplastic cell death across a wider range of tumours. In this regard, it seems rational that the activity of apical DDR kinases, such as ATR and ATM, that regulate replication stress signalling and DNA break repair mechanisms might cooperate to limit the cytotoxicity of temozolomide in cancer cells. Interestingly, activation of both ATR and ATM following temozolomide chemotherapy occurs in an MMR-dependent manner [156], which likely reflects the nature of the cytotoxicity associated with O6meG compared with N7-meG and N3-meA lesions, which do not activate MMR. Previous work by Eich and colleagues showed that siRNA-mediated knockdown of either ATR or ATM sensitised an MGMT-negative glioma cell line to temozolomide [157], with similar findings for ATM also reported by Nadkarni et al. [158]. Interestingly, the degree of sensitisation conferred by ATR knockdown in the Eich study was more than double that observed following ATM siRNA, indicating that impaired resolution of DNA replication stress generated from O6MeG lesions may be a key driver of sensitisation in this context, especially given that simultaneous ATR and ATM knockdown did not provide any additional sensitisation relative to ATR alone and that the enhanced temozolomide sensitisation could be rescued by ectopic expression of MGMT [157]. These findings were also recently corroborated by Jackson et al., who established that MGMT-deficient glioma cells are profoundly susceptible to temozolomide sensitisation using small-molecule ATR inhibitors both in vitro and in vivo [159].

3.3. The FA pathway as a foundation for future DDR-centric combinatorial strategies

In addition to potential compounding genetic and/or epigenetic alterations within glioblastomas that might affect DDR-targeting strategies, and the interplay within the BER, MMR, MGMT, FA and HR pathways (highlighted above), interactions between the FA pathway and other DDR elements have been characterised that are also important to consider with regard to potential FA-based combination targeting strategies within glioblastomas [160]. For example, as mentioned previously, both ATM and ATR phosphorylate several proteins within the various FA subcomplexes [161–163] and components of the FA pathway have also been shown to promote activation of ATR and suppress potentially deleterious repair of DNA ICLs by NHEJ [164–166]. Additionally, as the FA pathway promotes HR-mediated DNA repair processes around stalled/collapsed replication forks [167], HR-deficient cells have been shown to be sensitive to disruption of
FA pathway function [168,169]. Given this functional interplay, or has been postulated that FA dysfunctional tumours, or targeting of the FA pathway, may render them sensitive to PARP1 inhibitors [170–172], as well as a multitude of other drug targeting strategies within the FA and related DDR pathways [170]. Indeed, we have recently generated data in clinically relevant GSC 3D cell culture model systems highlighting profound radio- and chemosensitisation through the combined targeting of the FA pathway in combination with ATM, ATR or PARP1 inhibitors (Rominiyi et al, manuscript under preparation).

Unfortunately, there have been no reported rationally designed inhibitors of the FA pathway to date; however, recent structural insights into key regulatory components of the FA pathway together with current efforts by several groups to identify FA pathway inhibitors (including our own group) [173–178] should hopefully lead to the emergence and further development of such compounds in the near future that could be of great clinical significance for glioblastoma treatment regimes. Apart from exhibiting high potency and specificity, such compounds would also need to demonstrate high biological efficacy within the correct brain regions or be adaptable to novel delivery mechanisms in order to maximise their effectiveness in any such therapeutic regimes [3]. Encouragingly, tumour margin penetration within a biologically active drug concentration range has been recently reported for the PARP1 inhibitor olaparib (Lynparza) in combination with temozolomide chemotherapy as part of the OPARATIC trial [145], and it will be interesting to see the results from other DDR inhibitor trials for glioblastoma when they are reported (Table 1).

3.4. Expanding the preclinical evidence base for parallel targeting of the DDR

As highlighted previously, there has been intense research into the development of small-molecule inhibitors of DDR proteins [82]; to date, however, only a handful of published preclinical studies have examined combined inhibition of multiple DDR elements simultaneously in glioblastoma. Given that ATM and PARP1 inhibition each individually possess some utility in sensitising GSCs to radiotherapy [179,180], Ahmed et al. [181] investigated parallel inhibition of the DDR targets ATM, ATR, CHK1 and PARP1 in primary patient-derived glioblastoma cell lines. Firstly, in agreement with previous findings by Bao et al. [48], this study demonstrated that these DDR factors were upregulated in the inherently treatment-resistant subpopulation of GSCs compared to bulk populations. This further confirms the importance of targeting multiple DDR pathways and the potential for functional redundancy within the DDR to contribute to the treatment-refractory nature of glioblastoma. Secondly, the study by Ahmed et al. demonstrated that combined inhibition of PARP1 and ATR resulted in a profound radiosensitisation of GSCs, with effects greater than any single inhibitor used in isolation [181].

The rationale for a combination DDR inhibitory strategy in glioblastoma is also supported by the work of Signore and colleagues, who performed a simultaneous multipathway approach with subsequent reverse-phase protein microarrays and kinase inhibitor library screening to identify dual inhibition of CHK1 and PDK1, resulted in profound retardation of GSC growth in both in vitro and in vivo (subcutaneous and intracranial mouse) models [182]. Although this study is highly informative, the lack of target specificity with use of a drug such as UCN-01 severely limits potential clinical utility due to the high likelihood of dose-limiting toxicities mediated by known off-target effects associated with this compound. Nevertheless, this work provides further preclinical proof-of-concept data in support of such combinatorial approaches within heterogeneous glioblastoma tumour populations. More recently, Rasmussen et al. [183] demonstrated that reduced DDR capacity through PARP1 inhibition (olaparib) in conjunction with epigenetic-downregulation-induced oxidative stress through histone deacetylase (HDAC) inhibition (vorinostat) led to reduced glioblastoma cell survival, induced apoptosis and impaired cell cycle progression.

As demonstrated through these examples, the principle of multimodal targeting within the DDR network based on an in-depth mechanistic understanding of functional interplay and regulatory mechanisms offers a potentially powerful approach to combat biological complexity and functional redundancy within the DDR, as well as intratumour heterogeneity within glioblastoma tumour subpopulations (Fig. 3). Indeed, preclinical research investigating such dual DDR inhibition approaches outside of glioblastoma research is supportive of this concept, for example CHK1 and PARP1 inhibition in pancreatic cancer [184], CHK2 and PARP1 inhibition in lymphoma [185], CHK1 and PARP1 inhibition in breast cancer [186], ATR and PARP1 inhibition in breast and ovarian cancer [187], and ATM and PARP1 inhibition in lung cancer [188].

3.5. Indirect DDR-targeting strategies in glioblastoma

In addition to direct combination targeting of DDR network factors, a comparable approach can be to
Table 1. DNA damage response inhibitor trials in high-grade glioma. AEs (G3-4), grade 3-4 adverse events; AEs, adverse events; and WBRT, whole brain radiotherapy; CR, complete response; DIPG, diffuse intrinsic pontine glioma; DLTs, dose-limiting toxicities; EFS, event-free survival; F/U, follow-up; IMRT, intensity-modulated radiation therapy; IR, radiotherapy; MTD, maximum tolerated dose; nGBM, newly diagnosed GBM; NIRA, niraparib; OLAP, olaparib; ORR, overall response rate (proportion of patients with a PR or CR); OS, median overall survival; PAMI, pamiparib; PFS, median progression-free survival; PR, partial response; QoL, quality of life; rGBM, recurrent GBM; rHGG, recurrent high-grade glioma; RP2D, recommended phase II dose – highest dose with acceptable toxicity (producing a rate of around 20% DLTs); Rx, treatment; SAEs, severe adverse events; SD, stable disease; SoC, standard-of-care; TALA, talazoparib; TMZ, temozolomide; TTF, tumour-treating fields; VELI, veliparib.

| Trial (reference) & indication | Design & n (rec dates) | Treatment(s) | 1st Endpoint | 2nd Endpoint(s) | Results/remarks & conclusions |
|-------------------------------|-------------------------|--------------|--------------|----------------|-----------------------------|
| NCT0770471 [228] nGBM Veliparib (ABT-888), radiation therapy, and temozolomide in treating patients with newly diagnosed glioblastoma multiforme | Phase I Single arm 24 patients (2009-2012) | VELI + IR + TMZ | Phase I: VELI MTD Phase II: OS (with VELI MTD) | A) Safety/toxicity B) Pharmacokinetics | Following initial safety groups and planned dosing steps, 3/6 pts (50%) had DLTs (2 thrombocytopenia, 1 neutropenia) with 10mg BD VELI+IR+TMZ accrual discontinued F) VELI at this dose with standard dosing regimen of IR+TMZ deemed not tolerable F) Further development of appropriate dosing regimen needed |
| NCT01390571 [229,230] rGBM Olaparib and temozolomide in treating patients with relapsed glioblastoma (OPARATIC) | Phase 0/I Single arm 48 patients (2011-2017) | Stage I: OLAP for 3/7 prior to surgery then usual Rx Stage II: Escalating OLAP 3/7 prior to surgery then OLAP + TMZ post-op | Phase 0: Tumour penetration via BBB/BTB Phase I: Safety | A) BBB disruption/permeability B) Preliminary antitumour activity of OLAP + TMZ | OLAP detected in 73/74 tumour specimens from 27 pts, mean conc. 588nM (range = 97–1374 nM) Mean tumour margin: core ratio = 1.2 (0.2-3.9) Mean tumour: plasma ratio = 0.25 (0.01-0.8) 24/35 pts (67%) AEs (G3-4) 45% PFS at 6 m FAU OLAP penetrates tumour core/margins and is safe with extended low-dose TMZ |
| NCT01294735 [230] nGBM/Melanoma/solid cancers | Phase I Single arm 19 patients | NIRA + TMZ | No. of DLTs | A) ORR within 30 days of | MTD & RP2D = 40 mg OD NIRA with 150 mg.m⁻² TMZ, |
Table 1. (Continued).

| Trial (reference) & indication | Design & n (rec dates) | Treatment(s) 1 ° | 1° Endpoint | 2° Endpoint(s) | Results/remarks & conclusions |
|--------------------------------|------------------------|------------------|-------------|---------------|-------------------------------|
| Niraparib (MK-4827) given with temozolomide in participants with advanced cancer | (2011–2012) | last dose & 2m intervals | B) PFS | ● 2/10 pts (20%) had Grade 4 thrombocytopenia at this dose |
| nGBM [231] | | Safety/toxicity (MTD & optimum scheduling) | A) Define DLTs (+/- TMZ) | ● 1 PR (glioblastoma) & 2 SD out of 16 evaluable pts |
| Two parallel phase I studies of olaparib and radiotherapy or olaparib and radiotherapy plus temozolomide in patients with newly diagnosed glioblastoma, with treatment stratified by MGMT status (PARADIGM-2) | Phase I Parallel Estimated patients: 25-40 methylated; 19-28 unmethylated (2016-2021) | Methylated: OLAP + IR + TMZ | Phase I: MTD. Phase II: PFS at 6m | ● NIRA tolerable in combination with TMZ |
| Phase I/II studies | NCT01026493 [232] rGBM | Arm 1: VELI + TMZ 75 mg·m⁻² (both 21/28 day cycle) | A) ORR |
| A randomized phase I/II study of veliparib (ABT-888) in combination with temozolomide in recurrent (temozolomide resistant) glioblastoma (RTOG0929) | Phase II Randomised 225 patients: 151 BEV naïve (BEV-N); 74 BEV refractory (BEV-R) (2009-2017) | Arm 2: VELI + TMZ 150 mg·m⁻² (both 5/28 day cycle) | B) OS | ● Myelosuppression AE(G3-4) in 20% of pts |
| NCT01514201 [214,233] DIPG | Phase I Single arm 65 patients (2012-2018) | VELI + IR + TMZ | Phase I: RP2D/DLTs. Phase II: OS | ● PFS at 6 m = 17.0% (BEV-N) & 4.4% (BEV-R) – median PFS ~ 2 m (95% CI, 1.9-2.1 m) in both groups |
| Veliparib, radiation therapy, and temozolomide in treating younger patients with newly diagnosed diffuse pontine | | Phase I: RP2D/DLTs. | A) PFS | ● Median OS = 10.3 m (8.4–12.0 m, BEV-N) & 4.7 m (3.5–5.6 m, BEV-R) |
| | | Phase II: OS | B) Pseudoprogression | ● Concluded addition of VELI ‘did not significantly improve PFS at 6m’ relative to historic controls |
| | | Pharmacokinetics | C) Pharmacokinetics | ● Note: MGMT status was not included or analysed |

Note: MGMT status was not included or analysed.
Table 1. (Continued).

| Trial (reference) & indication | Design & n (rec dates) | Treatment(s) | 1° Endpoint | 2° Endpoint(s) | Results/remarks & conclusions |
|-------------------------------|------------------------|--------------|-------------|---------------|-------------------------------|
| gliomas: a paediatric brain tumor consortium study |       | TALA + TMZ | Phase I/II |     | Day 4 average VELI (65 mg·m⁻² day⁻¹) plasma C_max = 3 µM |
|                               | Phase I: MTD/RP2D; Safety/toxicity; Pharmacokinetics Phase II: ORR |             |             |               | VELI DLTs inc: intratumoural haemorrhage (1 pt, Grade 2); rash (2 pts, Grade 3); neurological (1 pt, Grade 3) |
|                               |                        |             |             |               | Additional intrapatient TMZ dose escalation could not be tolerated |
|                               |                        |             |             |               | OS at 1 and 2 years = 37.2% and 5.3% respectively |
|                               |                        |             |             |               | Accrual stopped early due to futility at interim analysis |
| Solid & haematological cancers Talazoparib and temozolomide in treating younger patients with refractory or recurrent malignancies | Phase I/II Single arm 40 patients (2014–2018) | TALA + TMZ | Phase I: MTD/RP2D; Safety/toxicity; Pharmacokinetics Phase II: ORR (Ewing/PNET) | A) ORR all solid tumours (RECIST) | RP2D = TALA 600 µg·m⁻² BD on day 1 then OD days 2–6/28 with TMZ 30 mg·m⁻² day 2–6/28 cycle |
|                               |                        |             |             |               | Majority of patients had Ewing sarcoma (EWS), but one patient with a malignant glioma experienced a PR |
|                               |                        |             |             |               | During Phase II, no response observed out of 10 EWS pts |
|                               |                        |             |             |               | No efficacy in EWS but may warrant further study in CNS tumours |
| nGBM/rGBM Pamiparib (BGB-290) with radiation and/or temozolomide (TMZ) in newly diagnosed or recurrent glioblastoma | Phase Ib/II Parallel Estimated patients: 116 (2017–2021) | nGBM (unmethylated) Arm 1: PAMI + IR Arm 2: PAMI + IR + TMZ nGBM (un- & methylated) Arm 3: PAMI + TMZ | Phase I: Safety/toxicity Phase II: Disease response/control | A) Pharmacokinetics B) PFS C) OS D) ORR | RP2D for Arm 1 = PAMI 60 mg BD for 6 weeks alongside IR |
|                               |                        |             |             |               | RP2D for Arm 3 = PAMI 60 mg BD day 1–28 + TMZ 60 mg·m⁻² 7/28 day cycle |
|                               |                        |             |             |               | Well tolerated – no Grade 4/5 toxicities; Grade 3 – Arm 1 -nausea (2%), Arm |

NCT02167777 [234]  
NCT03150862 [235]  
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| Trial (reference) & indication | Design & n (rec dates) | Treatment(s) | 1° Endpoint | 2° Endpoint(s) | Results/remarks & conclusions |
|--------------------------------|------------------------|--------------|-------------|---------------|-----------------------------|
| **Phase I/Ia studies**        |                        |              |             |               |                             |
| NCT03212742 [236]             | Phase I/Ia Sequential  | OLAP + IR + TMZ | Phase I: RP2D for both IR-period and maintenance period. Phase II: OS | A) PFS  
B) ORR  
C) Neurocognitive function  
D) Morphological and functional MRI findings | 2 – decreased WBC count (11%). Arm 3 none  
• PAM1 + IR + TMZ well tolerated – trial ongoing – recruitment ends October 2021, final results awaited |
| Unresectable HGG              | Estimated patients: 79 |              |             |               |                             |
| Study of concomitant radiotherapy with olaparib and temozolomide in unresectable high-grade gliomas patients (OLA-TMZ-RTE-01) | (2017-2022) |              |             |               |                             |
|                                |                        |              |             |               |                             |
| **Phase II studies**          |                        |              |             |               |                             |
| NCT02974621                   | Phase II Randomised    | Arm 1: OLAP + cediranib Arm 2: BEV | PFS | A) OS  
B) Safety/toxicity  
C) Circulating biomarkers (inc DDR and cytokines) | 14-

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| Trial (reference) & indication | Design & n (rec dates) | Treatment(s) | 1° Endpoint | 2° Endpoint(s) | Results/remarks & conclusions |
|-------------------------------|------------------------|--------------|--------------|---------------|-----------------------------|
| NCT03212274                  | Phase II               | OLAP only    | ORR (3 cohorts) | A) PFS       | ● Trial ongoing – recruitment ends September 2024 |
| rGlioma (WHO Grade II-IV) / cholangiocarcinoma / solid tumours with IDH1/2 mutation Olaparib in treating patients with advanced glioma, cholangiocarcinoma, or solid tumours with IDH1 or IDH2 mutations | Single arm Estimated patients: 145 (2018-2021) | | B) OS | ● Dosing schedule = OLAP BD on day 1-28/28 cycle |
|                              |                        |              | C) Other solid tumours | C) Safety/toxicity | ● Builds on preclinical studies demonstrating ‘BRCAness’ with IDH1/2 mutation and elevated 2HG [238] |
|                              |                        |              | D) Exploratory objectives inc correlation between baseline 2HG and response | D) Exploratory objectives inc: relationship between BRCA 1/2 alterations and features of HRD inc. large-scale translocations, mutational signature 3; penetrance of HRD genes inc. HR genes, FA genes, ATM, CHK2, and MMR genes | | |
| NCT03581292                  | Phase II               | VELI + IR + TMZ | PFS | A) ORR | ● Trial ongoing – recruitment ends October 2024 |
| nHGG (H3K27M- BRAFV600-) Veliparib (ABT-888), radiation therapy, and temozolomide in treating patients with newly diagnosed malignant glioma without H3 K27M or BRAFV600 mutations | Single arm Estimated patients: 115 Age 3-25 (2018-2024) | | B) OS | | ● Dosing schedule = daily VELI BD during chemoradiotherapy phase then 4 weeks after completion → daily VELI BD + maintenance TMZ on days 1-5/28 cycle |
|                              |                        |              |               |               | ● Incorporates longitudinal assessment of ctDNA |
|                              |                        |              |               |               | ● Exploratory objectives inc: relationship between BRCA 1/2 alterations and features of HRD inc. large-scale translocations, mutational signature 3; penetrance of HRD genes inc. HR genes, FA genes, ATM, CHK2, and MMR genes |
| NCT04221503                  | Phase II               | All patients receive NIRA + TTFields | Disease control (CR/PR or SD) | A) Safety/toxicity | ● Trial ongoing – recruitment ends October 2024 |
| rGBM Evaluating the efficacy and safety of niraparib and tumor-treating fields in recurrent glioblastoma (Niraparib/TTFields) [240] | Parallel Estimated patients: 30 (2019-2023) | Cohort 1: surgical resection indicated Cohort 2: resection not indicated | B) ORR | | ● Cohort 1: initiate and continue TTFields for 5–7 days prior to starting NIRA |
|                              |                        |              | C) PFS | | ● Cohort 2: receive TTFields for 5–7 before planned |
|                              |                        |              | D) OS | | |
Table 1. (Continued).

| Trial Reference) & indication | Design & n (rec dates) | Treatment(s) | 1° Endpoint | 2° Endpoint(s) | Results/remarks & conclusions |
|-------------------------------|------------------------|--------------|-------------|---------------|-----------------------------|
| NCT03561870                   | Phase II Single arm    | OLAP only    | PFS         | n/a           | Builds on preclinical studies demonstrating 'BRCAness' induced by TTFields [118,239] |
| rHGG (IDH<sup>mut</sup>) Olaparib in Recurrent IDH-mutant High Grade Gliomas (OLAGLI) | Estimated patients: 35 (2020–2021) |                          |             |               | Trial ongoing – expected completion September 2021 |
| NCT03991832                   | Phase II Parallel      | All pts receive OLAP + durvalumab | ORR         | A) PFS        | Dosing schedule = OLAP 300 mg BD on days 1–28/28 cycle |
| IDH<sup>mut</sup> solid tumours Olaparib and durvalumab in patients with IDH-mutated solid tumors (MEDI 4736) | Estimated patients: 78 (2020–2022) | Cohort 1: Glioma Cohort 2: Cholangio Cohort 3: Other solid tumours |             | B) OS         | Based on preclinical studies demonstrating 'BRCAness' with IDH1/2 mutation and elevated 2HG [238] |
|                              |                        |              |             | C) Safety/toxicity | Trial ongoing – expected completion September 2021 |

Phase II/III studies

| Trial Reference) & indication | Design & n (rec dates) | Treatment(s) | 1° Endpoint | 2° Endpoint(s) | Results/remarks & conclusions |
|-------------------------------|------------------------|--------------|-------------|---------------|-----------------------------|
| NCT02152982                   | Phase II RCT           | After SoC IR and concomitant TMZ: Arm 1: VELI + TMZ Arm 2: PLACEBO + TMZ | OS           | A) 'Interaction' with TTFields (for pts receiving this) | Patients permitted to receive TTFields alongside trial therapies. No other additional therapies permitted |
| nGBM (MGMT promoter hypermethylated) Temozolomide with or without veliparib in treating patients with newly diagnosed glioblastoma multiforme | Randomised 447 patients (2014–2021) |                          |             | B) PFS        | Studies will also assess whether genetic/epigenetic |

"The DDR in glioblastoma biology and therapy"

O. Rominiyi and S. J. Collis

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| Trial (reference) & indication | Design & n (rec dates) | Treatment(s) | 1° Endpoint | 2° Endpoint(s) | Results/remarks & conclusions |
|-----------------------------|----------------------|--------------|--------------|---------------|-------------------------------|
| NCT03423628 [241,242] nGBM/rGBM/brain metastases Safety and tolerability of AZD1390 given with radiation therapy in patients with brain cancer | Phase I Parallel Estimated patients: 132 (2018-2023) | AZD1390 + SoC IR: nGBM: IMRT 60 Gy over 6 weeks rGBM: IMRT 35 Gy over 2 weeks Mets: WBRT 30 Gy over 2 weeks | Safety/toxicity | A) EFS B) ORR C) Pharmacokinetics | Dosing schedule = AZD1390 administered in 3 ‘cycles’ – (1) 1 dose prior to starting IR; (2) intermittent if continuous administration during IR; (3) 2-week adjuvant ATMi after IR. Based on preclinical studies demonstrating BBB penetration and improved survival with AZD1390 in mouse models [243] |
| NCT02977780 nGBM INdividualized Screening Trial of Innovative Glioblastoma Therapy (INSIGhT) | Phase II Parallel RCT Estimated patients: 250 (2017-2021) | After SoC IR and concomitant TMZ: Arm 1: TMZ Arm 2: Neratinib + TMZ Arm 3: CC115 + TMZ Arm 4: Abemaciclib + TMZ | OS | A) Safety/toxicity B) PFS C) Biomarkers & survival associations | Compares SoC therapy with 3 novel regimens each adding an additional drug: neratinib (tyrosine kinase inhibitor); CC115 (DNA-PK inhibitor); abemaciclib (cyclin-dependent kinase 4 and 6 inhibitor). Details on CC115 dosing schedule not available. Trial ongoing – expected completion December 2022. |

Summary of key ATM inhibitor in high-grade glioma clinical trials – ongoing

Summary of key DNA-PK inhibitor in high-grade glioma clinical trials – ongoing

alternations to DDR genes influence outcomes
● Trial ongoing – recruitment completed November 2020, results awaited

Trial ongoing – expected completion February 2023

Trial ongoing – expected completion December 2022
Table 1. (Continued).

| Trial (reference) & indication | Design & n (rec dates) | Treatment(s) | 1st Endpoint | 2nd Endpoint(s) | Results/remarks & conclusions |
|-------------------------------|------------------------|--------------|--------------|----------------|-------------------------------|
| **Summary of key WEE1 inhibitor in high-grade glioma clinical trials – ongoing** |
| **Phase I studies** |
| NCT02207010 [225] rGBM | Phase 0/I Single arm 20 patients (2015–2019) | Single dose of AZD1775 (100mg, 200mg or 400mg) prior to surgery | A) Plasma concentration | B) Intratumoural concentration | Tissue biomarker analysis |
| ● Mean peak total AZD1775 plasma concentration over 100 ng·mL⁻¹ with single 200 mg or 400 mg dose |
| ● Mean unbound AZD1775 tumour concentration of 85 ng·g⁻¹ at 2–24 h exceeding the in vitro IC₅₀ (40 ng·mL⁻¹) for WEE1 inhibition |
| ● Confirmation of target effects including elevated γH2AX, pH3 and cleaved caspase-3 |
| NCT01849146 [244] nGBM/rGBM | Phase I Nonrandomised Estimated patients: 114 (2013–2021) | Arm 1: AZD1775 during initial IR + TMZ and maintenance TMZ Arm 2: AZD1775 during maintenance TMZ | A) MTD | B) Safety/toxicity | A) OS B) PFS |
| ● Preliminary data suggests AZD1775 in combination with initial IR + TMZ at 150 mg QDS and 425 mg QDS alongside maintenance TMZ for 5 days in each 28 day cycle had acceptable toxicity |
| ● Trial recruitment completed – estimated study completion December 2021 |
target other signalling pathways that impact on DDR activity and/or capacity. For example, Gomez-Roman et al. [64] recently demonstrated in 3D GSC models that disruption to functional VEGF and AKT signalling pathways impacted the balance between NHEJ and HR DNA DSB break repair activities to increase radiation sensitivity. This is particularly interesting given that targeted therapies against VEGF (such as bevacizumab) have generally failed to improve patient overall survival in large clinical trials [51].

In a similar manner to identifying nonclassical DDR pathway targeting strategies, we recently identified ERK5/MAPK5 through a kinome-wide RNAi screen as a novel temozolomide resistance factor, with abrogation of ERK5 activity in glioma cells leading to defective DNA repair capacity, likely through inappropriate NHEJ activity prior to mitosis [189]. Interestingly, ERK5 has recently been identified as a key factor in promoting cell growth and cell survival in the aggressive diffuse intrinsic pontine gliomas [190], supporting recent evidence around ERK5 as an emerging novel oncology drug target [191–193]. As such, we are currently further assessing the potential of ERK5 targeting in glioblastomas as part of various combinatorial approaches together with current standard-of-care therapy.

On the topic on non-DDR signalling kinases that impact the DDR, Riess et al. [194] recently assessed targeting of the CDK family of signalling kinases given their common dysregulation in glioblastoma and the recent advancement of a new generation of clinically approved compounds. Using a CDK-based monotherapy approach in various 3D glioblastoma preclinical models, they showed that CDK inhibitors could negatively affect tumour growth, but also that some CDK inhibitors were able to effectively combine with DNA damaging regimes such as radiation and temozolomide treatments. However, they also showed that not all tested CDK inhibitors behaved in the same way, with some conferring antagonistic properties when combined with temozolomide, potentially through differential effects on global gene expression patterns [194]. This study further highlights the need to understand the intricate functional interplay and regulatory mechanisms within the DDR as part of preclinical studies to help maximise the therapeutic potential of such new combinatorial regimes within the clinic.

In addition to CDK dysregulation, another common feature of cancer cells is a heightened level of replication stress due to the activation of oncogenes [92,195]. Oncogene-induced replication stress has been shown to be present in GSCs [49] and is capable of triggering the DDR during early tumorigenesis [196]. Recent work from Ning et al. [197] revealed that heightened MYC activity in GSCs can lead to suppression of...
ATR-mediated replication stress signalling through transcriptional repression of CDK18. This is consistent with recent findings from our laboratory that identified CDK18 as a novel component of the ATR-mediated replication stress signalling module that promotes cellular resistance to a variety of replication stress-inducing chemotherapeutic agents [198,199]. In keeping with the aforementioned interplay between ATR signalling and PARP1 activity (see above), Ning et al. [197] further showed that GSCs with reduced CDK18 expression and subsequent retarded ATR activity, as a consequence of oncogenic MYC action, were rendered sensitive to PARP1 inhibitors. In keeping with a heightened S-phase fraction within a subpopulation of GSCs, a recent study by Zhou et al. [200] showed that purine metabolism was increased in aggressive/high-grade tumours and represents a potential target to improve the effectiveness of radiotherapy regimes. This is especially compelling given that, as highlighted within this study, there are currently FDA-approved inhibitors of GTP synthesis, although obviously the efficient delivery of therapeutic doses within the brain will be key to the success of such strategies.

Finally, another common feature of cancer cells, particularly solid tumours, is an imbalance between oxygen supply and demand from active aerobic metabolism, causing regional hypoxia defined as regions of reduced oxygen concentration. This presents both challenges in terms of cell death mechanisms, which are less effective in the context of hypoxia, such as those elicited by radiotherapy, but also potentially exploitable therapeutic opportunities given the effects hypoxia has on several DDR factors [201–204]. Recent discoveries outside of glioblastoma have revealed key molecular and functional links between the DDR, replication stress signalling and the cGAS-STING immune pathways [205,206], and that targeting of replication stress signalling may synergise with immuno-oncology (IO) therapies [207,208]. However, the propensity of glioblastoma to escape immunosurveillance, potentially poor receptor expression and anatomical considerations have so far limited progress in the development of effective immunotherapies for glioblastoma compared to other cancers [209–212]. However, strategies to circumvent such immuno-surveillance escape in gliomas have recently been reported [213] and, as more mechanistic understanding around how these pathways interact becomes available, further therapeutic opportunities for gliomas will hopefully be developed. Together, these studies raise the possibility that oncogene-induced replication stress within residual GSC populations following surgical resection may be targeted with agents that exploit such defective ATR signalling, for example. However, as is unfortunately all too common in glioblastoma research, promising preclinical studies do not necessarily translate into clinical benefit for patients [3,214] so expectations in this regard need to be measured.

4. Conclusions and future perspectives

As highlighted in this Review, there is a critical need for new and more effective treatment strategies to combat the long-standing dismal survival rates experienced by patients diagnosed with high-grade brain tumours such as glioblastoma. Through the combined acquisition of fundamental biological insights into DDR mechanisms and interplay within this network and associated pathways, together with continued progress in imaging, surgical technologies and radiochemotherapy delivery within the clinic, it is expected that targeting of the DDR has a potential to tackle the historic lack of treatment options for these tumours. It will be particularly interesting to see the results of DDR inhibitor clinical trials currently in various phases around the world (Table 1), as this will give an indication of how successful such approaches may or may not be [145,215]. There is, of course, the risk, given the extensive intratumoural heterogeneity of glioblastoma and inherently treatment-resistant GSCs within these tumours, that any single targeted therapy may in fact be ‘too targeted’, leading to inevitable resurgence of resistant subclones and tumour repopulation. In a similar manner to other difficult-to-treat diseases such as HIV and multidrug-resistant tuberculosis [216], novel drug combinations may be required to overcome the extensive genetic heterogeneity and resistance mechanisms observed in glioblastoma. Consequently, as highlighted within this Review, targeting multiple DDR constituents in parallel has the potential to provide new effective treatment paradigms that might help prevent disease progression by counteracting complex and overarching phenotypic delinquency within the DDR of cancerous cells (Fig. 3).

In addition to the aforementioned needed improvement in preclinical models that better reflect postsurgical residual disease, an important factor alongside the development of such multimodal strategies will be further improvements in the efficient delivery of therapeutically active doses of drugs beyond the BBB, which remains a significant challenge in glioblastoma therapy [3]. Potential innovations on the horizon include the use of MRI-directed magnetic nanoparticles [217,218], surgical delivery of in situ gelling agents [219,220] and enhanced intrathecal/cerebrospinal fluid delivery using...
novel viral vectors, antibody ligands or exosomes [221–223]. Such approaches, in addition to traditional oral or intravenous delivery approaches, coupled with novel ways to disrupt the BBB, such as ultrasound [224,225] or TTFields [226]-based approaches, will hopefully provide the best chance for DDR-targeting approaches to provide much-needed clinical benefit to patients and families faced with the devastating diagnosis of a high-grade glioma.

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Conflict of interest

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Author contributions

OR and SJC conceived and wrote the manuscript following an invitation from Prof. Kevin Ryan (co-EIC).

References

1 Burnet NG, Jefferies SJ, Benson RJ, Hunt DP & Treasure FP (2005) Years of life lost (YLL) from cancer is an important measure of population burden— and should be considered when allocating research funds. B J Cancer 92, 241–245.
2 Patel AP, Fisher JL, Nichols E, Abd-Allah F, Abdelalim A, Abrah N, Agius D, Alahdab F, Alam T et al. (2019) Global, regional, and national burden of brain and other CNS cancer, 1990–2016: a systematic analysis for the Global Burden of Disease Study 2016. Lancet Neurol 18, 376–393.
3 Aldape K, Brindle KM, Chels L, Chopra R, Gajjar A, Gilbert MR, Gottardo N, Gutmann DH, Hargrave D, Holland EC et al. (2019) Challenges to curing primary brain tumours. Nat Rev Clin Oncol 16, 509–520.
4 Oststrom QT, Patil N, Cioffi G, Waite K, Kruchko C & Barnholtz-Sloan JS (2020) CBTRUS statistical report: primary brain and other central nervous system tumors diagnosed in the United States in 2013–2017. Neuro Oncol 22, iv1–iv96.
5 Reifenberger G, Wirsching HG, Knobbe-Thomsen CB & Weller M (2017) Advances in the molecular genetics of gliomas – implications for classification and therapy. Nat Rev Clin Oncol 14, 434–452.
6 Bi J, Chowdhry S, Wu S, Zhang W, Masui K & Mischel PS (2020) Altered cellular metabolism in gliomas – an emerging landscape of actionable co-dependency targets. Nat Rev Cancer 20, 57–70.
7 Stupp R, Hugi ME, Mason WP, van den Bent MJ, Taphoorn MJB, Janzer RC, Ludwin SK, Allgeier A, Fisher B, Belanger K et al. (2009) Effects of radiotherapy with concomitant and adjuvant temozolomide versus radiotherapy alone on survival in glioblastoma in a randomised phase III study: 5-year analysis of the EORTC-NCIC trial. Lancet Oncol 10, 459–466.
8 Stupp R, Mason WP, van den Bent MJ, Weller M, Fisher B, Taphoorn MJB, Belanger K, Brandes AA, Marosi C, Bogdahn U et al. (2005) Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. N Engl J Med 352, 987–996.
9 Weller M, van den Bent M, Preusser M, Le Rhun E, Tonn JC, Minniti G, Bendszus M, Balana C, Chint O, Dirven L et al. (2021) EANO guidelines on the diagnosis and treatment of diffuse gliomas of adulthood. Nat Rev Clin Oncol 18, 170–186.
10 Hugi ME, Diserens AC, Gorlia T, Hamou MF, de Tribolet N, Weller M, Kros JM, Hainfellner JA, Mason W, Mariani L et al. (2005) MGMT gene silencing and benefit from temozolomide in glioblastoma. N Engl J Med 352, 997–1003.
11 Chintol OL, Wick W, Mason W, Henriksson R, Saran F, Nishikawa R, Carpenter AF, Houang-Xuan K, Kavan P, Cernea D et al. (2014) Bevacizumab plus radiotherapy-temozolomide for newly diagnosed glioblastoma. N Engl J Med 370, 709–722.
12 Gilbert MR, Wang M, Aldape KD, Stupp R, Hegi ME, Jaeckle KA, Armstrong TS, Wefel JS, Won M, Blumenthal DT et al. (2013) Dose-dense temozolomide for newly diagnosed glioblastoma: a randomized phase III clinical trial. J Clin Oncol 31, 4085–4091.
13 Khasraw M, McDonald KL, Rosenthal M, Lwin Z, Ashley DM, Wheeler H, Barnes E, Foote MC, Koh ES, Sulman EP et al. (2019) A randomized phase II trial of veliparib (V), radiotherapy (RT) and temozolomide (TMZ) in patients (pts) with unmethylated MGMT (uMGMT) glioblastoma (GBM). J Clin Oncol 37, 2011.
14 Oronszyk B, Reid TR, Oronszyk A, Sandhu N & Knox SJ (2020) A review of newly diagnosed glioblastoma. Front Oncol 10, 574012.
15 Chaudhry A, Benson L, Varshaver M, Farber O, Weinberg U, Kirson E & Palti Y (2015) NovoTTF-
100A system (Tumor Treating Fields) transducer array layout planning for glioblastoma: a NovoTAL system user study. World J Surg Oncol 13, 316.

16 Rominyyi O, Vanderlinden A, Clenton SJ, Bridgewater C, Al-Tamimi Y & Collis SJ (2021) Tumour treating fields therapy for glioblastoma: current advances and future directions. Br J Cancer 124, 697–709.

17 Stupp R, Taillibert S, Kanner A, Read W, Steinberg D, Lhermitte B, Toms S, Idbaih A, Ahluwalia MS, Fink K et al. (2017) Effect of tumor-treating fields plus maintenance temozolomide maintenance temozolomide alone on survival in patients with glioblastoma: a randomized clinical trial. JAMA 318, 2306–2316.

18 Stupp R, Wong ET, Kanner AA, Steinberg D, Engelhard H, Heidecke V, Kirson ED, Taillibert S, Liebermann F, Dabby V et al. (2022) NovoTTF-100A versus physician’s choice chemotherapy in recurrent glioblastoma: a randomised phase III trial of a novel treatment modality. Eur J Cancer 48, 2192–2202.

19 Lacroix M, Abi-Said D, Fourney DR, Gokaslan ZL, Shi W, DeMonte F, Lang FF, McCutcheon IE, Hassenbusch SJ, Holland E et al. (2001) A multivariate analysis of 416 patients with glioblastoma multiforme: prognosis, extent of resection, and survival. J Neurosurg 95, 190–198.

20 Ferraro N, Barbarite E, Albert TR, Berchmans E, Shah AH, Bregy A, Ivan ME, Brown T & Komotar RJ (2016) The role of 5-aminolevulinic acid in brain tumor surgery: a systematic review. Neurosurg Rev 39, 545–555.

21 Kuhnt D, Becker A, Ganslandt O, Bauer M, Buchfelder M & Nimsky C (2011) Correlation of the extent of tumor volume resection and patient survival in surgery of glioblastoma multiforme with high-field intraoperative MRI guidance. Neuro Oncol 13, 1339–1348.

22 Prada F, Bene MD, Fornaro R, Vetrano IG, Martegani A, Aiani L, Sconfienza LM, Mauri G, Solbiati L, Pollo B et al. (2016) Identification of residual tumor with intraoperative contrast-enhanced ultrasound during glioblastoma resection. Neurosurg Focus 40, E7.

23 Stummer W, Novotny A, Stepp H, Goetz C, Bise K & Reulen HJ (2000) Fluorescence-guided resection of glioblastoma multiforme by using 5-aminolevulinic acid-induced porphyrins: a prospective study in 52 consecutive patients. J Neurosurg 93, 1003–1013.

24 Stummer W, Pichlmeyer U, Meinel T, Wiestler OD, Zanella F, Reulen HJ & Group AL-GS (2006) Fluorescence-guided surgery with 5-aminolevulinic acid for resection of malignant glomema: a randomised controlled multicentre phase III trial. Lancet Oncol 7, 392–401.

25 Alexander J, Gildea L, Balog J, Speller A, McKenzie J, Muirhead L, Scott A, Kontoukounisios C, Rasheed S, Teare J et al. (2017) A novel methodology for in vivo endoscopic phenotyping of colorectal cancer based on real-time analysis of the mucosal lipidome: a prospective observational study of the iKnife. Surg Endosc 31, 1361–1370.

26 Balog J, Sasi-Szabo L, Kinross J, Lewis MR, Muirhead LJ, Veselkov K, Mirnezami R, Dezso B, Damjanovich L, Darzi A et al. (2013) Intraoperative tissue identification using rapid evaporative ionization mass spectrometry. Sci Transl Med 5, 194ra93.

27 Depciuich J, Tolpa B, Witek P, Szmac K, Kaznowska E, Osuchowski M, Krol P & Cebulski J (2020) Raman and FTIR spectroscopy in determining the chemical changes in healthy brain tissues and glioblastoma tumor tissues. Spectrochim Acta A Mol Biomol Spectrosc 225, 117526.

28 Livermore LJ, Isabelle M, Bell IM, Edgar O, Voets NL, Stacey R, Ansgore O, Vallance C & Plaha P (2020) Raman spectroscopy to differentiate between fresh tissue samples of glioma and normal brain: a comparison with 5-ALA-induced fluorescence-guided surgery, J Neurosurg 1–11. https://doi.org/10.3171/ 2020.5.JNS20376.

29 Livermore LJ, Isabelle M, Bell IM, Scott C, Walsby-Tickle J, Gannon J, Plaha P, Vallance C & Ansorge O (2019) Rapid intraoperative molecular genetic classification of gliomas using Raman spectroscopy. Neurooncol Adv 1, vdz008.

30 Riva M, Sciortino T, Secoli R, D’Amico E, Moccia S, Fernandes B, Conti Nibali M, Gay L, Rossi M, De Momi E et al. (2021) Glioma biopsies classification using Raman spectroscopy and machine learning models on fresh tissue samples. Cancers 13, 1073.

31 Ludmir EB, Mahajan A, Ahern V, Ajithkumar T, Alapetite C, Bernier-Chastagner V, Bindra RS, Bishop AJ, Bolle S, Brown PD et al. (2019) Assembling the brain trust: the multidisciplinary imperative in neuro-oncology. Nat Rev Clin Oncol 16, 521–522.

32 Thompson MK, Poortmans P, Chalmers AJ, Faivre-Finn C, Hall E, Huddart RA, Lievens Y, Sebag-Montefiore D & Coles CE (2018) Practice-changing radiation therapy trials for the treatment of cancer: where are we 150 years after the birth of Marie Curie? Br J Cancer 119, 389–407.

33 Arvanitis CD, Ferraro GB & Jain RK (2020) The blood–brain barrier and blood–tumour barrier in brain tumours and metastases. Nat Rev Cancer 20, 26–41.

34 Brennan CW, Verhaak RG, McKenna A, Campos B, Noshmehr H, Salama SR, Zheng S, Chakravarty D, Sanborn JZ, Berman SH et al. (2013) The somatic genomic landscape of glioblastoma. Cell 155, 462–477.

35 Nefed C, Laffy J, Filbin MG, Hara T, Shore ME, Rahme GJ, Richman AR, Silverbush D, Shaw ML, Hebert CM et al. (2019) An integrative model of
The DDR in glioblastoma biology and therapy

36 Ohgaki H & Kleihues P (2013) The definition of primary and secondary glioblastoma. Clin Cancer Res 19, 764–772.

37 Le Rhun E, Preussner M, Roth P, Reardon DA, van den Bent M, Wen P, Reifenberger G & Weller M (2019) Molecular targeted therapy of glioblastoma. Cancer Treat Rev 80, 101896.

38 Patel AP, Tirosh I, Trombetta JJ, Shalek AK, Gillespie SM, Wakimoto H, Cahill DP, Nahed BV, Curry WT, Martuza RL et al. (2014) Single-cell RNA-seq highlights intratumoral heterogeneity in primary glioblastoma. Science 344, 1396–1401.

39 Piccirillo SGM, Colman S, Potter NE, van Delft FW, Lillis S, Carnicer MJ, Kearney L, Watts C & Greaves M (2015) Genetic and functional diversity of propagating cells in glioblastoma. Stem Cell Rep 4, 7–15.

40 Sotterriva A, Spiteri I, Piccirillo SG, Touloumis A, Collins VP, Marioni JC, Curtis C, Watts C & Tavare S (2013) Intratumor heterogeneity in human glioblastoma reflects cancer evolutionary dynamics. Proc Natl Acad Sci USA 110, 4009–4014.

41 Spiteri I, Caravagna G, Cresswell GD, Vatsiou A, Nichol D, Acar A, Ermini L, Chkhaidze K, Werner B, Mair R et al. (2019) Evolutionary dynamics of residual disease in human glioblastoma. Ann Oncol 30, 456–463.

42 Castellan M, Guarnieri A, Fujimura A, Zanconato F, Battilana G, Panciera T, Sladitschek HL, Contessotto P, Citron A, Grilli A et al. (2021) Single-cell analyses reveal YAP/TAZ as regulators of stemness and cell plasticity in Glioblastoma. Nat Cancer 2, 174–188.

43 Galli R, Binda E, Orfanelli U, Cipelletti B, Gritti A, De Vitis S, Fiocco R, Foroni C, Dimeco F & Vescovi A (2004) Isolation and characterization of tumorigenic, stem-like neural precursors from human glioblastoma. Cancer Res 64, 7011–7021.

44 Lathia JD, Mack SC, Mulkearns-Hubert EE, Valentim CL & Rich NJ (2015) Cancer stem cells in glioblastoma. Genes Dev 29, 1203–1217.

45 Mitchell K, Troike K, Silver DJ & Lathia JD (2021) The evolution of the cancer stem cell state in glioblastoma: emerging insights into the next generation of functional interactions. Neuro Oncol 23, 199–213.

46 Prager BC, Bhargava S, Mahadev V, Hubert CG & Rich NJ (2020) Glioblastoma stem cells: driving resilience through chaos. Trends Cancer 6, 223–235.

47 Singh SK, Hawkins C, Clarke ID, Squire JA, Bayani J, Hide T, Henkelman RM, Cusimano MD & Dirks PB (2004) Identification of human brain tumour initiating cells. Nature 432, 396–401.

48 Bao S, Wu Q, McLendon RE, Hao Y, Shi Q, Hjelmeland AB, Dewhirst MW, Bigner DD & Rich NJ (2006) Glioma stem cells promote radioresistance by preferential activation of the DNA damage response. Nature 444, 756–760.

49 Carruthers RD, Ahmed SU, Ramachandran S, Strathdee K, Kurian KM, Hedley A, Gomez-Roman N, Kalna G, Neilson M, Gilmour L et al. (2018) Replication stress drives constitutive activation of the DNA damage response and radioresistance in glioblastoma stem-like cells. Cancer Res 78, 5060–5071.

50 Chen J, Li Y, Yu TS, McKay RM, Burns DK, Kernie SG & Parada LF (2012) A restricted cell population propagates glioblastoma growth after chemotherapy. Nature 488, 522–526.

51 Stupp R, Lukas RV & Hegi ME (2019) Improving survival in molecularly selected glioblastoma. Lancet 393, 615–617.

52 Bedard PL, Hansen AR, Ratain MJ & Siu LL (2013) Tumour heterogeneity in the clinic. Nature 501, 355–364.

53 Johansson P, Krona C, Kundu S, Doroszko M, Baskaran S, Schmidt L, Vinel C, Almstedt E, Elgendy R, Elifineh L et al. (2020) A patient-derived cell atlas informs precision targeting of glioblastoma. Cell Rep 32, 107897.

54 Phillips HS, Kharbanda S, Chen R, Forrest WF, Soriano RH, Wu TD, Misra A, Nigro JM, Colman H, Sorocenau L et al. (2006) Molecular subclasses of high-grade glioma predict prognosis, delineate a pattern of disease progression, and resemble stages in neurogenesis. Cancer Cell 9, 157–173.

55 Verhaak RG, Hoadley KA, Purdom E, Wang V, Qi Y, Wilkerson MD, Miller CR, Ding L, Golub T, Mesirov JP et al. (2010) Integrated genomic analysis identifies clinically relevant subtypes of glioblastoma characterized by abnormalities in PDGFRA, IDH1, EGFR, and NF1. Cancer Cell 17, 98–110.

56 Wang Q, Hu B, Hu X, Kim H, Squatrito M, Scarpace L, deCarvalho AC, Lyu S, Li P, Li Y et al. (2017) Tumor evolution of glioma-intrinsic gene expression subtypes associates with immunological changes in the microenvironment. Cancer Cell 32, 42–56.

57 Soeda A, Hara A, Kunisada T, Yoshimura S, Iwama T & Park DM (2015) The evidence of glioblastoma heterogeneity. Sci Rep 5, 7979.

58 Vartanian A, Singh SK, Agnihotri S, Jalali S, Burrell K, Aldape KD & Zadeh G (2014) GBM’s multifaceted landscape: highlighting regional and microenvironmental heterogeneity. Neuro Oncol 16, 1167–1175.

59 Caragher S, Chalmers AJ & Gomez-Roman N (2019) Glioblastoma’s next top model: novel culture systems for brain cancer radiotherapy research. Cancers 11, 44.

60 Rominiyi O, Al-Tamimi Y & Collis SJ (2019) The ‘Ins and Outs’ of early preclinical models for brain tumor research: are they valuable and have we been doing it wrong? Cancers 11, 426.
61 De Witt Hamer PC, Van Tilborg AA, Eijk PP, Sminia P, Troost D, Van Noorden CJ, Ylstra B & Leenstra S (2008) The genomic profile of human malignant glioma is altered early in primary cell culture and preserved in spheroids. Oncogene 27, 2091–2096.

62 Fael Al-Mayhani TM, Ball SL, Zhao JW, Fawcett J, Ichimura K, Collins PV & Watts C (2009) An efficient method for derivation and propagation of glioblastoma cell lines that conserves the molecular profile of their original tumours. J Neurosci Methods 176, 192–199.

63 Gomez-Roman N & Chalmers AJ (2019) Patient-specific 3D-printed glioblastomas. Nat Biomed Eng 3, 498–499.

64 Gomez-Roman N, Chong MY, Chahal SK, Caragher SP, Jackson MR, Stevenson KH, Dongre SA & Chalmers AJ (2020) Radiation responses of 2D and 3D glioblastoma cells: a novel, 3D-specific radioprotective role of VEGF/Akt signaling through functional activation of NHEJ. Mol Cancer Ther 19, 575–589.

65 Gomez-Roman N, Stevenson K, Gilmour L, Hamilton G & Chalmers AJ (2017) A novel 3D human glioblastoma cell culture system for modeling drug and radiation responses. Neuro Oncol 19, 229–241.

66 Hubert CG, Rivera M, Spangler LC, Wu Q, Mack SC, Prager BC, Cousse M, McLendon RE, Sloan AE & Rich JN (2016) A three-dimensional organoid culture system derived from human glioblastomas recapitulates the hypoxic gradients and cancer stem cell heterogeneity of tumors found in vivo. Cancer Res 76, 2465–2477.

67 Iorio F, Knijnenburg TA, Vis DJ, Bignell GR, Menden MP, Schubert M, Aben N, Goncales E, Barthorpe S, Lightfoot H et al. (2016) A landscape of pharmacogenomic interactions in cancer. Cell 166, 740–754.

68 Linkous A, Balamatsias D, Snuderl M, Edwards L, Miyaguchi K, Milner T, Reich B, Cohen-Gould L, Storaska A, Nakayama Y et al. (2019) Modeling patient-derived glioblastoma with cerebral organoids. Cell Rep 26, 3203–3211.

69 Ogawa J, Pao GM, Shokhirev MN & Verma IM (2018) Glioblastoma model using human cerebral organoids. Cell Rep 23, 1220–1229.

70 Pine AR, Cirigliano SM, Nicholson JG, Hu Y, Linkous A, Miyaguchi K, Edwards L, Singhania R, Schwartz TH, Ramakrishna R et al. (2020) Tumor microenvironment is critical for the maintenance of cellular states found in primary glioblastomas. Cancer Discov 10, 964–979.

71 Pollard SM, Yoshikawa K, Clarke ID, Danovi D, Stricker S, Russell R, Bayani J, Head R, Lee M, Bernstein M et al. (2009) Glioma stem cell lines expanded in adherent culture have tumor-specific phenotypes and are suitable for chemical and genetic screens. Cell Stem Cell 4, 568–580.

72 Rahman M, Reyner K, Deleyrolle L, Millette S, Azari H, Day BW, Stringer BW, Boyd AW, Johns TG, Blot V et al. (2015) Neurosphere and adherent culture conditions are equivalent for malignant glioma stem cell lines. Anat Cell Biol 48, 25–35.

73 Yi HG, Jeong YH, Kim Y, Choi YJ, Moon HE, Park SH, Kang KS, Bae M, Jang J, Youn H et al. (2019) A bioprinted human-glioblastoma-on-a-chip for the identification of patient-specific responses to chemoradiotherapy. Nat Biomed Eng 3, 509–519.

74 Ben-David U, Ha G, Tseng YY, Greenwald NF, Oh C, Shih J, McFarland JM, Wong B, Boehm JS, Beroukhim R et al. (2017) Patient-derived xenografts undergo mouse-specific tumor evolution. Nat Genet 49, 1567–1575.

75 Holland EC (2000) A mouse model for glioma: biology, pathology, and therapeutic opportunities. Toxicol Pathol 28, 171–177.

76 Lenting K, Verhaak R, Ter Laan M, Wesseling P & Leenders W (2017) Glioma: experimental models and reality. Acta Neuropathol 133, 263–282.

77 Oh T, Fakurnejad S, Sayegh ET, Clark AJ, Ivan ME, Sun MZ, Safaee M, Bloch O, James CD & Parsa AT (2014) Immunocompetent murine models for the study of glioblastoma immunotherapy. J Transl Med 12, 107.

78 Goldstein M & Kastan MB (2015) The DNA damage response: implications for tumor responses to radiation and chemotherapy. Annu Rev Med 66, 129–143.

79 Hoeijmakers JH (2001) Genome maintenance mechanisms for preventing cancer. Nature 411, 366–374.

80 Lindahl T (1993) Instability and decay of the primary structure of DNA. Nature 362, 709–715.

81 Ciccia A & Elledge SJ (2010) The DNA damage response: making it safe to play with knives. Mol Cell 40, 179–204.

82 Curtin NJ (2012) DNA repair dysregulation from cancer driver to therapeutic target. Nat Rev Cancer 12, 801–817.

83 Jeggo PA, Pearl LH & Carr AM (2016) DNA repair, genome stability and cancer: a historical perspective. Nat Rev Cancer 16, 35–42.

84 Olivieri M, Cho T, Alvarez-Quilon A, Li K, Schellenberg MJ, Zimmermann M, Hustedt N, Rossi SE, Adam S, Melo H et al. (2020) A genetic map of the response to DNA damage in human cells. Cell 182, 481–496.

85 Smith HL, Southgate H, Tweddle DA & Curtin NJ (2020) DNA damage checkpoint kinases in cancer. Expert Rev Mol Med 22, e2.

86 Gourley C, Balmana J, Ledermann JA, Serra V, Dent R, Loibl S, Pujade-Lauraine E & Boulton SJ (2019) Moving from poly (ADP-ribose) polymerase inhibition
to targeting DNA repair and DNA damage response in cancer therapy. J Clin Oncol 37, 2257–2269.
87 Jackson SP & Helleday T (2016) Drugging DNA repair. Science 352, 1178–1179.
88 Pearl LH, Schierz AC, Ward SE, Al-Lazikani B & Pearl FM (2015) Therapeutic opportunities within the DNA damage response. Nat Rev Cancer 15, 166–180.
89 Pilie PG, Tang C, Mills GB & Yap TA (2019) State-of-the-art strategies for targeting the DNA damage response in cancer. Nat Rev Clin Oncol 16, 81–104.
90 Blackford AN & Jackson SP (2017) ATM, ATR, and DNA-PK: the trinity at the heart of the DNA damage response. Mol Cell 66, 801–817.
91 Mavragani IV, Nikitaki Z, Kalospyros SA & Georgakilas AG (2019) Ionizing radiation and complex DNA damage: from prediction to detection challenges and biological significance. Cancers 11, 1789.
92 Kotsantis P, Petermann E & Boulton SJ (2018) Mechanisms of oncogene-induced replication stress: jigsaw falling into place. Cancer Discov 8, 537–555.
93 Primo LMF & Teixeira LK (2019) DNA replication stress: oncogenes in the spotlight. Genet Mol Biol 43, e20190138.
94 Shiloh Y & Ziv Y (2013) The ATM protein kinase: regulating the cellular response to genotoxic stress, and more. Nat Rev Mol Cell Biol 14, 197–210.
95 Saldívar JC, Cortez D & Cimprich KA (2017) The essential kinase ATR: ensuring faithful duplication of a challenging genome. Nat Rev Mol Cell Biol 18, 622–636.
96 Marechal A & Zou L (2013) DNA damage sensing by the ATM and ATR kinases. Cold Spring Harb Perspect Biol 5, a012716.
97 Beckta JM, Bindra RS & Chalmers AJ (2019) Targeting DNA repair in gliomas. Curr Opin Neurol 32, 878–885.
98 Bradbury A, Hall S, Curtin N & Drew Y (2020) Targeting ATR as cancer therapy: a new era for synthetic lethality and synergistic combinations? Pharmacol Ther 207, 107450.
99 Chapman JR, Taylor MR & Boulton SJ (2012) Playing the end game: DNA double-strand break repair pathway choice. Mol Cell 47, 497–510.
100 Jeggo PA & Downs JA (2014) Roles of chromatin remodellers in DNA double strand break repair. Exp Cell Res 329, 69–77.
101 Jeggo PA, Downs JA & Gasser SM (2017) Chromatin modifiers and remodellers in DNA repair and signalling. Philos Trans R Soc Lond B Biol Sci 372, 20160279.
102 Panier S & Boulton SJ (2014) Double-strand break repair: 53BP1 comes into focus. Nat Rev Mol Cell Biol 15, 7–18.
103 Kaina B, Christmann M, Naumann S & Roos WP (2007) MGMT: key node in the battle against genotoxicity, carcinogenicity and apoptosis induced by alkylating agents. DNA Repair 6, 1079–1099.
104 Jiricny J (2013) Postreplicative mismatch repair. Cold Spring Harb Perspect Biol 5, a012633.
105 Fu D, Calvo JA & Samson LD (2012) Balancing repair and tolerance of DNA damage caused by alkylating agents. Nature Rev Cancer 12, 104–120.
106 Barthel FP, Johnson KC, Varn FS, Moskalik AD, Tanner G, Kocakavuk E, Anderson KJ, Abioli O, Aldape K, Alfaro KD et al. (2019) Longitudinal molecular trajectories of diffuse glioma in adults. Nature 576, 112–120.
107 Daniel P, Sabri S, Chaddad A, Meehan B, Jean-Claude B, Rak J & Abdulkarim BS (2019) Temozolomide induced hypermutation in glioma: evolutionary mechanisms and therapeutic opportunities. Front Oncol 9, 41.
108 MacLeod G, Bozek DA, Rajakulendran N, Monteiro V, Ahmadi M, Steinhart Z, Kushida MM, Yu H, Coutinho FJ, Cavalli FMG et al. (2019) Genome-wide CRISPR-Cas9 screens expose genetic vulnerabilities and mechanisms of temozolomide sensitivity in glioblastoma stem cells. Cell Rep 27, 971–986.
109 Wick W, Weller M, van den Bent M, Sanson M, Weiler M, von Deimling A, Plass C, Hegi M, Platten M & Reifenberger G (2014) MGMT testing—the challenges for biomarker-based glioma treatment. Nat Rev Neurol 10, 372–385.
110 Frosina G (2000) Overexpression of enzymes that repair endogenous damage to DNA. Eur J Biochem 267, 2135–2149.
111 Johannessen TC, Bjerkvig R & Tysnes BB (2008) DNA repair and cancer stem-like cells—potential partners in glioma drug resistance? Cancer Treat Rev 34, 558–567.
112 Trivedi RN, Almeida KH, Fornsaglio JL, Schamus S & Sobol RW (2005) The role of base excision repair in the sensitivity and resistance to temozolomide-mediated cell death. Cancer Res 65, 6394–6400.
113 Martejn JA, Lans H, Vermeulen W & Hoeijmakers JH (2014) Understanding nucleotide excision repair and its roles in cancer and ageing. Nat Rev Mol Cell Biol 15, 465–481.
114 Harrison D, Gravells P, Thompson R & Bryant HE (2020) Poly(ADP-ribose) glycohydrolase (PARG) vs. Poly(ADP-ribose) polymerase (PARP) – function in genome maintenance and relevance of inhibitors for anti-cancer therapy. Front Mol Biosci 7, 191.
115 Liao H, Ji F, Helleday T & Ying S (2018) Mechanisms for stalled replication fork stabilization: new targets for synthetic lethality strategies in cancer treatments. EMBO Rep 19, e46263.
116 Lord CJ & Ashworth A (2016) BRCAness revisited. Nat Rev Cancer 16, 110–120.
The DDR in glioblastoma biology and therapy

117 Sung P & Klein H (2006) Mechanism of homologous recombination: mediators and helicases take on regulatory functions. Nat Rev Mol Cell Biol 7, 739–750.

118 Giladi M, Munster M, Schneiderman RS, Voloshin T, Porat Y, Blat R, Zielinska-Chomej K, Haag P, Bomzon Z, Kirson ED et al. (2017) Tumor treating fields (TTFields) delay DNA damage repair following radiation treatment of glioma cells. Radiat Oncol 12, 206.

119 Chang HHY, Pannunzio NR, Adachi N & Lieber MR (2017) Non-homologous DNA end joining and alternative pathways to double-strand break repair. Nat Rev Mol Cell Biol 18, 495–506.

120 Scully R, Panday A, Elango R & Willis NA (2019) DNA double-strand break repair-pathway choice in somatic mammalian cells. Nat Rev Mol Cell Biol 20, 698–714.

121 Yata K & Esashi F (2009) Dual role of CDKs in DNA repair: to be, or not to be. DNA Repair 8, 6–18.

122 Malumbres M & Barbacid M (2009) Cell cycle, CDKs and cancer: a changing paradigm. Nat Rev Cancer 9, 153–166.

123 Vijayaraghavan S, Moulder S, Keyomarsi K & Layman RM (2018) Inhibiting CDK in cancer therapy: current evidence and future directions. Target Oncol 13, 21–38.

124 Degan P, Cappelli E, Regis S & Ravera S (2019) New insights and perspectives in Fanconi Anemia research. Trends Mol Med 25, 167–170.

125 Kennedy RD & D’Andrea AD (2005) The Fanconi Anemia/BRCA pathway: new faces in the crowd. Genes Dev 19, 2925–2940.

126 Nalepa G & Clapp DW (2018) Fanconi anemia and cancer: an intricate relationship. Nat Rev Cancer 18, 168–185.

127 Niraj J, Farkkila A & D’Andrea AD (2019) The Fanconi anemia pathway in cancer. Annu Rev Cancer Biol 3, 457–478.

128 Wang AT & Smogorzewska A (2015) SnapShot: Fanconi anemia and associated proteins. Cell 160, 354–354.e1.

129 Andreassen PR, D’Andrea AD & Taniguchi T (2004) ATR couples FANCD2 monoubiquitination to the DNA-damage response. Genes Dev 18, 1958–1963.

130 Elia AE, Wang DC, Willis NA, Boardman AP, Hajdu I, Adeyemi RO, Lowry E, Gygi SP, Scully R & Ellledge SJ (2015) RFWD3-dependent ubiquitination of RPA regulates repair at stalled replication forks. Mol Cell 60, 280–293.

131 Feeney L, Munoz IM, Lachaud C, Toth R, Appleton PL, Schindler D & Rouse J (2017) RPA-mediated recruitment of the E3 ligase RFWD3 is vital for interstrand crosslink repair and human health. Mol Cell 66, 610–621.

132 Inano S, Sato K, Katsuki Y, Kobayashi W, Tanaka H, Nakajima K, Nakada S, Miyoshi H, Knies K, Takaori-Kondo A et al. (2017) RFWD3-mediated ubiquitination promotes timely removal of both RPA and RAD51 from DNA damage sites to facilitate homologous recombination. Mol Cell 66, 622–634.

133 Ishiai M, Kitao H, Smogorzewska A, Tomida J, Kinomura A, Uchida E, Saberi A, Kinoshita E, Kinoshita-Kikutake E, Koike T et al. (2008) FANCI phosphorylation functions as a molecular switch to turn on the Fanconi anemia pathway. Nat Struct Mol Biol 15, 1138–1146.

134 Lossaint G, Larroque M, Ribeyre C, Bec N, Larroque C, Decaillot C, Gari K & Constantinou A (2013) FANCD2 binds MCM proteins and controls replisome function upon activation of s phase checkpoint signaling. Mol Cell 51, 678–690.

135 Walden H & Deans AJ (2014) The Fanconi anemia DNA repair pathway: structural and functional insights into a complex disorder. Annu Rev Biophys 43, 257–278.

136 Kaelin WG Jr (2005) The concept of synthetic lethality in the context of anticancer therapy. Nat Rev Cancer 5, 689–698.

137 Ashworth A & Lord CJ (2018) Synthetic lethal therapies for cancer: what’s next after PARP inhibitors? Nat Rev Clin Oncol 15, 564–576.

138 Bryant HE, Schultz N, Thomas HD, Parker KM, Flower D, Lopez E, Kyle S, Meuth M, Curtin NJ & Helleday T (2005) Specific killing of BRCA2-deficient tumours with inhibitors of poly(ADP-ribose) polymerase. Nature 434, 913–917.

139 Farmer H, McCabe N, Lord CJ, Tutt AN, Johnson DA, Richardson TB, Santarosa M, Dillon KJ, Hickson I, Knights C et al. (2005) Targeting the DNA repair defect in BRCA mutant cells as a therapeutic strategy. Nature 434, 917–921.

140 Mateo J, Lord CJ, Serra V, Tutt A, Balmana J, Castroviejo-Bermejo M, Cruz C, Oaknin A, Kaye SB e de Bono JS (2019) A decade of clinical development of PARP inhibitors in perspective. Ann Oncol 30, 1437–1447.

141 Ning J & Wakimoto H (2020) Therapeutic application of PARP inhibitors in neuro-oncology. Trends Cancer 6, 147–159.

142 Yap TA, Plummer R, Azad NS & Helleday T (2019) The DNA damaging revolution: PARP inhibitors and beyond. Am Soc Clin Oncol Educ Book 39, 185–195.

143 Sulkowski PL, Oek S, Dow J, Economos NG, Mirfakhraie L, Liu Y, Noronha K, Bao X, Li J, Shuch BM et al. (2020) Oncometabolites suppress DNA repair by disrupting local chromatin signalling. Nature 582, 586–591.

144 Wang Y, Wild AT, Turcan S, Wu WH, Sigel C, Klimstra DS, Ma X, Gong Y, Holland EC, Huse JT et al. (2020) Targeting therapeutic vulnerabilities with PARP inhibition and radiation in IDH-mutant
gliomas and cholangiocarcinomas. Sci Adv 6, eaa3221.

145 Hanna C, Kurian KM, Williams K, Watts C, Jackson A, Carruthers R, Strathdee K, Cruickshank G, Dunn L, Erridge S et al. (2020) Pharmacokinetics, safety, and tolerability of olaparib and temozolomide for recurrent glioblastoma: results of the phase I OPARATIC trial. Neuro Oncol 22, 1840–1850.

146 Meyer M, Reimand J, Lan X, Head R, Zhu X, Kushida M, Bayani J, Pressey JC, Lionel AC, Clarke ID et al. (2015) Single cell-derived clonal analysis of human glioblastoma links functional and genomic heterogeneity. Proc Natl Acad Sci USA 112, 851–856.

147 Xie Y, Bergstrom T, Jiang Y, Johansson P, Marinescu VD, Lindberg N, Segerman A, Wichet G, Niklasson M, Baskaran S et al. (2015) The human glioblastoma cell culture resource: validated cell models representing all molecular subtypes. EBioMedicine 2, 1351–1363.

148 Chen CC, Taniguchi T & D’Andrea A (2007) The Fanconi anemia (FA) pathway confers glioma resistance to DNA alkylating agents. J Mol Med 85, 497–509.

149 Kondo N, Takahashi A, Mori E, Noda T, Zdzenicka MZ, Thompson LH, Helleday T, Suzuki M, Kinashi Y, Masunaga S et al. (2011) FANCD1/BRA2 plays predominant role in the repair of DNA damage induced by ACNU or TMZ. PLoS One 6, e19659.

150 Patil AA, Sayal P, Depondt ML, Beveridge RD, Roylance A, Kriplani DH, sundaram RK, Kalathil AN, Ganesa S, Jia L, Breslin H, Burgenske DM, Gilad O, Sarkaria JN et al. (2019) Temozolomide sensitizes MGMT-deficient tumor cells to ATR inhibitors. Cancer Res 79, 4331–4338.

151 Gupta SK, Kizilbash SH, Carlson BL, Mladek AC, Boakye-Agyeman F, Bakken KK, Pokorny JL, Schroeder MA, Decker PA, Cen L et al. (2016) Delineation of MGMT hypermethylation as a biomarker for veliparib-mediated temozolomide-sensitizing therapy of glioblastoma. J Natl Cancer Inst 108, djv369.

152 Gupta SK, Smith EJ, Mladek AC, Tian S, Decker PA, Kizilbash SH, Kitange GJ & Sarkaria JN (2018) PARP inhibitors for sensitization of alkylating chemotherapy in glioblastoma: impact of blood-brain barrier and molecular heterogeneity. Front Oncol 8, 670.

153 Helleday T, Petermann E, Lundin C, Hodgson B & Sharma RA (2008) DNA repair pathways as targets for cancer therapy. Nat Rev Cancer 8, 193–204.

154 D’Atri S, Tentori L, Lucal PM, Graziani G, Pagani E, Benincasa E, Zambruno G, Bonmassar E & Jiricny J (1998) Involvement of the mismatch repair system in temozolomide-induced apoptosis. Mol Pharmacol 54, 334–341.

155 Sarkaria JN, Kitange GJ, James CD, Plummer R, Calvert H, Weller M & Wick W (2008) Mechanisms of chemoresistance to alkylating agents in malignant glioma. Clin Cancer Res 14, 2900–2908.

156 Caporali S, Falcinelli S, Starace G, Russo MT, Bonmassar E, Jiricny J & D’Atri S (2004) DNA damage induced by temozolomide signals to both ATM and ATR: role of the mismatch repair system. Mol Pharmacol 66, 478–491.

157 Eich M, Roos WP, Nikolova T & Kaina B (2013) Contribution of ATM and ATR to the resistance of glioblastoma and malignant melanoma cells to the methylating anticancer drug temozolomide. Mol Cancer Ther 12, 2529–2540.

158 Nadkarni A, Shrivastav M, Mladek AC, Schwingler PM, Grogan PT, Chen J & Sarkaria JN (2012) ATM inhibitor KU-55933 increases the TMZ responsiveness of only inherently TMZ sensitive GBM cells. J Neuro Oncol 110, 349–357.

159 Jackson CB, Noorbakhsh SI, Sundaram RK, Kalathil AN, Ganesa S, Jia L, Breslin H, Burgenske DM, Gilad O, Sarkaria JN et al. (2019) Temozolomide sensitizes MGMT-deficient tumor cells to ATR inhibitors. Cancer Res 79, 4331–4338.

160 Lopez-Martinez D, Liang CC & Cohn MA (2016) Cellular response to DNA interstrand crosslinks: the Fanconi anemia pathway. Cell Mol Life Sci 73, 3097–3114.

161 Boisvert RA & Howlett NG (2014) The Fanconi anemia ID2 complex: dueling saxes at the crossroads. Cell Cycle 13, 2999–3015.

162 Ceccaldi R, Sarangi P & D’Andrea AD (2016) The Fanconi anaemia pathway: new players and new functions. Nat Rev Mol Cell Biol 17, 337–349.

163 Kim H & D’Andrea AD (2012) Regulation of DNA cross-link repair by the Fanconi anemia/BRCA pathway. Genes Dev 26, 1393–1408.

164 Adamo A, Collis SJ, Adelman CA, Silva N, Horejsi Z, Ward JD, Martinez-Perez E, Boltun SJ & La Volpe A (2010) Preventing nonhomologous end joining suppresses DNA repair defects of Fanconi anemia. Mol Cell 39, 25–35.

165 Collis SJ, Ciccia A, Deans AJ, Horejsi Z, Martin JS, Maslen SL, Skehel JM, Elledge SJ, West SC & Boltun SJ (2008) FANCM and FAAP24 function in ATM-mediated checkpoint signaling independently of the Fanconi anemia core complex. Mol Cell 32, 313–324.

166 Pace P, Mosedale G, Hodkinson MR, Rosado IV, Sivasubramaniam M & Patel KJ (2010) Ku70 corrupts DNA repair in the absence of the Fanconi anemia pathway. Science 329, 219–223.

167 Eccles LJ, Bell AC & Powell SN (2018) Inhibition of non-homologous end joining in Fanconi Anemia cells results in rescue of survival after interstrand crosslinks.
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but sensitization to replication associated double-strand breaks. DNA Repair 64, 1–9.

168 Kai Z, Rondinelli B, Holmes A, O’Leary C, Kozono D, D’Andrea AD & Ceccaldi R (2016) FANCD2 maintains fork stability in BRCA1/2-deficient tumors and promotes alternative end-joining DNA repair. Cell Rep 15, 2488–2499.

169 Michl J, Zimmer J, Buffa FM, McDermott U & Tarsounas M (2016) FANCD2 limits replication stress and genome instability in cells lacking BRCA2. Nat Struct Mol Biol 23, 755–757.

170 Cleary JM, Aguirre AJ, Shapiro GI & D’Andrea AD (2018) Mechanisms of PARP inhibitor sensitivity and resistance. DNA Repair 71, 172–176.

171 Zimmermann M, Murina O, Rejns MAM, Agathanggelou A, Challis R, Tarnauskaite Z, Muir M, Fluteau A, Aregger M, McCowan A et al. (2018) CRISPR screens identify genomic ribonucleotides as a source of PARP-trapping lesions. Nature 559, 285–289.

172 Alcón P, Shakese S, Chen ZA, Rappisiler J, Patel KJ & Passmore LA (2020) FANCD2-FANCI is a clamp stabilized on DNA by monoubiquitination of FANCD2 during DNA repair. Nat Struct Mol Biol 27, 240–248.

173 Rennie ML, Lemonidis K, Arkinson C, Chaugule VK, Clarke M, Streetley J, Spagnolo L & Walden H (2020) Differential functions of FANCI and FANCD2 ubiquitination stabilize ID2 complex on DNA. EMBO Rep 21, e50133.

174 Sharp MF, Murphy VJ, Twest SV, Tan W, Lui J, Simpson KJ, Deans AJ & Crismani W (2020) Methodology for the identification of small molecule inhibitors of the Fanconi Anaemia ubiquitin E3 ligase complex. Sci Rep 10, 7959.

175 Tan W, van Twest S, Leis A, Bythell-Douglas R, Murphy VJ, Sharp M, Parker MW, Crismani W & Deans AJ (2020) Monoubiquitination by the human Fanconi anemia core complex clamps FANCI: FANCD2 on DNA in filamentous arrays. eLife 9, e54128.

176 van Twest S, Murphy VJ, Hodson C, Tan W, Swuec P, O’Rourke JJ, Heierhorst J, Crismani W & Deans AJ (2017) Mechanism of ubiquitination and deubiquitination in the Fanconi anemia pathway. Mol Cell 65, 247–259.

177 Wang S, Wang R, Peralta C, Yaseen A & Pavletich NP (2021) Structure of the FA core ubiquitin ligase closing the ID clamp on DNA. Nat Struct Mol Biol 28, 300–309.

178 Carruthers R, Ahmed SU, Strathdee K, Gomez-Roman N, Amoah-Buahin E, Watts C & Chalmers AJ (2015) Abrogation of radiosensitivity in glioblastoma stem-like cells by inhibition of ATM kinase. Mol Oncol 9, 192–203.

179 Venere M, Hamerlik P, Wu Q, Rasmussen RD, Song LA, Vasanji A, Tenley N, Flavahan WA, Hjelmeeland AB, Bartek J et al. (2014) Therapeutic targeting of constitutive PARP activation compromises stem cell phenotype and survival of glioblastoma-initiating cells. Cell Death Differ 21, 258–269.

180 Ahmed SU, Carruthers R, Gilmour L, Yildirim S, Watts C & Chalmers AJ (2015) Selective inhibition of parallel DNA damage response pathways optimizes radiosensitization of glioblastoma stem-like cells. Cancer Res 75, 4416–4428.

181 Signore M, Pelacchi F, di Martino S, Runci D, Biffoni M, Giannetti S, Morgante L, De Majo M, Petricoin EF, Stancato L et al. (2014) Combined PDK1 and CHK1 inhibition is required to kill glioblastoma stem-like cells in vitro and in vivo. Cell Death Differ 21, e1223.

182 Rasmussen RD, Gajjar MK, Jensen KE & Hamerlik P (2016) Enhanced efficacy of combined HDAC and PARP targeting in glioblastoma. Mol Oncol 10, 751–763.

183 Vance S, Liu E, Zhao L, Parsels JD, Parsels LA, Brown JL, Maybaum J, Lawrence TS & Morgan MA (2011) Selective radiosensitization of p53 mutant pancreatic cancer cells by combined inhibition of Chk1 and PARP1. Cell Cycle 10, 4321–4329.

184 Hoglund A, Stromvall K, Li Y, Forsell LP & Nilsson JA (2011) Chk2 deficiency in Myc overexpressing lymphoma cells elicits a synergistic lethal response in combination with PARP inhibition. Cell Cycle 10, 3598–3607.

185 Booth L, Cruickshanks N, Ridder T, Dai Y, Grant S & Dent P (2013) PARP and CHK inhibitors interact to cause DNA damage and cell death in mammary carcinoma cells. Cancer Biol Ther 14, 458–465.

186 Peasland A, Wang LZ, Rowling E, Kyle S, Chen T, Hopkins A, Cliby WA, Sarkaria J, Beale G, Edmondson RJ et al. (2011) Identification and evaluation of a potent novel ATR inhibitor, NU6027, in breast and ovarian cancer cell lines. Br J Cancer 105, 372–381.

187 Riches LC, Trinidad AG, Hughes G, Jones GN, Hughes AM, Thomason AG, Gavine P, Cui A, Ling S, Stott J et al. (2020) Pharmacology of the ATM inhibitor AZD0156: potentiation of irradiation and olaparib responses preclinically. Mol Cancer Ther 19, 13–25.

188 Carmell N, Rominiyi O, Myers KN, McGarry–Cottrell C, Vanderlinden A, Lad N, Perroux-David E, El-Khamisy SF, Fernando M, Finegan KG et al. (2021) Identification and validation of ERK5 as a DNA damage modulating drug target in glioblastoma. Cancers 13, 944.

189 Koncar RF, Dey BR, Stanton AJ, Agrawal N, Wassell ML, McCarl LH, Locke AL, Sanders L, Morozova-
Vaske O, Myers MI et al. (2019) Identification of novel RAS signaling therapeutic vulnerabilities in diffuse intrinsic pontine gliomas. *Cancer Res* 79, 4026–4041.

Pereira DM & Rodrigues CMP (2020) Targeted avenues for cancer treatment: the MEK5-ERK5 signaling pathway. *Trends Mol Med* 26, 394–407.

Siomes AE, Rodrigues CM & Borralho PM (2016) The MEK5/ERK5 signalling pathway in cancer: a promising novel therapeutic target. *Drug Discov Today* 21, 1654–1663.

Stecca B & Rovida E (2019) Impact of ERK5 on the signaling pathway. *Avenues for cancer treatment: the MEK5-ERK5 kinase inhibitors exert distinct effects on patient-derived 2D and 3D glioblastoma cell culture models. Cell Death Discov* 7, 54.

Gaillard H, García-Muse T & Aguilera A (2015) Replication stress and cancer. *Nat Rev Cancer* 15, 276–289.

Bartkova J, Hamerlik P, Stockhausen MT, Ehrmann J, Hlobilıkova A, Laursen H, Kalita O, Kolar Z, Poulsen HS, Broholm H et al. (2010) Replication stress and oxidative damage contribute to aberrant constitutive activation of DNA damage signalling in human gliomas. *Oncogene* 29, 5095–5102.

Ning JF, Stanciu M, Humphrey MR, Gorham J, Wakimoto H, Nishihara R, Lees J, Zou L, Martuza RL, Wakimoto H et al. (2019) Myc targeted CDK18 promotes ATR and homologous recombination to mediate PARP inhibitor resistance in glioblastoma. *Nat Commun* 10, 2910.

Barone G, Arora A, Ganesh A, Abdel-Fatah T, Moseley P, Ali R, Chan SY, Savva C, Schiavone K, Carmell N et al. (2018) The relationship of CDK18 expression in breast cancer to clinicopathological parameters and therapeutic response. *Oncotarget* 9, 29508–29524.

Barone G, Staples CJ, Ganesh A, Patterson KW, Bryne DP, Myers KN, Patil AA, Evers CE, Maslen S, Skehel JM et al. (2016) Human CDK18 promotes replication stress signaling and genome stability. *Nucleic Acids Res* 44, 8772–8785.

Zhou W, Yao Y, Scott AJ, Wilder-Romans K, Dresser JJ, Werner CK, Sun H, Pratt D, Sajjakulunikit P, Zhao SG et al. (2020) Purine metabolism regulates DNA repair and therapy resistance in glioblastoma. *Nat Commun* 11, 3811.

Bader SB, Dewhirst MW & Hammond EM (2020) Cyclic hypoxia: an update on its characteristics, methods to measure it and biological implications in cancer. *Cancers* 13, 23.

Bindra RS, Chalmers AJ, Evans S & Dewhirst M (2017) GBM radiosensitizers: dead in the water...or just the beginning? *J Neurooncol* 134, 513–521.

Chedeville AL & Madureira PA (2021) The role of hypoxia in glioblastoma radiotherapy resistance. *Cancers* 13, 542.

Ng N, Purshouse K, Foskolou IP, Olcina MM & Hammond EM (2018) Challenges to DNA replication in hypoxic conditions. *FEBS J* 285, 1563–1571.

Feng X, Tubbs A, Zhang C, Tang M, Srirahar S, Wang C, Jiang D, Su D, Zhang H, Chen Z et al. (2020) ATR inhibition potentiates ionizing radiation-induced interferon response via cytosolic nucleic acid-sensing pathways. *EMBO J* 39, e104036.

Motwani M, Pesiridis S & Fitzgerald KA (2019) DNA sensing by the cGAS-STING pathway in health and disease. *Nat Rev Genet* 20, 657–674.

Forment JV & O’Connor MJ (2018) Targeting the replication stress response in cancer. *Pharmacol Ther* 188, 155–167.

Sheridan C (2019) Drug developers switch gears to inhibit STING. *Nat Biotechnol* 37, 199–201.

Akhavan D, Alizadeh D, Wang D, Weist MR, Shephard JK & Brown CE (2019) CAR T cells for brain tumors: lessons learned and road ahead. *Immuno Rev* 290, 60–84.

Chan HY, Choi J, Jackson C & Lim M (2021) Combination immunotherapy strategies for glioblastoma. *J Neurooncol* 151, 375–391.

Chuntova P, Chow F, Watchmaker P, Galvez M, Heimberger AB, Newell EW, Diaz A, DePinho RA, Li MO, Wherry EJ et al. (2021) Unique challenges for glioblastoma immunotherapy – discussions across neuro-oncology and non-neuro-oncology experts in cancer immunology. *Neuro Oncol* 23, 356–375.

Jackson CM, Choi J & Lim M (2019) Mechanisms of immunotherapy resistance: lessons from glioblastoma. *Nat Immunol* 20, 1100–1109.

von Roemeling CA, Wang Y, Qie Y, Yuan H, Zhao H, Liu X, Yang Z, Yang M, Deng W, Bruno KA et al. (2020) Therapeutic modulation of phagocytosis in glioblastoma can activate both innate and adaptive antitumour immunity. *Nat Commun* 11, 1508.

Baxter PA, Su JM, Onar-Thomas A, Billups CA, Li XN, Poussaint TY, Smith ER, Thompson P, Adesina A, Ansell P et al. (2020) A phase I/II study of veliparib (ABT-888) with radiation and temozolomide in newly diagnosed diffuse pontine glioma: a Pediatric Brain Tumor Consortium study. *Neuro Oncol* 22, 875–885.

Sanai N, Li J, Boerner J, Stark K, Wu J, Kim S, Deragotis A, Mehta S, Dhruv HD, Heilbrun LK et al. (2018) Phase 0 trial of AZD1775 in first-recurrence glioblastoma patients. *Clin Cancer Res* 24, 3820–3828.

Calcagno A, Di Perri G & Bonora S (2017) Treating gliomas: dead in the water...or just the beginning? *Drugs* 77, 145–157.

Sur-Erdem I, Muslu K, Pinarbası N, Altunbek M, Seker-Polat F, Cingoz A, Aydin SO, Kahraman M,
Culha M, Solaroglu I et al. (2020) TRAIL-conjugated silver nanoparticles sensitize glioblastoma cells to TRAIL by regulating CHK1 in the DNA repair pathway. *Neural Res* **42**, 1061–1069.

Zhang H, van Os WL, Tian X, Zu G, Ribovski L, Bron R, Bussmann J, Kros A, Liu Y & Zuhorn IS (2021) Development of curcumin-loaded zein nanoparticles for transport across the blood-brain barrier and inhibition of glioblastoma cell growth. *Biomater Sci.* https://doi.org/10.1039/d0bm01536a

McCorrie P, Vasey CE, Smith SJ, Marlow M, Alexander C & Rahman R (2020) Biomedical engineering approaches to enhance therapeutic delivery for malignant glioma. *J Control Release* **328**, 917–931.

Vasey CE, Cavanagh RJ, Taresco V, Moloney C, Smith S, Rahman R & Alexander C (2021) Polymer pro-drug nanoparticles for sustained release of cytotoxic drugs evaluated in patient-derived glioblastoma cell lines and in situ gelling formulations. *Pharmaceutics* **13**, 208.

D’Amico RS, Aghi MK, Vogelbaum MA & Bruce JN (2021) Convection-enhanced drug delivery for glioblastoma: a review. *J Neurooncol* **151**, 415–427.

Mozhei O, G. Teschemacher A & Kasparov S (2020) Viral vectors as gene therapy agents for treatment of glioblastoma. *Cancers* **12**, 3724.

Romano E, Netti PA & Torino E (2020) Exosomes in gliomas: biogenesis, isolation, and preliminary applications in nanomedicine. *Pharmaceuticals* **13**, 319.

Etame AB, Diaz RJ, Smith CA, Mainprize TG, Hynynen K & Rutka JT (2012) Focused ultrasound disruption of the blood-brain barrier: a new frontier for therapeutic delivery in molecular neurooncology. *Neurosurg Focus* **32**, E3.

Xie F, Boska MD, LoF J, Uberti MG, Tsutsui JM & Porter TR (2008) Effects of transcranial ultrasound and intravenous microbubbles on blood brain barrier permeability in a large animal model. *Ultrasound Med Biol* **34**, 2028–2034.

Chang E, Patel CB, Pohling C, Young C, Song J, Flores TA, Zeng Y, Joubert LM, Arami H, Natarajan A et al. (2018) Tumor treating fields increases membrane permeability in glioblastoma cells. *Cell Death Discov* **4**, 113.

Carruthers RD & Chalmers AJ (2019) DNA repair in Radiation Oncology. *Radiat Oncol* 1–16. Available at: https://link.springer.com/referenceworkentry/10.1007/978-3-319-52619-5_111-1

Kleinberg L, Supko JG, Mikkelsen T, Blakeley JON, Stevens G, Ye X, Desideri S, Ryu S, Desai B, Giranda VL et al. (2013) Phase I adult brain tumor consortium (ABTC) trial of ABT-888 (veliparib), temozolomide (TMZ), and radiotherapy (RT) for newly diagnosed glioblastoma multiforme (GBM) including pharmacokinetic (PK) data. *J Clin Oncol* **31**, 2065.

Halford SER, Cruickshank G, Dunn L, Erridge S, Godfrey L, Herbert C, Jefferies S, Lopez JS, Mc Bain C, Pittman M et al. (2017) Results of the OPARATIC trial: A phase I dose escalation study of olaparib in combination with temozolomide (TMZ) in patients with relapsed glioblastoma (GBM). *J Clin Oncol* **35**, 2022.

Kurzrock R, Galanis E, Johnson DR, Kansra V, Wilcoxon K, McClure T, Martell RE & Agarwal S (2014) A phase I study of niraparib in combination with temozolomide (TMZ) in patients with advanced cancer. *J Clin Oncol* **32**, 2092.

Fulton B, Short SC, James A, Nowicki S, McBain C, Jefferies S, Kelly C, Stobo J, Morris A, Williamson A et al. (2017) PARADIGM-2: Two parallel phase I studies of olaparib and radiotherapy or olaparib and radiotherapy plus temozolomide in patients with newly diagnosed glioblastoma, with treatment stratified by MGMT status. *Clin Transl Radiat Oncol* **8**, 12–16.

Robins HI, Zhang P, Gilbert M, Chakravarti A, deGroot J, Grimm S, Wang F, Lieberman F, Krauze A, Sharma A et al. (2015) ATCT-27: NRG oncology/ RTOG 0929: a randomized phase I/II study of ABT-888 in combination with temozolomide in recurrent temozolomide resistant glioblastoma. *Neuro Onco* **17**, v7.

Baxter PA, Su JM, Li X-N, Onar-Thomas A, Billups CA, Thompson PA, Goldman S, Gururangan S, Young-Poussaint T, McKeegan EM et al. (2015) A phase I/II clinical trial of veliparib (ABT-888) and radiation followed by maintenance therapy with veliparib and temozolomide in patients with newly diagnosed diffuse intrinsic pontine glioma (DIPG): A Pediatric Brain Tumor Consortium Interim Report of Phase I Study. *J Clin Oncol* **33**, 10053.

Schafer ES, Rau RE, Berg SL, Liu X, Minard CG, Bishop AJR, Romero JC, Hicks MJ, Nelson MD Jr et al. (2020) Phase 1/2 trial of talazoparib (ABT-888) in combination with temozolomide in children and adolescents with refractory/recurrent solid tumors including Ewing sarcoma: A Children’s Oncology Group Phase 1 Consortium study (ADV14111). *Pediatr Blood Cancer* **67**, e28073.

Piotrowski A, Puduvalli V, Wen P, Campian J, Colman H, Pearlman M, Butowski N, Battiste J, Glass J, Cloughesy T et al. (2019) ACTR-39, pamiparib in combination with radiation therapy (RT) and/or temozolomide (TMZ) in patients with newly diagnosed or recurrent/refractory (R/R) glioblastoma (GBM); phase 1b/2 study update. *Neuro Oncol* **21**, vi21–vi22.

Lesueur P, Lequesne J, Grellard JM, Dugue A, Coquan E, Brachet PE, Geffrelot J, Kao W, Emery E, Berro DH et al. (2019) Phase I/IIa study of concomitant radiotherapy with olaparib and
temozolomide in unresectable or partially resectable glioblastoma: OLA-TMZ-RTE-01 trial protocol. BMC Cancer 19, 198.

237 Parsons DW, Janeway KA, Patton D, Coffey B, Williams PM, Hamilton SR, Purkayastha A, Tsongalis GJ, Routbort M, Gastier-Foster JM et al. (2019) Identification of targetable molecular alterations in the NCI-COG Pediatric MATCH trial. J Clin Oncol 37, 10011.

238 Sulkowski PL, Corso CD, Robinson ND, Scanlon SE, Purshouse KR, Bai H, Liu Y, Sundaram RK, Hegan DC, Fons NR et al. (2017) 2-Hydroxyglutarate produced by neomorphic IDH mutations suppresses homologous recombination and induces PARP inhibitor sensitivity. Sci Transl Med 9, eaal2463.

239 Karanam NK, Ding L, Aroumougame A & Story MD (2020) Tumor treating fields cause replication stress and interfere with DNA replication fork maintenance: Implications for cancer therapy. Transl Res 217, 33–46.

240 Karanam NK, Srinivasan K, Ding L, Sishc B, Saha D & Story MD (2017) Tumor-treating fields elicit a conditional vulnerability to ionizing radiation via the downregulation of BRCA1 signaling and reduced DNA double-strand break repair capacity in non-small cell lung cancer cell lines. Cell Death Dis 8, e2711.

241 Cancer Research UK (2020) A trial of AZD1390 and radiotherapy for some types of brain tumour. Available at: https://www.cancerresearchuk.org/about-cancer/find-a-clinical-trial/a-trial-of-azd1390-and-radiotherapy-for-some-types-of-brain-tumour.

242 Reddy VP, Sykes A, Colclough N, Durant ST, Connor LO, Hoch M, Bruna NB, Vita SD, Merchant M & Pass M (2019) A preclinical PK/PD model based on a mouse glioblastoma survival model for AZD1390, a novel, brain-penetrant ATM kinase inhibitor, to predict the inhibition of DNA damage response induced by radiation and the human efficacious dose. Cancer Res 79, 4868.

243 Durant ST, Zheng L, Wang Y, Chen K, Zhang L, Zhang T, Yang Z, Riches L, Trinidad AG, Fok JHL et al. (2018) The brain-penetrant clinical ATM inhibitor AZD1390 radiosensitizes and improves survival of preclinical brain tumor models. Sci Adv 4, eaaat1719.

244 Alexander B, Supko J, Agar N, Ahluwalia M, Desai A, Dietrich J, Kaley T, Peereboom D, Takebe N, Desideri S et al. (2018) ACTR-14. Phase I study of AZD1775 with radiation therapy (RT) and temozolomide (TMZ) in patients with newly diagnosed glioblastoma (GBM) and evaluation of intratumoral drug distribution in patients with recurrent GBM. Neuro Oncol 20, vi13–vi14.