Targeting K-Ras-mediated DNA damage response in radiation oncology: Current status, challenges and future perspectives

Mahmoud Toulany

Division of Radiobiology & Molecular Environmental Research, Department of Radiation Oncology, University of Tuebingen, Roentgenweg 11, 72076 Tuebingen, Germany

ABSTRACT

Approximately 60% of cancer patients receive curative or palliative radiation. Despite the significant role of radiotherapy (RT) as a curative approach for many solid tumors, tumor recurrence occurs, partially because of intrinsic radioresistance. Accumulating evidence indicates that the success of RT is hampered by activation of the DNA damage response (DDR). The intensity of DDR signaling is affected by multiple parameters, *e.g.,* loss-of-function mutations in tumor suppressor genes, gain-of-function mutations in protooncogenes as well as radiation-induced alterations in signal-transduction pathways. Therefore, the response to irradiation differs in tumors of different types, which makes the individualization of RT as a rational but challenging goal. One contributor to tumor cell radiation survival is signaling through the Ras pathway. Three RAS genes encode 4 Ras isoforms: K-Ras4A, K-Ras4B, H-Ras, and N-Ras. RAS family members are found to be mutated in approximately 19% of human cancers. Mutations in RAS lead to constitutive activation of the gene product and activation of multiple Ras-dependent signal-transduction cascades. Preclinical studies have shown that the expression of mutant KRAS affects DDR and increases cell survival after irradiation. Approximately 70% of RAS mutations occur in KRAS. Thus, applying targeted therapies directly against K-Ras as well as K-Ras upstream activators and downstream effectors might be a tumor-specific approach to overcome K-Ras-mediated RT resistance. In this review, the role of K-Ras in the activation of DDR signaling will be summarized. Recent progress in targeting DDR in KRAS-mutated tumors in combination with radiochemotherapy will be discussed.

1. Introduction

Conventional fractionated radiotherapy (RT) is a curative approach that significantly contributes to human tumor treatment. Nevertheless, treatment failure still occurs, and overall survival for certain tumor types remains dismal. One of the major mechanisms of tumor survival after irradiation is hyperactivation of the survival signaling pathways regulated by different oncogenes. Among these, RAS (rat sarcoma viral oncogene homolog) encodes a small molecular weight protein with intrinsic GTPase activity. Ras proteins cycle between inactivated GDP-bound and active GTP-bound states and couple extracellular signals to intracellular effector pathways [1]. The three RAS genes in humans consist of KRAS (Kirsten rat sarcoma virus), HRAS (Harvey RAS) and NRAS (Neuroblastoma RAS). The KRAS gene has two splice variants, KRAS4A and KRAS4B, with differential expression levels of the two variants in tumors [2]. Approximately 19 % of human cancers harbor mutations in one of the RAS isoforms, equivalent to approximately 3.4 million new cases per year worldwide [3]. Mutations in RAS genes are

mainly at hotspots, such as codons 12, 13 and 61, reviewed elsewhere [4]. Nearly 70 % of the mutations occur in KRAS, mainly in pancreatic cancers (86 %), colorectal cancers (CRC; 41 %) and lung cancer (32 %) [4].

Anchoring Ras to the cell membrane via a variety of posttranslational modifications (PTMs) is essential for the localization of Ras in the cell membrane, subsequent activation and carcinogenic potency. PTMs on K-Ras have been reviewed in [5]. Essential steps in K-Ras PTM are prenylation of CAAX box by the enzymes farnesyltransferase or geranylgeranyl transferase, removing the last 3 amino acids AAX through proteolysis by endoplasmic reticulum located Ras-converting enzyme and, finally, methylation of the cysteine residue of the CAAX box catalyzed by the enzyme isoprenylcysteine carboxyl methyltransferase. Both proteolysis and methylation are essential for efficient membrane binding of prenylated K-Ras [6], a prerequisite for its biological activity. Activation of Ras occurs by changing GDP-bound inactive Ras to the GTP-bound active state. Guanine nucleotide exchange factors (GEFs) and GTPase activating proteins (GAPs), such as neurofibromin 1 (NF1)

and p120-GAP, promote nucleotide exchange by Ras (Fig. 1). Due to RAS point mutations, GTPase stimulation by GAPs is greatly reduced, and intrinsic GTPase activity is also reduced. Under these conditions, Ras inactivation is suppressed and it predominantly stays bound to the cell membrane in a constitutively active form [7].

Among the upstream activators of Ras, the epidermal growth factor receptor (EGFR)/HER family consisting of four members, is the most important among the membrane-bound receptor tyrosine kinases. Stimulation of these receptors by their respective ligands results in homo- and heterodimerization, leading to the activation of signaling cascades. Ras transmits these signals, which regulate a variety of cellular functions. Growth, proliferation, survival, tumorigenesis, and metastasis are among the important cellular functions that are stimulated by the EGFR [8]. KRAS mutation leads to constitutive K-Ras activity in association with stimulated autocrine production of the EGFR ligand as reported in panc-1 pancreatic carcinoma cells [9], A549 lung cancer cells and MDA-MB-231 breast cancer cells [10,11] as well as in CRC cell lines LIM1215, OXCO-2 and DiFi that develop resistance to cetuximab, presumably due to a secondary KRAS mutation[12] (Fig. 1). KRAS mutation promotes post-irradiation cell survival through EGFR activity in non-small-cell lung cancers (NSCLC) as demonstrated in NCI-H1703 cells *in vitro* and *in vivo* [13,14] and possibly through wild-type HRAS, which has been shown in DLD-1 colorectal and MiaPaCa-2 pancreatic cancer cells harboring frequent mutations in KRAS [15]. Additionally, *in vitro* and *in vivo* studies have shown that EGFR signaling in KRAS-mutant NSCLC cell line A549 promotes chromatin condensation in interphase

cells, thereby restricting the number of DNA double-strand breaks (DSB) produced by 1 Gy ionizing radiation (IR) [13]. In addition to point mutations and stimulation with receptor ligands, exposure to a clinically relevant dose of IR rapidly activates K-Ras in KRAS wild-type head and neck squamous cell carcinoma FaDu cells [10].

GTP-bound Ras stimulates several cytoplasmic signaling cascades. Among these pathways, mitogen-activated protein kinase (MAPK), phosphoinositide 3-kinase (PI3K), protein kinase C and RAL guanine nucleotide dissociation stimulator (RALGDS) are the most important pathways [16,17]. K-Ras triggers the Raf family of serine/threonine kinases, which in turn stimulates mitogen-activated protein kinase kinase 1/2 (MEK1/2). MEK1/2 stimulate MAPK/extracellular signal-regulated kinases (ERK1/2). Activated ERK1/2 phosphorylate their target substrates, such as multifunctional protein Y-Box binding protein-1 (YB-1) [18,19]. Oncogenic K-Ras hyperactivates the PI3K/AKT pathway. The PI3K/AKT pathway is the major survival pathway, which is hyperactivated in human tumors and is involved in DNA damage response (DDR) signaling, as reviewed elsewhere [20,21]. In terms of cell survival after RT, constitutive K-Ras activity due to RAS mutation or IR-induced Ras activation leads to accelerated repair of radiation-induced DSB and increased survival in solid tumors from different tissues [18,22–25]. In line with the preclinical data on the role of K-Ras in therapy resistance, the association of KRAS mutation with worse treatment outcome has been well documented. In this regard, pancreatic cancers, as the most lethal cancer with frequent mutations in KRAS, have a 5-year survival rate of approximately 10 % [26]. In a study in Chinese
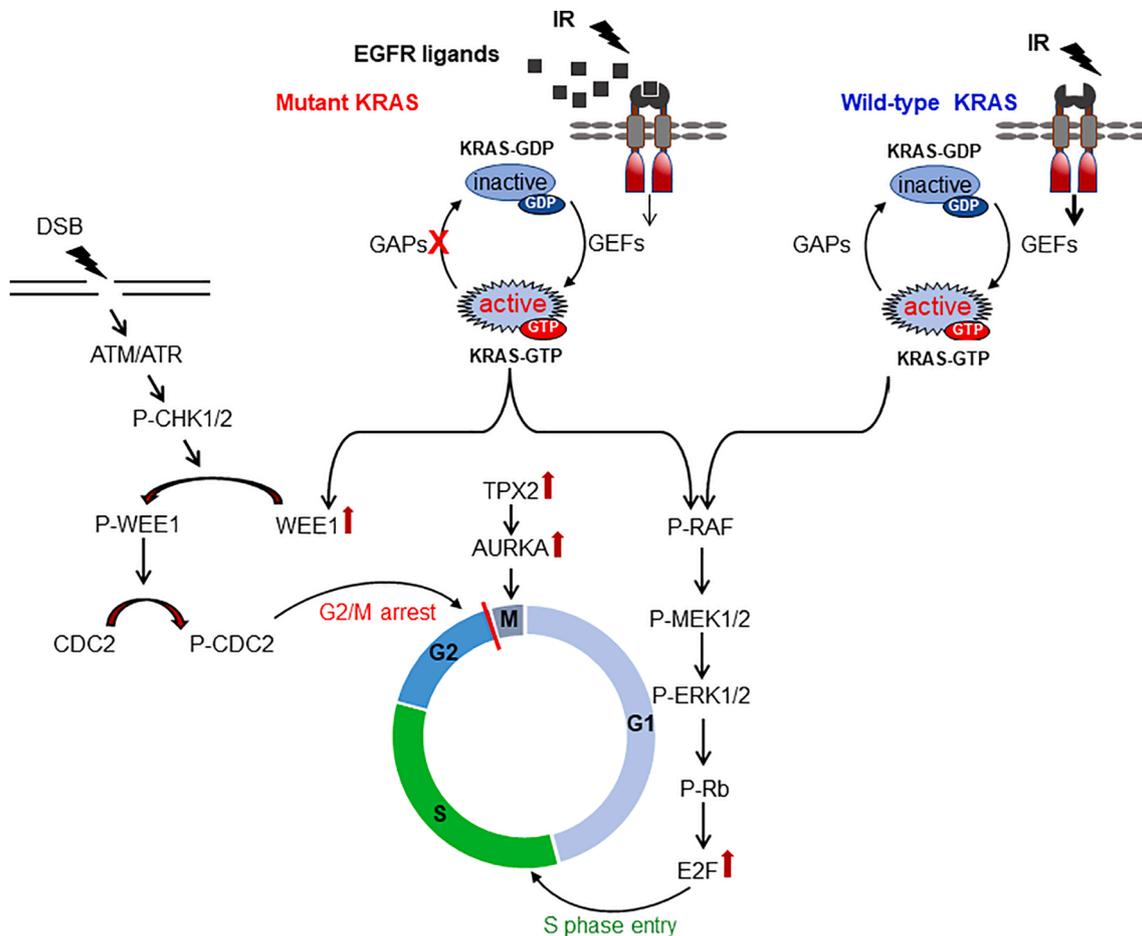
Fig. 1. K-Ras controls cell cycle progression after irradiation. A point mutation in KRAS results in autocrine secretion of EGFR ligands but maintains K-Ras in a continuous GTP-bound active state, independent of EGFR. In contrast, ionizing radiation stimulates K-Ras in wild-type cells in an EGFR-dependent manner but independent of the EGFR ligand. Activation of K-Ras stimulates ERK1/2 phosphorylation/activation. ERK1/2 activity stimulates the transition of cells from G1 to S phase. In parallel, KRAS mutation increases the expression of WEE1, which is activated after DNA damage induction. Activated WEE1 phosphorylates CDC2 and prevents mitotic entry by inhibiting the cyclin B/CDC2 complex.

patients with advanced pancreatic ductal adenocarcinoma, patients with KRAS mutation showed worse overall survival than patients with KRAS wild-type [27]. Likewise, KRAS wild type pancreatic adenocarcinoma patients exibited a survival benefit, both, in overall cohorts and in patients treated with chemotherapy agents [28]. The second most common KRAS-mutated human tumor is CRC, which is resistant to chemoradiation [29,30]. KRAS-mutated cervical cancer has significantly worse recurrence-free survival and distant metastases after RT [31]. The lung adenocarcinoma subgroup of NSCLC is the third cancer category with the most frequent mutations in KRAS. NSCLC has a 5-year survival rate of approximately 25 %, and KRAS mutation has been shown to be associated with radioresistance [32] and decreased cancer-specific survival after lung stereotactic RT [33]. Thus, accumulating evidence from preclinical and clinical studies indicates that mutation in KRAS diminishes the effect of RT.

2. Role of K-Ras in DDR signaling

2.1. K-Ras cascades regulate cell-cycle progression

Upon induction of DNA damage, the DDR is activated, which is a complex signal-transduction network responsible for sensing and responding to specific types of DNA damage, encompassing specific machineries mediating cell-cycle regulation and DNA repair. Exposure to IR (2 Gy) rapidly stimulates Ras-dependent activation of ERK1/2 by phosphorylation of the threonine and tyrosine residues in different tumor cells lacking mutations in the components of the Ras/MAPK pathway [34,35]. ERK1/2 activity stimulates the expression of immediate target genes, which in turn, by phosphorylating retinoblastoma protein (RB), enhances the expression of the transcription factor E2F and prepares cells for transition from the G1 to S phase [36] along with sequestering and degrading cyclin-dependent kinases (CDKs), such as p27 [37,38]. ERK1/2, as a regulator of the G1- to S-phase transition, has been extensively reviewed previously [39]. Continuous activation of ERK1/2, in parallel to stimulating the expression of proliferation-associated genes, suppresses the expression of antiproliferative genes as well [40,41]. IR-induced ERK phosphorylation promotes cell proliferation by stimulating the G1 to S transition, as shown in KRAS[G13D]-mutated colorectal cancer HCT116 cells after irradiation with 1 Gy [17]. The transition of cells from G1 to S after irradiation is important in the context of DSB repair. Radiation-induced DSB in G1 phase in cells with wild-type TP53 results in ataxia telangiectasia mutated (ATM)/ checkpoint kinase 2 (CHK2)-dependent G1 arrest until the damage is repaired through NHEJ. G1 progression in cells with loss of p53 function makes DSB repair solely dependent on G2/M arrest. G2/M arrest through ataxia telangiectasia and Rad3 related (ATR)/checkpoint kinase 1 (CHK1) arrest is crucial since the majority of tumors with KRAS mutations also harbor TP53 mutations, as shown in pancreatic ductal carcinomas [42]. The expression of mutated KRAS and TP53 in NSCLC was also shown to be associated with poor clinical outcomes [43]. Due to the role of CHK1 in G2 arrest and, consequently, HR repair of DSB, CHK1 and ATR have been described to be targets in combination with DNA damage-inducing agents as treatment approaches for pancreatic cancers [44,45]. The p53-independent expression of the CDK inhibitor p21 in response to mitogen-activated ERK1/2 has been described in early G1 phase. However, it remains to be investigated whether in TP53-mutated cells IR-induced activation of ERK1/2 also induces p21 expression [40]. Hyperactivation of ERK1/2 in KRAS-mutated cells also stimulates cell proliferation. In this context, the stimulated proliferation of KRAS[G12D]-transformed pancreatic ductal cells harboring common TP53 and p16(INK4a) mutations was shown to be dependent on ERK2 downstream of K-Ras [46].

KRAS mutation increases the expression of WEE1 kinase, which drives cell-cycle progression [47]. Upon induction of DNA damage, WEE1 becomes activated, presumably by CHK1 and CHK2 as ATR and ATM downstream kinases [48]. Mitotic entry is prevented by inhibition

of the cyclin-B/CDC2 complex, in which inhibitory phosphorylation of CDC2 plays a fundamental role and is stimulated by WEE1 activity [49]. Thus, the function of WEE1 in G2/M arrest is crucial in all tumor cells, especially in TP53-mutated tumors with a lack of G1 arrest. Accordingly, targeting WEE1 can be an efficient approach to sensitize tumor cells to DNA-damaging agents [50,51], particularly in TP53-mutated cells, based on the concept of synthetic lethality [52].

Aurora kinase A (AURKA) is involved in the G2/M transition by promoting centrosome maturation and mitotic spindle assembly and is thus involved in chromosomal stability [53]. Oncogenic K-Ras stimulates the expression of both AURKA and its activator,TPX2, as shown in pancreatic ductal adenocarcinomas (PDACs) cell lines KRAS[G12D] mutated PANC-1 and KRAS[G12C] mutated PaCa-2 [54]. In PDACs, high expression levels of AURKA and TPX2 were associated with shorter patient survival and the presence of oncogenic KRAS [54]. The expression of AURKA is stimulated by IR in a dose-dependent manner and induces radioresistance in preclinical studies [55–57]. Likewise, it has been shown that AURKA expression diminishes RT outcome in cancer patients with different tumor types [58–60]. Thus, it can be concluded that K-Ras-mediated radiochemotherapy resistance occurs partially through regulating cell-cycle progression. The function of oncogenic K-Ras in cell-cycle progression is outlined in Fig. 1.

2.2. K-Ras downstream cascades stimulate DNA DSB repair

Direct evidence for oncogenic K-Ras-mediating radioresistance was initially shown by Bernhard et al. [24] and Kim et al. [61], who demonstrated that knockdown of K-Ras by siRNA in KRAS[G12V] SW480 cells resulted in radiosensitization by a radiation dose enhancement ratio (DER) of 1.22. PI3K/AKT and MAPK/ERK are the two major pathways downstream of K-Ras that are hyperactivated by mutated KRAS and stimulated by irradiation. The impact of these two pathways on DDR repair has been investigated mainly in the context of stimulating the 3 key kinases, *i.e.*, ATM, DNA-dependent protein kinase catalytic subunit (DNA-PKcs) and ATR. Activation of ATM following 53BP1 focus formation at the DNA damage site seems to be one of the early steps in the DDR after irradiation [62]. ATM induces CHK2 activity, leading to p53-dependent responses that promote G1 cell-cycle arrest, as a prerequisite event for DSB repair through NHEJ. Activation of p53 also induces chromatin remodeling and enhances the induction of DNA-repair genes [63]. If radiation-induced DNA damage is not repaired, cell death occurs mainly due to mitotic catastrophe but not senescence or autophagy [64,65]. ATM functionally interacts with K-Ras. ATM deficiency markedly increases the proportion of chromosomal alterations in pancreatic primary tumors with KRAS[G12D] and renders pancreatic tumors highly sensitive to radiation in association with increased residual DSB 24 h post-4 Gy [66]. This observation indicates a link between the K-Ras protooncogene and the activation of ATM. Several studies have shown that the activation of ERK and AKT depends on ATM kinase activity and that ATM forms a complex with ERK1/2 [67]. Thus, the K-Ras-ATM-AKT-ERK signaling pathway can be one of the underlying signaling pathways involved in DSB repair in KRAS-mutated cells. This conclusion is supported by the literature indicating that DSB repair is stimulated by HR and NHEJ through the AKT and ERK pathways [68–75]. In further support of this conclusion, ATM was shown to mediate crosstalk between the prosurvival MEK/ERK and AKT/mTOR pathways as the two major pathways regulated by K-Ras [76]. The key function of K-Ras in this interaction is also supported by the enhanced sensitivity of KRAS-mutant lung cancer cells to MEK inhibition after ATM loss [76] and radiosensitization of KRAS-mutated pancreatic cancer cells after MEK targeting through inhibition of HR- and NHEJ-dependent DSB repair [77]. KRAS mutation also activates NRF2 antioxidant signaling [78,79] that suppresses p53 expression [78] and increases the expression of 53BP1 [79]. Upregulation of 53BP1 stimulates NHEJ repair and mediates radioresistance, as shown in CRC cells SW48 and HCT116 after single doses of IR up to 8 Gy [79]. In

KRAS^G12V-mutated NSCLC cell line A549 cells, AKT1-dependent expression of RAD51 has also been reported [80]. This indicates that KRAS-mutated cells may produce an effective HR repair of DSB, as described previously in lung cancer cells [81] (Fig. 2).

YB-1 is highly expressed in human solid tumors from different tissues and is involved in various cellular processes, *e.g.*, cell cycle progression, maintaining cancer stemness and DDR as reviewed in [82,83]. The cancer hallmarks described thus far, *i.e.*, resisting cell death such as after RT, are also regulated by YB-1 [84]. YB-1 expression is associated with a limited response to radiochemotherapy [18,83,85]. Serine 102 (S102) is one of the major phosphorylation sites in YB-1 that is necessary for its cellular functions. p90 ribosomal S6 kinase (RSK) and AKT are the key kinases within the oncogenic K-Ras downstream pathway that directly activate YB-1 [18,86]. YB-1 is constitutively phosphorylated at S102 in KRAS-mutated tumor cells, *i.e.*, in CRC cells [85] and in triple-negative breast cancer (TNBC) cells [18]. In KRAS wild-type breast cancer cells, exposure to IR (4 Gy) induces YB-1 phosphorylation as early as 5 min post-IR [18]. Delayed YB-1 phosphorylation was also detected in CRC cells 6 h as well as 12 h after IR (5 Gy) [87]. Similar to the knockdown of K-Ras, knockdown of YB-1 inhibits the repair of IR-induced DSB in KRAS^G13D-mutated MDA-MB-231 breast cancer cells after irradiation with single doses of 2, 4 and 6 Gy, resulting in an enhanced radiosensitivity [18]. Likewise, blocking YB-1 with an S102 blocking peptide [83] or by impairing its interaction with RSK [88] blocks DSB repair after 4 Gy irradiation in breast cancer cells. Thus, YB-1 is one of the components that is stimulated by the K-Ras protooncogene and enhances DSB repair, mediating RT resistance. The function of oncogenic K-Ras in DSB repair is outlined in Fig. 2.

3. Targeting strategies to improve RT of KRAS-mutated tumors

3.1. Targeting the K-Ras-regulated components of DDR signaling

As reviewed above, KRAS mutation interferes with cell-cycle progression by stimulating the G1 transition and activating G2/M arrest (Fig. 1). After induction of DSB, arrested cells initiate DSB repair through HR and NHEJ in KRAS-mutated cells (Fig. 2). According to the function of K-Ras in DDR signaling, targeting K-Ras signaling will partially interfere with DSB repair after irradiation either by overwhelming cell-cycle arrest or by hampering the components of repair pathways downstream of K-Ras. Thus, a selective approach to overcome RT resistance of KRAS-mutated cells would be targeting the components of cell-cycle progression and DNA repair that are known to be affected by mutated KRAS. In preclinical studies, the classical components of cell-cycle progression and DNA-damage repair, such as ATM, DNA-PKcs, ATR, CHK1, CHK2 and WEE1, have shown to be very promising targets for radiosensitizing tumor cells by applying small-molecule inhibitors [89–92]. Since the majority of tumors with KRAS mutations also harbor *TP53* mutations, targeting the G2/M checkpoint might be the most effective approach to interfere with the repair of DSBs in KRAS-mutated cells. Thus, among a variety of targets involved in DDR and regulated by K-Ras, the current status of targeting DDR signaling involved in the G2/M checkpoint will be summarized. The major focus will be on those clinical trials performed specifically in 3 tumor entities, *i.e.,* pancreatic, CRC and lung cancers with frequent mutations in the KRAS gene.

3.1.1. Targeting ATR

ATR is activated upon DNA damage and in turn activates CHK1 to induce G2/M cell-cycle arrest. The mechanism of ATR function in DDR has been extensively investigated in preclinical studies. Currently, specific small-molecule inhibitors of ATR, *e.g.*, VE-821, M6620 (berzosertib, VX-970), and AZD6738 (ceralasertib), are available, and the sensitizing effect of ATR inhibitors has been successfully demonstrated after combination with RT or chemotherapy agents. In PSN-1, MiaPaCa-2 and primary PancM pancreatic cancer cells, VE-821, as the first highly selective and potent ATR inhibitor, increased sensitivity to radiation doses of 2, 4 and 6 Gy *in vitro* [93]. VE-821 also induced sensitivity of the pancreatic cancer cells to gemcitabine [93]. Likewise, ATR inhibition by VE-821 enhanced the response to a single dose irradiation of 6 Gy in a PSN-1 tumor xenograft model *in vivo* [94]. A study by Baschnagel *et al.* demonstrated that inhibition of ATR by M6620 radiosensitizes NSCLC brain metastasis patient-derived xenografts [95].

3.1.2. Targeting CHK1

ATR stimulating CHK1 activation protects oncogenic K-Ras-expressing pancreatic cancer cells from DNA damage induced by the
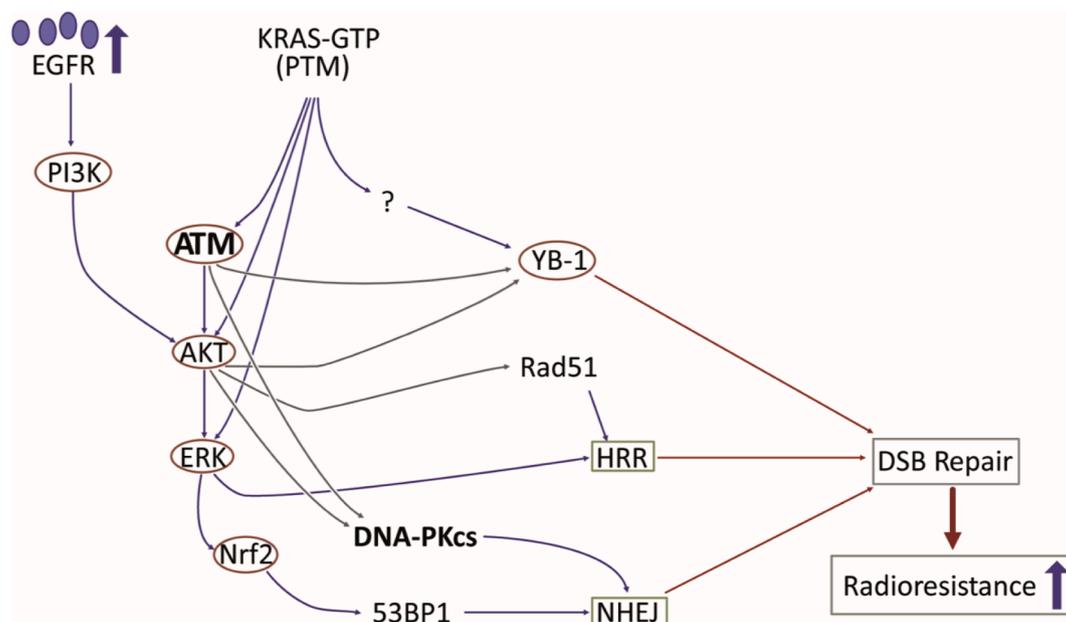
Fig. 2. K-Ras stimulates repair of IR-induced DSB. The PI3K/AKT and MAPK/ERK pathways are the major signaling pathways activated either directly by K-Ras-GTP or downstream to EGFR by K-Ras-dependent autocrine secretion of EGFR ligands. Activation of these two pathways accelerates DSB repair by stimulating key components of the HR and NHEJ repair pathways. For further details, please see the text.

irradiation mimic neocarzinostatin [44], potentially through interference with G2 arrest and successful DSB repair. AZD7762, MK-8776 (SCH 900776) and LY2606368 (prexasertib) are the most studied CHK1 inhibitors that have been investigated in combination with DNA-damaging agents in a variety of tumor entities. In a study by Dinkelborg et al, targeting CHK1 in combination with single doses of irradiation (2, 4, 6, and 8 Gy) resulted in radiosensitization in KRAS mutant/hyperactivated TNBC cells but not in KRAS wild-type non-TNBC cells. [96]. Targeting CHK1 as a potential approach to induce radiosensitization of KRAS-mutated cells has also been demonstrated in tumor cells with frequent KRAS mutations. In pancreatic cancer cells, targeting CHK1 by applying AZD7762 or MK-8776 was shown to improve the effect of chemotherapy agents [97,98], radiation [99,100], and chemoradiation [101] *in vitro* and/or *in vivo*. In KRAS-mutated rectal cancer cells, AZD7762 was shown to be an effective compound to induce radiosensitization after 2 Gy irradiation [102]. Likewise, lung cancer cells could also be radiosensitized by CHK1 inhibitors in preclinical studies *in vitro* and *in vivo* [103,104]. Currently, there are no clinical studies published applying CHK1 inhibitors in combination with chemoradiation in tumors with frequent KRAS mutations. However, based on the results published from preclinical studies, the combination of CHK1 inhibitors in patients with KRAS-mutated tumors may be beneficial and should be tested in a clinical setting. Table 1 presents the clinical trials in which CHK1 targeting was combined with chemotherapy or RT in 3 tumor entities most frequently harboring KRAS mutations, *i.e.*, pancreatic cancer, CRC and lung cancers.

3.1.3. Targeting WEE1

WEE1 is the key kinase and the direct target of CHK1, and its expression is enhanced by KRAS mutation, as shown in pancreatic cancer cells [47]. In irradiated cells, activation of WEE1 stimulates G2/M cell-cycle arrest and, consequently, DSB repair through both HR and NHEJ. Thus, WEE1 inhibition can boost the cells harboring residual DSB through replication, resulting in cell death through mitotic catastrophe. The WEE1 kinase inhibitor AZD1775 (adavosertib) has been investigated in preclinical studies and has shown radio/chemosensitization. In pancreatic cancer cells, as a representative KRAS-mutated tumor entity, targeting WEE1 induced sensitization to radiation with a DER of 1.3 ± 0.1 in MiaPaCa-2 cells and gemcitabine chemoradiation *in vitro* and *in vivo* through inhibition of the HR repair pathway and abrogation of the G2 checkpoint [105,106]. Abrogation of the G2 checkpoint as a potential mechanism of radiosensitization in pancreatic cancer cells has also been shown *in vitro* and *in vivo* through controlling translational regulation of WEE1 and RAD51 by metformin [107]. Furthermore, WEE1 targeting was shown to be more efficient in KRAS-mutant NSCLC expressing mutated TP53 [108], indicating that the G2 checkpoint is the major target of WEE1 inhibitors. The data obtained from clinical trials of the WEE1 kinase inhibitor AZD1775 are promising. In a phase II randomized trial, AZD1775 improved the progression-free survival of CRC patients with KRAS- and TP53-mutated tumors and was well-tolerated [109]. Together, pharmacological inhibitors targeting the components of the ATR/CHK1/WEE1 pathway in 3 tumor entities expressing most KRAS mutations have reached phase II clinical trials. However, except

Table 1

Clinical studies targeting K-Ras and K-Ras-regulated DDR signaling. The studies in combination with RT are marked in bold.

Target	Drug	Trial	Combination	Cancer type	Major findings	Ref.
ATR	M6620	phase I	*GEM*	advanced NSCLC	- well tolerated.	[129]
			+/- CB	ST including CRC	- well tolerated.	[130]
					- complete response in ATM loss CRC	
			TPT	ST including NSCLC and PaCa	- MTD of combination was well tolerated	[131]
					- enhanced DNA DSB in combination	
					- partially active in TPT-non-responding NSCLC	
CHK1	AZD7762	phase I	*GEM*	ST including CRC and lung cancer	- MTD of 21 mg, stable disease	[132]
		phase II	*GEM*	PaCa	- not superior to *GEM*	[133]
	LY26063618	phase II	*GEM* + CDDP	advanced nonsquamous NSCLC	- improved PFS	[134]
					- increased risk of thromboembolism	
			pemetrexed	advanced or metastatic NSCLC	- partial response (9.1 %)	[135]
					- stable disease (36.4 %)	
					- no association between p53 status and response	
WEE1	AZD1775	phase II	**GEM + RT**	**LAPaCa**	**- well tolerated**	[110]
					- improved OS	
			–	RAS and TP53 mutations mCRC	- improved PFS	[109]
FTase	tipifarnib	phase I	–	brainstem glioma	- MTD (125 mg/m^2 twice-daily)	[136]
			RT+/-TMZ	**GBM**	**- MTD (300 mg/m^2 twice-daily)**	[137]
			RT	**GBM**	**- MTD (200 mg/m^2/day)**	[138]
		phase II	**RT**	**brainstem glioma**	**- no clinical advantage**	[115]
			RT	**GBM**	**- no clinical advantage**	[116]
FTase + GGTase-1	L-778,123	phase I	**RT**	**HNSCC and NSCLC**	**- acceptable toxicity**	[118]
					- partial to complete response	
					- radiosensitization of PD cell line	
					- accumulated in G2/M after L-778,123	
			PaCa		**- acceptable toxicity.**	[119]
					- radiosensitization of PD cell line	
KRAS^{G12C}	AMG 510	phase I	–	KRAS ^{G12C} ST	- encouraging anticancer activity	[128]
					- Grade 3 or 4 treatment-related toxic effects occurred in 11.6 %	
		phase II	–	KRAS ^{G12C} NSCLC, previously treated with standard therapies	- durable clinical benefit	[139]
					- partial and complete response in 37.1 %	
					- adverse events in 69.8 %	
				KRAS ^{G12C} CRC, previously treated with standard therapies	- 9.7 % overall response	[140]

ATR: ataxia telangiectasia and Rad3 related, **CB:** carboplatin, **CDDP:** *cis*-diammindichloridoplatin, **CHK1:** checkpoint kinase 1, **CRC:** colorectal cancer, **DSB:** double-strand break, **FTase:** farnesyltransferase**, GBM:** glioblastoma multiforme, *GEM*: gemcitabine, **GGTase-1:** geranylgeranyl transferase type-1, **HNSCC:** head and neck squamous cell carcinoma**, LAPaCa:** locally advanced pancreatic cancer, **mCRC:** metastatic colorectal cancer **MTD:** maximum tolerated dose, **NSCLC:** non-small-cell lung cancer, **PaCa:** pancreatic cancer, **PD:** patient derived, **PFS:** progression-free survival, **OS:** overall survival, **RT:** radiotherapy, **ST:** solid tumors, **TMZ:** temozolomide, **TPT:** topotecan.

for one study, nearly all other studies were in combination with chemotherapy, and combination studies with RT or radiochemotherapy should be conducted. To date, the data of only one study have been published combining the WEE1 inhibitor AZD1775 with gemcitabine and RT in pancreatic cancers [110]. In this study, AZD1775 in combination with gemcitabine and RT was well tolerated, and the overall survival was described to be substantially higher than prior results combining gemcitabine with radiation [110]. The result of this study [110] is of special importance because it targets WEE1, whose expression is expected to be higher in KRAS-mutated tumors than in KRAS wild-type tumors [47]. This difference might be an advantage for targeting WEE1 instead of ATM and CHK2 to block G2/M arrest in terms of tumor specificity and limited normal tissue toxicity issues.

3.2. Direct targeting of KRAS PTMs

Prenylation, palmitoylation/depalmitoylation, phosphorylation, acetylation, nitrosylation, ubiquitination and SUMOylation are the major PTMs of K-Ras [5]. These PTMs regulate K-Ras membrane localization and, consequently, its activity and oncogenic capacity. Thus, inhibitors of PTMs were supposed to be the first approach to overcome the radioresistance of KRAS-mutated tumors. Prenylation by farnesyl-protein transferase as the first step in K-Ras maturation occurs by adding the lipid farnesyl group to the cysteine near the end of the target protein, which is necessary for localization of K-Ras to the plasma membrane [111]. Targeting farnesyltransferase was one of the first approaches proposed to target Ras by applying farnesyltransferase inhibitors (FTIs). Several FTIs, *i.e.*, L-744,832, L-778,123, FTI-276 and R115777 (tipifarnib), have been preclinically investigated in combination with RT. A study by Cohen-Jonathan et al. was one of the first studies to demonstrate that inhibition of farnesyltransferase by L-744,832 and FTI-276 radiosensitizes T24 bladder cancer cell lines expressing HRAS mutations but not the human colon carcinoma cell line HT-29, which expresses wild-type RAS, and both cell lines express TP53 mutations [112]. A separate study from the same group demonstrated that FTI can change the oxygenation of HRAS mutated but not HRAS wild-type tumors [113]. In a further study of L-744,832 in pancreatic cancer cells, it was demonstrated that L-744,832 enhanced the cytotoxic effect of IR, apparently by overriding G2/M checkpoint activation [114]. Although the effect of the studied FTIs on DSB repair has not been investigated in any of these studies, the results confirmed the potential radiosensitizing effect of FTIs in pancreatic cancer in combination with clinically relevant doses of IR. However, cell-cycle regulation by L-744,832 was associated with changes in PTM of H-Ras and *N*-Ras, but not K-Ras [114]. From this study, it could be concluded that FTIs can also interfere with the activation and function of other farnesylated proteins.

Most clinical trials on the combination of FTIs with RT have been conducted in brain tumors (Table 1). Tipifarnib entered a phase II trial in combination with RT of gliomas and glioblastomas [115,116]. Administration of RT did not offer a clinical advantage over historical controls in pontine gliomas [115]. A similar result was observed from the combination of tipifarnib with RT in glioblastomas. The lack of benefit of the combination of tipifarnib with RT in brain tumors is not surprising since only 2 % of glioblastomas harbor RAS mutations [117]. Thus, RAS mutation seems to be a prerequisite for selecting patients for the combination of RT and FTIs. This conclusion is supported by the results of the combination of FTI L-778,123 in NSCLC [118] and pancreatic cancers [119]. Although RAS mutation was not essential for study enrollment, L-778,123 in a phase I trial led to local responses without an increase in RT-associated toxicities in four NSCLC patients [118]. In a study of four pancreatic cancers (3 with KRAS mutation), a combination of L-778,123 and RT at dose level 1 showed acceptable toxicity. In this study, L-778,123 radiosensitized a patient-derived cancer cell line [119].

Since only very limited clinical trials on the combination of FTIs with

RT exist, no solid conclusion can be made on the effectiveness of such a combination. Thus, the combination of FTIs with standard RT is reasonable, and in such a trial, stratification on the basis of mutation or pathway activation would be more informative. Evidence from preclinical studies indicates that in farnesyltransferase-inhibited cells, prenylation of K-Ras can occur by geranylgeranyl transferase type-1 (GGTase-1) [120]. Therefore, the applied FTI should also be able to block GGTase-1 with tolerable toxicity when combined with RT.

3.3. Other approaches to block prosurvival effect of K-Ras protooncogene

Discovering small-molecule inhibitors that bind irreversibly in the switch-II pocket of oncogenic KRAS[G12C] [121,122] accounts for the latest progress in targeting the K-Ras protooncogene. AMG510 (sotorasib) is among those K-Ras[G12C] inhibitors that has been approved by the FDA for the treatment of patients with NSCLC with KRAS[G12C] mutations. To date, most of the studies with AMG510 have been performed on the antitumor activity of the compound as a single treatment or in combination with DNA damage-inducing chemotherapy agents. The mutation-specific cellular activity of AMG510 has been shown across a panel of KRAS[G12C]-mutated cell lines compared to non-KRAS[G12C] lines [123]. AMG510 was shown to selectively target KRAS[G12C] mutated tumors, with a durable tumor regression effect as a monotherapy and with a synergistic effect in combination with chemotherapy and targeted therapy agents [123]. Several clinical trials with AMG510 are enrolling patients for study. The results of previously published trials are summarized in Table 1. The effect of K-Ras[G12C] inhibitors in terms of an objective response in NSCLC and CRC patients is limited, which may be due to the reported resistance to these inhibitors. In this context, the development of resistance to AMG510 has been demonstrated to be due to a variety of different mechanisms. Deregulation of upstream receptor tyrosine kinases, activation of the MAPK/ERK and PI3K/AKT pathways due to mutations in the regulatory components of these pathways, KRAS secondary mutation in codons 12, 13, 61, 68, 95, 96 and amplification of the KRAS[G12C] allele are among those resistance mechanisms [124–127]. Additionally, applying these inhibitors is limited to a small number of tumors with KRAS mutation, *i.e.*, in 13 % of KRAS mutated NSCLCs, in 1 to 3 % of KRAS mutated colorectal cancers and in 1 to 2 % of KRAS mutated pancreatic cancers [128]. Thus, due to the diverse mechanisms of resistance to KRAS[G12C] inhibitors and applicability of this strategy to very limited tumors, there is uncertainty regarding the combination of this strategy with radiotherapy.

Targeting upstream and downstream effectors of K-Ras, *e.g.*, EGFR, PI3K, Raf, AKT and MEK, is not restricted to KRAS-mutated cells. These targeting strategies were not discussed in this review since they have been extensively reviewed by other investigators.

4. Conclusions and future directions

In addition to targeting the components of K-Ras downstream cascades, two major strategies have been used to overcome K-Ras-induced radioresistance, which were reviewed here. Targeting the PTM of K-Ras is the most well-studied approach. In this context, preclinical studies showed that the use of FTI in combination with RT may be an effective approach to radiosensitize KRAS-mutated cells. However, the applied FTIs should also be able to block prenylation of K-Ras not only by blocking farnesyltransferase but also by inhibiting GGTase-1 as an acquired resistance mechanism to FTIs. Due to the lack of benefit of the combination of FTIs with RT of KRAS wild-type tumors, KRAS mutation should be a prerequisite for enrolling patients in future trials.

The role of the protooncogene K-Ras in the DNA-damage response and G2/M cell-cycle arrest has been well described. This function of KRAS may well be of special importance in tumors with TP53 mutations that rely on the G2/M checkpoint for DNA repair after RT, based on the concept of synthetic lethality. The results of early-phase clinical trials applying inhibitors of ATR, CHK1 and WEE1 are promising and suggest

that suppressing G2/M arrest might be the most effective approach to combine with RT in KRAS-mutated tumors.

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.

References

[1] Maertens O, Cichowski K. An expanding role for ras gtpase activating proteins (ras gaps) in cancer. Adv Biol Regul 2014;55:1–14.

[2] Nussinov R, Tsai C-J, Chakrabarti M, Jang H. A new view of ras isoforms in cancers. Cancer Res 2016;76(1):18–23.

[3] Prior IA, Hood FE, Hartley JL. The frequency of ras mutations in cancer. Cancer Res 2020;80(14):2969–74.

[4] Moore AR, Rosenberg SC, McCormick F, Malek S. Ras-targeted therapies: Is the undruggable drugged? Nat Rev Drug Discov 2020;19(8):533–52.

[5] Wang W-H, Yuan T, Qian M-J, Yan F-J, Yang L, He Q-J, et al. Post-translational modification of kras: Potential targets for cancer therapy. Acta Pharmacol Sin 2021;42(8):1201–11.

[6] Hancock JF, Cadwallader K, Marshall CJ. Methylation and proteolysis are essential for efficient membrane binding of prenylated p21k-ras(b). EMBO J 1991;10(3):641–6.

[7] Reuter CW, Morgan MA, Bergmann L. Targeting the ras signaling pathway: A rational, mechanism-based treatment for hematologic malignancies? Blood 2000;96:1655–69.

[8] Lo HW. Nuclear mode of the egfr signaling network: Biology, prognostic value, and therapeutic implications. Discov Med 2010;10:44–51.

[9] Watanabe M, Nobuta A, Tanaka J, Asaka M. An effect of k-ras gene mutation on epidermal growth factor receptor signal transduction in panc-1 pancreatic carcinoma cells. Int J Cancer 1996;67(2):264–8.

[10] Toulany M, Baumann M, Rodemann HP. Stimulated pi3k-akt signaling mediated through ligand or radiation-induced egfr depends indirectly, but not directly, on constitutive k-ras activity. Mol Cancer Res 2007;5(8):863–72.

[11] Toulany M, Minjgee M, Saki M, Holler M, Meier F, Eicheler W, et al. Erk2-dependent reactivation of akt mediates the limited response of tumor cells with constitutive k-ras activity to pi3k inhibition. Cancer Biol Ther 2014;15(3):317–28.

[12] Hobor S, Van Emburgh BO, Crowley E, et al. Tgfalpha and amphiregulin paracrine network promotes resistance to egfr blockade in colorectal cancer cells. Clin Cancer Res 2014;20:6429–38.

[13] Wang M, Kern AM, Hülskötter M, Greninger P, Singh A, Pan Y, et al. Egfr-mediated chromatin condensation protects kras-mutant cancer cells against ionizing radiation. Cancer Res 2014;74(10):2825–34.

[14] Gurtner K, Kryzmien Z, Koi L, Wang M, Benes CH, Hering S, et al. Radioresistance of kras/tp53-mutated lung cancer can be overcome by radiation dose escalation or egfr tyrosine kinase inhibition in vivo. Int J Cancer 2020;147(2):472–7.

[15] Cengel KA, Voong KR, Chandrasekaran S, Maggiorella L, Brunner TB, Stanbridge E, et al. Oncogenic k-ras signals through epidermal growth factor receptor and wild-type h-ras to promote radiation survival in pancreatic and colorectal carcinoma cells. Neoplasia 2007;9(4):341–8.

[16] Khan AQ, Kuttikrishnan S, Siveen KS, Prabhu KS, Shanmugakonar M, Al-Naemi HA, et al. Ras-mediated oncogenic signaling pathways in human malignancies. Semin Cancer Biol 2019;54:1–13.

[17] Caron RW, Yacoub A, Mitchell C, Zhu X, Hong Y, Sasazuki T, et al. Radiation-stimulated erk1/2 and jnk1/2 signaling can promote cell cycle progression in human colon cancer cells. Cell Cycle 2005;4(3):456–64.

[18] Toulany M, Schickfluß T-A, Eicheler W, Kehlbach R, Schittek B, Rodemann HP. Impact of oncogenic k-ras on yb-1 phosphorylation induced by ionizing radiation. Breast Cancer Res 2011;13(2).

[19] Donaubauer EM, Hunzicker-Dunn ME. Extracellular signal-regulated kinase (erk)-dependent phosphorylation of y-box-binding protein 1 (yb-1) enhances gene expression in granulosa cells in response to follicle-stimulating hormone (fsh). J Biol Chem 2016;291(23):12145–60.

[20] Szymonowicz K, Oeck S, Malewicz NM, et al. New insights into protein kinase b/akt signaling: Role of localized akt activation and compartment-specific target proteins for the cellular radiation response. Cancers (Basel) 2018;10.

[21] Toulany M, Rodemann HP. Phosphatidylinositol 3-kinase/akt signaling as a key mediator of tumor cell responsiveness to radiation. Semin Cancer Biol 2015;35:180–90.

[22] Minjgee M, Toulany M, Kehlbach R, Giehl K, Rodemann HP. K-ras(v12) induces autocrine production of egfr ligands and mediates radioresistance through egfr-dependent akt signaling and activation of DNA-pkcs. Int J Radiat Oncol Biol Phys 2011;81(5):1506–14.

[23] Affolter A, Drigotas M, Fruth K, Schmidtmann I, Brochhausen C, Mann WJ, et al. Increased radioresistance via g12s k-ras by compensatory upregulation of mapk and pi3k pathways in epithelial cancer. Head Neck 2013;35(2):220–8.

[24] Bernhard EJ, Stanbridge EJ, Gupta S, et al. Direct evidence for the contribution of activated n-ras and k-ras oncogenes to increased intrinsic radiation resistance in human tumor cell lines. Cancer Res 2000;60:6597–600.

[25] Gillies McKenna W, Muschel RJ, Gupta AK, Hahn SM, Bernhard EJ. The ras signal transduction pathway and its role in radiation sensitivity. Oncogene 2003;22(37):5866–75.

[26] Nature 2014;511:543–50.

[27] Shui L, Li X, Peng Y, Tian J, Li S, He Du, et al. The germline/somatic DNA damage repair gene mutations modulate the therapeutic response in chinese patients with advanced pancreatic ductal adenocarcinoma. J Transl Med 2021;19(1).

[28] Philip PA, Azar I, Xiu J, et al. Molecular characterization of kras wild-type tumors in patients with pancreatic adenocarcinoma. Clin Cancer Res 2022;28:2704-2714.

[29] Duldulao MP, Lee W, Nelson RA, Li W, Chen Z, Kim J, et al. Mutations in specific codons of the kras oncogene are associated with variable resistance to neoadjuvant chemoradiation therapy in patients with rectal adenocarcinoma. Ann Surg Oncol 2013;20(7):2166–71.

[30] Yang J, Lin Y, Huang Y, Jin J, Zou S, Zhang X, et al. Genome landscapes of rectal cancer before and after preoperative chemoradiotherapy. Theranostics 2019;9(23):6856–66.

[31] Wegman P, Ahlin C, Sorbe B. Genetic alterations in the k-ras gene influence the prognosis in patients with cervical cancer treated by radiotherapy. Int J Gynecol Cancer 2011;21(1):86–91.

[32] Zhu DQ, Liu Y, Yu ZJ, Zhang RH, Li AW, Gong FY, et al. The diverse analysis identifies mutated kras associated with radioresistance in non-small cell lung cancer. World J Oncol 2022;13(2):84–95.

[33] Mak RH, Hermann G, Lewis JH, et al. Outcomes by tumor histology and kras mutation status after lung stereotactic body radiation therapy for early-stage non-small-cell lung cancer. Clin Lung Cancer 2015;16:24–32.

[34] Graves PR, Din SU, Ashamalla M, Ashamalla H, Gilbert TSK, Graves LM. Ionizing radiation induces epha2 s897 phosphorylation in a mek/erk/rsk-dependent manner. Int J Radiat Biol 2017;93(9):929–36.

[35] Toulany M, Dittmann K, Baumann M, Rodemann HP. Radiosensitization of ras-mutated human tumor cells in vitro by the specific egf receptor antagonist bibx1382bs. Radiother Oncol 2005;74(2):117–29.

[36] Torii S, Yamamoto T, Tsuchiya Y, et al. Erk map kinase in g cell cycle progression and cancer. Cancer Sci 2006;97:697–702.

[37] Tong W, Pollard JW. Genetic evidence for the interactions of cyclin d1 and p27 (kip1) in mice. Mol Cell Biol 2001;21(4):1319–28.

[38] Cardozo T, Pagano M. The scf ubiquitin ligase: Insights into a molecular machine. Nat Rev Mol Cell Biol 2004;5(9):739–51.

[39] Meloche S, Pouysségur J. The erk1/2 mitogen-activated protein kinase pathway as a master regulator of the g1- to s-phase transition. Oncogene 2007;26(22):3227–39.

[40] Botta GP, Reichert M, Reginato MJ, Heeg S, Rustgi AK, Lelkes PI. Erk2-regulated timp1 induces hyperproliferation of k-ras(g12d)-transformed pancreatic ductal cells. Neoplasia 2013;15(4):359–IN1.

[41] Yamamoto T, Ebisuya M, Ashida F, Okamoto K, Yonehara S, Nishida E. Continuous erk activation downregulates antiproliferative genes throughout g1 phase to allow cell-cycle progression. Curr Biol 2006;16(12):1171–82.

[42] Network CGAR. Integrated genomic characterization of pancreatic ductal adenocarcinoma. Cancer Cell 2017;32(185–203):e13.

[43] Wang Y, Wang Z, Piha-Paul S, Janku F, Subbiah V, Shi N, et al. Outcome analysis of phase i trial patients with metastatic kras and/or tp53 mutant non-small cell lung cancer. Oncotarget 2018;9(70):33258–70.

[44] Klomp JE, Lee YS, Goodwin CM, Papke B, Klomp JA, Waters AM, et al. Chk1 protects oncogenic kras-expressing cells from DNA damage and is a target for pancreatic cancer treatment. Cell Rep 2021;37(9).

[45] Wallez Y, Dunlop CR, Johnson TI, et al. The atr inhibitor azd6738 synergizes with gemcitabine in vitro and in vivo to induce pancreatic ductal adenocarcinoma regression. Mol Cancer Ther 2018;17:1670–82.

[46] Schreiber FS, Deramaudt TB, Brunner TB, Boretti MI, Gooch KJ, Stoffers DA, et al. Successful growth and characterization of mouse pancreatic ductal cells: Functional properties of the ki-ras(g12v) oncogene. Gastroenterology 2004;127(1):250–60.

[47] Diehl JN, Klomp JE, Snare KR, Hibshman PS, Blake DR, Kaiser ZD, et al. The kras-regulated kinome identifies wee1 and erk coinhibition as a potential therapeutic strategy in kras-mutant pancreatic cancer. J Biol Chem 2021;297(5).

[48] Ghelli Luserna di Rora' A, Iacobucci I, Martinelli G. The cell cycle checkpoint inhibitors in the treatment of leukemias. J Hematol Oncol 2017;10(1).

[49] PosthumaDeBoer J, Würdinger T, Graat HCA, van Beusechem VW, Helder MN, van Royen BJ, et al. Wee1 inhibition sensitizes osteosarcoma to radiotherapy. BMC Cancer 2011;11(1).

[50] De Witt Hamer PC, Mir SE, Noske D, Van Noorden CJF, Würdinger T. Wee1 kinase targeting combined with DNA-damaging cancer therapy catalyzes mitotic catastrophe. Clin Cancer Res 2011;17(13):4200–7.

[51] Li J, Wang Y, Sun Yi, Lawrence TS. Wild-type tp53 inhibits g(2)-phase checkpoint abrogation and radiosensitization induced by pd0166285, a wee1 kinase inhibitor. Radiat Res 2002;157(3):322–30.

[52] Hirai H, Iwasawa Y, Okada M, Arai T, Nishibata T, Kobayashi M, et al. Small-molecule inhibition of wee1 kinase by mk-1775 selectively sensitizes p53-deficient tumor cells to DNA-damaging agents. Mol Cancer Ther 2009;8(11):2992–3000.

[53] Willems E, Dedobbeleer M, Digregorio M, Lombard A, Lumapat PN, Rogister B. The functional diversity of aurora kinases: A comprehensive review. Cell Div 2018;13(1).

[54] Gomes-Filho SM, dos Santos EO, Bertoldi ERM, Scalabrini LC, Heidrich V, Dazzani B, et al. Aurora a kinase and its activator tpx2 are potential therapeutic targets in kras-induced pancreatic cancer. Cell Oncol (Dordr) 2020;43(3):445–60.

[55] Liu JB, Hu L, Yang Z, et al. Aurora-a/nf-kb signaling is associated with radio-resistance in human lung adenocarcinoma. Anticancer Res 2019;39:5991–8.

[56] Shen ZT, Chen Y, Huang GC, et al. Aurora-a confers radioresistance in human hepatocellular carcinoma by activating nf-kappab signaling pathway. BMC Cancer 2019;19:1075.

[57] Li M, Liu H, Zhao Q, Han S, Zhou Li, Liu W, et al. Targeting aurora b kinase with tanshinone iia suppresses tumor growth and overcomes radioresistance. Cell Death Dis 2021;12(2).

[58] Ma Y, Yang J, Wang R, Zhang Z, Qi X, Liu C, et al. Aurora-a affects radiosentivity in cervical squamous cell carcinoma and predicts poor prognosis. Oncotarget 2017;8(19):31509–20.

[59] Tamotsu K, Okumura H, Uchikado Y, Kita Y, Sasaki K, Omoto I, et al. Correlation of aurora-a expression with the effect of chemoradiation therapy on esophageal squamous cell carcinoma. BMC Cancer 2015;15(1).

[60] Barton VN, Foreman NK, Donson AM, Birks DK, Handler MH, Vibhakar R. Aurora kinase a as a rational target for therapy in glioblastoma. J Neurosurg Pediatr 2010;6(1):98–105.

[61] Kim IA, Bae SS, Fernandes A, et al. Selective inhibition of ras, phosphoinositide 3 kinase, and akt isoforms increases the radiosensitivity of human carcinoma cell lines. Cancer Res 2005;65:7902–10.

[62] Schultz LB, Chehab NH, Malikzay A, Halazonetis TD. P53 binding protein 1 (53bp1) is an early participant in the cellular response to DNA double-strand breaks. J Cell Biol 2000;151(7):1381–90.

[63] Bartek J, Lukas J. DNA repair: Damage alert. Nature 2003;421(6922):486–8.

[64] Kobayashi D, Oike T, Shibata A, Niimi A, Kubota Y, Sakai M, et al. Mitotic catastrophe is a putative mechanism underlying the weak correlation between sensitivity to carbon ions and cisplatin. Sci Rep 2017;7(1).

[65] Shang Z-F, Huang Bo, Xu Q-Z, Zhang S-M, Fan R, Liu X-D, et al. Inactivation of DNA-dependent protein kinase leads to spindle disruption and mitotic catastrophe with attenuated checkpoint protein 2 phosphorylation in response to DNA damage. Cancer Res 2010;70(9):3657–66.

[66] Drosos Y, Escobar D, Chiang M-Y, Roys K, Valentine V, Valentine MB, et al. Atm-deficiency increases genomic instability and metastatic potential in a mouse model of pancreatic cancer. Sci Rep 2017;7(1).

[67] Heo J-I, Oh S-J, Kho Y-J, Kim J-H, Kang H-J, Park S-H, et al. Atm mediates interdependent activation of p53 and erk through formation of a ternary complex with p-p53 and p-erk in response to DNA damage. Mol Biol Rep 2012;39(8):8007–14.

[68] Szymonowicz K, Oeck S, Krysztofiak A, van der Linden J, Iliakis G, Jendrossek V. Restraining akt1 phosphorylation attenuates the repair of radiation-induced DNA double-strand breaks and reduces the survival of irradiated cancer cells. Int J Mol Sci 2018;19(8):2233.

[69] Golding SE, Rosenberg E, Neill S, Dent P, Povirk LF, Valerie K. Extracellular signal-related kinase positively regulates ataxia telangiectasia mutated, homologous recombination repair, and the DNA damage response. Cancer Res 2007;67(3):1046–53.

[70] Hawkins AJ, Golding SE, Khalil A, Valerie K. DNA double-strand break - induced pro-survival signaling. Radiother Oncol 2011;101(1):13–7.

[71] Toulany M, Kehlbach R, Florczak U, Sak A, Wang S, Chen J, et al. Targeting of akt1 enhances radiation toxicity of human tumor cells by inhibiting DNA-pkcs-dependent DNA double-strand break repair. Mol Cancer Ther 2008;7(7):1772–81.

[72] Toulany M, Lee K-J, Fattah KR, Lin Y-F, Fehrenbacher B, Schaller M, et al. Akt promotes post-irradiation survival of human tumor cells through initiation, progression, and termination of DNA-pkcs-dependent DNA double-strand break repair. Mol Cancer Res 2012;10(7):945–57.

[73] Toulany M, Maier J, Iida M, Rebholz S, Holler M, Grottke A, et al. Akt1 and akt3 but not akt2 through interaction with DNA-pkcs stimulate proliferation and post-irradiation cell survival of k-ras-mutated cancer cells. Cell Death Discov 2017;3(1).

[74] Schötz U, Balzer V, Brandt F-W, Ziemann F, Subtil FSB, Rieckmann T, et al. Dual pi3k/mtor inhibitor nvp-bez235 enhances radiosensitivity of head and neck squamous cell carcinoma (hnscc) cell lines due to suppressed double-strand break (dsb) repair by non-homologous end joining. Cancers (Basel) 2020;12(2):467.

[75] Kang KB, Zhu C, Wong YL, Gao Q, Ty A, Wong MC. Gefitinib radiosensitizes stem-like glioma cells: Inhibition of epidermal growth factor receptor-akt-DNA-pk signaling, accompanied by inhibition of DNA double-strand break repair. Int J Radiat Oncol Biol Phys 2012;83(1):e43–52.

[76] Smida M, Fece de la Cruz F, Kerzendorfer C, Uras IZ, Mair B, Mazouzi A, et al. Mek inhibitors block growth of lung tumours with mutations in ataxia-telangiectasia mutated. Nat Commun 2016;7(1).

[77] Estrada-Bernal A, Chatterjee M, Haque SJ, Yang L, Morgan MA, Kotian S, et al. Mek inhibitor gsk1120212-mediated radiosensitization of pancreatic cancer cells involves inhibition of DNA double-strand break repair pathways. Cell Cycle 2015;14(23):3713–24.

[78] Yang H, Xiang S, Kazi A, Sebti SM. The gtpase kras suppresses the p53 tumor suppressor by activating the nrf2-regulated antioxidant defense system in cancer cells. J Biol Chem 2020;295(10):3055–63.

[79] Yang L, Shen C, Estrada-Bernal A, Robb R, Chatterjee M, Sebastian N, et al. Oncogenic kras drives radioresistance through upregulation of nrf2-53bp1-mediated non-homologous end-joining repair. Nucleic Acids Res 2021;49(19):11067–82.

[80] Mueck K, Rebholz S, Harati M, Rodemann H, Toulany M. Akt1 stimulates homologous recombination repair of DNA double-strand breaks in a rad51-dependent manner. Int J Mol Sci 2017;18(11):2473.

[81] Hu J, Zhang Z, Zhao L, Li Li, Zuo W, Han L. High expression of rad51 promotes DNA damage repair and survival in kras-mutant lung cancer cells. BMB Rep 2019;52(2):151–6.

[82] Kosnopfel C, Sinnberg T, Schittek B. Y-box binding protein 1–a prognostic marker and target in tumour therapy. Eur J Cell Biol 2014;93(1-2):61–70.

[83] Lettau K, Zips D, Toulany M. Simultaneous targeting of rsk and akt efficiently inhibits yb-1-mediated repair of ionizing radiation-induced DNA double-strand breaks in breast cancer cells. Int J Radiat Oncol Biol Phys 2021;109(2):567–80.

[84] Lasham A, Print C, Woolley A, Dunn S, Braithwaite A. Yb-1: Oncoprotein, prognostic marker and therapeutic target? Biochem J 2013;449(1):11–23.

[85] Maier E, Attenberger F, Tiwari A, et al. Dual targeting of y-box binding protein-1 and akt inhibits proliferation and enhances the chemosensitivity of colorectal cancer cells. Cancers (Basel) 2019;11(4):562.

[86] Lettau K, Khozooei S, Kosnopfel C, Zips D, Schittek B, Toulany M. Targeting the y-box binding protein-1 axis to overcome radiochemotherapy resistance in solid tumors. Int J Radiat Oncol Biol Phys 2021;111(4):1072–87.

[87] Kim A, Shim S, Kim YH, et al. Inhibition of y box binding protein 1 suppresses cell growth and motility in colorectal cancer. Mol Cancer Ther 2020;19:479–89.

[88] Khozooei S, Lettau K, Barletta F, Jost T, Rebholz S, Veerappan S, et al. Fisetin induces DNA double-strand break and interferes with the repair of radiation-induced damage to radiosensitize triple negative breast cancer cells. J Exp Clin Cancer Res 2022;41(1).

[89] Pilié PG, Tang C, Mills GB, Yap TA. State-of-the-art strategies for targeting the DNA damage response in cancer. Nat Rev Clin Oncol 2019;16(2):81–104.

[90] Zenke FT, Zimmermann A, Sirrenberg C, Dahmen H, Kirkin V, Pehl U, et al. Pharmacologic inhibitor of DNA-pk, m3814, potentiates radiotherapy and regresses human tumors in mouse models. Mol Cancer Ther 2020;19(5):1091–101.

[91] Zhang Y, Lai J, Du Z, Gao J, Yang S, Gorityala S, et al. Targeting radioresistant breast cancer cells by single agent chk1 inhibitor via enhancing replication stress. Oncotarget 2016;7(23):34688–702.

[92] Gill SJ, Wijnhoven PWG, Fok JHL, et al. Radiopotentiation profiling of multiple inhibitors of the DNA damage response for early clinical development. Mol Cancer Ther 2021;20:1614–26.

[93] Prevo R, Fokas E, Reaper PM, Charlton PA, Pollard JR, McKenna WG, et al. The novel atr inhibitor ve-821 increases sensitivity of pancreatic cancer cells to radiation and chemotherapy. Cancer Biol Ther 2012;13(11):1072–81.

[94] Fokas E, Prevo R, Pollard JR, Reaper PM, Charlton PA, Cornelissen B, et al. Targeting atr in vivo using the novel inhibitor ve-822 results in selective sensitization of pancreatic tumors to radiation. Cell Death Dis 2012;3(12).

[95] Baschnagel AM, Elnaggar JH, VanBeek HJ, Kromke AC, Skiba JH, Kaushik S, et al. Atr inhibitor m6620 (vx–970) enhances the effect of radiation in non-small cell lung cancer brain metastasis patient-derived xenografts. Mol Cancer Ther 2021;20(11):2129–9.

[96] Dinkelborg PH, Wang M, Gheorghiu L, Gurski JM, Hong TS, Benes CH, et al. A common chk1-dependent phenotype of DNA double-strand break suppression in two distinct radioresistant cancer types. Breast Cancer Res Treat 2019;174(3):605–13.

[97] Venkatesha VA, Parsels LA, Parsels JD, Zhao L, Zabludoff SD, Simeone DM, et al. Sensitization of pancreatic cancer stem cells to gemcitabine by chk1 inhibition. Neoplasia 2012;14(6):519–25.

[98] Parsels LA, Parsels JD, Tanska DM, et al. The contribution of DNA replication stress marked by high-intensity, pan-nuclear gammah2ax staining to chemosensitization by chk1 and wee1 inhibitors. Cell Cycle 2018;17:1076–86.

[99] Vance S, Liu E, Zhao L, Parsels JD, Parsels LA, Brown JL, et al. Selective radiosensitization of p53 mutant pancreatic cancer cells by combined inhibition of chk1 and parp1. Cell Cycle 2011;10(24):4321–9.

[100] Morgan MA, Parsels LA, Zhao L, Parsels JD, Davis MA, Hassan MC, et al. Mechanism of radiosensitization by the chk1/2 inhibitor azd7762 involves abrogation of the g2 checkpoint and inhibition of homologous recombinational DNA repair. Cancer Res 2010;70(12):4972–81.

[101] Engelke CG, Parsels LA, Qian Y, Zhang Q, Karnak D, Robertson JR, et al. Sensitization of pancreatic cancer to chemoradiation by the chk1 inhibitor mk8776. Clin Cancer Res 2013;19(16):4412–21.

[102] Kleiman LB, Krebs AM, Kim SY, Hong TS, Haigis KM, Xie J. Comparative analysis of radiosensitizers for k-ras mutant rectal cancers. PLoS ONE 2013;8(12):e82982.

[103] Grabauskiene S, Bergeron EJ, Chen G, Thomas DG, Giordano TJ, Beer DG, et al. Checkpoint kinase 1 protein expression indicates sensitization to therapy by

checkpoint kinase 1 inhibition in non-small cell lung cancer. J Surg Res 2014;187 (1):6–13.

[104] Yang H, Yoon SJ, Jin J, Choi SH, Seol HJ, Lee J-I, et al. Inhibition of checkpoint kinase 1 sensitizes lung cancer brain metastases to radiotherapy. Biochem Biophys Res Commun 2011;406(1):53–8.

[105] Karnak D, Engelke CG, Parsels LA, et al. Combined inhibition of wee1 and parp1/2 for radiosensitization in pancreatic cancer. Clin Cancer Res 2014;20:5085–96.

[106] Kausar T, Schreiber JS, Karnak D, Parsels LA, Parsels JD, Davis MA, et al. Sensitization of pancreatic cancers to gemcitabine chemoradiation by wee1 kinase inhibition depends on homologous recombination repair. Neoplasia 2015; 17(10):757–66.

[107] Wang Z, Lai S-T, Ma N-Y, Deng Y, Liu Y, Wei D-P, et al. Radiosensitization of metformin in pancreatic cancer cells via abrogating the g2 checkpoint and inhibiting DNA damage repair. Cancer Lett 2015;369(1):192–201.

[108] Ku BM, Bae Y-H, Koh J, Sun J-M, Lee S-H, Ahn JS, et al. Mutational status of tp53 defines the efficacy of wee1 inhibitor azd1775 in kras-mutant non-small cell lung cancer. Oncotarget 2017;8(40):67526–37.

[109] Seligmann JF, Fisher DJ, Brown LC, Adams RA, Graham J, Quirke P, et al. Inhibition of wee1 is effective in tp53- and ras-mutant metastatic colorectal cancer: A randomized trial (focus4-c) comparing adavosertib (azd1775) with active monitoring. J Clin Oncol 2021;39(33):3705–15.

[110] Cuneo KC, Morgan MA, Sahai V, Schipper MJ, Parsels LA, Parsels JD, 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 2019;37(29):2643–50.

[111] Cox AD. Farnesyltransferase inhibitors: Potential role in the treatment of cancer. Drugs 2001;61(6):723–32.

[112] Cohen-Jonathan E, Muschel RJ, Gillies McKenna W, et al. Farnesyltransferase inhibitors potentiate the antitumor effect of radiation on a human tumor xenograft expressing activated hras. Radiat Res 2000;154:125–32.

[113] Cohen-Jonathan E, Evans SM, Koch CJ, et al. The farnesyltransferase inhibitor l744,832 reduces hypoxia in tumors expressing activated h-ras. Cancer Res 2001; 61:2289–93.

[114] Song SY, Meszoely IM, Coffey RJ, Pietenpo JA, Leach SD. K-ras-independent effects of the farnesyl transferase inhibitor l-744,832 on cyclin b1/cdc2 kinase activity, g2/m cell cycle progression and apoptosis in human pancreatic ductal adenocarcinoma cells. Neoplasia 2000;2(3):261–72.

[115] Haas-Kogan DA, Banerjee A, Poussaint TY, Kocak M, Prados MD, Geyer JR, et al. Phase ii trial of tipifarnib and radiation in children with newly diagnosed diffuse intrinsic pontine gliomas. Neuro Oncol 2011;13(3):298–306.

[116] Lustig R, Mikkelsen T, Lesser G, Grossman S, Ye X, Desideri S, et al. Phase ii preradiation r115777 (tipifarnib) in newly diagnosed gbm with residual enhancing disease. Neuro Oncol 2008;10(6):1004–9.

[117] Cancer Genome Atlas Research N. Comprehensive genomic characterization defines human glioblastoma genes and core pathways. Nature 2008;455:1061–8.

[118] Hahn SM, Bernhard EJ, Regine W, et al. A phase i trial of the farnesyltransferase inhibitor l-778,123 and radiotherapy for locally advanced lung and head and neck cancer. Clin Cancer Res 2002;8:1065–72.

[119] Martin NE, Brunner TB, Kiel KD, DeLaney TF, Regine WF, Mohiuddin M, et al. A phase i trial of the dual farnesyltransferase and geranylgeranyltransferase inhibitor l-778,123 and radiotherapy for locally advanced pancreatic cancer. Clin Cancer Res 2004;10(16):5447–54.

[120] Whyte DB, Kirschmeier P, Hockenberry TN, Nunez-Oliva I, James L, Catino JJ, et al. K- and n-ras are geranylgeranylated in cells treated with farnesyl protein transferase inhibitors. J Biol Chem 1997;272(22):14459–64.

[121] Ostrem JM, Peters U, Sos ML, Wells JA, Shokat KM. K-ras(g12c) inhibitors allosterically control gtp affinity and effector interactions. Nature 2013;503 (7477):548–51.

[122] Ostrem JML, Shokat KM. Direct small-molecule inhibitors of kras: From structural insights to mechanism-based design. Nat Rev Drug Discov 2016;15(11):771–85.

[123] Canon J, Rex K, Saiki AY, Mohr C, Cooke K, Bagal D, et al. The clinical kras(g12c) inhibitor amg 510 drives anti-tumour immunity. Nature 2019;575(7781):217–23.

[124] Basaki Y, Taguchi K-I, Izumi H, Murakami Y, Kubo T, Hosoi F, et al. Y-box binding protein-1 (yb-1) promotes cell cycle progression through cdc6-dependent pathway in human cancer cells. Eur J Cancer 2010;46(5):954–65.

[125] Koga T, Suda K, Fujino T, et al. Kras secondary mutations that confer acquired resistance to kras g12c inhibitors, sotorasib and adagrasib, and overcoming strategies: Insights from in vitro experiments. J Thorac Oncol 2021;16:1321–32.

[126] Misale S, Fatherree JP, Cortez E, et al. Kras g12c nsclc models are sensitive to direct targeting of kras in combination with pi3k inhibition. Clin Cancer Res 2019;25:796–807.

[127] Ryan MB, Fece de la Cruz F, Phat S, et al. Vertical pathway inhibition overcomes adaptive feedback resistance to kras(g12c) inhibition. Clin Cancer Res 2020;26: 1633–43.

[128] Hong DS, Fakih MG, Strickler JH, Desai J, Durm GA, Shapiro GI, et al. Kras(g12c) inhibition with sotorasib in advanced solid tumors. N Engl J Med 2020;383(13): 1207–17.

[129] Plummer R, Dean E, Arkenau H-T, Redfern C, Spira AI, Melear JM, et al. A phase 1b study evaluating the safety and preliminary efficacy of berzosertib in combination with gemcitabine in patients with advanced non-small cell lung cancer. Lung Cancer 2022;163:19–26.

[130] Yap TA, O'Carrigan B, Penney MS, Lim JS, Brown JS, de Miguel Luken MJ, et al. Phase i trial of first-in-class atr inhibitor m6620 (vx–970) as monotherapy or in combination with carboplatin in patients with advanced solid tumors. J Clin Oncol 2020;38(27):3195–204.

[131] Thomas A, Redon CE, Sciuto L, Padiernos E, Ji J, Lee M-J, et al. Phase i study of atr inhibitor m6620 in combination with topotecan in patients with advanced solid tumors. J Clin Oncol 2018;36(16):1594–602.

[132] Seto T, Esaki T, Hirai F, Arita S, Nosaki K, Makiyama A, et al. Phase i, dose-escalation study of azd7762 alone and in combination with gemcitabine in japanese patients with advanced solid tumours. Cancer Chemother Pharmacol 2013;72(3):619–27.

[133] Laquente B, Lopez-Martin J, Richards D, Illerhaus G, Chang DZ, Kim G, et al. A phase ii study to evaluate ly2603618 in combination with gemcitabine in pancreatic cancer patients. BMC Cancer 2017;17(1).

[134] Wehler T, Thomas M, Schumann C, Bosch-Barrera J, Viñolas Segarra N, Dickgreber NJ, et al. A randomized, phase 2 evaluation of the chk1 inhibitor, ly2603618, administered in combination with pemetrexed and cisplatin in patients with advanced nonsquamous non-small cell lung cancer. Lung Cancer 2017;108:212–6.

[135] Scagliotti G, Kang JH, Smith D, Rosenberg R, Park K, Kim S-W, et al. Phase ii evaluation of ly2603618, a first-generation chk1 inhibitor, in combination with pemetrexed in patients with advanced or metastatic non-small cell lung cancer. Invest New Drugs 2016;34(5):625–35.

[136] Haas-Kogan DA, Banerjee A, Kocak M, Prados MD, Geyer JR, Fouladi M, et al. Phase i trial of tipifarnib in children with newly diagnosed intrinsic diffuse brainstem glioma. Neuro Oncol 2008;10(3):341–7.

[137] Nghiemphu PL, Wen PY, Lamborn KR, Drappatz J, Robins HI, Fink K, et al. A phase i trial of tipifarnib with radiation therapy, with and without temozolomide, for patients with newly diagnosed glioblastoma. Int J Radiat Oncol Biol Phys 2011;81(5):1422–7.

[138] Cohen-Jonathan Moyal E, Laprie A, Delannes M, Poublanc M, Catalaa I, Dalenc F, et al. Phase i trial of tipifarnib (r115777) concurrent with radiotherapy in patients with glioblastoma multiforme. Int J Radiat Oncol Biol Phys 2007;68(5): 1396–401.

[139] Skoulidis F, Li BT, Dy GK, Price TJ, Falchook GS, Wolf J, et al. Sotorasib for lung cancers with kras p. G12c mutation. N Engl J Med 2021;384(25):2371–81.

[140] Fakih MG, Kopetz S, Kuboki Y, Kim TW, Munster PN, Krauss JC, et al. Sotorasib for previously treated colorectal cancers with kras(g12c) mutation (codebreak100): A prespecified analysis of a single-arm, phase 2 trial. Lancet Oncol 2022;23(1):115–24.