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Developing H3K27M mutant selective radiosensitization strategies in diffuse intrinsic pontine glioma

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ABSTRACT

Diffuse intrinsic pontine glioma (DIPG) is a rare but highly lethal pediatric and adolescent tumor located in the pons of the brainstem. DIPGs harbor unique and specific pathological and molecular alterations, such as the hallmark lysine 27-to-methionine (H3K27M) mutation in histone H3, which lead to global changes in the epigenetic landscape and drive tumorigenesis. While fractionated radiotherapy, the current standard of care, improves symptoms and delays tumor progression, DIPGs inevitably recur, and despite extensive efforts chemotherapy-driven radiosensitization strategies have failed to improve survival. Advances in our understanding of the role of epigenetics in the cellular response to radiation-induced DNA damage, however, offer new opportunities to develop combinational therapeutic strategies selective for DIPGs expressing H3K27M. In this review, we provide an overview of preclinical studies that explore potential radiosensitization strategies targeting the unique epigenetic landscape of H3K27M mutant DIPG. We further discuss opportunities to selectively radiosensitize DIPG through strategic inhibition of the radiation-induced DNA damage response. Finally, we discuss the potential for using radiation to induce anti-tumor immune responses that may be potentiated in DIPG by radiosensitizing-therapeutic strategies.

Introduction

Diffuse intrinsic pontine gliomas are devastating pediatric brain tumors that affect hundreds of children in the US each year [1]. Children diagnosed with DIPG have a median survival of 10-11 months from onset, and the 2- and 5-year survival rates are less than 10% and 1%, respectively [2,3]. Sequencing efforts have revealed that in addition to commonly altered genetic events such as loss of p53 or amplification of platelet-derived growth factor receptor alpha (PDGFRA), 80-90% of DIPGs harbor a monoallelic point mutation in the genes encoding histone H3.3 or H3.1 which converts lysine 27 to a methionine (termed the H3K27M mutation) [4,5]. Both the 2016 and the 2021 World Health Organization (WHO) classifications of CNS tumors list DIPG with the histone H3K27M mutation as a grade 4 glioma, regardless of histological features, and coined the tumor entity "diffuse midline glioma, H3K27M-mutant," which for simplicity we refer to herein as DIPG [6,7].

Due in part to the anatomical location and infiltrative characteristics of DIPGs that prohibit surgical resection, as well as the lack of efficacy of chemotherapies used in the treatment of adult gliomas, fractionated radiation therapy continues to be the standard of care [8]. Unfortunately, DIPGs inevitably recur within the high dose radiation field and uniformly prove fatal [9]. Simply escalating the radiation dose has not been beneficial, likely because of increased toxicity to critical normal neural structures in the brainstem and thalamus [10], and while re-irradiation at first progression may have some initial effect on tumor growth, eventually these tumors recur as well [11]. It has therefore been of particular interest to develop radiosensitizing strategies in DIPG that maximize the therapeutic ratio by targeting tumor-specific vulnerabilities to achieve selective, synergistic tumor cell killing at lower radiation doses, thereby sparing nearby normal, healthy tissues from radiotherapy-related side effects [12,13].

Ionizing radiation kills cells through a variety of lesions including direct induction of both DNA single-strand (SSBs) and double-strand breaks (DSBs), formation of bulky DNA adducts, and/or increased levels of reactive oxygen species (ROS). In response to these radiation-induced lesions, multiple DNA repair pathways are triggered to prevent cell death. The coordination of these processes with pathways that regulate cell cycle progression, DNA synthesis and DNA repair are collectively known as the DNA damage response (DDR). The DDR promotes tumor cell survival following radiation by limiting DNA damage that otherwise promotes both direct cell killing and an anti-tumoral innate immune response.

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Previous radiosensitization strategies based on either direct DNA damaging agents [14] or drugs that increase formation of ROS after treatment with radiation [15] failed to improve overall survival in patients with DIPG. In addition, nearly all DIPGs with the H3K27M mutation lack DNA methylation of the O6-methyl-guanine-DNA methyltransferase (MGMT) gene. This mark is a key predictor for benefit from the alkylating chemotherapeutic agent temozolomide (TMZ) in adult glioma. It is therefore not surprising that compared to RT alone, combined treatment with TMZ and RT affords limited survival benefit in patients with DIPG [16]. Targeting the DNA damage response instead of directly damaging DNA, however, may be a better strategy. In other malignancies, radiotherapy-DDR inhibitor combination strategies designed to target tumor-specific alterations in the DNA damage response have demonstrated promising efficacy in early phase clinical trials [17,18]. Similarly, leveraging the unique biology of H3K27M tumor cells to selectively potentiate the efficacy of radiation-based therapies has the potential to meaningfully improve outcomes for patients with DIPG.

Although the H3K27M mutation accounts for only 5-10% of total cellular histone H3 proteins in DIPG cells, it causes *genome-wide* histone H3 hypomethylation through inhibition of polycomb repressive complex 2 (PRC2) activity [19–21]. As a result, DIPG cells have distinct genetic and epigenetic characteristics, such as elevated H3K27 acetylation (H3K27ac) [20], that may have profound effects on both DDR signaling and overall DNA damage repair. In this review we will discuss both the impact of the H3K27M mutation on the radiation-induced DNA damage response, and progress in the field to pharmacologically target H3K27M DIPG-specific biology to enhance radiation therapy. Unless otherwise noted, this review will focus on DIPGs with the H3.3 K27M mutation.

Epigenetic regulation of DNA double strand break repair and the DNA damage response

Histones are among the most abundant and highly conserved proteins in cells, providing the foundation for the basic unit of chromatin, the nucleosome. Each nucleosome consists of approximately 150 base pairs of DNA wrapped around an octameric core of paired histone proteins H2A, H2B, H3 and H4. Post-translational modifications of these histones, including methylation, acetylation, phosphorylation and ubiquitination, act in both a combinatorial and a sequential fashion to modulate both DNA accessibility and recruitment of the molecular machinery required for transcription, replication, and repair [22]. Thus, in addition to their structural role packaging DNA, histone modifications play a dynamic role in the regulation of both gene expression and genome integrity [23]. The importance of histone epigenetics for normal cell development and differentiation is well-established, and histone mutations that lead to dysfunction of these processes, such as H3K27M, can drive tumorigenesis [24,25]. Understanding the net effect of histone post-translational modifications on the radiation-induced DNA damage response is an area of intense research.

While ionizing radiation induces a variety of DNA lesions, unresolved DSBs are the primary lesions responsible for radiation-mediated cell death [26,27]. The coordinated abilities of both DDR and direct DNA repair proteins to sense, signal and repair DSBs are key determinants of radiation sensitivity, while tumor-specific expression of DDR proteins and increased capacity for DNA repair are key factors in acquired radioresistance [28,29]. Nonhomologous end-joining (NHEJ) and homologous recombination repair (HR) are two mechanistically distinct DSB repair pathways central to the radiation-induced DNA damage response [30]. NHEJ, the primary DSB repair mechanism in G1 phase cells, is a rapid, error-prone, 'quick fix' mechanism active throughout the cell cycle that catalyzes sequence-independent DNA end-joining [31]. A distinguishing feature of NHEJ is binding of 53BP1 and Ku70/80 to DNA ends which prevents the extensive end resection required for other DNA repair pathways to proceed. Homologous recombination repair, on the other hand, is a relatively slow, high-fidelity process confined to late S-phase or G2/M that repairs complex DSBs by using an undamaged sister chromatid as a template for repair [32]. Key features of HR are 5′ to 3′ end resection initiated by the MRE11/RAD50/NBS1 (MRN) complex and CtBP interacting protein (CtIP), replication protein A (RPA) binding to the resultant single-stranded 3′ DNA ends, and BRCA2-mediated displacement of RPA by RAD51, which mediates strand exchange for recombination.

Emerging evidence suggests that H3K27M-induced changes to the epigenetic landscape could affect both DNA repair capacity and NHEJ versus HR pathway choice. At a global level, the overall net effect of H3K27 hypomethylation and increased H3K27ac is a relaxation of chromatin structure and de-repressed transcription of genes involved in stemness and neurogenesis, conferring a cancer stem cell-like phenotype associated with constitutive activation of the DDR and radioresistance [21,19,33,34]. At the genomic level, however, exon sequencing analysis revealed that nearly half of the primary H3K27M DIPG tumor samples surveyed had heterozygous mutations in a diverse set of DDR genes including *ATM, ATR, BRCA2, PALB2, RAD50* and *RAD51C* [35]. Additionally, Zarghooni et al. reported a high frequency of loss of heterozygosity and/or deletions of the HR pathway gene *BRCA2* in a small cohort of DIPG samples [36]. Whether HR-deficiency is a common phenotype in DIPG is unclear, but these genetic analyses suggest radiosensitizing-therapeutics with synthetic lethality in HR defective tumors may provide additional therapeutic benefit in combination with radiation in these DIPGs.

In addition to changes at the genomic and transcriptional levels, H3K27M-associated changes in chromatin structure may directly alter DNA repair efficiency and pathway choice. NHEJ and HR rely on distinct chromatin remodeling processes that, through post-translational modification of histones, dynamically regulate chromatin accessibility, DNA repair factor recruitment, and pathway choice [37]. For example, decondensation of heterochromatin at DSBs (mediated in part by the apical DDR kinase ataxia telangiectasia mutated (ATM) via effector protein KRAB-associated protein (KAP-1)) [38] facilitates DSB repair by promoting accessibility of NHEJ repair proteins. Multiple groups have reported that H3K36me2 is elevated in H3K27M DIPG cells [34,39] and may be expected to facilitate HR efficiency. The p75 splice variant of lens epithelium derived growth factor (LEDGF) constitutively binds chromatin at H3K36me2/3 through its PWWP (Pro-Trp-Trp-Pro) domain [40]. In response to radiation-induced DNA damage, LEDGF recruits CtBP, a protein required for resection of DSBs, to the damage site, facilitating HR repair [40]. Furthermore, expression of the E3 ubiquitin ligase BMI1, a core subunit of the polycomb repressive complex 1 (PRC1), is upregulated in DIPG [41]. Correspondingly, levels of monoubiquitinated histone H2A lysine 119 (H2A119ub), a product of BMI1, were higher in cells expressing H3K27M compared to those expressing wild-type histone H3 [41,34]. Like LEDGF, BMI1 and H2AK119ub have been reported to facilitate CtIP recruitment and DNA end resection at DSBs [42]. Epigenetic changes associated with the H3K27M mutation and their reported roles in DNA repair are summarized in Table 1.

Despite evidence that PRC2 subunits accumulate at DNA lesions and promote DSB repair and cell survival in response to ionizing radiation, whether H3K27me2/3 marks themselves are *directly* involved in DSB repair remains unclear [43,44]. Zhang et al. reported that H3K27me2/3 marks are required for 53BP1 focus formation and NHEJ repair of radiation-induced DSBs in human dermal fibroblasts [45]. They further demonstrated that the DDR protein FANCD2 directly binds both hypo-H3K27me2/3 and H3.1K27M, recruiting the histone acetyltransferase Tip60, which then acetylates histone H4 at lysine 16, sterically blocking access of 53BP1 to its H4K20me2 binding site. While further investigation is required to determine whether H3K27me2/3 plays a direct role in DSB repair, current evidence does suggest that the susceptibility of DIPG cells to radiation-induced cell death is in part attributable to the global effects of the H3K27M mutation on chromatin architecture, which has down-stream effects on induction of DNA damage and subsequent DNA repair [46].

Table 1
Histone marks in the setting of H3K27M and their roles in DSB repair.

Histone marks	Up(+)/Down(-) upon H3K27M mutation	Up(+)/Down(-) Upon DSB	Function in DSB repair	Ref
H3K27me2/3	—	Conflicting, increase or no change	Possibly suppresses gene expression at DNA lesions	[44]
H3K27Ac	+++	+	Facilitates permissive chromatin environment at DSBs, and supports HR	[20,21]
H3K36me2/3	+	+	Binds to LEDGF/p75, and promotes CtIP-mediated end resection and HR	[34,39]
H4K20me2/3	-	+	Binds to the Tudor domain of 53BP1 and facilitates NHEJ	[45]
H4K16Ac	+	Conflicting (increase, no change)	Blocks 53BP1 binding to H4K20me, possibly promotes DNA resection and BRCA1 recruitment	[98]
H3K4me3	+	Conflicting (increase, no change, decrease)	Promotes chromatin de-condensation and HR	[99]
H3K9me2/3	+	Conflicting (no change, increase, decrease)	Facilitates 53BP1 binding as well as promotes ATM activation and HR	[100,101]
H3K64Ac	+	n/a	Facilitates nucleosome eviction and transcription	[102]
H2AK119ub	+	+	Promotes DNA end resection and HR	[41,42]
H2BK120ub	n/a	-	H2BK120 undergoes a transition from Ub to acetylation upon DSB formation. Deubiquitination of H2BK120 is essential for proper DNA repair. Enrichment of H2BK120Ac at HR-prone DSBs	[37,103]
H2BK120Ac	n/a	+		

Targeting H3K27M-associated epigenetic changes to radiosensitize H3K27M DIPGs

Work defining H3K27M-initiated epigenetic changes has nominated novel, DIPG-selective targets for radiosensitizing these cells (Table 2). In one preclinical study, Katagi et al. assessed the ability of GSK-J4, an inhibitor of the histone H3 demethylase JMJD3/UTX, to radiosensitize in H3K27M DIPG models [47]. Previous studies had established that GSK-J4 restored cellular H3K27me2/3 levels and changed the transcriptional profiles of H3K27M DIPG cells [48]. Using gene set enrichment analysis of RNA-Seq data generated from DIPG cell lines, Katagi et al. found that GSK-J4 reduced expression of DNA repair genes selectively in H3K27M mutant-expressing DIPG [47]. Furthermore, they found GSK-J4 decreased DNA accessibility and inhibited homologous recombination repair of radiation-induced DSBs. This persistent DNA damage correlated with tumor cell radiosensitization both *in vitro* and *in vivo,* and was specific to H3K27M DIPG cells, highlighting the role of global H3K27 methylation status in determining radiosensitivity. While these preclinical results are promising, GSK-J4 itself is not suitable for clinical development due to its rapid conversion *in vivo* to the active metabolite GSK-J1, which has poor cell membrane permeability [48,47].

In addition to loss of H3K27me2/3, multiple reports document the profound gain of H3K27ac, a transcription enhancer element that co-localizes with H3K27M at actively transcribed genes in DIPG cells [20,49,34]. This epigenetic mark is read by the bromodomain protein 4 (BRD4), which specifically binds to H3K27ac and facilitates RNA polymerase II-mediated transcription and gene expression [50]. Inhibition of BRD4 with the small molecule JQ1 has been reported to decrease expression of DSB repair genes and slow repair of radiation-induced DNA damage signaling [51]. Furthermore, JQ1 radiosensitized both DIPG cells *in vitro* and orthotopic tumors *in vivo* [51]. Like GSK-J4, the pharmacodynamic profile of JQ1 limits its clinical applicability [52]. These preclinical data, however, provide a rationale for the development of second generation BRD4 inhibitors such as TEN-010 or OTX015, in combination strategies with radiation for DIPG.

Chemical screening and *in vivo* preclinical testing have also identified combination therapies with the pan-histone deacetylase (HDAC) inhibitor panobinostat as promising strategies for treating DIPG [53–55]. Meel et. al. recently reported that panobinostat in combination with the receptor tyrosine kinase AXL inhibitor bemcentinib (BGB324) reversed the mesenchymal phenotype associated with radiotherapy resistance in DIPG cells, and decreased expression of genes associated with

Table 2
Molecular targets for DIPG radiosensitization in preclinical studies.

Target	Inhibitor/Drug	In vitro model	In vivo model	Remarks	Ref
BCL2	Venetoclax	SU-DIPG04, BT245, SU-DIPGXVII	BT245-Luc2 and SU-DIPGXIII-Luc2 mouse pons xenograft models	Augmentation of mitochondrial ROS and apoptosis upon combination of Venetoclax and radiation	[104]
mTORC1/2	TAK228	JHH-DIPG1, SF7761, SU-DIPG-XIII	PKC-L (murine DIPG cells expressing luciferase)	Suppression of cell proliferation, induction of apoptosis, and radiosensitization effects	[105]
MRK/γ-Secretase	MRK003	JHH-DIPG1, SF7761, SU-DIPG-XIII	n/a	Inhibition of growth and induction of apoptosis	[106]
PLK1	BI 6727	DIPG IV, DIPG VI	n/a	Inhibition of growth and induction of G2/M arrest and apoptosis	[107]
ALX and HDAC	BGB324 and panobinostat	VUMC-DIPG, SU-DIPG, HSJD-DIPG, JHH-DIPG1, SF7761, SF8628	HSJD-DIPG-07-Fluc xenograft model, UC-8D2 allograft model	Inhibition of viability and migration, suppression of mesenchymal, stem cell, and DNA damage repair gene expression	[55]
JMJD3 demethylase	GSK-J4	SF8628, SF9427, DIPG-007	SF8628 xenograft	Suppression of DNA repair gene expression and HR repair	[47]
BRD4	JQ1	n/a	n/a	Suppression of DNA repair gene expression	[51]
HDAC and PI3K	CUDC-907	SF188, SF8628, SF9427, BT245, BT869	n/a	Inhibition of radiation-induced DNA repair by suppressing NFκB/ FOXM1 recruitment to promoters of DNA damage response genes	[56]
BMI-1	PTC-209	CCHMC-DIPG-1, CCHMC-DIPG-2, SU-DIPG-IV	n/a	Pretreatment with PTC-209 sensitizes DIPG neurospheres to radiomimetic drug Bleocin	[108]

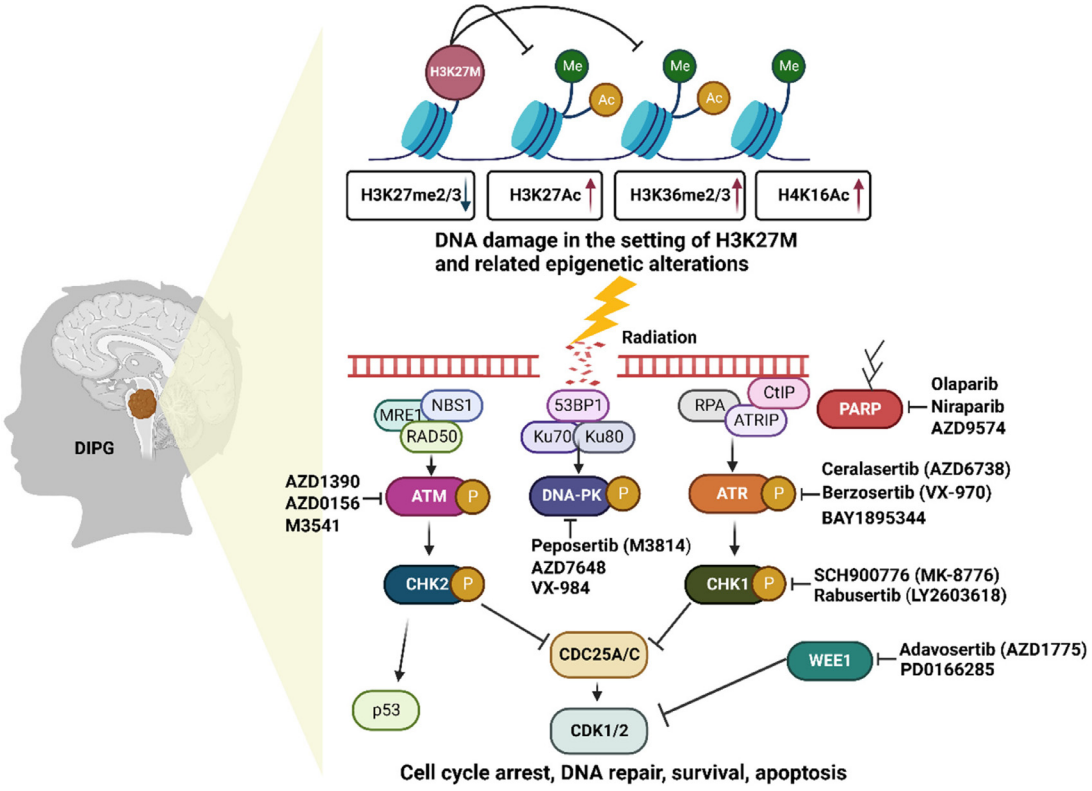

Fig. 1. Targeting the radiation-induced DNA damage response pathway signaling to **radiosensitize in DIPG.** DIPG tumors with the H3K27M mutation have specific and unique epigenetic characteristics and chromatin structure. In response to radiation, DNA damage response (DDR) pathways are activated to trigger DNA repair, cell cycle arrest, and/or cellular senescence or apoptosis. Targeted inhibition of the DNA damage response has the potential to augment the efficacy of radiation treatment for patients with DIPG. The impact of the epigenetic changes associated with H3K27M on the DNA damage response, however, as well as the radiosensitization effect of DDR inhibitors in pre-clinical models of DIPG, require further investigation.

repair of radiation-induced DNA damage [55]. Furthermore, they found that while DIPG neurospheres eventually recovered from panobinostat-mediated radiosensitization (similar to the resistance that develops in response to panobinostat monotherapy), combined treatment with bemcentinib resulted in complete inhibition of neurosphere regrowth up to three months after treatment [55]. Preclinical studies with the first-in-class dual pan-HDAC and pan-PI3K inhibitor CUDC-907 further support the concept of targeting chromatin remodeling and DNA repair gene promoter accessibility to radiosensitize in DIPG [56].

In summary, while there is currently little evidence that the H3K27M mutation directly affects transcription of DDR-related genes or capacity for DNA repair, multiple lines of preclinical evidence support the hypothesis that *reversing* the epigenetic changes associated with the H3K27M mutation inhibits repair of radiation-induced DNA damage and re-sensitizes DIPG tumor cells to radiation-induced cytotoxicity, both *in vitro* and *in vivo*. Whether this sensitization results from global, constitutive changes in the epigenetic landscape, changes in specific epigenetic marks directly linked to DNA repair, or both, remains to be determined.

Direct inhibition of the DNA damage response to radiosensitize in K27M DIPG

A core feature of the DNA damage response is the interconnected signaling network regulated by three apical phosphotidylinositol (PI) 3-kinase-like kinases – DNA-dependent protein kinase, catalytic subunit (DNA-PKcs), ataxia telangiectasia mutated (ATM), and ataxia telangiectasia and rad3 related (ATR) [57] (Fig. 1). Upon activation, these kinases phosphorylate a number of partially overlapping substrates, including themselves, to coordinate DNA repair with other cellular processes such as cell cycle arrest, DNA transcription and replication. In response to radiation-induced DSBs, recruitment and activation of the DNA-PK complex, consisting of Ku70/80 and DNA-PKcs, initiates NHEJ while ATM, recruited to sites of DSBs by the MRN complex, regulates HR. In contrast, ATR primarily mediates the response to radiation-induced replication stress through RPA and the ATR interacting protein (ATRIP). Though there is some redundancy in function, the essential roles of ATM and DNA-PK in mediating the radiation-induced DNA damage response are evidenced by the efficacy of ATM and DNA-PK inhibitors to radiosensitize in a variety of malignancies [58,59]. ATR inhibitors, on the other hand, have been used to confer a synthetically lethal phenotype similar to HR deficiency to target for radiosensitization. In addition, inhibition of these central DDR kinases promotes innate immune signaling and anti-tumor immune responses [60–63]. Using a novel dual recombinase Cre-FlpO strategy to delete *ATM* in primary mouse models of DIPG, Deland et al. demonstrated that tumor-cell specific ATM depletion selectively sensitized p53-deficient, but not p53 wild-type, DIPG cells to radiation [64]. p53 is a direct target of ATM, and the p53-mediated G1 checkpoint is critical to executing an effective, high-fidelity DNA damage response. As a consequence, tumor cells that have lost p53 function are often dependent on ATM- and/or ATR-mediated DDR pathway signaling, and the intra-S phase and G2/M cell cycle checkpoints, for survival post-radiation. *TP53* is the second most frequently mutated gene in H3.3K27M DIPG [65], and inactivation of *TP53* has been identified as the main driver of H3.3K27M DIPG radioresistance [66]. Furthermore, approximately 80% of H3.3K27M/$TP53^{WT}$ DIPG patient samples express a gain-of-function mutation in *PPM1D* (*Protein Phosphatase Mg2+/Mn2+ Dependent 1D*) which confers a p53-deficient phenotype [67]. These findings highlight the importance of characterizing the distinct mutations that co-occur with either H3.3 K27M or H3.1 K27M in developing DDR inhibitor-based therapeutic strategies for DIPG [68], and furthermore

suggest that p53 mutation status may serve as a biomarker for the subset of H3.3K27M DIPG patients that best respond to radiation plus ATM inhibitor combination therapy. Preclinical studies have established that AZD1390, a CNS-penetrant clinical grade ATM inhibitor, radiosensitizes in both a syngeneic mouse model of glioblastoma (GBM; GL261) and patient-derived subcutaneous xenograft models of GBM [58]. Based in part on this work, AZD1390 plus fractionated radiotherapy is currently under investigation in a phase I clinical trial for patients with recurrent grade 4 glioma (NCT05182905). Preclinical studies addressing the efficacy of AZD1390 in DIPG are a current priority and will be critical for the next generation of clinical trials.

Downstream of the apical DDR kinases ATM and ATR are cell cycle checkpoint kinases which transiently arrest cell cycle progression and DNA replication in response to radiation-induced DSBs. In addition to the *ATM* depletion described above, Werbrouck et al. identified loss of several of these DDR proteins, including *CHK1* and *WEE1*, as synthetically lethal with radiation in multiple H3.3K27M/*TP53*^{MUT} glioma stem cell (GSC) models [66]. CHK1 and WEE1 each activate the intra-S-phase and G2/M checkpoints through distinct mechanisms that result in the inhibitory phosphorylation of CDK1 and CDK2 at Y15 [69]. The negative regulation of CDK1/2 by CHK1 and/or WEE1 also facilitates HR-mediated DSB repair [69]. As a result, cells treated with CHK1 or WEE1 inhibitors fail to arrest in S-phase or G2/M in response to radiation-induced DNA damage, have diminished capacity to repair DSBs and may progress through mitosis with incompletely replicated and/or damaged DNA [70]. In preclinical studies, treatment with the CHK1/2 inhibitor prexasertib (LY2606368) abrogated radiation-induced G2 arrest, delayed repair of radiation-induced DNA damage and significantly sensitized p53-deficient GSC cells to radiation-induced cytotoxicity [66]. Similarly, WEE1 inhibition with adavosertib (AZD1775) suppressed the radiation-induced G2/M checkpoint, attenuated repair of radiation-induced DSBs, and radiosensitized in H3K27M DIPG preclinical models [71]. Interestingly, two independent studies reported that *WEE1* mRNA levels were significantly higher in DIPG than in either normal brain or brainstem tissues, or low-grade gliomas, further nominating WEE1 as a therapeutic target in DIPG [71,72]. In addition, the lack of toxicity of adavosertib in preclinical GSC models [66] suggests this compound may have a broader therapeutic window than prexasertib as a radiosensitizing agent in DIPG.

In general, both prexasertib and adavosertib are well-tolerated in pediatric patients with CNS tumors [73,74]. In a recently published phase I consortium study, however, adavosertib in combination with cranial radiation therapy (CRT), though well-tolerated, failed to improve overall survival in children with newly-diagnosed DIPG [74]. As the authors noted, this result was not surprising given the conservative dosing schedule used in the study. While the recommended phase 2 dose (RP2D) of adavosertib given concurrently with focal CRT at the current standard dose (54 Gy in 180 cGy fractions) was defined by the highest dose administered, 200 mg/m^2/day, a relatively lower starting dose of adavosertib (50 mg/m^2/day) was given for most of the treatment regimen. Furthermore, adavosertib was administered *after* daily CRT rather than before, and it is unclear whether this dosing schedule provided optimal inhibition of the WEE1-mediated DNA damage response. Since local radiation therapy does not induce a systemic DNA damage response, previously validated biomarkers that assessed the effects of WEE1 inhibition on DDR signaling in surrogate tissues were uninformative. These results highlight the need to develop pharmacodynamic biomarkers relevant to DIPG that verify DDR target inhibition in tumor cells.

Preclinical evidence also supports development of drugs targeting DDR proteins outside the canonical ATM or ATR-mediated signaling pathways, such as PARP1, as radiosensitizing strategies in DIPG. PARP1 is a nuclear enzyme that promotes repair of both radiation-induced SSBs and DSBs [75]. It acts primarily through the poly(ADP-ribosyl)ation (PARylation) of several proteins, including histones and PARP1 itself. This PARylation signals the subsequent recruitment of DDR proteins to the damage site, and facilitates release of the PARP1 protein from DNA, a step necessary for subsequent repair [76]. The therapeutic activities of individual PARP inhibitors have been attributed in part to their capacity for a distinct mechanism termed *PARP trapping*, which is hypothesized to result from both inhibition of PARP catalytic activity, and thus the autoPARylation required for dissociation of PARP from DNA, as well as drug-induced allosteric changes in the PARP1 protein [77]. Increased PARP1 expression and activity have been demonstrated in pediatric high-grade gliomas (pHGG), including DIPG, and correlated with poor overall survival [78].

PARP inhibition as a therapeutic target in DIPG was first proposed by Zarghooni et al. whose SNP-based microarray analysis found both low-level gains in *PARP1* copy number (3/11, 27%) and PARP1 expression via immunohistochemistry staining (6/11, 55%), as well as loss of heterozygosity and/or deletion of the HR pathway gene *BRCA2*, in their cohort of DIPG patient samples [36]. Given that PARP inhibitors are synthetically lethal with germline mutations in *BRCA1* or *BRCA2* and HR-deficiency [79], PARP inhibitors were hypothesized to be efficacious in DIPGs with HR gene mutation or deletion. In subsequent studies the same group confirmed the expression of PARP1 in DIPG tissue microarray samples (76%), found elevated levels of PARP1 and PARylation in multiple DIPG cell lines and, importantly, reported that the PARP inhibitor niraparib both inhibited repair of radiation-induced DNA damage and sensitized DIPG cells to radiation-induced cytotoxicity [80]. Interestingly, the presence of PARP1 protein was required for niraparib-mediated radiosensitization, and the PARP inhibitor veliparib, which does not effectively trap PARP1 on damaged DNA, was ineffective in their model systems. The critical role of PARP trapping for therapeutic efficacy may partially explain the failure of veliparib to benefit overall survival in a phase I/II clinical trial of veliparib and radiation followed by veliparib and temozolomide in patients with newly diagnosed DIPG [81]. In this study, patients received dose-escalated veliparib (50-85 mg/m^2, b.i.d) with the morning dose administered 60–120 minutes prior to radiation therapy. The ability of veliparib to inhibit PARP was assessed by PAR levels in peripheral blood mononuclear cells but produced heterogeneous results across patients and no correlation with overall survival. Monitoring of cerebrospinal fluid pharmacokinetics might present a better strategy for optimizing dosing in patients with DIPG. Despite a lack of survival benefit concurrent veliparib and radiation was tolerable (RP2D 65mg/m^2) although with more toxicity than that observed with the WEE1 inhibitor adavosertib (described above) including grade 3 and higher lymphopenia and neutropenia.

While the radioresistance and recurrence of DIPG tumors are universal, multiple studies suggest re-irradiation therapy provides some clinical benefit, with minimal risk of serious toxicity, in patients with recurrent DIPG [82]. To our best of knowledge, the potential impact of DDR inhibitors on the efficacy of re-irradiation therapy in either the pre-clinical or clinical setting is largely unknown. Identification of DDR-related radioresistance mechanisms and their associated biomarkers could provide compelling rationales for the combination of DDR therapeutics with re-irradiation therapy.

Targeting the DDR to enhance the radiation-induced antitumor immune response in H3K27M mutant DIPG

Analysis of the DIPG tumor immune microenvironment has led to the identification of a substantial proportion of macrophage/microglia but few infiltrating lymphocytes [83], indicating a paucity of immune surveillance in the process of DIPG formation and progression. Both *in vitro* cell culture and *in vivo* RNAseq analysis revealed minimal immunogenicity of untreated DIPG cells; the basal expression levels of inflammatory cytokines essential for lymphocyte recruitment, such as IL2, IL4, CCL2-5, were similar in DIPG and nontumor control samples [84]. Furthermore, the immunomodulatory ligands PD-1 and PD-L1 were not detectable [84,83]. It is therefore not surprising that initial clinical trials with immune checkpoint blockade failed to provide any survival benefit, with one cohort study even suggesting a worse outcome in

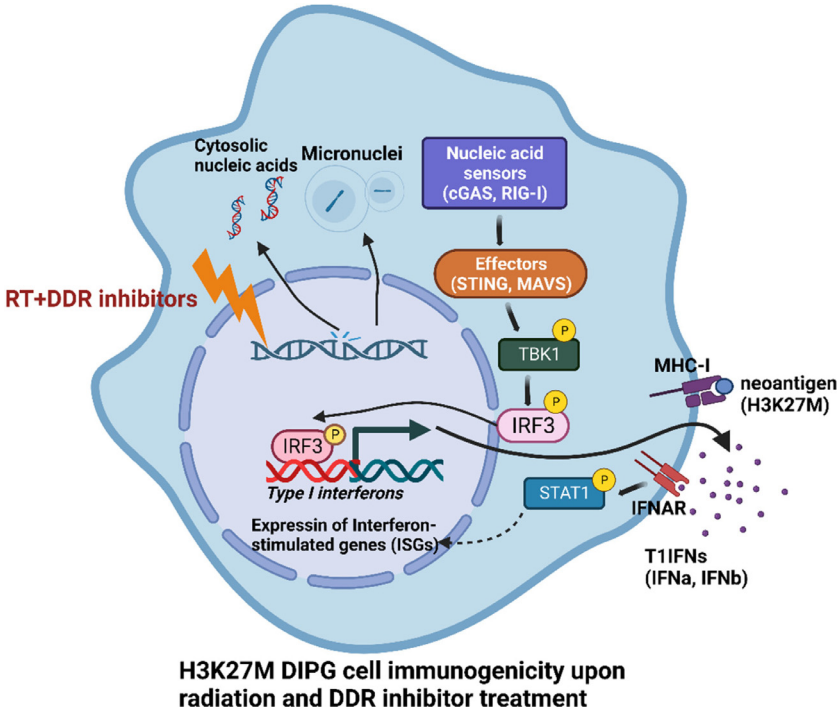

Fig. 2. Generation of immunogenicity in H3K27M DIPG cells upon radiation and DDR inhibitor treatment. DDR inhibitors may increase radiation-induced cytoplasmic self-nucleic acids and micronucleus production in DIPG cells, which in turn activate innate immune signaling pathways (cGAS/STING/TBK1/IRF3 and/or RIG-I/MAVS/TBK1/IRF3) and Type I interferon (T1IFN) production. Interferon/STAT1 signaling induces the expression of a wide array of IFN-stimulated genes (ISGs) which mediate both neoantigen presentation and immune cell infiltration.

DIPG patients upon anti-PD-1 treatment [85,86]. In other "immune-cold" malignancies, however, radiotherapy-induced DNA damage has been shown to increase tumor antigenicity, mitigate immunosuppression, and generate both an adaptive and an innate antitumor immune response [87,60,88,63]. Radiation can increase MHC-I expression and antigen presentation on tumor cells, which primes an antitumor cytotoxic CD8+ T cell response. Given that H3K27M mutant DIPG cells express MHC-I and potentially harbor H3K27M-specific neoantigens, it is plausible that radiation treatment augments presentation of the neoantigens and antigen-specific antitumor CD8+ cytotoxicity.

Radiation stimulates type I interferon (T1IFN) pro-inflammatory responses in both tumor and innate immune cells via formation of micronuclei and release of damaged DNA and chromosome fragments into the cytoplasm [89]. The activation of cytosolic DNA and/or RNA sensors and effectors, such as the cyclic GMP-AMP synthase/stimulator of interferon gene (cGAS/STING) and the retinoic acid-inducible gene-I and mitochondrial antiviral-signaling protein (RIG-I/MAVS) pathways, are key components in this radiation-induced innate immune response [88]. Both STING and MAVS can recruit TANK binding kinase-1 (TBK1) and nuclear factor-kappa B (NF-κB) to phosphorylate the interferon regulatory factor 3 (IRF3) and initiate the transcription of T1IFNs [90]. T1IFNs in turn elicit a broad interferon stimulated gene response that promotes antigen presentation, cytokine production and cytotoxic T cells, but is counter-balanced by induction of the immune checkpoint (PD-1 and PD-L1) (Fig. 2). Whether these immunogenic DNA damage response pathways are intact or functional in DIPG cells remains to be determined. In other malignancies, the persistent DNA damage that results from inhibition of DDR proteins such as ATM, DNA-PKcs, ATR, CHK1/2, or PARP in irradiated cells has been shown to further increase cytosolic DNA/RNA, chromosomal fragments, and downstream immunostimulatory signaling [91]. Future studies are warranted to evaluate the combinatorial effects of DDR inhibition and radiation on the anti-tumor immune responses in preclinical syngeneic mouse models of DIPG. In addition, as an interferon stimulated gene, the immune checkpoint ligand PD-L1 may also be upregulated in DIPG in response to radiation and DDR inhibitors, suggesting a potential for therapeutic efficacy of combinatorial strategies using immune checkpoint and DDR inhibitors and radiation.

The lack of infiltrating immune cells in the DIPG tumor microenvironment has prompted the development of chimeric antigen receptor T cell (CAR T cell) strategies specific for H3K27M-expressing cells which leverage genetically modified T cells to activate the immune system to specifically recognize and kill DIPG tumor cells. Compared to H3 wild-type pHGG, patient-derived DIPG cells with the H3K27M mutation have higher expression of genes encoding ganglioside synthesis enzymes, as well as elevated levels of the disialoganglioside GD2 [92]. Furthermore, treatment with CAR T cells engineered to present anti-GD2 antibodies exhibited promising outcomes in preclinical mouse models [92], and a recent first-in-human phase I clinical trial (NCT04196413) showed radiographic and clinical benefit from initial intravenous and subsequent intracranial infusion of GD2-CAR T cells in three of four DIPG patients expressing mutant H3K27M [93]. The same group subsequently released the outcome of a 3 + 3 phase I dose escalation trial testing GD2-CAR T cell therapy in 11 patients with H3K27M DIPG or spinal diffuse midline glioma (DMG) (NCT04196413) after standard radiotherapy [94]. Nine patients showed radiographic and/or clinical benefit, with two experiencing a near-complete reduction in tumor volume (1 DIPG and 1 spinal DMG). The side effects of CAR T cell therapy, such as cytokine release syndrome and tumor inflammation-associated neurotoxicity, however, are major challenges for future DIPG management. As clinical trials and preclinical data related to the DIPG tumor immune microenvironment and CAR T cell treatment progress, further delineation of dynamic changes in pro-inflammatory cytokines and neo-antigens generated and elevated by radiation will be critical for optimal treatment strategies which may also incorporate DDR therapeutics in the future. Furthermore, preclinical studies are needed to establish the effects of radiation on both ganglioside synthesis enzyme expression and GD2 regulation, as well as therapeutic efficacy and neuroinflammatory related side effects of GD2 CAR T cell therapy, before additional therapeutics can be introduced.

As aforementioned, DIPG cells do not express significant levels of the immune checkpoint ligand PD-L1 (also known as B7-H1) [84]. This result suggests the lack of immune cells in the DIPG tumor microenvironment does not result from activation of the PD-1/PD-L1 immune checkpoint axis. However, expression of another B7 superfamily mem-

ber and immune checkpoint molecule, B7 homolog 3 protein (B7-H3, also known as CD276), was 2.4-fold higher in a cohort of DIPG tumor samples than in normal control tissue [84], suggesting B7-H3 may be another attractive target for DIPG-selective CAR T cell therapy. In a variety of other tumor types, B7-H3-based immunotherapy strategies have displayed effective antitumor activity, with mild side effects, in both preclinical models and clinical trials [95]. The safety and practicality of administering CAR T cells transduced to express B7-H3 to pediatric patients with CNS tumors is currently under clinical investigation (BrainChild-03, NCT04185038). Outcomes for the first 3 patients with recurrent DIPG showed that serial administration of B7-H3 CAR T cells via infusion was both feasible and well tolerated with one patient experiencing clinical improvement and lack of progression through 12 months on study [96]. Although the regulation of B7-H3 expression by radiation is not clear, a recent preclinical study in triple negative breast cancer indicated that the efficacy of B7-H3 CAR-T cell mediated immunotherapy was enhanced by radiation [97]. Therefore, it will be important in future studies to explore the effects of radiation (and DDR signaling) on B7-H3 expression and the potential for therapeutic strategies combining of B7-H3 CAR T cells, radiotherapy, and DDR inhibitors as a novel immunotherapy strategy for DIPG.

Future perspectives

Discovery of the hallmark H3K27M alteration as the major driver of DIPG has inspired the development of novel therapeutic strategies including radiosensitizing-therapeutics to leverage the unique underlying biology of H3K27M with the goal of improving survival for this deadliest of pediatric malignancies. Among these, therapeutics targeting the epigenome or the altered DDR in these H3K27M mutant tumors are areas of intense investigation. Several issues, however, must be addressed in order to improve the likelihood of success of these strategies such as understanding the DDR and DSB repair processes in the context of H3K27M, the contributions of other common DDR-related molecular changes (such as p53, PPM1D), and characterization of both tumor heterogeneity and the tumor immune microenvironment in H3K27M mutant DIPGs. Advances related to these issues are critical for combining radiation and experimental therapeutics in a precision manner. Furthermore, characterization of the effects of radiation on the tumor immune microenvironment will be crucial to develop therapies to further enhance radiation-induced anti-tumor immune responses. While CAR T clinical trials in DIPG have usually been carried out after radiotherapy in patients with recurrent tumors, future studies focused on identifying interactions between radiation and CAR T therapy are needed to determine optimal treatment schedules and whether radiosensitizing-therapeutics could further potentiate anti-tumor immune responses to K27M mutant DIPGs. In sum, harnessing this information in the development of combination treatment modalities is necessary for improving outcomes for patients with this most aggressive pediatric cancer.

Author contributions

Q.Z., L.A.P. and M.A.M. wrote the manuscript, which was then edited by all of the co-authors. All of the co-authors approved the final version of the manuscript before submission.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

[1] N. Patil, et al., Epidemiology of brainstem high-grade gliomas in children and adolescents in the United States, 2000-2017, Neuro. Oncol. 23 (6) (2021) 990–998.
[2] M.H. Jansen, et al., Survival prediction model of children with diffuse intrinsic pontine glioma based on clinical and radiological criteria, Neuro. Oncol. 17 (1) (2015) 160–166.
[3] T. Cooney, et al., Contemporary survival endpoints: an international diffuse intrinsic pontine glioma registry study, Neuro. Oncol. 19 (9) (2017) 1279–1280.
[4] J. Schwartzentruber, et al., Driver mutations in histone H3.3 and chromatin remodelling genes in paediatric glioblastoma, Nature 482 (7384) (2012) 226–231.
[5] G. Wu, et al., Somatic histone H3 alterations in pediatric diffuse intrinsic pontine gliomas and non-brainstem glioblastomas, Nat. Genet. 44 (3) (2012) 251–253.
[6] D.N. Louis, et al., The 2016 World Health Organization classification of tumors of the central nervous system: a summary, Acta Neuropathol. 131 (6) (2016) 803–820.
[7] D.N. Louis, et al., The 2021 WHO classification of tumors of the central nervous system: a summary, Neuro. Oncol. 23 (8) (2021) 1231–1251.
[8] M. Gallitto, et al., Role of radiation therapy in the management of diffuse intrinsic pontine glioma: a systematic review, Adv. Radiat. Oncol. 4 (3) (2019) 520–531.
[9] C.L. Tinkle, et al., Defining optimal target volumes of conformal radiation therapy for diffuse intrinsic pontine glioma, Int. J. Radiat. Oncol. Biol. Phys. 106 (4) (2020) 838–847.
[10] C.R. Freeman, et al., Final results of a study of escalating doses of hyperfractionated radiotherapy in brain stem tumors in children: a Pediatric Oncology Group study, Int. J. Radiat. Oncol. Biol. Phys. 27 (2) (1993) 197–206.
[11] G.O. Janssens, et al., Survival benefit for patients with diffuse intrinsic pontine glioma (DIPG) undergoing re-irradiation at first progression: a matched-cohort analysis on behalf of the SIOP-E-HGG/DIPG working group, Eur. J. Cancer 73 (2017) 38–47.
[12] Z.R. Reichert, D.R. Wahl, M.A. Morgan, Translation of targeted radiation sensitizers into clinical trials, Semin. Radiat. Oncol. 26 (4) (2016) 261–270.
[13] C.J. Shen, S.A. Terezakis, The evolving role of radiotherapy for pediatric cancers with advancements in molecular tumor characterization and targeted therapies, Front. Oncol. 11 (2021) 679701.
[14] S. Bailey, et al., Diffuse intrinsic pontine glioma treated with prolonged temozolomide and radiotherapy–results of a United Kingdom phase II trial (CNS 2007 04), Eur. J. Cancer 49 (18) (2013) 3856–3862.
[15] K.A. Bradley, et al., Motexafin-gadolinium and involved field radiation therapy for intrinsic pontine glioma of childhood: a children's oncology group phase 2 study, Int. J. Radiat. Oncol. Biol. Phys. 85 (1) (2013) e55–e60.
[16] A.R. Pandya, et al., Retrospective analysis of radiotherapy with or without concurrent and adjuvant temozolomide in newly diagnosed paediatric diffuse intrinsic pontine glioma, Int. J. Radiat. Oncol. Biol. Phys. 105 (1) (2019) E627–E628.
[17] K.C. Cuneo, et al., Dose escalation trial of the WEE1 Inhibitor Adavosertib (AZD1775) in combination with gemcitabine and radiation for patients with locally advanced pancreatic cancer, J. Clin. Oncol. 37 (29) (2019) 2643–2650.
[18] R. Tuli, et al., A phase 1 study of veliparib, a PARP-1/2 inhibitor, with gemcitabine and radiotherapy in locally advanced pancreatic cancer, EBioMedicine 40 (2019) 375–381.
[19] A.S. Harutyunyan, et al., H3K27M induces defective chromatin spread of PRC2-mediated repressive H3K27me2/me3 and is essential for glioma tumorigenesis, Nat. Commun. 10 (1) (2019) 1262.
[20] P.W. Lewis, et al., Inhibition of PRC2 activity by a gain-of-function H3 mutation found in pediatric glioblastoma, Science 340 (6134) (2013) 857–861.
[21] S. Bender, et al., Reduced H3K27me3 and DNA hypomethylation are major drivers of gene expression in K27M mutant pediatric high-grade gliomas, Cancer Cell 24 (5) (2013) 660–672.
[22] T. Jenuwein, C.D. Allis, Translating the histone code, Science 293 (5532) (2001) 1074–1080.
[23] P. Caron, E. Pobega, S.E. Polo, DNA double-strand break repair: all roads lead to HeterochROMAtin marks, Front. Genet. 12 (2021) 730696.
[24] W.A. Flavahan, E. Gaskell, B.E. Bernstein, Epigenetic plasticity and the hallmarks of cancer, Science 357 (6348) (2017).
[25] N. Furth, E. Shema, It's all in the combination: decoding the epigenome for cancer research and diagnostics, Curr. Opin. Genet. Dev. 73 (2022) 101899.
[26] E. Mladenov, et al., DNA double-strand break repair as determinant of cellular radiosensitivity to killing and target in radiation therapy, Front. Oncol. 3 (2013) 113.

[27] I.V. Mavragani, et al., Ionizing radiation and complex DNA damage: from prediction to detection challenges and biological significance, Cancers 11 (11) (2019).
[28] M. Goldstein, M.B. Kastan, The DNA damage response: implications for tumor responses to radiation and chemotherapy, Annu. Rev. Med. 66 (2015) 129–143.
[29] R.X. Huang, P.K. Zhou, DNA damage response signaling pathways and targets for radiotherapy sensitization in cancer, Signal Transduct. Target Ther. 5 (1) (2020) 60.
[30] R. Ceccaldi, B. Rondinelli, A.D. D'Andrea, Repair pathway choices and consequences at the double-strand break, Trends Cell Biol. 26 (1) (2016) 52–64.
[31] H.H.Y. Chang, et al., Non-homologous DNA end joining and alternative pathways to double-strand break repair, Nat. Rev. Mol. Cell Biol. 18 (8) (2017) 495–506.
[32] A. Kakarougkas, P.A. Jeggo, DNA DSB repair pathway choice: an orchestrated handover mechanism, Br. J. Radiol. 87 (1035) (2014) 20130685.
[33] S. Bao, et al., Glioma stem cells promote radioresistance by preferential activation of the DNA damage response, Nature 444 (7120) (2006) 756–760.
[34] N. Harpaz, et al., Single-cell epigenetic analysis reveals principles of chromatin states in H3.3-K27M gliomas, Mol. Cell 82 (14) (2022) 2696–2713 e9.
[35] A. Mackay, et al., Integrated molecular meta-analysis of 1,000 pediatric high-grade and diffuse intrinsic pontine glioma, Cancer Cell 32 (4) (2017) 520–537 e5.
[36] M. Zarghooni, et al., Whole-genome profiling of pediatric diffuse intrinsic pontine gliomas highlights platelet-derived growth factor receptor alpha and poly (ADP-ribose) polymerase as potential therapeutic targets, J. Clin. Oncol. 28 (8) (2010) 1337–1344.
[37] T. Clouaire, et al., Comprehensive mapping of histone modifications at DNA double-strand breaks deciphers repair pathway chromatin signatures, Mol. Cell 72 (2) (2018) 250–262 e6.
[38] Y. Ziv, et al., Chromatin relaxation in response to DNA double-strand breaks is modulated by a novel ATM- and KAP-1 dependent pathway, Nat. Cell Biol. 8 (8) (2006) 870–876.
[39] J.R. Yu, et al., The H3K36me2 writer-reader dependency in H3K27M-DIPG, Sci. Adv. 7 (29) (2021).
[40] M. Daugaard, et al., LEDGF (p75) promotes DNA-end resection and homologous recombination, Nat. Struct. Mol. Biol. 19 (8) (2012) 803–810.
[41] I. Balakrishnan, et al., Senescence induced by BMI1 inhibition is a therapeutic vulnerability in H3K27M-mutant DIPG, Cell Rep. 33 (3) (2020) 108286.
[42] A. Fitieh, et al., BMI-1 regulates DNA end resection and homologous recombination repair, Cell Rep. 38 (12) (2022) 110536.
[43] D.M. Chou, et al., A chromatin localization screen reveals poly (ADP ribose)-regulated recruitment of the repressive polycomb and NuRD complexes to sites of DNA damage, Proc. Natl. Acad. Sci. USA 107 (43) (2010) 18475–18480.
[44] S. Campbell, et al., Polycomb repressive complex 2 contributes to DNA double-strand break repair, Cell Cycle 12 (16) (2013) 2675–2683.
[45] Y. Zhang, et al., Histone H3K27 methylation modulates the dynamics of FANCD2 on chromatin to facilitate NHEJ and genome stability, J. Cell Sci. 131 (12) (2018).
[46] H. Pedersen, K. Schmiegelow, P. Hamerlik, Radio-resistance and DNA repair in pediatric diffuse midline gliomas, Cancers 12 (10) (2020).
[47] H. Katagi, et al., Radiosensitization by histone H3 demethylase inhibition in diffuse intrinsic pontine glioma, Clin. Cancer Res. 25 (18) (2019) 5572–5583.
[48] R. Hashizume, et al., Pharmacologic inhibition of histone demethylation as a therapy for pediatric brainstem glioma, Nat. Med. 20 (12) (2014) 1394–1396.
[49] A. Piunti, et al., Therapeutic targeting of polycomb and BET bromodomain proteins in diffuse intrinsic pontine gliomas, Nat. Med. 23 (4) (2017) 493–500.
[50] A. Zippo, et al., Histone crosstalk between H3S10ph and H4K16ac generates a histone code that mediates transcription elongation, Cell 138 (6) (2009) 1122–1136.
[51] T. Sasaki, et al., PDTM-34. Radiosensitization by BRD4 inhibition in diffuse intrinsic pontine glioma, Neuro. Oncol. 21 (2019) vi194–vi195.
[52] T. Shorstova, W.D. Foulkes, M. Witcher, Achieving clinical success with BET inhibitors as anti-cancer agents, Br. J. Cancer 124 (9) (2021) 1478–1490.
[53] C.S. Grasso, et al., Functionally defined therapeutic targets in diffuse intrinsic pontine glioma, Nat. Med. 21 (7) (2015) 827.
[54] S. Nagaraja, et al., Transcriptional dependencies in diffuse intrinsic pontine glioma, Cancer Cell 31 (5) (2017) 635–652 e6.
[55] M.H. Meel, et al., Combined therapy of AXL and HDAC inhibition reverses mesenchymal transition in diffuse intrinsic pontine glioma, Clin. Cancer Res. 26 (13) (2020) 3319–3332.
[56] S. Pal, et al., Dual HDAC and PI3K inhibition abrogates NFκB- and FOXM1-mediated DNA damage response to radiosensitize pediatric high-grade gliomas, Cancer Res. 78 (14) (2018) 4007–4021.
[57] A.N. Blackford, S.P. Jackson, DNA-PK, The trinity at the heart of the DNA damage response, Mol. Cell 66 (6) (2017) 801–817.
[58] S.T. Durant, et al., The brain-penetrant clinical ATM inhibitor AZD1390 radiosensitizes and improves survival of preclinical brain tumor models, Sci. Adv. 4 (6) (2018) eaat1719.
[59] F.T. Zenke, et al., Pharmacologic inhibitor of DNA-PK, M3814, potentiates radiotherapy and regresses human tumors in mouse models, Mol. Cancer Ther. 19 (5) (2020) 1091–1101.
[60] Q. Zhang, et al., Inhibition of ATM increases interferon signaling and sensitizes pancreatic cancer to immune checkpoint blockade therapy, Cancer Res. 79 (15) (2019) 3940–3951.
[61] K. Nakamura, et al., Inhibition of DNA-PK with AZD7648 sensitizes tumor cells to radiotherapy and induces type I IFN-dependent durable tumor control, Clin. Cancer Res. 27 (15) (2021) 4353–4366.
[62] E.C. Patin, et al., Harnessing radiotherapy-induced NK-cell activity by combining DNA damage-response inhibition and immune checkpoint blockade, J. Immunother. Cancer 10 (3) (2022).
[63] W. Wang, et al., DNA-PK inhibition and radiation promote antitumoral immunity through RNA polymerase III in pancreatic cancer, Mol. Cancer Res. 20 (7) (2022) 1137–1150.
[64] K. Deland, et al., Tumor genotype dictates radiosensitization after Atm deletion in primary brainstem glioma models, J. Clin. Invest. 131 (1) (2021).
[65] H. Nikbakht, et al., Spatial and temporal homogeneity of driver mutations in diffuse intrinsic pontine glioma, Nat. Commun. 7 (2016) 11185.
[66] C. Werbrouck, et al., TP53 pathway alterations drive radioresistance in Diffuse Intrinsic Pontine Gliomas (DIPG), Clin. Cancer Res. 25 (22) (2019) 6788–6800.
[67] G. Wu, et al., The genomic landscape of diffuse intrinsic pontine glioma and pediatric non-brainstem high-grade glioma, Nat. Genet. 46 (5) (2014) 444–450.
[68] I.J. Findlay, et al., Pharmaco-proteogenomic profiling of pediatric diffuse midline glioma to inform future treatment strategies, Oncogene 41 (4) (2022) 461–475.
[69] S. Hauge, et al., Expanding roles of cell cycle checkpoint inhibitors in radiation oncology, Int. J. Radiat. Biol. (2021) 1–10.
[70] M.A. Morgan, et al., Improving the efficacy of chemoradiation with targeted agents, Cancer Discov. 4 (3) (2014) 280–291.
[71] V. Caretti, et al., WEE1 kinase inhibition enhances the radiation response of diffuse intrinsic pontine gliomas, Mol. Cancer Ther. 12 (2) (2013) 141–150.
[72] S. Mueller, et al., Targeting WEE1 for the treatment of pediatric high-grade gliomas, Neuro. Oncol. 16 (3) (2014) 352–360.
[73] T. Cash, et al., A phase 1 study of prexasertib (LY2606368), a CHK1/2 inhibitor, in pediatric patients with recurrent or refractory solid tumors, including CNS tumors: a report from the Children's Oncology Group Pediatric Early Phase Clinical Trials Network (ADVL1515), Pediatr. Blood Cancer 68 (9) (2021) e29065.
[74] S. Mueller, et al., WEE1 kinase inhibitor adavosertib with radiation in newly diagnosed diffuse intrinsic pontine glioma: a children's oncology group phase I consortium study, Neurooncol. Adv. 4 (1) (2022) vdac073.
[75] N.J. Curtin, C. Szabo, Poly(ADP-ribose) polymerase inhibition: past, present and future, Nat. Rev. Drug Discov. 19 (10) (2020) 711–736.
[76] M. Javle, N.J. Curtin, The role of PARP in DNA repair and its therapeutic exploitation, Br. J. Cancer 105 (8) (2011) 1114–1122.
[77] J. Murai, et al., Trapping of PARP1 and PARP2 by Clinical PARP Inhibitors, Cancer Res. 72 (21) (2012) 5588–5599.
[78] D.G. van Vuurden, et al., PARP inhibition sensitizes childhood high grade glioma, medulloblastoma and ependymoma to radiation, Oncotarget 2 (12) (2011) 984–996.
[79] C.J. Lord, A. Ashworth, PARP inhibitors: synthetic lethality in the clinic, Science 355 (6330) (2017) 1152–1158.
[80] Y. Chornenkyy, et al., Poly-ADP-ribose polymerase as a therapeutic target in pediatric diffuse intrinsic pontine glioma and pediatric high-grade astrocytoma, Mol. Cancer Ther. 14 (11) (2015) 2560–2568.
[81] P.A. Baxter, et al., 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 (6) (2020) 875–885.
[82] V.M. Lu, et al., Reirradiation for diffuse intrinsic pontine glioma: a systematic review and meta-analysis, Childs Nerv. Syst. 35 (5) (2019) 739–746.
[83] G.L. Lin, et al., Non-inflammatory tumor microenvironment of diffuse intrinsic pontine glioma, Acta Neuropathol. Commun. 6 (1) (2018) 51.
[84] N.A.P. Lieberman, et al., Characterization of the immune microenvironment of diffuse intrinsic pontine glioma: implications for development of immunotherapy, Neuro. Oncol. 21 (1) (2019) 83–94.
[85] N.A. Vitanza, M. Monje, Diffuse intrinsic pontine glioma: from diagnosis to next–generation clinical trials, Curr. Treat. Options Neurol. 21 (8) (2019) 37.
[86] C. Kline, et al., Reirradiation and PD-1 inhibition with nivolumab for the treatment of recurrent diffuse intrinsic pontine glioma: a single-institution experience, J. Neurooncol. 140 (3) (2018) 629–638.
[87] S.A. Patel, A.J. Minn, Combination cancer therapy with immune checkpoint blockade: mechanisms and strategies, Immunity 48 (3) (2018) 417–433.
[88] X. Feng, et al., ATR inhibition potentiates ionizing radiation-induced interferon response via cytosolic nucleic acid-sensing pathways, EMBO J. 39 (14) (2020) e104036.
[89] S.M. Harding, et al., Mitotic progression following DNA damage enables pattern recognition within micronuclei, Nature 548 (7668) (2017) 466–470.
[90] J. Wu, Z.J. Chen, Innate immune sensing and signaling of cytosolic nucleic acids, Annu. Rev. Immunol. 32 (2014) 461–488.
[91] Q. Storozynsky, M.M. Hitt, The impact of radiation-induced DNA damage on cGAS-STING-mediated immune responses to cancer, Int. J. Mol. Sci. 21 (22) (2020).
[92] C.W. Mount, et al., Potent antitumor efficacy of anti-GD2 CAR T cells in H3-K27M, Nat. Med. 24 (5) (2018) 572–579.
[93] R.G. Majzner, et al., GD2-CAR T cell therapy for H3K27M-mutated diffuse midline gliomas, Nature 603 (7903) (2022) 934–941.
[94] R.G. Majzner, et al., Abstract CT001: major tumor regressions in H3K27M-mutated diffuse midline glioma (DMG) following sequential intravenous (IV) and intracerebroventricular (ICV) delivery of GD2-CAR T cells, Cancer Res. (2022) 82.
[95] W.T. Zhou, W.L. Jin, B7-H3/CD276: an emerging cancer immunotherapy, Front. Immunol. 12 (2021) 701006.
[96] N.A. Vitanza, et al., Intraventricular B7-H3 CAR T cells for diffuse intrinsic pontine glioma: preliminary first-in-human bioactivity and safety, Cancer Discov. (2022).
[97] F. Kontos, et al., Radiation enhances the efficacy of B7-H3 Chimeric Antigen Receptor (CAR) T-cell based immunotherapy for Triple Negative Breast Cancer (TNBC), J. Am. Coll. Surg. 231 (4) (2020) S40–S41.
[98] S. An, et al., Histone tail analysis reveals H3K36me2 and H4K16ac as epigenetic signatures of diffuse intrinsic pontine glioma, J. Exp. Clin. Cancer Res. 39 (1) (2020) 261.

[99] Y. Chen, et al., Refined spatial temporal epigenomic profiling reveals intrinsic connection between PRDM9-mediated H3K4me3 and the fate of double-stranded breaks, Cell Res. 30 (3) (2020) 256–268.
[100] M.K. Ayrapetov, et al., DNA double-strand breaks promote methylation of histone H3 on lysine 9 and transient formation of repressive chromatin, Proc. Natl. Acad. Sci. USA 111 (25) (2014) 9169–9174.
[101] A.S. Kovarikova, et al., H3K9me3 and H4K20me3 represent the epigenetic landscape for 53BP1 binding to DNA lesions, Aging-Us 10 (10) (2018) 2585–2605.
[102] V. Di Cerbo, et al., Acetylation of histone H3 at lysine 64 regulates nucleosome dynamics and facilitates transcription, Elife 3 (2014) e01632.
[103] J.J. Kim, et al., Systematic bromodomain protein screens identify homologous recombination and R-loop suppression pathways involved in genome integrity, Genes Dev. 33 (23-24) (2019) 1751–1774.
[104] K. Madhavan, et al., Venetoclax cooperates with ionizing radiation to attenuate diffuse midline glioma tumor growth, Clin. Cancer Res. 28 (11) (2022) 2409–2424.
[105] H. Miyahara, et al., The dual mTOR kinase inhibitor TAK228 inhibits tumorigenicity and enhances radiosensitization in diffuse intrinsic pontine glioma, Cancer Lett. 400 (2017) 110–116.
[106] I.C. Taylor, et al., Disrupting NOTCH slows diffuse intrinsic pontine glioma growth, enhances radiation sensitivity, and shows combinatorial efficacy with bromodomain inhibition, J. Neuropathol. Exp. Neurol. 74 (8) (2015) 778–790.
[107] V. Amani, et al., Polo-like kinase 1 as a potential therapeutic target in diffuse intrinsic pontine glioma, BMC Cancer 16 (2016) 647.
[108] S.S. Kumar, et al., BMI-1 is a potential therapeutic target in diffuse intrinsic pontine glioma, Oncotarget 8 (38) (2017) 62962–62975.