REVIEW

Radiotherapy and radio-sensitization in $H3^{K27M}$-mutated diffuse midline gliomas

Chao Liu[1,2] [iD] | Shuwen Kuang[1] | Lei Wu[3] | Quan Cheng[4] [iD] | Xuan Gong[4] | Jun Wu[4] | Longbo Zhang[2,4,5]

[1]Departments of Oncology, Xiangya Hospital, Central South University, Changsha, China

[2]National Clinical Research Center for Geriatric Disorders, Xiangya Hospital, Central South University, Changsha, China

[3]Department of Neurosurgery, The Second Affiliated Hospital of Nanchang University, Nanchang, China

[4]Departments of Neurosurgery, Xiangya Hospital, Central South University, Changsha, China

[5]Departments of Neurosurgery, Yale School of Medicine, New Haven, Connecticut, USA

Correspondence
Xuan Gong, Jun Wu and Longbo Zhang, Departments of Neurosurgery, Xiangya Hospital, Central South University, Changsha, 410008, China.
Email: longbo.zhang@yale.edu, gong.xuan@csu.edu.cn and jianjuncs@foxmail.com

Abstract

Background: $H3^{K27M}$ mutated diffuse midline gliomas (DMGs) are extremely aggressive and the leading cause of cancer-related deaths in pediatric brain tumors with 5-year survival <1%. Radiotherapy is the only established adjuvant treatment of $H3^{K27M}$ DMGs; however, the radio-resistance is commonly observed.

Methods: We summarized current understandings of the molecular responses of $H3^{K27M}$ DMGs to radiotherapy and provide crucial insights into current advances in radiosensitivity enhancement.

Results: Ionizing radiation (IR) can mainly inhibit tumor cell growth by inducing DNA damage regulated by the cell cycle checkpoints and DNA damage repair (DDR) system. In H3K27M DMGs, the aberrant genetic and epigenetic changes, stemness genotype, and epithelial-mesenchymal transition (EMT) disrupt the cell cycle checkpoints and DDR system by altering the associated regulatory signaling pathways, which leads to the development of radio-resistance.

Conclusions: The advances in mechanisms of radio-resistance in $H3^{K27M}$ DMGs promote the potential targets to enhance the sensitivity to radiotherapy.

KEYWORDS
cell checkpoints, DMG, DNA damage and repair, H3K27M, radio-sensitization

1 | INTRODUCTION

Glioma is one of the most common brain malignancies. Although the integrative therapies including surgeries and chemoradiotherapies are applied in clinical treatment, the outcomes of glioma patients are unsatisfied.[1,2] Recent progress in glioma genomic sequencing suggests that somatic mutations are closely associated with the biology of tumors and the prognoses of patients, which results in the integration of both histopathology and molecular abnormalities in the WHO classification of central nervous system tumor in 2016.[3]

$H3^{K27M}$ mutations are prevalent in diffuse midline gliomas (DMGs) leading poor survival of less than 12 months[4–6] (All of the abbreviations are listed in Table 1). Current clinical management

TABLE 1 Abbreviations.

Abbreviations	Descriptions
ACVR1	Activin A receptor, type I
APE-1	Apurinic/apyrimidinic endonuclease 1
ATM	Ataxia telangiectasia mutated
ATR	Ataxia telangiectasia and Rad3 related
ATRIP	ATR-interacting protein
AURKA	Aurora kinase A
BBB	Brain blood barrier
BRD	Bromodomain protein
BMI1	B lymphoma moloney murine leukemia virus insertion region 1
BRCA1	Breast cancer type 1 susceptibility protein
BRCA2	Breast cancer type 2 susceptibility protein
CAK	CDK activated kinase
CDKs	Cyclin dependent kinases
CED	Convection-enhanced delivery
CHKs	Checkpoint kinases
CKI	Cyclin-dependent kinase inhibitor
CSCs	Cancer stem cells
CtIP	Ctbp-interacting protein
CTV	Clinical tumor volume
DDR	DNA damage repair
DIPG	Diffuse intrinsic pontine glioma
DMGs	Diffuse midline gliomas
DNA pol	DNA polymerase
DNA-PKcs	DNA-dependent protein kinase catalytic subunit
DRD2	Dopamine receptor D2
E2F	Early 2 factor
EGFR	Epidermal growth factor receptor
EMT	Epithelial-mesenchymal transition
ETAA1	ETAA1 activator of ATR kinase
EZH2	Zeste homolog 2
FANCD2	FA complementation group D2
FEN-1	Free endonuclease 1
GTV	Gross tumor volume
HDAC	Histone deacetylases
HR	Homologous recombination
IR	Ionizing radiation
LigIII	Ligase III
LigIV	Ligase IV
MDC1	Mediator of DNA damage checkpoint 1
MGMT	O6-methylguanine-DNA methyltransferase
MRE11	Meiotic recombination 11 homolog 1
MRN	MRE11/RAD50/NBS1

TABLE 1 (Continued)

Abbreviations	Descriptions
NBS1	Phosphopeptide-binding Nijmegen break-age syndrome protein 1
NHEJ	Nonhomologous end-joining
NQO1	NAD(P)H quinone oxidoreductase 1
PALB2	Partner and localizer of BRCA2
PARP1	Poly (ADP-ribose) polymerase 1
PCNA	Proliferation cell nuclear antigen
PDGFRα	Platelet-derived growth factor receptor α
PEK-1	Poly nucleotide kinase 1
PLK1i	Polo-like kinase 1 inhibitor
PNK	Poly nucleotide kinase
polyB	DNA polymerase
PPM1D	Protein phosphatase magnesium-dependent 1 delta
PRC1	Polycomb repressive complex 1
PRC2	Polycomb repressive complex 2
PTIP	Pax transactivation domain interacting protein
PTMs	Post-translational modifications
RAD50	ATP-binding cassette (ABC)-ATPase
RAD51	RAD51 recombinase
RB	Retinoblastoma
RECQ4	RecQ helicase
RFC	Replication factor C
RIF1	Replication timing regulatory factor 1
RNF8	RING finger protein 8
ROS	Reactive oxygen species
RT	Radiation therapy
RTKs	Receptor tyrosine kinases
SHH	Sonic hedgehog
SSBR	Single-strand breaks repair
ssDNA	Single strand DNA
TMZ	Temozolomide
TOPBP1	DNA topoisomerase 2-binding protein 1
WRN	Werner syndrome helicase
XRCC1	X-ray repair cross complementing protein1
XRCC4	X-ray repair cross complementing protein 4
γH2AX	Histone H2A.X
53BP1	P53 binding protein1

of $H3^{K27M}$-mutated DMGs include surgical resections and postoperative adjuvant therapies such as radiotherapy and chemotherapies.[7] However, due to the infiltrative and diffuse biological

behavior of $H3^{K27M}$-mutated DMGs,[8,9] as well as the anatomical adjacent with most important brain structures such as thalamus, brainstem and medulla oblongata, the total resection of tumors is impractical.[8-12] Thus, the adjuvant therapies are essential to prevent tumor recurrence and metastasis in $H3^{K27M}$ DMGs. Although the resistance of first-line temozolomide has commonly observed in $H3^{K27M}$ DMGs due to the universal O6-methylguanine-DNA methyltransferase (MGMT) promotor unmethylation and intact blood–brain barrier (BBB).[13,14] Radiotherapy, as a standard and efficient strategy, can significantly improve the life qualities of $H3^{K27M}$ DMGs with 70%–80% of patients obtaining temporary symptom relief and increased survival.[15-18] Ionizing radiation (IR) can inhibit tumor growth by inducing DNA damage directly or through reactive oxygen species (ROS).[19] The cell cycle checkpoints and DNA damage repair (DDR) system are essential for regulating the process.[20] In $H3^{K27M}$ DMGs, the aberrant genetic and epigenetic changes, stemness genotype, and epithelial–mesenchymal transition (EMT) disrupt the cell cycle checkpoints and DDR system by altering the associated regulatory signaling pathways,[9,21-23] which could lead to the development of radio-resistance. In clinical, a large proportion of $H3^{K27M}$ DMGs relapses within 7 months after radiotherapy.[4,24] To improve the sensitivity of tumor cells to radiation, extensive investigations have been conducted to understand the mechanisms of radio-resistance and discover the approach to enhance the radiosensitivity including modulating radiotherapy doses and fractions and a combination of immunotherapy and chemotherapy. Here, we provide crucial insights into the molecular responses of $H3^{K27M}$ DMGs to radiotherapy and current advances in radiosensitivity enhancement.

2 | ONCOGENIC MECHANISMS OF $H3^{K27M}$ MUTATION

2.1 | Histones and cancers

Histones including four core proteins H3, H4, H2A and H2B are the major structural components of chromatins which play important roles in gene expression and epigenetic modification.[25,26] The protruding N-terminal amino acid tail of a histone is subject to various post-translational modifications (PTMs) such as methylation, acetylation, phosphorylation, ubiquitylation, and SUMOylation.[27-31] Histone PTMs can active or decrease gene transcription through a local structural alteration of chromatin conducted by the interactions between histones and DNA.[32] The alterations of histones are demonstrated to be associated with various cancers such as gliomas, sarcomas, head and neck cancers and carcinosarcomas.[33,34] Of note, "oncohistones", the cancer-associated histone mutations, are highly specific in certain type of cancers. For instance, $H3^{K27M}$ and $H3^{G34R/V}$ mutations are specifically detected in brain tumors, $H3^{K36M}$ and $H3^{G34W/L}$ are mostly detected in bone cancers, while $H1$ mutations are commonly found in lymphomas.[35]

2.2 | H3 mutations in DMGs

The mutations in the histone $H3$ family including canonical $H3.1$ ($H3C1$) and variant $H3.3$ ($H3F3A$) dominantly occur in approximately 50% of DMGs and 80% of diffuse intrinsic pontine glioma (DIPG) with lysine to methionine substitution at position 27.[4,15,36] However, $H3.3$ and $H3.1$ mutations differ in their pathological and molecular features, as well as clinical prognoses. Unlike the $H3.1$-mutant tumors are usually restrictedly located in pons, the $H3.3$-mutant tumors can occur along the brain midline.[37,38] In addition, $H3.3$ alteration often co-occur with $TP53$ mutation and platelet-derived growth factor receptor α ($PDGFRα$) upregulation, while activin A receptor, type I ($ACVR1$) mutation is detected only in $H3.1$-mutant tumors.[37,39,40] Moreover, $H3.3$ mutation is associated with more aggressive tumor with early relapse and frequent metastases, and exhibit poor response to radiotherapy.[37,41,42]

2.3 | Molecular mechanisms and signaling transduction in $H3^{K27M}$ tumor

In DMGs, $H3^{K27M}$ mutation can induce a global reduction of H3K27me3, an epigenetic mark to H3 indicating the trimethylation of lysine 27 on histone H3 protein, which is associated with transcriptional silencing of the key genes regulating cell differentiation via the formation of heterochromatic regions.[43-45] H3K27me3 is catalyzed by the histone methyltransferase enhancer of zeste homolog 2 (EZH2), a subunit of polycomb repressive complex 2 (PRC2), which is the central modulator of H3K27me3-decorated facultative heterochromatin.[46,47] The crystal structure analysis suggests that K27M-mutant H3 can bind directly to the active SET domain of EZH2 with 16-fold higher affinity than wildtype H3 leading to the sequestration of EZH2 and further impairment of PRC2 methyltransferase activity and the reduction of H3K27me3.[4,15,48,49] In addition, $H3^{K27M}$ mutation can decrease the auto-methylation of EZH2/PRC2 which is required for histone methyltransferase activity, resulting the detention of H3K27me2 to H3K27me3.[50,51] Another epigenetic mark to H3 in $H3^{K27M}$ mutation cells is that the increased acetylation of H3K27 (H3K27ac), which is considered to be a super-enhancer to promote gene transcription involved in stem cell differentiation and tumorigenesis[45,52] (Figure 1).

Beyond epigenetic signatures, the $H3^{K27M}$ mutation has been demonstrated to be associated with aberrant amplifications and overexpression of receptor tyrosine kinases (RTKs), a group of membrane proteins such as epidermal growth factor receptor (EGFR) and platelet-derived growth factor receptor α (PDGFRα) regulating a set of biological functions including cell growth, survival, differentiation and migration.[53-57] Overexpression of PDGFRα is commonly found in aggressive glioma tumors exhibiting upregulated proliferation and migration of cancer cells, and significantly associated to poor survival in $H3^{K27M}$ DMGs.[38,54,57-59] In addition, $ACVR1$ mutation, for example, is prevalent in $H3.1^{K27M}$ tumor which could upregulate
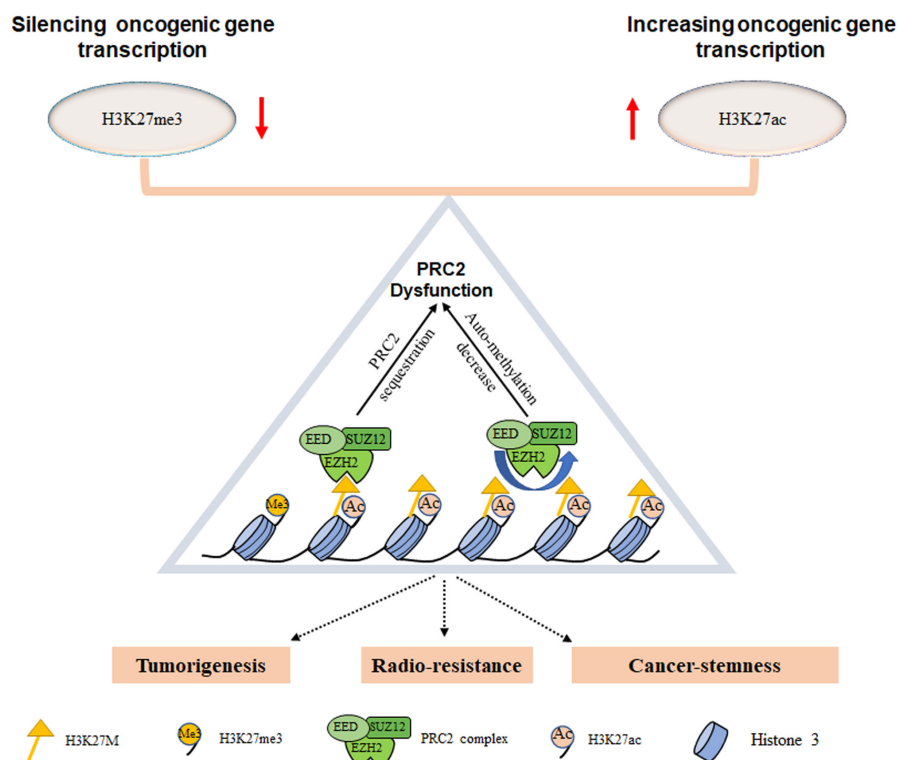
Silencing oncogenic gene transcription

Increasing oncogenic gene transcription

FIGURE 1 Diagram illustrating the epigenetic changes induced by $H3^{K27M}$ in DMGs.

SMAD1/5/8 signaling leading to cancer cell proliferation.[23,40,60–62] PI3K/AKT/mTOR and MEK/ERK pathway, as downstream signaling of RTKs, are commonly activated in $H3^{K27M}$ mutation DMGs22,[9,22,63] which contribute to tumorigenesis, metastasis, angiogenesis and therapy resistance resulting in poor clinical prognosis.[64–69]

3 | RADIOTHERAPIES AND MECHANISMS

Radiotherapy serves as cornerstone in the adjuvant treatment of $H3^{K27M}$ DMGs, which significantly improve the clinical outcomes in 70%–80% of patients.[16–18] Currently, the recommend total dose setting of radiotherapy is 54–60 Gy with a daily fraction of 1.8–2.0 Gy over 6 weeks.[70] Mechanically, radiation mainly inhibits tumor growth by inducing DNA damage.[19] The damaged DNA would trigger tumor cell death once failure in the repairment. However, DNA damage detection and repair are complicated regulated by intricate intracellular and extracellular networks. Under certain circumstances, tumor cells can exhibit radio-resistance due to cell-intrinsic mechanisms and microenvironment resulting treatment failure and recurrence.[71,72] Thus, understanding the mechanisms underlying radio-resistance and developing mechanism-based radio sensitization to enhance radiotherapy responses would improve the outcomes of glioma patients. A set of sophisticated and highly regulated cellular processes can be triggered by the exposure of radiation. Among these, the cell cycle checkpoints and DDR system are the main components coordinated by complex signal transduction.[73,74] Mechanically, the sensors recognizing the radiation induced DNA damage can initiate the cascade activation of a series of kinases involved in the cell cycle checkpoints and DDR networks to complete DNA repairs.

3.1 | Cell cycle checkpoints

Cell cycle checkpoints consisting of G1, intra-S, G2 and spindle phase act as surveillance mechanisms and regulators to monitor the major events among the process of cell cycle (such as DNA damage). Once aberrant events are detected by the checkpoints during segregation at mitosis, a pulse would be placed to arrest the cell cycle which allows to provide a time for DNA repair in order to accurately passage bioinformation into daughter cells and maintain the genomic stability. For example, G1 phase checkpoint assure the damaged DNA cannot enter S phase, and G2 checkpoint could prevent the passing of abnormal DNA to future generations.[75] The dysfunction of cell cycle checkpoints or failure in the repairment would trigger cell death eventually.

Cyclins and cyclin dependent kinases (CDKs) are the core members regulating cell cycle through the modulation of cell cycle checkpoints.[76] In the condition of DNA damage, recruited MRE11/RAD50/NBS1 (MRN) complex and ATR-interacting protein (ATRIP) activate ataxia telangiectasia mutated (ATM) kinase and ataxia telangiectasia- and Rad3-related protein (ATR), respectively.[74,77] Activated ATM and ATR then phosphorylate checkpoint kinases (CHKs), which can further phosphorylate the Cdc25 leading to the inactivation of CDKs, as a result, causing G1 and G2 phase arrest.[75,78–80] The function of CDKs and Cdc25 could be regulated by the modulator CDK activated kinase (CAK), Wee1, PLK1, 14–3-3 σ, and cyclin-dependent kinase inhibitor (CKI).[81–84] In addition, p53 could execute G1 phase arrest via p21 generation and accumulation[85–88] (Figure 2). Radiotherapy as one of the most common treatments for gliomas can damage cancer cells DNA by IR leading to mitotic catastrophe. However, glioma cells could get the radiation-induced DNA damages repaired via G1 and G2 arrest and continue the cell
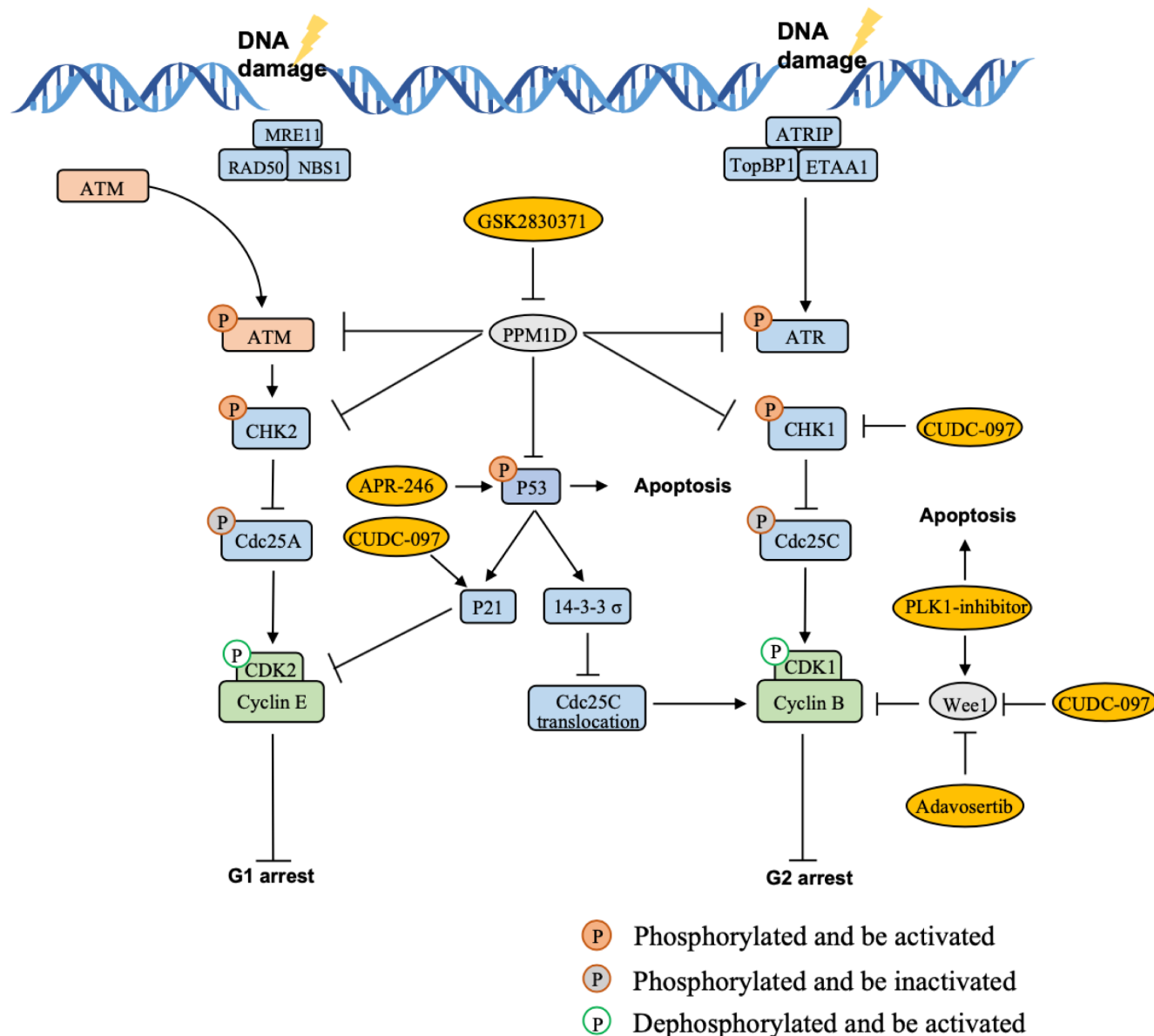
FIGURE 2 The regulation of cell cycle checkpoints in response to the irradiation-induced DNA damage. Molecules in yellow ovals are suggested to restore the radiosensitivity in $H3^{K27M}$-mutated DMGs.

cycle and proliferation to avoid cells death or senescence presenting radiotherapy resistance. Thus, targeting the checkpoints could be a promising strategy of enhancing radio-sensitivity.[89,90]

3.2 | DNA damage and repair system

3.2.1 | DNA double-strand breaks (DSBs) repair

Homologous recombination (HR) and nonhomologous end-joining (NHEJ) are two main pathways involved in DSBs damage repairing. HR occurs in the S and G2 phases requiring a sister chromatid as template. NHEJ could participate in all cell cycle phases, predominantly in G1 phase.[19] Different from HR, NHEJ can directly ligate the broken DNA ends without resection.[20]

3.2.2 | Homologous recombination (HR)

HR repair can restore damaged DNA using an undisturbed sister chromatid as template during S and G2 phases. During DSBs repair process, MRN complex, as the sensor of the DNA breaks, can firstly recruit and active ATM to initiate a phosphorylation cascade of mediators, such as mediator of DNA damage checkpoint 1 (MDC1) and breast cancer type 1 susceptibility protein (BRCA1).[19,91] MDC1 is essential for signal transduction and
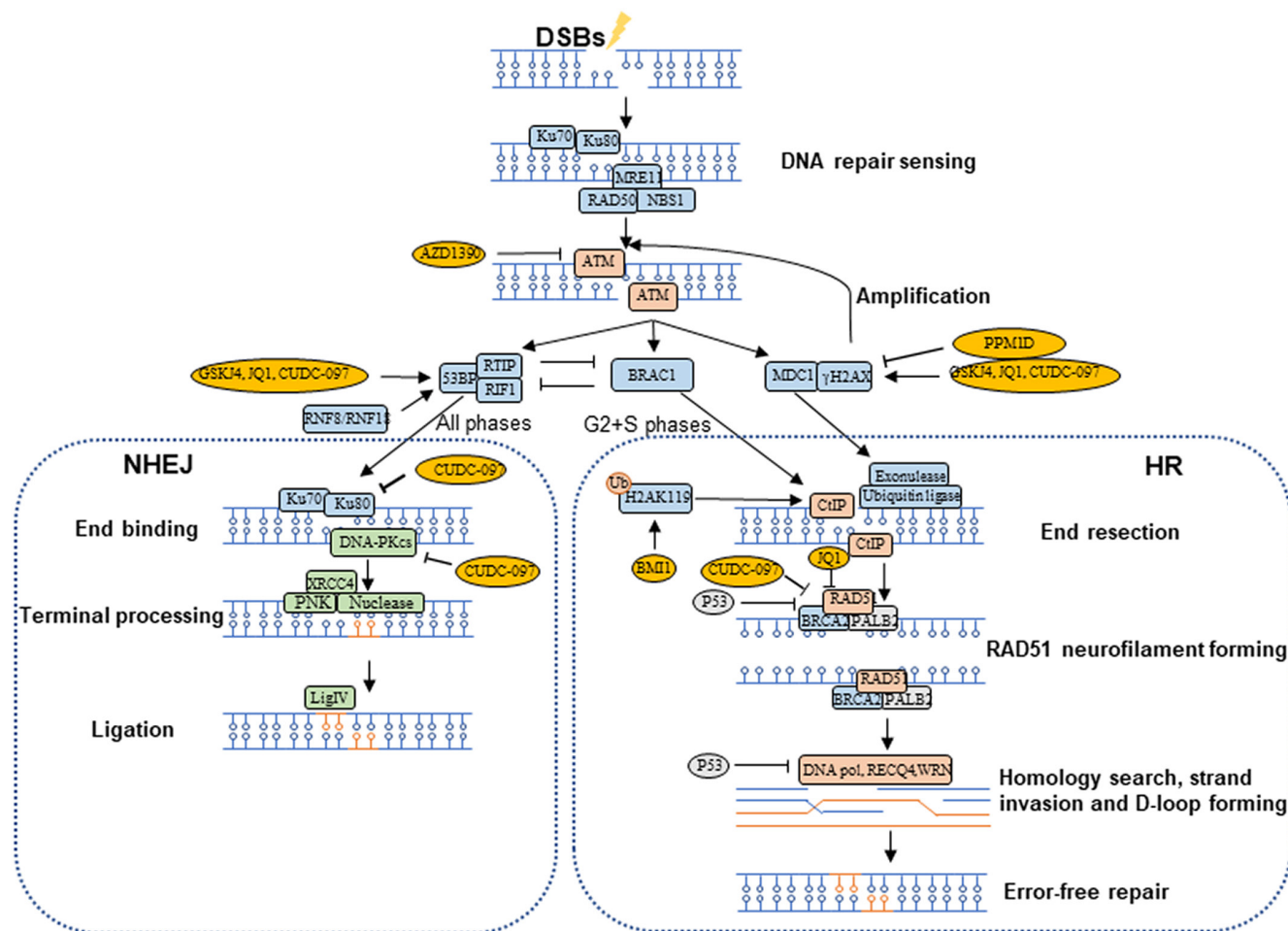
FIGURE 3 The illustration of DNA double-strand breaks (DSBs) repair. Yellow ovals indicate the molecules potentially increasing the radiosensitivity in *H3*[K27M]-mutated DMGs.

amplification in DNA damage repair networks. For example, MDC1 can recruit a series of enzymes participating in damaged DNA ends resection including helicase, exonuclease, ubiquitin ligase, recombinase, and DNA end decorating factors.[20,92–95] Meanwhile, MDC1 combined with γH2AX, a substrate of ATM,[96] can further recruit additional MRN complex and ATM at the DSBs locus, resulting in the amplification of ATM signaling. BRCA1 as a scaffold protein can recruit multiple repair proteins such as CtBP-interacting protein (CtIP) to the DNA break sites promoting the single-strand excision of DNA ends.[73,97] CtIP then promotes an accumulation of RAD51 which can bind single-strand DNA (ssDNA) after end resection to recognize and invade the sister chromatid forming D-loop for the assurance of the DNA synthesis fidility.[73,77] In addition, recent studies have suggested that B lymphoma Moloney murine leukemia virus insertion region 1 (BMI1), a core member of polycomb repressive complex 1 (PRC1), could regulate the progression of HR by depositing H2A ubiquitylation at K119N (H2AK119ub) which is able to recruit CtIP and promote CtIP-BRCA1 complex formation and consequential signaling transduction[98] (Figure 3).

3.2.3 | Nonhomologous end-joining (NHEJ)

NHEJ reaction can detect and ligate the damaged double-strand DNA ends to restore the strand continuity without template. The Ku70/80 heterodimer firstly recognize and bind to the broken DNA ends with the recruitment and activation of DNA-dependent protein kinase catalytic subunit (DNA-PKcs). DNA-PKcs then interacts with multiple enzymes including the ARTEMIS and APLF nucleases and the poly nucleotide kinase (PNK) to initiate the terminal processing by removing non-ligatable end group at DNA damage sites.[99] Meanwhile, DNA-PKcs can activate x-ray repair cross complementing protein4 (XRCC4) to keep the two broken DNA ends closely aligned.[58] Finally, XRCC4 binds with ligase IV (LigIV) to form the XRCC4-LigIV complex leading to the DNA ends ligation.[73]

P53 binding protein1 (53BP1) is essential to determine the initiation of either HR or NHEJ pathways. γH2AX, as a trigger of DNA damage repair, binds directly with MDC1 to activate RING finger protein 8 (RNF8)-RNF168-mediated ubiquitination of histone, which can induce the 53BP1 recruitment to the DNA damage site. 53BP1 further interacts with replication timing regulatory factor 1 (RIF1)
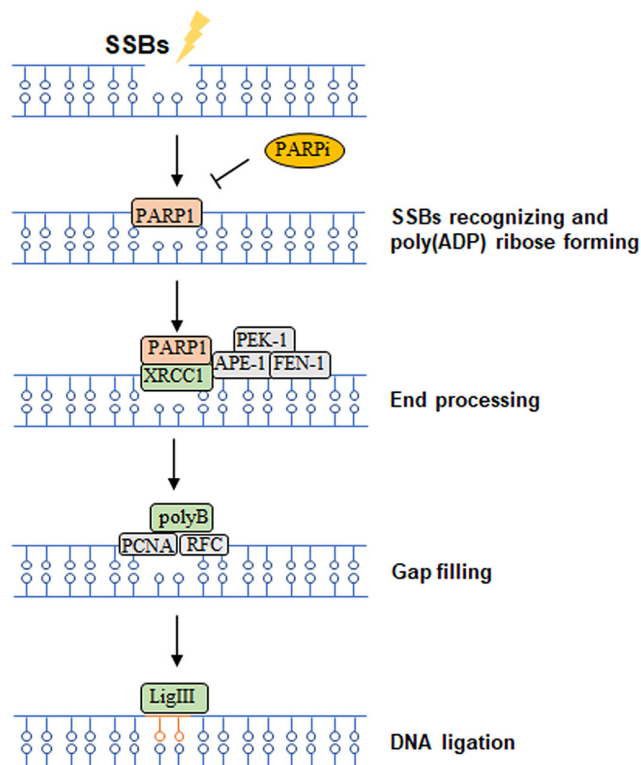
FIGURE 4 The diagram of DNA single-strand breaks repair (SSBR) and potential targets to increase radiosensitivity in $H3^{K27M}$-mutated DMGs (yellow oval).

and pax transactivation domain interacting protein (PTIP) to inhibit HR-associated DNA end resection. Importantly, the function of 53BP1-RIF1 complex and 53BP1-PTIP complex could be negatively regulated by BRCA1[100–102] (Figure 3).

3.3 | DNA single-strand break repair (SSBR)

SSBR responding to single-strand breaks (SSBs) consist of SSBs recognition, end processing, gap filling and ligation. Poly (ADP-ribose) polymerase 1 (PARP1) is the key enzyme to detect the DNA damage and induce poly-ADP-ribosylation (PARylation) to initiate SSBR.[103] PARP1 then interacts with X-ray repair cross complementing protein1 (XRCC1) resulting in the recruitment of multiple DNA repair factors such as proliferation cell nuclear antigen (PCNA), replication factor C (RFC) and DNA polymerase (DNA pol) to facilitate the end processing and gap filling. Finally, XRCC1 can bind DNA ligase III (LigIII) to execute ligation to repair the broken DNAs[104] (Figure 4).

3.4 | Signaling pathways regulate DNA damage repair

DDR process receives regulations from multiple intra- and extra-cellular signalings.[105] RTKs and their signaling pathways including PI3K/AKT/mTOR and MEK/ERK can regulate DDR by interacting

with the components in both HR and NHEJ, such as PCNA, ATM, and DNA-PKcs.[106,107] For example, in DSBs, AKT can activate Rad51 and DNA-PKcs to facilitate DNA damage repair.[108] mTOR is able to mediate DDR by regulating RNF168 ubiquitination and P53.[109,110] In addition, mTOR can increase FA complementation group D2 (FANCD2) to promote ATM-CHKs checkpoints by activating NF-κB pathway.[111,112] MEK/ERK pathway has been shown to interfere with cell cycle checking points and DDR by enhancing ATM and ATR activation[113–115] (Figure 5).

4 | RADIO-RESISTANCE AND CURRENT RADIO-SENSITIZATION STRATEGIES

In $H3^{K27M}$ DMGs, the genetic and epigenetic abnormalities could disrupt the cell cycle checkpoints and DDR system by directly or indirectly altering the associated regulatory signaling pathways.[9,21–23,72,116]

Which results in the common development of radio-resistance. Thus, current strategies of enhancing radiosensitivity of $H3^{K27M}$ DMGs mainly focus on the amendment of the $H3^{K27M}$ epigenic and genetic alterations and the aberrant cell cycle checkpoints and DDR system (Table 2), as well as the combination therapies with immunotherapy and chemotherapy.

4.1 | Targeting epigenetic alterations

A global reduction of a H3K27me3 and increased H3K27ac are two hallmarks in $H3^{K27M}$ DMGs.[9,21] Recent studies suggest that targeting the epigenetic abnormalities could increase the responses of K27M tumors to radiotherapy. The inhibitor of H3K27 demethylase JMJD3, BET bromodomain protein (BRD) and a dual inhibition of histone deacetylases (HDAC) and PI3K have been demonstrated to sensitize the radiotherapy via enhancing radiation damage and inhibiting DNA damage repair.[117–119] A recent study showed targeting EZH2 had a tumor suppressor function in DMG tumor bearing mice, which could potentially enhance the radiotherapy.[120]

4.2 | Targeting cell cycle checkpoints and DDR system

Cell cycle checkpoints and DDR intertwined signaling networks can arrest cell cycles, identify, and repair DNA damages induced by radiation. Targeting the components of cell cycle checkpoints and DDR is one of the main strategies to rescue radio-resistance. In $H3^{K27M}$ DMGs, the abnormalities of G1/S checkpoint regulators such as CDK4/6 and cyclinD and G2/S regulators such as Wee1, PLK1 were commonly detected.[121–124] Although the block of CDK4/6 could result in G1 arrest which potentially decrease the sensitivity of radiotherapy, CDK4/6 inhibition alone or following radiotherapy have been suggested to significantly reduce tumor
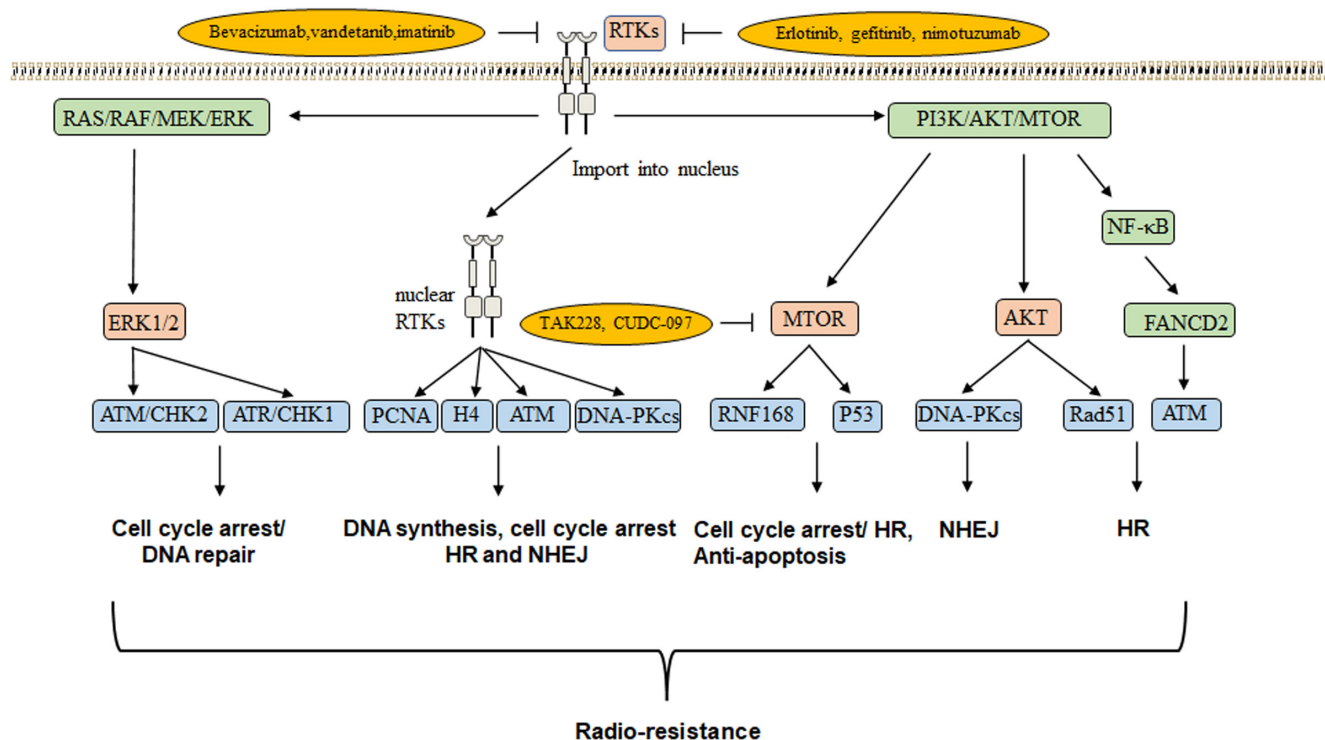
FIGURE 5 RTKs and downstream signaling associated with radio-resistance in $H3^{K27M}$-mutated DMGs. Yellow ovals indicate the molecules enhancing radiosensitivity in clinical and investigation.

growth and improve survival in DIPGs,[125–127] which is possibly due to the increased sensitivity to radiation of G1 arrested tumor cells, and failing in the requirement of sustained DNA damage during cell cycle stocked.[89,128] Wee1 acting as a regulator of G2 cycle could repair irradiation-induced DNA damage by suppressing Cdc25 and CDK1 activation.[129] The Wee1 inhibitor adavosertib has been demonstrated to promote the radiation responses in DIPGs though the suppression of G2 cycle arrest.[130] Although PLK1 negatively regulates Wee1 activation, its inhibition induces accelerated apoptosis and enhanced DNA damage of DIPG cells during radiotherapy.[131] Besides, the inhibition of Aurora kinase A (AURKA), as a key cell cycle kinase in M phase, could block cell cycle progression synergistically combined with *PLK1* inhibitor in DMG.[132] ATM as a key modulator in the networks of cell cycle checkpoints and DDR initiates a series phosphorylation cascades including CHK1/2, BRAC1/2, MDC1, γ-H2AX and 53BP1[74,133] ATM deletion is proved to enhance tumor radiosensitivity in p53-deficient DIPG,[134] and its inhibitor AZD1390 is currently involved in a clinical trial of the brain cancer patients underwent radiation therapy (NCT03423628). In addition, the enzymes such as BMI1 and PARP1 are hyperactive in DIPG.[135,136] The inhibition of BMI1 could sensitize the DIPG cells response to radiotherapy via inhibiting damaged DNA ends resection in HR.[135] PARP1 is important for the recognition of SSBs and recruitment of XRCC1.[105] The inactivation of PARP1 exhibits enhanced radiosensitivity in DIPGs.[137,138] However, a phase I/II study of veliparib combined with chemoradiotherapy in younger patients with newly diagnosed DIPG could not improve the the survival.[139]

4.3 | Targeting P53 and PPM1D

P53 participates in the G1/S phase arrest via Retinoblastoma (RB) pathway and contributes to the G2 arrest via 14-3-3 σ induced sequestration of Cdc25/CDK2 complexes in the cytoplasm.[140] TP53 mutation frequently occurs in $H3^{K27M}$ DMGs.[141,142] The lost-of-function of P53 in $H3^{K27M}$ DMGs can decrease Bax-induced apoptosis and promote HR process in DDR resulting in a general poor response to radiotherapy.[134,143–151] Thus, restoring P53 could be a potential treatment of mutant TP53-mutated $H3^{K27M}$ DMGs. A recent study suggests that reactivation of P53 combined with *H3K27M* demethylase inhibitor augments the therapeutic efficacy of irradiation in $H3^{K27M}$ DIPGs.[152]

PPM1D amplification has been identified in DMGs,[153–156] which present resistance to radiotherapy. PPM1D can dephosphorylate and inactivate P53 and a series of key enzymes in cell cycle checkpoints and DDR, including ATM, ATR, Chk1/2 and γH2AX, leading to cell cycle escape and failing in DDR.[155,157–161] Recent studies show that inhibition of gain-of-function of mutated PPM1D can increase radiosensitivity of DIPG tumors through restoring the activation of p53,[156,162] and targeting PPM1D would sensitize DIPG cells to the *PARP* inhibitor due to dual suppression of HR and SSBR.[163]

4.4 | Targeting RTKs and downstream signaling pathways

RTKs and their downstream pathways regulate the cell cycle checkpoints and DDR process involving in the response of tumor cells to

TABLE 2 Potential targets to increase radiosensitivity in *H3*K27M DMGs.

Targets	Molecules	Molecular mechanisms	References
H3K27M	GSKJ4	Sustain high level of 53BP1 and γH2AX	117
BET	JQ1	Sustain high level of 53BP1 and γH2AX; decrease RAD51	118
H3K27ac	CUDC-097	Sustain high level of 53BP1 and γH2AX; decrease RAD51, DNA PKcs, KU70/80, Wee1 and CHK1	119
TP53	APR-246	Inhibit antioxidants; increase apoptosis; inhibit HR	5,74,89,134,246–248
ATM	AZD1390	Inhibit phosphorylation cascades including CHK1/2, BRAC1/2, γH2AX, MDC1 and 53BP1	74,133,134
PPM1D	GSK2830371	Restore the activation of P53	153–156,159–161
PARP1	Niraparib	Inhibit SSBR	103,105
Wee1	Adavosertib	Promote G2 to M transition	123,249,250
PLK1	BI6727	Enhance DNA damage; increase apoptosis	131,250
CDK4/6	Ribociclib	Sustain high level of γH2AX; increase apoptosis; decrease ATM and RAD51	85,124,126,128
BMI1	PTC-209	Inhibit DNA end resection in HR	98,135
RTKs	Nimotuzumab, Erlotinib, Gefitinib, Bevacizumab, Vandetanib, Imatinib	Inhibit mTOR and MAPK pathways	9,164,165,251,252
PI3K/AKT/MTOR	TAK228, CUDC-097	Induce sustained DNA damage and apoptosis; reduce DDR	119,176

radiotherapy and radio-resistance in DMGs.[22,23,72] Although the clinical benefit of targeting RTKs such as EGFR and vascular endothelial growth factor receptor (VEGFR) in combination with radiotherapy are still not conclusive in *H3*K27M DMGs or DIPGs.[9,56,164–170] The inhibition of their downstream PI3K/AKT/mTOR and MEK/ERK pathways can significantly sensitize glioma cells to radiotherapy exhibiting increased cell apoptosis and reduced cancer stemness.[171–177] The mTOR inhibitor TAK228 has been suggested to induce cell apoptosis and enhance radiosensitivity in DIPG.[176] In addition, combined targeting strategy is under estimation. A study of dual inhibition of PI3K and HDACs in DIPGs shows an increased radiosensitivity though abrogating NFκB and Forkhead box M1 (FOXM1) mediated in DNA damage responses.[119,178,179] FOXM1 is a transcription factor regulating the expression of cell cycle genes essential for DNA replication and mitosis, and DNA damage repair.[178]

4.5 | Targeting cancer-stemness and EMT

Accumulating evidence suggests that cancer stem cells (CSCs) in DMGs impact the sensitivity of radiotherapies.[177,180,181] CSCs are a small subpopulation of cells within tumors with capabilities of self-renewal, differentiation, and tumorigenicity. The stemness of CSCs regulated by intrinsic factors such as cell survival and self-renewal pathways[182–189] and extrinsic causes such as hypoxia microenvironment[190–194] allow to activate cell cycle check points and DDR to decrease the DNA damage from radiation and develop radio-resistance.[182] Notch pathway is considered to be an essential self-renewal signaling to maintain CSCs stemness in *H3*K27M gliomas, while its inhibitor, MRK003, presents an enhanced irradiation-induced apoptosis of tumor cells.[195] Recently, targeting mitochondrial metabolism and tumor hypoxia microenvironment are suggested to effectively ameliorate radio-resistance in CSCs in high-grade gliomas.[196]

EMT is commonly involved in tumor initiation, invasion and metastasis in a broad set of cancers, which presents a transition of epithelial cells to mesenchymal cells with the lost function of cell–cell junctions and cell polarity.[197–203] EMT requires a robust reprogramming of gene expression which is associated with maintenance of cancer stemness leading chemo- and radio-resistance.[204–207] EMT programs are regulated by multiple pathways including JAK/STAT pathway, TGF-β/SMAD, NF-κB, PI3K/AKT signaling, and Notch

signaling,[208–210] which offer the potential targets to enhance the sensitivity of radiotherapy. Recently, several preclinical studies suggested that the inhibition of STAT3 and Notch pathways significantly increase the therapeutic response to radiotherapy in DIPGs.[211,212] In addition, sonic hedgehog (SHH) pathway is considered as an EMT-promoting signaling promoting tumor cell differentiation and invasion in gliomas.[213] Its inhibition is shown to effectively arrest tumor growth in various types of cancers.[214–217] A clinical trial about SHH pathway inhibitor, Vismodegib, in the refractory pontine glioma patients was on going.[218] Pursuing further studies in DMGs would be appreciated.

4.6 | Other potential targets

Recent clinical trials suggested that ONC201, a dopamine receptor D2 (DRD2) inhibitor, combined with radiotherapy can significantly improve the prognosis of $H3^{K27M}$ DMGs.[219,220] Although ONC201 was reported to activate ATF4/CHOP-mediated integrated stress response and inhibit Akt/ERK signaling leading to the apoptosis in cancer cells, the mechanism of radio-sensitization is still unknown. Further studies are be appreciated. BCL2 inhibitor venetoclax could reduce BCL2-BIM association and increase ROS resulting in tumor apoptosis. Radiotherapy combined with venetoclax was reported to suppress tumor growth in DMGs.[221] Future investigation in radio-sensitization in $H3^{K27M}$ DMGs treatment are encouraging.

4.7 | Combination with immunotherapy and chemotherapy

$H3^{K27M}$ DMGs are considered as "immune cold" tumors with reduced residency of immune cells and lack of factors or chemokines recruiting immune cells.[222–224] Radiation can stimulate the release of tumor antigens and pro-inflammatory factors and immune cells infiltration to enhance immune responses in the tumor microenvironment.[225–227] Conversely, radiosensitivity can be affected by the activation of immune cells such as macrophages and fibroblasts in tumor and tumor microenvironment.[228] Chimeric antigen receptor (CAR)-T cell therapy targets tumor via endowing T cells with the specific tumoral antigens,[229] which have been used in the treatment of $H3^{K27M}$ DMGs. The newest clinical trials showed that GD2 and B7-H3 CAR-T cells therapies following standard radiotherapy are beneficial and safe for $H3^{K27M}$ DMGs or DIPGs.[230,231] Additionally, a latest randomized clinical trial demonstrated the patients undergoing concurrent radio-chemotherapy with interferon α had a longer survival time than those without interferon α in high-grade gliomas.[232] The oncolytic virus Delta-24-RGD is recently reported to elicit an elevated sensitivity of $H3^{K27M}$ DMGs to radiotherapy by increasing the trafficking of CD4$^+$ and CD8$^+$ cells to the tumor niche, and the downregulation of kinase activities in DDR system.[233,234] Another oncolytic virus DNX-2401

followed by radiotherapy can reduce and stabilize tumor size in pediatric DIPGs clinical trial.[235] Some other immunotherapeutic options also include vaccines and checkpoint blockade; however, it was still in clinal trial phase.[218] To date, the interacted mechanisms between radiotherapy and immunotherapy have not completely unknown in $H3^{K27M}$ DMGs. Further studies would be warranted to explore the synergetic mechanism on radiation combined with immunotherapy.

As for chemotherapy, the combination of temozolomide (TMZ) and radiotherapy is recommended in $H3^{K27M}$ DMGs. However, the clinical of outcomes are not satisfied due to the unmethylation of the O^6-methylguanine-DNA methyltransferase (MGMT) associated facilitation of DNA damage repair, and relatively intact blood–brain barrier (BBB).[13,14,236–242] Recently, the development of nanotechnologies allows to reformulate TMZ to enhance therapeutic efficiency with the evasion of MGMT enzyme.[243] In addition, pulsed ultrasound and convection-enhanced delivery (CED) have been applied to temporally disrupt the BBB and increase the concentration of drug delivery in tumor area, as a result, to enhance the sensitivity of chemotherapies and radiotherapy.[244,245]

5 | CONCLUSIONS

The clinical treatment of $H3^{K27M}$ DMGs remains challenging. Beyond maximal safe tumor resection, adjuvant radiotherapy is reliable treatment to improve patients' outcomes. However, the genetic and epigenetic alterations and signaling dysregulation participating in cell cycle checkpoints and DDR in $H3^{K27M}$ DMGs frequently associates with radio-resistance resulting in unsatisfied prognoses. Recent advances in these mechanisms have tremendously improved our understandings of radio-resistance in $H3^{K27M}$ DMGs and allowed to develop the potential targets to enhance the sensitivity to radiotherapy. Further clinical trials would be appreciated to precede successful radiotherapy-enhancing interventions and eventually improve the survival of $H3^{K27M}$ DMGs.

AUTHOR CONTRIBUTIONS
Chao Liu and Longbo Zhang searched the literature and wrote the manuscript; all authors contribute to reviewing and editing.

CONFLICT OF INTEREST STATEMENT
The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT
No new data were created.

INSTITUTIONAL REVIEW BOARD STATEMENT

Not applicable.

INFORMED CONSENT STATEMENT

Not applicable.

ORCID

Chao Liu https://orcid.org/0000-0002-3277-0750
Quan Cheng https://orcid.org/0000-0003-2401-5349

REFERENCES

1. Ostrom QT, Gittleman H, Truitt G, Boscia A, Kruchko C, Barnholtz-Sloan JS. CBTRUS statistical report: primary brain and other central nervous system tumors diagnosed in the United States in 2011–2015. *Neuro Oncol*. 2018;20(suppl_4):iv1-iv86.
2. Zhao Z, Zhang KN, Wang Q, et al. Chinese Glioma Genome Atlas (CGGA): a comprehensive resource with functional genomic data from Chinese glioma patients. *Genomics Proteomics Bioinformatics*. 2021;19(1):1-12.
3. Louis DN, Perry A, Reifenberger G, et al. The 2016 World Health Organization classification of tumors of the central nervous system: a summary. *Acta Neuropathol*. 2016;131(6):803-820.
4. Wu G, Broniscer A, McEachron TA, et al. Somatic histone H3 alterations in pediatric diffuse intrinsic pontine gliomas and non-brainstem glioblastomas. *Nat Genet*. 2012;44(3):251-253.
5. Khuong-Quang DA, Buczkowicz P, Rakopoulos P, et al. K27M mutation in histone H3.3 defines clinically and biologically distinct subgroups of pediatric diffuse intrinsic pontine gliomas. *Acta Neuropathol*. 2012;124(3):439-447.
6. Hoffman LM, Veldhuijzen van Zanten SEM, Colditz N, et al. Clinical, radiologic, pathologic, and molecular characteristics of long-term survivors of Diffuse Intrinsic Pontine Glioma (DIPG): a collaborative report from the international and European Society for Pediatric Oncology DIPG registries. *J Clin Oncol*. 2018;36(19):1963-1972.
7. Expert consensus of China on radiation therapy for gliomas in 2017. *Chin J Radiat Oncol*. 2018;27(2):123-131.
8. El-Khouly FE, Veldhuijzen van Zanten SEM, Santa-Maria Lopez V, et al. Diagnostics and treatment of diffuse intrinsic pontine glioma: where do we stand? *J Neurooncol*. 2019;145(1):177-184.
9. Mathew RK, Rutka JT. Diffuse intrinsic pontine glioma: clinical features, molecular genetics, and novel targeted therapeutics. *J Korean Neurosurg Soc*. 2018;61(3):343-351.
10. Khan DA, Laghari AA, Bari ME, Shamim MS. Treatment options for paediatric brainstem gliomas. *J Pak Med Assoc*. 2019;69(9):1400-1402.
11. Campello C, Tabouret E, Chinot O. Challenges in diagnosis and management of adult spinal cord gliomas. *Rev Neurol*. 2021;177(5):515-523.
12. Ozek MM, Ture U. Surgical approach to thalamic tumors. *Childs Nerv Syst*. 2002;18(8):450-456.
13. Abe H, Natsumeda M, Kanemaru Y, et al. MGMT expression contributes to temozolomide resistance in H3K27M-mutant diffuse midline gliomas and MGMT silencing to temozolomide sensitivity in IDH-mutant gliomas. *Neurol Med Chir*. 2018;58(7):290-295.
14. Abe H, Natsumeda M, Okada M, et al. MGMT expression contributes to temozolomide resistance in H3K27M-mutant diffuse midline gliomas. *Front Oncol*. 2019;9:1568.
15. Schwartzentruber J, Korshunov A, Liu XY, et al. Driver mutations in histone H3.3 and chromatin remodelling genes in paediatric glioblastoma. *Nature*. 2012;482(7384):226-231.
16. Long W, Yi Y, Chen S, Cao Q, Zhao W, Liu Q. Potential new therapies for pediatric diffuse intrinsic pontine glioma. *Front Pharmacol*. 2017;8:495.
17. Frappaz D, Schell M, Thiesse P, et al. Preradiation chemotherapy may improve survival in pediatric diffuse intrinsic brainstem gliomas: final results of BSG 98 prospective trial. *Neuro Oncol*. 2008;10(4):599-607.
18. Frazier JL, Lee J, Thomale UW, Noggle JC, Cohen KJ, Jallo GI. Treatment of diffuse intrinsic brainstem gliomas: failed approaches and future strategies. *J Neurosurg Pediatr*. 2009;3(4):259-269.
19. Santivasi WL, Xia F. Ionizing radiation-induced DNA damage, response, and repair. *Antioxid Redox Signal*. 2014;21(2):251-259.
20. Thompson LH. Recognition, signaling, and repair of DNA double-strand breaks produced by ionizing radiation in mammalian cells: the molecular choreography. *Mutat Res*. 2012;751(2):158-246.
21. Panditharatna E, Yaeger K, Kilburn LB, Packer RJ, Nazarian J. Clinicopathology of diffuse intrinsic pontine glioma and its redefined genomic and epigenomic landscape. *Cancer Genet*. 2015;208(7–8):367-373.
22. Duchatel RJ, Jackson ER, Alvaro F, Nixon B, Hondermarck H, Dun MD. Signal transduction in diffuse intrinsic pontine glioma. *Proteomics*. 2019;19(21–22):e1800479.
23. Zhang L, Nesvick CL, Day CA, et al. STAT3 is a biologically relevant therapeutic target in H3K27M-mutant diffuse midline glioma. *Neuro Oncol*. 2022;24(10):1700-1711.
24. Jansen MH, Veldhuijzen van Zanten SE, Sanchez Aliaga E, et al. Survival prediction model of children with diffuse intrinsic pontine glioma based on clinical and radiological criteria. *Neuro Oncol*. 2015;17(1):160-166.
25. Cheung P, Allis CD, Sassone-Corsi P. Signaling to chromatin through histone modifications. *Cell*. 2000;103(2):263-271.
26. Ilango S, Paital B, Jayachandran P, Padma PR, Nirmaladevi R. Epigenetic alterations in cancer. *Front Biosci*. 2020;25(6):1058-1109.
27. Black JC, Van Rechem C, Whetstine JR. Histone lysine methylation dynamics: establishment, regulation, and biological impact. *Mol Cell*. 2012;48(4):491-507.
28. Kouzarides T. Chromatin modifications and their function. *Cell*. 2007;128(4):693-705.
29. Suganuma T, Workman JL. Signals and combinatorial functions of histone modifications. *Annu Rev Biochem*. 2011;80:473-499.
30. Vaughan RM, Kupai A, Rothbart SB. Chromatin regulation through ubiquitin and ubiquitin-like histone modifications. *Trends Biochem Sci*. 2021;46(4):258-269.
31. Ryu HY, Hochstrasser M. Histone sumoylation and chromatin dynamics. *Nucleic Acids Res*. 2021;49(11):6043-6052.
32. Millan-Zambrano G, Burton A, Bannister AJ, Schneider R. Histone post-translational modifications – cause and consequence of genome function. *Nat Rev Genet*. 2022;23(9):563-580.
33. Nacev BA, Feng L, Bagert JD, et al. The expanding landscape of 'oncohistone' mutations in human cancers. *Nature*. 2019;567(7749):473-478.
34. Quenet D. Histone variants and disease. *Int Rev Cell Mol Biol*. 2018;335:1-39.
35. Qiu L, Hu X, Jing Q, Zeng X, Chan KM, Han J. Mechanism of cancer: oncohistones in action. *J Genet Genomics*. 2018;45(5):227-236.
36. Manjunath N, Jha P, Singh J, et al. Clinico-pathological and molecular characterization of diffuse midline gliomas: is there a prognostic significance? *Neurol Sci*. 2021;42(3):925-934.
37. Castel D, Philippe C, Calmon R, et al. Histone H3F3A and HIST1H3B K27M mutations define two subgroups of diffuse intrinsic pontine gliomas with different prognosis and phenotypes. *Acta Neuropathol*. 2015;130(6):815-827.
38. Mackay A, Burford A, Carvalho D, et al. Integrated molecular meta-analysis of 1,000 pediatric high-grade and diffuse intrinsic pontine glioma. *Cancer Cell*. 2017;32(4):520-537 e525.
39. Taylor KR, Mackay A, Truffaux N, et al. Recurrent activating ACVR1 mutations in diffuse intrinsic pontine glioma. *Nat Genet*. 2014;46(5):457-461.

40. Wu G, Diaz AK, Paugh BS, et al. The genomic landscape of diffuse intrinsic pontine glioma and pediatric non-brainstem high-grade glioma. *Nat Genet*. 2014;46(5):444-450.

41. Vuong HG, Ngo TNM, Le HT, Dunn IF. The prognostic significance of HIST1H3B/C and H3F3A K27M mutations in diffuse midline gliomas is influenced by patient age. *J Neurooncol*. 2022;158(3):405-412.

42. Karremann M, Gielen GH, Hoffmann M, et al. Diffuse high-grade gliomas with H3 K27M mutations carry a dismal prognosis independent of tumor location. *Neuro Oncol*. 2018;20(1):123-131.

43. Duan R, Du W, Guo W. EZH2: a novel target for cancer treatment. *J Hematol Oncol*. 2020;13(1):104.

44. Pereira JD, Sansom SN, Smith J, Dobenecker MW, Tarakhovsky A, Livesey FJ. Ezh2, the histone methyltransferase of PRC2, regulates the balance between self-renewal and differentiation in the cerebral cortex. *Proc Natl Acad Sci USA*. 2010;107(36):15957-15962.

45. Fang D, Gan H, Cheng L, et al. H3.3K27M mutant proteins reprogram epigenome by sequestering the PRC2 complex to poised enhancers. *Elife*. 2018;7:e36696.

46. Cooney TM, Lubanszky E, Prasad R, Hawkins C, Mueller S. Diffuse midline glioma: review of epigenetics. *J Neurooncol*. 2020;150(1):27-34.

47. Larson JD, Kasper LH, Paugh BS, et al. Histone H3.3 K27M accelerates spontaneous brainstem glioma and drives restricted changes in bivalent gene expression. *Cancer Cell*. 2019;35(1):140-155 e147.

48. Justin N, Zhang Y, Tarricone C, et al. Structural basis of oncogenic histone H3K27M inhibition of human polycomb repressive complex 2. *Nat Commun*. 2016;7:11316.

49. Lewis PW, Muller MM, Koletsky MS, et al. Inhibition of PRC2 activity by a gain-of-function H3 mutation found in pediatric glioblastoma. *Science*. 2013;340(6134):857-861.

50. Lee CH, Yu JR, Granat J, et al. Automethylation of PRC2 promotes H3K27 methylation and is impaired in H3K27M pediatric glioma. *Genes Dev*. 2019;33(19–20):1428-1440.

51. Wang X, Long Y, Paucek RD, et al. Regulation of histone methylation by automethylation of PRC2. *Genes Dev*. 2019;33(19–20):1416-1427.

52. Piunti A, Hashizume R, Morgan MA, et al. Therapeutic targeting of polycomb and BET bromodomain proteins in diffuse intrinsic pontine gliomas. *Nat Med*. 2017;23(4):493-500.

53. Du Z, Lovly CM. Mechanisms of receptor tyrosine kinase activation in cancer. *Mol Cancer*. 2018;17(1):58.

54. Dufour C, Perbet R, Leblond P, et al. Identification of prognostic markers in diffuse midline gliomas H3K27M-mutant. *Brain Pathol*. 2020;30(1):179-190.

55. Filbin MG, Tirosh I, Hovestadt V, et al. Developmental and oncogenic programs in H3K27M gliomas dissected by single-cell RNA-seq. *Science*. 2018;360(6386):331-335.

56. Fleischhack G, Massimino M, Warmuth-Metz M, et al. Nimotuzumab and radiotherapy for treatment of newly diagnosed diffuse intrinsic pontine glioma (DIPG): a phase III clinical study. *J Neurooncol*. 2019;143(1):107-113.

57. Grasso CS, Tang Y, Truffaux N, et al. Functionally defined therapeutic targets in diffuse intrinsic pontine glioma. *Nat Med*. 2015;21(6):555-559.

58. Puget S, Philippe C, Bax DA, et al. Mesenchymal transition and PDGFRA amplification/mutation are key distinct oncogenic events in pediatric diffuse intrinsic pontine gliomas. *PLoS One*. 2012;7(2):e30313.

59. Meel MH, Sewing ACP, Waranecki P, et al. Culture methods of diffuse intrinsic pontine glioma cells determine response to targeted therapies. *Exp Cell Res*. 2017;360(2):397-403.

60. Fontebasso AM, Papillon-Cavanagh S, Schwartzentruber J, et al. Recurrent somatic mutations in ACVR1 in pediatric midline high-grade astrocytoma. *Nat Genet*. 2014;46(5):462-466.

61. Hoeman CM, Cordero FJ, Hu G, et al. ACVR1 R206H cooperates with H3.1K27M in promoting diffuse intrinsic pontine glioma pathogenesis. *Nat Commun*. 2019;10(1):1023.

62. Buczkowicz P, Hoeman C, Rakopoulos P, et al. Genomic analysis of diffuse intrinsic pontine gliomas identifies three molecular subgroups and recurrent activating ACVR1 mutations. *Nat Genet*. 2014;46(5):451-456.

63. Georgescu MM, Islam MZ, Li Y, et al. Global activation of oncogenic pathways underlies therapy resistance in diffuse midline glioma. *Acta Neuropathol Commun*. 2020;8(1):111.

64. Porta C, Paglino C, Mosca A. Targeting PI3K/Akt/mTOR signaling in cancer. *Front Oncol*. 2014;4:64.

65. Guo YJ, Pan WW, Liu SB, Shen ZF, Xu Y, Hu LL. ERK/MAPK signalling pathway and tumorigenesis. *Exp Ther Med*. 2020;19(3):1997-2007.

66. Rodriguez EF, Scheithauer BW, Giannini C, et al. PI3K/AKT pathway alterations are associated with clinically aggressive and histologically anaplastic subsets of pilocytic astrocytoma. *Acta Neuropathol*. 2011;121(3):407-420.

67. Chakravarti A, Zhai G, Suzuki Y, et al. The prognostic significance of phosphatidylinositol 3-kinase pathway activation in human gliomas. *J Clin Oncol*. 2004;22(10):1926-1933.

68. Mizoguchi M, Betensky RA, Batchelor TT, Bernay DC, Louis DN, Nutt CL. Activation of STAT3, MAPK, and AKT in malignant astrocytic gliomas: correlation with EGFR status, tumor grade, and survival. *J Neuropathol Exp Neurol*. 2006;65(12):1181-1188.

69. Brlek P, Kafka A, Bukovac A, Pecina-Slaus N. Integrative cBioPortal analysis revealed molecular mechanisms that regulate EGFR-PI3K-AKT-mTOR pathway in diffuse gliomas of the brain. *Cancers*. 2021;13(13):3247.

70. Cohen KJ, Jabado N, Grill J. Diffuse intrinsic pontine gliomas-current management and new biologic insights. Is there a glimmer of hope? *Neuro Oncol*. 2017;19(8):1025-1034.

71. Valtorta S, Salvatore D, Rainone P, Belloli S, Bertoli G, Moresco RM. Molecular and cellular complexity of glioma. Focus on tumour microenvironment and the use of molecular and imaging biomarkers to overcome treatment resistance. *Int J Mol Sci*. 2020;21(16):5631.

72. Pedersen H, Schmiegelow K, Hamerlik P. Radio-resistance and DNA repair in pediatric diffuse midline gliomas. *Cancers*. 2020;12(10):2813.

73. Huang R, Zhou PK. DNA damage repair: historical perspectives, mechanistic pathways and clinical translation for targeted cancer therapy. *Signal Transduct Target Ther*. 2021;6(1):254.

74. Marechal A, Zou L. DNA damage sensing by the ATM and ATR kinases. *Cold Spring Harb Perspect Biol*. 2013;5(9):a012716.

75. Matthews HK, Bertoli C, de Bruin RAM. Cell cycle control in cancer. *Nat Rev Mol Cell Biol*. 2022;23(1):74-88.

76. Barnum KJ, O'Connell MJ. Cell cycle regulation by checkpoints. *Methods Mol Biol*. 2014;1170:29-40.

77. Smith HL, Southgate H, Tweddle DA, Curtin NJ. DNA damage checkpoint kinases in cancer. *Expert Rev Mol Med*. 2020;22:e2.

78. Smith J, Tho LM, Xu N, Gillespie DA. The ATM-Chk2 and ATR-Chk1 pathways in DNA damage signaling and cancer. *Adv Cancer Res*. 2010;108:73-112.

79. Shiloh Y, Ziv Y. The ATM protein kinase: regulating the cellular response to genotoxic stress, and more. *Nat Rev Mol Cell Biol*. 2013;14(4):197-210.

80. Simoneau A, Zou L. An extending ATR-CHK1 circuitry: the replication stress response and beyond. *Curr Opin Genet Dev*. 2021;71:92-98.

81. Mani S, Wang C, Wu K, Francis R, Pestell R. Cyclin-dependent kinase inhibitors: novel anticancer agents. *Expert Opin Investig Drugs*. 2000;9(8):1849-1870.

82. Aressy B, Ducommun B. Cell cycle control by the CDC25 phosphatases. *Anticancer Agents Med Chem*. 2008;8(8):818-824.

83. Elbaek CR, Petrosius V, Sorensen CS. WEE1 kinase limits CDK activities to safeguard DNA replication and mitotic entry. *Mutat Res.* 2020;819-820:111694.

84. Hashimoto Y, Tanaka H. Mre11 exonuclease activity promotes irreversible mitotic progression under replication stress. *Life Sci Alliance.* 2022;5(6):e202101249.

85. Ou YH, Chung PH, Sun TP, Shieh SY. p53 C-terminal phosphorylation by CHK1 and CHK2 participates in the regulation of DNA-damage-induced C-terminal acetylation. *Mol Biol Cell.* 2005;16(4):1684-1695.

86. Abbas T, Dutta A. p21 in cancer: intricate networks and multiple activities. *Nat Rev Cancer.* 2009;9(6):400-414.

87. Karimian A, Ahmadi Y, Yousefi B. Multiple functions of p21 in cell cycle, apoptosis and transcriptional regulation after DNA damage. *DNA Repair.* 2016;42:63-71.

88. Waldman T, Kinzler KW, Vogelstein B. p21 is necessary for the p53-mediated G1 arrest in human cancer cells. *Cancer Res.* 1995;55(22):5187-5190.

89. Pawlik TM, Keyomarsi K. Role of cell cycle in mediating sensitivity to radiotherapy. *Int J Radiat Oncol Biol Phys.* 2004;59(4):928-942.

90. Vlatkovic T, Veldwijk MR, Giordano FA, Herskind C. Targeting cell cycle checkpoint kinases to overcome intrinsic radioresistance in brain tumor cells. *Cancers.* 2022;14(3):701.

91. Kastan MB, Bartek J. Cell-cycle checkpoints and cancer. *Nature.* 2004;432(7015):316-323.

92. Bartek J, Lukas J. DNA damage checkpoints: from initiation to recovery or adaptation. *Curr Opin Cell Biol.* 2007;19(2):238-245.

93. Mailand N, Bekker-Jensen S, Faustrup H, et al. RNF8 ubiquitylates histones at DNA double-strand breaks and promotes assembly of repair proteins. *Cell.* 2007;131(5):887-900.

94. Doil C, Mailand N, Bekker-Jensen S, et al. RNF168 binds and amplifies ubiquitin conjugates on damaged chromosomes to allow accumulation of repair proteins. *Cell.* 2009;136(3):435-446.

95. Stucki M, Clapperton JA, Mohammad D, Yaffe MB, Smerdon SJ, Jackson SP. MDC1 directly binds phosphorylated histone H2AX to regulate cellular responses to DNA double-strand breaks. *Cell.* 2005;123(7):1213-1226.

96. Yuan J, Adamski R, Chen J. Focus on histone variant H2AX: to be or not to be. *FEBS Lett.* 2010;584(17):3717-3724.

97. Parameswaran B, Chiang HC, Lu Y, et al. Damage-induced BRCA1 phosphorylation by Chk2 contributes to the timing of end resection. *Cell Cycle.* 2015;14(3):437-448.

98. Fitieh A, Locke AJ, Mashayekhi F, Khaliqdina F, Sharma AK, Ismail IH. BMI-1 regulates DNA end resection and homologous recombination repair. *Cell Rep.* 2022;38(12):110536.

99. Ciccia A, Elledge SJ. The DNA damage response: making it safe to play with knives. *Mol Cell.* 2010;40(2):179-204.

100. Panier S, Boulton SJ. Double-strand break repair: 53BP1 comes into focus. *Nat Rev Mol Cell Biol.* 2014;15(1):7-18.

101. Shibata A, Jeggo PA. Roles for 53BP1 in the repair of radiation-induced DNA double strand breaks. *DNA Repair.* 2020;93:102915.

102. Zhang F, Gong Z. Regulation of DNA double-strand break repair pathway choice: a new focus on 53BP1. *J Zhejiang Univ Sci B.* 2021;22(1):38-46.

103. Oei SL, Keil C, Ziegler M. Poly(ADP-ribosylation) and genomic stability. *Biochem Cell Biol.* 2005;83(3):263-269.

104. Caldecott KW. Mammalian single-strand break repair: mechanisms and links with chromatin. *DNA Repair.* 2007;6(4):443-453.

105. Meel MH, Kaspers GJL, Hulleman E. Preclinical therapeutic targets in diffuse midline glioma. *Drug Resist Updat.* 2019;44:15-25.

106. Tomas A, Futter CE, Eden ER. EGF receptor trafficking: consequences for signaling and cancer. *Trends Cell Biol.* 2014;24(1):26-34.

107. Chabot T, Cheraud Y, Fleury F. Relationships between DNA repair and RTK-mediated signaling pathways. *Biochim Biophys Acta Rev Cancer.* 2021;1875(1):188495.

108. Toulany M, Targeting DNA. Double-Strand break repair pathways to improve radiotherapy response. *Genes.* 2019;10(1):25.

109. Xie X, Hu H, Tong X, et al. The mTOR-S6K pathway links growth signalling to DNA damage response by targeting RNF168. *Nat Cell Biol.* 2018;20(3):320-331.

110. Lai KP, Leong WF, Chau JF, et al. S6K1 is a multifaceted regulator of Mdm2 that connects nutrient status and DNA damage response. *EMBO J.* 2010;29(17):2994-3006.

111. Guo F, Li J, Du W, et al. mTOR regulates DNA damage response through NF-kappaB-mediated FANCD2 pathway in hematopoietic cells. *Leukemia.* 2013;27(10):2040-2046.

112. Shen C, Oswald D, Phelps D, et al. Regulation of FANCD2 by the mTOR pathway contributes to the resistance of cancer cells to DNA double-strand breaks. *Cancer Res.* 2013;73(11):3393-3401.

113. Wei F, Xie Y, Tao L, Tang D. Both ERK1 and ERK2 kinases promote G2/M arrest in etoposide-treated MCF7 cells by facilitating ATM activation. *Cell Signal.* 2010;22(11):1783-1789.

114. Wu D, Chen B, Parihar K, et al. ERK activity facilitates activation of the S-phase DNA damage checkpoint by modulating ATR function. *Oncogene.* 2006;25(8):1153-1164.

115. Yan Y, Black CP, Cowan KH. Irradiation-induced G2/M checkpoint response requires ERK1/2 activation. *Oncogene.* 2007;26(32):4689-4698.

116. Findlay IJ, De Iuliis GN, Duchatel RJ, et al. Pharmaco-proteogenomic profiling of pediatric diffuse midline glioma to inform future treatment strategies. *Oncogene.* 2022;41(4):461-475.

117. Katagi H, Louis N, Unruh D, et al. Radiosensitization by histone H3 demethylase inhibition in diffuse intrinsic pontine glioma. *Clin Cancer Res.* 2019;25(18):5572-5583.

118. Sasaki T, Katagi H, Unruh D, et al. PDTM-34. RADIOSENSITIZATION by BRD4 inhibition IN diffuse intrinsic pontine glioma. *Neuro Oncol.* 2019;21(Suppl 6):vi194-vi195.

119. Pal S, Kozono D, Yang X, et al. Dual HDAC and PI3K inhibition abrogates NFkappaB- and FOXM1-mediated DNA damage response to radiosensitize pediatric high-grade gliomas. *Cancer Res.* 2018;78(14):4007-4021.

120. Dhar S, Gadd S, Patel P, et al. A tumor suppressor role for EZH2 in diffuse midline glioma pathogenesis. *Acta Neuropathol Commun.* 2022;10(1):47.

121. Buczkowicz P, Zarghooni M, Bartels U, et al. Aurora kinase B is a potential therapeutic target in pediatric diffuse intrinsic pontine glioma. *Brain Pathol.* 2013;23(3):244-253.

122. Meel MH, de Gooijer MC, Guillen Navarro M, et al. MELK inhibition in diffuse intrinsic pontine glioma. *Clin Cancer Res.* 2018;24(22):5645-5657.

123. Mueller S, Hashizume R, Yang X, et al. Targeting Wee1 for the treatment of pediatric high-grade gliomas. *Neuro Oncol.* 2014;16(3):352-360.

124. Paugh BS, Broniscer A, Qu C, et al. Genome-wide analyses identify recurrent amplifications of receptor tyrosine kinases and cell-cycle regulatory genes in diffuse intrinsic pontine glioma. *J Clin Oncol.* 2011;29(30):3999-4006.

125. Sun Y, Sun Y, Yan K, et al. Potent anti-tumor efficacy of palbociclib in treatment-naive H3.3K27M-mutant diffuse intrinsic pontine glioma. *EBioMedicine.* 2019;43:171-179.

126. DeWire M, Fuller C, Hummel TR, et al. A phase I/II study of ribociclib following radiation therapy in children with newly diagnosed diffuse intrinsic pontine glioma (DIPG). *J Neurooncol.* 2020;149(3):511-522.

127. Barton KL, Misuraca K, Cordero F, et al. PD-0332991, a CDK4/6 inhibitor, significantly prolongs survival in a genetically engineered mouse model of brainstem glioma. *PLoS One.* 2013;8(10):e77639.

128. Yang Y, Luo J, Chen X, et al. CDK4/6 inhibitors: a novel strategy for tumor radiosensitization. *J Exp Clin Cancer Res.* 2020;39(1):188.

129. Esposito F, Giuffrida R, Raciti G, Puglisi C, Forte S. Wee1 kinase: a potential target to overcome tumor resistance to therapy. *Int J Mol Sci*. 2021;22(19):10689.

130. Caretti V, Hiddingh L, Lagerweij T, et al. WEE1 kinase inhibition enhances the radiation response of diffuse intrinsic pontine gliomas. *Mol Cancer Ther*. 2013;12(2):141-150.

131. Amani V, Prince EW, Alimova I, et al. Polo-like kinase 1 as a potential therapeutic target in diffuse intrinsic pontine glioma. *BMC Cancer*. 2016;16:647.

132. Metselaar DS, du Chatinier A, Meel MH, et al. AURKA and PLK1 inhibition selectively and synergistically block cell cycle progression in diffuse midline glioma. *iScience*. 2022;25(6):104398.

133. Lavin MF, Kozlov S. ATM activation and DNA damage response. *Cell Cycle*. 2007;6(8):931-942.

134. Deland K, Starr BF, Mercer JS, et al. Tumor genotype dictates radiosensitization after Atm deletion in primary brainstem glioma models. *J Clin Invest*. 2021;131(1):e142158.

135. Kumar SS, Sengupta S, Lee K, et al. BMI-1 is a potential therapeutic target in diffuse intrinsic pontine glioma. *Oncotarget*. 2017;8(38):62962-62975.

136. Zarghooni M, Bartels U, Lee E, 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*. 2010;28(8):1337-1344.

137. Chornenkyy Y, Agnihotri S, Yu M, et al. Poly-ADP-ribose polymerase as a therapeutic target in pediatric diffuse intrinsic pontine glioma and pediatric high-grade astrocytoma. *Mol Cancer Ther*. 2015;14(11):2560-2568.

138. van Vuurden DG, Hulleman E, Meijer OL, et al. PARP inhibition sensitizes childhood high grade glioma, medulloblastoma and ependymoma to radiation. *Oncotarget*. 2011;2(12):984-996.

139. Baxter PA, Su JM, Onar-Thomas A, 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*. 2020;22(6):875-885.

140. Lee MH, Lozano G. Regulation of the p53-MDM2 pathway by 14-3-3 sigma and other proteins. *Semin Cancer Biol*. 2006;16(3):225-234.

141. Park C, Kim TM, Bae JM, et al. Clinical and genomic characteristics of adult diffuse midline glioma. *Cancer Res Treat*. 2021;53(2):389-398.

142. Vuong HG, Le HT, Ngo TNM, et al. H3K27M-mutant diffuse midline gliomas should be further molecularly stratified: an integrated analysis of 669 patients. *J Neurooncol*. 2021;155(3):225-234.

143. Chen J. The cell-cycle arrest and apoptotic functions of p53 in tumor initiation and progression. *Cold Spring Harb Perspect Med*. 2016;6(3):a026104.

144. Aubrey BJ, Kelly GL, Janic A, Herold MJ, Strasser A. How does p53 induce apoptosis and how does this relate to p53-mediated tumour suppression? *Cell Death Differ*. 2018;25(1):104-113.

145. Arias-Lopez C, Lazaro-Trueba I, Kerr P, et al. p53 modulates homologous recombination by transcriptional regulation of the RAD51 gene. *EMBO Rep*. 2006;7(2):219-224.

146. Kawamoto T, Araki K, Sonoda E, et al. Dual roles for DNA polymerase eta in homologous DNA recombination and translesion DNA synthesis. *Mol Cell*. 2005;20(5):793-799.

147. Liu G, Chen X. DNA polymerase eta, the product of the xeroderma pigmentosum variant gene and a target of p53, modulates the DNA damage checkpoint and p53 activation. *Mol Cell Biol*. 2006;26(4):1398-1413.

148. McIlwraith MJ, Vaisman A, Liu Y, Fanning E, Woodgate R, West SC. Human DNA polymerase eta promotes DNA synthesis from strand invasion intermediates of homologous recombination. *Mol Cell*. 2005;20(5):783-792.

149. Sengupta S, Shimamoto A, Koshiji M, et al. Tumor suppressor p53 represses transcription of RECQ4 helicase. *Oncogene*. 2005;24(10):1738-1748.

150. Yamabe Y, Shimamoto A, Goto M, Yokota J, Sugawara M, Furuichi Y. Sp1-mediated transcription of the Werner helicase gene is modulated by Rb and p53. *Mol Cell Biol*. 1998;18(11):6191-6200.

151. Entz-Werle N, Poidevin L, Nazarov PV, et al. A DNA repair and cell cycle gene expression signature in pediatric high-grade gliomas: prognostic and therapeutic value. *Cancers*. 2021;13(9):2252.

152. Nikolaev A, Fiveash JB, Yang ES. Combined Targeting of mutant p53 and Jumonji family histone demethylase augments therapeutic efficacy of radiation in H3K27M DIPG. *Int J Mol Sci*. 2020;21(2):490.

153. Nikbakht H, Panditharatna E, Mikael LG, et al. Spatial and temporal homogeneity of driver mutations in diffuse intrinsic pontine glioma. *Nat Commun*. 2016;7:11185.

154. Werbrouck C, Evangelista CCS, Lobon-Iglesias MJ, et al. TP53 pathway alterations drive radioresistance in diffuse intrinsic pontine gliomas (DIPG). *Clin Cancer Res*. 2019;25(22):6788-6800.

155. Zhang L, Chen LH, Wan H, et al. Exome sequencing identifies somatic gain-of-function PPM1D mutations in brainstem gliomas. *Nat Genet*. 2014;46(7):726-730.

156. Khadka P, Reitman ZJ, Lu S, et al. PPM1D mutations are oncogenic drivers of de novo diffuse midline glioma formation. *Nat Commun*. 2022;13(1):604.

157. Lowe J, Cha H, Lee MO, Mazur SJ, Appella E, Fornace AJ Jr. Regulation of the Wip1 phosphatase and its effects on the stress response. *Front Biosci*. 2012;17(4):1480-1498.

158. Lu X, Nguyen TA, Moon SH, Darlington Y, Sommer M, Donehower LA. The type 2C phosphatase Wip1: an oncogenic regulator of tumor suppressor and DNA damage response pathways. *Cancer Metastasis Rev*. 2008;27(2):123-135.

159. Fujimoto H, Onishi N, Kato N, et al. Regulation of the antioncogenic Chk2 kinase by the oncogenic Wip1 phosphatase. *Cell Death Differ*. 2006;13(7):1170-1180.

160. Lu X, Nannenga B, Donehower LA. PPM1D dephosphorylates Chk1 and p53 and abrogates cell cycle checkpoints. *Genes Dev*. 2005;19(10):1162-1174.

161. Shreeram S, Demidov ON, Hee WK, et al. Wip1 phosphatase modulates ATM-dependent signaling pathways. *Mol Cell*. 2006;23(5):757-764.

162. Akamandisa MP, Nie K, Nahta R, Hambardzumyan D, Castellino RC. Inhibition of mutant PPM1D enhances DNA damage response and growth suppressive effects of ionizing radiation in diffuse intrinsic pontine glioma. *Neuro Oncol*. 2019;21(6):786-799.

163. Wang Z, Xu C, Diplas BH, et al. Targeting mutant PPM1D sensitizes diffuse intrinsic pontine glioma cells to the PARP inhibitor Olaparib. *Mol Cancer Res*. 2020;18(7):968-980.

164. Crotty EE, Leary SES, Geyer JR, et al. Children with DIPG and high-grade glioma treated with temozolomide, irinotecan, and bevacizumab: the Seattle children's hospital experience. *J Neurooncol*. 2020;148(3):607-617.

165. El-Khouly FE, Veldhuijzen van Zanten SEM, Jansen MHA, et al. A phase I/II study of bevacizumab, irinotecan and erlotinib in children with progressive diffuse intrinsic pontine glioma. *J Neurooncol*. 2021;153(2):263-271.

166. Su JM, Murray JC, McNall-Knapp RY, et al. A phase 2 study of valproic acid and radiation, followed by maintenance valproic acid and bevacizumab in children with newly diagnosed diffuse intrinsic pontine glioma or high-grade glioma. *Pediatr Blood Cancer*. 2020;67(6):e28283.

167. Hummel TR, Salloum R, Drissi R, et al. A pilot study of bevacizumab-based therapy in patients with newly diagnosed high-grade gliomas and diffuse intrinsic pontine gliomas. *J Neurooncol*. 2016;127(1):53-61.

168. Kebudi R, Cakir FB. Management of diffuse pontine gliomas in children: recent developments. *Paediatr Drugs*. 2013;15(5):351-362.

169. Geoerger B, Hargrave D, Thomas F, et al. Innovative therapies for children with cancer pediatric phase I study of erlotinib in brainstem glioma and relapsing/refractory brain tumors. *Neuro Oncol.* 2011;13(1):109-118.

170. Pollack IF, Stewart CF, Kocak M, et al. A phase II study of gefitinib and irradiation in children with newly diagnosed brainstem gliomas: a report from the pediatric brain tumor consortium. *Neuro Oncol.* 2011;13(3):290-297.

171. Wang Z, Huang Y, Zhang J. Molecularly targeting the PI3K-Akt-mTOR pathway can sensitize cancer cells to radiotherapy and chemotherapy. *Cell Mol Biol Lett.* 2014;19(2):233-242.

172. Mardanshahi A, Gharibkandi NA, Vaseghi S, Abedi SM, Molavipordanjani S. The PI3K/AKT/mTOR signaling pathway inhibitors enhance radiosensivity in cancer cell lines. *Mol Biol Rep.* 2021;48(8):1-14.

173. Mortezaee K, Parwaie W, Motevaseli E, et al. Targets for improving tumor response to radiotherapy. *Int Immunopharmacol.* 2019;76:105847.

174. Chen YA, Tzeng DTW, Huang YP, et al. Antrocin sensitizes prostate cancer cells to radiotherapy through inhibiting PI3K/AKT and MAPK signaling pathways. *Cancers.* 2018;11(1):34.

175. Ciccarelli C, Vulcano F, Milazzo L, et al. Key role of MEK/ERK pathway in sustaining tumorigenicity and in vitro radioresistance of embryonal rhabdomyosarcoma stem-like cell population. *Mol Cancer.* 2016;15:16.

176. Miyahara H, Yadavilli S, Natsumeda M, et al. The dual mTOR kinase inhibitor TAK228 inhibits tumorigenicity and enhances radiosensitization in diffuse intrinsic pontine glioma. *Cancer Lett.* 2017;400:110-116.

177. Surowiec RK, Ferris SF, Apfelbaum A, et al. Transcriptomic analysis of diffuse intrinsic pontine glioma (DIPG) identifies a targetable ALDH-positive subset of highly tumorigenic cancer stem-like cells. *Mol Cancer Res.* 2021;19(2):223-239.

178. Zona S, Bella L, Burton MJ, Nestal de Moraes G, Lam EW. FOXM1: an emerging master regulator of DNA damage response and genotoxic agent resistance. *Biochim Biophys Acta.* 2014;1839(11):1316-1322.

179. Ding GR, Honda N, Nakahara T, et al. Radiosensitization by inhibition of IkappaB-alpha phosphorylation in human glioma cells. *Radiat Res.* 2003;160(2):232-237.

180. Da-Veiga MA, Rogister B, Lombard A, Neirinckx V, Piette C. Glioma stem cells in pediatric high-grade gliomas: from current knowledge to future perspectives. *Cancers.* 2022;14(9):2296.

181. Kfoury-Beaumont N, Prakasam R, Pondugula S, et al. The H3K27M mutation alters stem cell growth, epigenetic regulation, and differentiation potential. *BMC Biol.* 2022;20(1):124.

182. Bao S, Wu Q, McLendon RE, et al. Glioma stem cells promote radioresistance by preferential activation of the DNA damage response. *Nature.* 2006;444(7120):756-760.

183. Diehn M, Cho RW, Lobo NA, et al. Association of reactive oxygen species levels and radioresistance in cancer stem cells. *Nature.* 2009;458(7239):780-783.

184. Ishimoto T, Nagano O, Yae T, et al. CD44 variant regulates redox status in cancer cells by stabilizing the xCT subunit of system xc(−) and thereby promotes tumor growth. *Cancer Cell.* 2011;19(3):387-400.

185. Phillips TM, McBride WH, Pajonk F. The response of CD24(−/low)/CD44+ breast cancer-initiating cells to radiation. *J Natl Cancer Inst.* 2006;98(24):1777-1785.

186. Ropolo M, Daga A, Griffero F, et al. Comparative analysis of DNA repair in stem and nonstem glioma cell cultures. *Mol Cancer Res.* 2009;7(3):383-392.

187. Wang J, Wakeman TP, Lathia JD, et al. Notch promotes radioresistance of glioma stem cells. *Stem Cells.* 2010;28(1):17-28.

188. Yin H, Glass J. The phenotypic radiation resistance of CD44+/CD24(−or low) breast cancer cells is mediated through the enhanced activation of ATM signaling. *PLoS One.* 2011;6(9):e24080.

189. Zhang M, Atkinson RL, Rosen JM. Selective targeting of radiation-resistant tumor-initiating cells. *Proc Natl Acad Sci USA.* 2010;107(8):3522-3527.

190. Hardee ME, Marciscano AE, Medina-Ramirez CM, et al. Resistance of glioblastoma-initiating cells to radiation mediated by the tumor microenvironment can be abolished by inhibiting transforming growth factor-beta. *Cancer Res.* 2012;72(16):4119-4129.

191. Jamal M, Rath BH, Tsang PS, Camphausen K, Tofilon PJ. The brain microenvironment preferentially enhances the radioresistance of CD133(+) glioblastoma stem-like cells. *Neoplasia.* 2012;14(2):150-158.

192. Mao XG, Yan M, Xue XY, et al. Overexpression of ZNF217 in glioblastoma contributes to the maintenance of glioma stem cells regulated by hypoxia-inducible factors. *Lab Invest.* 2011;91(7):1068-1078.

193. Pistollato F, Abbadi S, Rampazzo E, et al. Intratumoral hypoxic gradient drives stem cells distribution and MGMT expression in glioblastoma. *Stem Cells.* 2010;28(5):851-862.

194. Pistollato F, Rampazzo E, Persano L, et al. Interaction of hypoxia-inducible factor-1alpha and Notch signaling regulates medulloblastoma precursor proliferation and fate. *Stem Cells.* 2010;28(11):1918-1929.

195. Taylor IC, Hutt-Cabezas M, Brandt WD, et al. Disrupting NOTCH slows diffuse intrinsic pontine glioma growth, enhances radiation sensitivity, and shows combinatorial efficacy with Bromodomain inhibition. *J Neuropathol Exp Neurol.* 2015;74(8):778-790.

196. Mudassar F, Shen H, O'Neill G, Hau E. Targeting tumor hypoxia and mitochondrial metabolism with anti-parasitic drugs to improve radiation response in high-grade gliomas. *J Exp Clin Cancer Res.* 2020;39(1):208.

197. Carro MS, Lim WK, Alvarez MJ, et al. The transcriptional network for mesenchymal transformation of brain tumours. *Nature.* 2010;463(7279):318-325.

198. Lamouille S, Xu J, Derynck R. Molecular mechanisms of epithelial-mesenchymal transition. *Nat Rev Mol Cell Biol.* 2014;15(3):178-196.

199. Park JK, Jang SJ, Kang SW, et al. Establishment of animal model for the analysis of cancer cell metastasis during radiotherapy. *Radiat Oncol.* 2012;7:153.

200. Wild-Bode C, Weller M, Rimner A, Dichgans J, Wick W. Sublethal irradiation promotes migration and invasiveness of glioma cells: implications for radiotherapy of human glioblastoma. *Cancer Res.* 2001;61(6):2744-2750.

201. Zhang X, Li X, Zhang N, Yang Q, Moran MS. Low doses ionizing radiation enhances the invasiveness of breast cancer cells by inducing epithelial-mesenchymal transition. *Biochem Biophys Res Commun.* 2011;412(1):188-192.

202. Kawamoto A, Yokoe T, Tanaka K, et al. Radiation induces epithelial-mesenchymal transition in colorectal cancer cells. *Oncol Rep.* 2012;27(1):51-57.

203. Moncharmont C, Levy A, Guy JB, et al. Radiation-enhanced cell migration/invasion process: a review. *Crit Rev Oncol Hematol.* 2014;92(2):133-142.

204. Iwadate Y. Epithelial-mesenchymal transition in glioblastoma progression. *Oncol Lett.* 2016;11(3):1615-1620.

205. Nantajit D, Lin D, Li JJ. The network of epithelial-mesenchymal transition: potential new targets for tumor resistance. *J Cancer Res Clin Oncol.* 2015;141(10):1697-1713.

206. Dudas J, Ladanyi A, Ingruber J, Steinbichler TB, Riechelmann H. Epithelial to mesenchymal transition: a mechanism that fuels cancer radio/chemoresistance. *Cell.* 2020;9(2):428.

207. Kurrey NK, Jalgaonkar SP, Joglekar AV, et al. Snail and slug mediate radioresistance and chemoresistance by antagonizing p53-mediated apoptosis and acquiring a stem-like phenotype in ovarian cancer cells. *Stem Cells.* 2009;27(9):2059-2068.

208. Ramachandran A, Vizan P, Das D, et al. TGF-beta uses a novel mode of receptor activation to phosphorylate SMAD1/5 and induce epithelial-to-mesenchymal transition. *Elife.* 2018;7:e31756.

209. Meel MH, Schaper SA, Kaspers GJL, Hulleman E. Signaling pathways and mesenchymal transition in pediatric high-grade glioma. *Cell Mol Life Sci*. 2018;75(5):871-887.

210. Natsuizaka M, Whelan KA, Kagawa S, et al. Interplay between Notch1 and Notch3 promotes EMT and tumor initiation in squamous cell carcinoma. *Nat Commun*. 2017;8(1):1758.

211. Park J, Lee W, Yun S, et al. STAT3 is a key molecule in the oncogenic behavior of diffuse intrinsic pontine glioma. *Oncol Lett*. 2020;20(2):1989-1998.

212. Saito N, Aoki K, Hirai N, et al. Effect of Notch expression in glioma stem cells on therapeutic response to chemo-radiotherapy in recurrent glioblastoma. *Brain Tumor Pathol*. 2015;32(3):176-183.

213. Carballo GB, Ribeiro JH, Lopes GPF, et al. GANT-61 induces autophagy and apoptosis in glioblastoma cells despite their heterogeneity. *Cell Mol Neurobiol*. 2021;41(6):1227-1244.

214. Hung HC, Liu CC, Chuang JY, Su CL, Gean PW. Inhibition of sonic hedgehog signaling suppresses glioma stem-like cells likely through inducing autophagic cell death. *Front Oncol*. 2020;10:1233.

215. Huo X, Li S, Shi T, Suo A, Ruan Z, Yao Y. Tripartite motif 16 inhibits epithelial-mesenchymal transition and metastasis by down-regulating sonic hedgehog pathway in non-small cell lung cancer cells. *Biochem Biophys Res Commun*. 2015;460(4):1021-1028.

216. Ishii A, Shigemura K, Kitagawa K, et al. Anti-tumor effect of hedgehog signaling inhibitor, Vismodegib, on castration-resistant prostate cancer. *Anticancer Res*. 2020;40(9):5107-5114.

217. Jeng KS, Jeng CJ, Jeng WJ, et al. Sonic hedgehog signaling pathway as a potential target to inhibit the progression of hepatocellular carcinoma. *Oncol Lett*. 2019;18(5):4377-4384.

218. Damodharan S, Lara-Velazquez M, Williamsen BC, Helgager J, Dey M. Diffuse intrinsic pontine glioma: molecular landscape, evolving treatment strategies and emerging clinical trials. *J Pers Med*. 2022;12(5):840.

219. Chi AS, Tarapore RS, Hall MD, et al. Pediatric and adult H3 K27M-mutant diffuse midline glioma treated with the selective DRD2 antagonist ONC201. *J Neurooncol*. 2019;145(1):97-105.

220. Duchatel RJ, Mannan A, Woldu AS, et al. Preclinical and clinical evaluation of German-sourced ONC201 for the treatment of H3K27M-mutant diffuse intrinsic pontine glioma. *Neurooncol Adv*. 2021;3(1):vdab169.

221. Madhavan K, Balakrishnan I, Lakshmanachetty S, et al. Venetoclax cooperates with ionizing radiation to attenuate diffuse midline glioma tumor growth. *Clin Cancer Res*. 2022;28(11):2409-2424.

222. Pachocki CJ, Hol EM. Current perspectives on diffuse midline glioma and a different role for the immune microenvironment compared to glioblastoma. *J Neuroinflammation*. 2022;19(1):276.

223. Persson ML, Douglas AM, Alvaro F, et al. The intrinsic and microenvironmental features of diffuse midline glioma: implications for the development of effective immunotherapeutic treatment strategies. *Neuro Oncol*. 2022;24(9):1408-1422.

224. Chen Y, Zhao C, Li S, Wang J, Zhang H. Immune microenvironment and immunotherapies for diffuse intrinsic pontine glioma. *Cancers (Basel)*. 2023;15(3):602.

225. Rodel F, Frey B, Multhoff G, Gaipl U. Contribution of the immune system to bystander and non-targeted effects of ionizing radiation. *Cancer Lett*. 2015;356(1):105-113.

226. Deng L, Liang H, Xu M, et al. STING-dependent cytosolic DNA sensing promotes radiation-induced type I interferon-dependent antitumor immunity in immunogenic tumors. *Immunity*. 2014;41(5):843-852.

227. Reits EA, Hodge JW, Herberts CA, et al. Radiation modulates the peptide repertoire, enhances MHC class I expression, and induces successful antitumor immunotherapy. *J Exp Med*. 2006;203(5):1259-1271.

228. Suwa T, Kobayashi M, Nam JM, Harada H. Tumor microenvironment and radioresistance. *Exp Mol Med*. 2021;53(6):1029-1035.

229. Ma S, Li X, Wang X, et al. Current Progress in CAR-T cell therapy for solid tumors. *Int J Biol Sci*. 2019;15(12):2548-2560.

230. Majzner RG, Ramakrishna S, Yeom KW, et al. GD2-CAR T cell therapy for H3K27M-mutated diffuse midline gliomas. *Nature*. 2022;603(7903):934-941.

231. Vitanza NA, Wilson AL, Huang W, et al. Intraventricular B7-H3 CAR T cells for diffuse intrinsic pontine glioma: preliminary first-in-human bioactivity and safety. *Cancer Discov*. 2023;13(1):114-131.

232. Guo C, Yang Q, Xu P, et al. Adjuvant temozolomide chemotherapy with or without interferon alfa among patients with newly diagnosed high-grade gliomas: a randomized clinical trial. *JAMA Netw Open*. 2023;6(1):e2253285.

233. Martinez-Velez N, Marigil M, Garcia-Moure M, et al. Delta-24-RGD combined with radiotherapy exerts a potent antitumor effect in diffuse intrinsic pontine glioma and pediatric high grade glioma models. *Acta Neuropathol Commun*. 2019;7(1):64.

234. Martinez-Velez N, Garcia-Moure M, Marigil M, et al. The oncolytic virus Delta-24-RGD elicits an antitumor effect in pediatric glioma and DIPG mouse models. *Nat Commun*. 2019;10(1):2235.

235. Gallego Perez-Larraya J, Garcia-Moure M, Labiano S, et al. Oncolytic DNX-2401 virus for pediatric diffuse intrinsic pontine glioma. *N Engl J Med*. 2022;386(26):2471-2481.

236. Bailey S, Howman A, Wheatley K, 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*. 2013;49(18):3856-3862.

237. Chassot A, Canale S, Varlet P, et al. Radiotherapy with concurrent and adjuvant temozolomide in children with newly diagnosed diffuse intrinsic pontine glioma. *J Neurooncol*. 2012;106(2):399-407.

238. Cohen KJ, Heideman RL, Zhou T, et al. Temozolomide in the treatment of children with newly diagnosed diffuse intrinsic pontine gliomas: a report from the Children's Oncology Group. *Neuro Oncol*. 2011;13(4):410-416.

239. Izzuddeen Y, Gupta S, Haresh KP, Sharma D, Giridhar P, Rath GK. Hypofractionated radiotherapy with temozolomide in diffuse intrinsic pontine gliomas: a randomized controlled trial. *J Neurooncol*. 2020;146(1):91-95.

240. Chua J, Nafziger E, Leung D. Evidence-based practice: temozolomide beyond glioblastoma. *Curr Oncol Rep*. 2019;21(4):30.

241. Hargrave D, Bartels U, Bouffet E. Diffuse brainstem glioma in children: critical review of clinical trials. *Lancet Oncol*. 2006;7(3):241-248.

242. Warren KE. Beyond the blood:brain barrier: the importance of central nervous system (CNS) pharmacokinetics for the treatment of CNS tumors, including diffuse intrinsic pontine glioma. *Front Oncol*. 2018;8:239.

243. Heravi Shargh V, Luckett J, Bouzinab K, et al. Chemosensitization of temozolomide-resistant pediatric diffuse midline glioma using potent nanoencapsulated forms of a N(3)-propargyl analogue. *ACS Appl Mater Interfaces*. 2021;13(30):35266-35280.

244. Bellat V, Alcaina Y, Tung CH, et al. A combined approach of convection-enhanced delivery of peptide nanofiber reservoir to prolong local DM1 retention for diffuse intrinsic pontine glioma treatment. *Neuro Oncol*. 2020;22(10):1495-1504.

245. Idbaih A, Canney M, Belin L, et al. Safety and feasibility of repeated and transient blood-brain barrier disruption by pulsed ultrasound in patients with recurrent glioblastoma. *Clin Cancer Res*. 2019;25(13):3793-3801.

246. Williams AB, Schumacher B. p53 in the DNA-damage-repair process. *Cold Spring Harb Perspect Med*. 2016;6(5):a026070.

247. Motea EA, Huang X, Singh N, et al. NQO1-dependent, tumor-selective radiosensitization of non-small cell lung cancers. *Clin Cancer Res*. 2019;25(8):2601-2609.

248. Speidel D. The role of DNA damage responses in p53 biology. *Arch Toxicol*. 2015;89(4):501-517.

249. Do K, Doroshow JH, Kummar S. Wee1 kinase as a target for cancer therapy. *Cell Cycle*. 2013;12(19):3159-3164.

250. Huang RX, Zhou PK. DNA damage response signaling pathways and targets for radiotherapy sensitization in cancer. *Signal Transduct Target Ther.* 2020;5(1):60.

251. Pollack IF, Jakacki RI, Blaney SM, et al. Phase I trial of imatinib in children with newly diagnosed brainstem and recurrent malignant gliomas: a pediatric brain tumor consortium report. *Neuro Oncol.* 2007;9(2):145-160.

252. Broniscer A, Baker JN, Tagen M, et al. Phase I study of vandetanib during and after radiotherapy in children with diffuse intrinsic pontine glioma. *J Clin Oncol.* 2010;28(31):4762-4768.