Review Article

Indian J Med Res 143, February 2016, pp 145-159
DOI:10.4103/0971-5916.180201

Targeting the AKT pathway: Repositioning HIV protease inhibitors as radiosensitizers

Jayant S. Goda*, Tejaswini Pachpor*, Trinanjan Basu*, Supriya Chopra* & Vikram Gota**

*Department of Radiation Oncology, Clinical Biology Laboratory, Department of Radiation Oncology & **Clinical Pharmacology Laboratory, Advance Centre for Treatment Research & Education in Cancer, Tata Memorial Center, Navi Mumbai, India

Received February 20, 2014

Cellular resistance in tumour cells to different therapeutic approaches has been a limiting factor in the curative treatment of cancer. Resistance to therapeutic radiation is a common phenomenon which significantly reduces treatment options and impacts survival. One of the mechanisms of acquiring resistance to ionizing radiation is the overexpression or activation of various oncogenes like the *EGFR* (epidermal growth factor receptor), *RAS* (rat sarcoma) oncogene or loss of *PTEN* (phosphatase and tensin homologue) which in turn activates the phosphatidyl inositol 3-kinase/protein kinase B (PI3-K)/ AKT pathway responsible for radiation resistance in various tumours. Blocking the pathway enhances the radiation response both *in vitro* and *in vivo*. Due to the differential activation of this pathway (constitutively activated in tumour cells and not in the normal host cells), it is an excellent candidate target for molecular targeted therapy to enhance radiation sensitivity. In this regard, HIV protease inhibitors (HPIs) known to interfere with PI3-K/AKT signaling in tumour cells, have been shown to sensitize various tumour cells to radiation both *in vitro* and *in vivo*. As a result, HPIs are now being investigated as possible radiosensitizers along with various chemotherapeutic drugs. This review describes the mechanisms by which PI3-K/AKT pathway causes radioresistance and the role of HIV protease inhibitors especially nelfinavir as a potential candidate drug to target the AKT pathway for overcoming radioresistance and its use in various clinical trials for different malignancies.

Key words Clinical trials - HIV protease - inhibitors - nelfinavir - radiosensitizer

Introduction

Radioresistance and chemoresistance are important contributing factors towards the failure of tumour cell kill and subsequent eradication of tumours. Strategies to overcome radioresistance or enhance radiation sensitivity include classically altering the radiation fractionation wherein a higher radiation dose is given to the tumour to overcome intrinsic radioresistance (hyperfractionation) or compensate for the tumour repopulation by reducing the overall treatment time (accelerated fractionation)[1-5]. A second approach is to use a combination of chemotherapy with radiotherapy, in particular concurrent chemoradiotherapy[6,7]. This approach has shown benefit in numerous solid cancers especially in head and neck and cervical carcinoma[7,8].

A third approach to overcome radioresistance is to modulate hypoxia in the tumour cells. This approach has been particularly useful in head and neck cancers where intrinsic hypoxia is a major factor contributing to tumour cell radioresistance. Trials using hypoxic sensitizers such as nitroimidazoles and hypoxic cytotoxins have been published[9-12]. Another promising approach is the use of targeted therapy concurrently with radiation, to enhance the efficacy of radiation, *e.g.*, epidermal growth factor receptor (EGFR) inhibitors like cetuximab in head and neck cancer[13,14], gefitinib, erlotinib and afatinib in lung cancer[15-18], and vascular endothelial growth factor (VEGF) inhibitor, bevacizumab in colon cancer[19,20]. The advantage of targeted therapy is that these have a reasonably high therapeutic ratio although drug specific toxicity may occur. In this respect targeting the phosphatidyl inositol 3-kinase/protein kinase B (PI3-K/AKT) signal transduction pathway considered to be a major pathway in radiation resistance may enhance the radiosensitivity of tumours[21,22]. The PI3-K/AKT pathway is overexpressed in a variety of tumours (Table I). Since this pathway is constitutively overexpressed in tumour cells, sparing the normal cells makes it an excellent target for enhancing the radiosensitivity.

Though the development in the field of targeted pharmacotherapy is ongoing, the process of developing novel agents that would block the PI3-K/AKT pathway and bringing these into the clinic as interventional agents is a relatively tardy process especially when starting from novel compounds not previously tested in humans. In contrast, drugs or agents which are already in clinical practice for other diseases could be used as molecular targeting agents for anti-cancer therapy and adopted in clinics after testing them in thoroughly designed clinical trials thereby avoiding any delay in the process of drug development. Currently, such off-label use of drugs is being followed with anti-retroviral [human immunodeficiency virus

Table I. Expression of AKT in various cancers

Type of tumour	% Tumours with active AKT	Predominant AKT isoform overexpressed	Reference
Thyroid carcinoma	80–100	AKT1 & AKT 2	23, 24
Anaplastic large-cell lymphoma	100	AKT2	25
Multiple myeloma	~90	Pan AKT	26
Bile duct carcinoma	~85	Pan AKT	27
Gastric carcinoma	~80	Pan AKT	28
Malignant mesothelioma	~80	-	29
Acute myeloid leukaemia	~70	Pan AKT	30
Gliomas	60	Pan AKT	31, 32
Small-cell lung carcinoma	~60	Pan AKT	33
Colorectal cancer	57%	AKT2	34
Head and neck carcinoma	35-92	Pan AKT	35, 36
Bronchial dysplasia & non-small-cell lung carcinoma	30-75	Pan AKT	37, 38
Ovarian carcinoma	40-70	AKT2	39-41
Pancreatic carcinoma	30-70	AKT2	42
Malignant melanoma	43-67	AKT3	43
Prostate carcinoma	45-55	AKT1	44
Renal cell carcinoma	~4	-	45
Hepatocellular carcinoma	~40	AKT2	46
Endometrial carcinoma	35	-	47
Gastrointestinal stromal tumours	~30	-	48
Carcinoma cervix	-	Pan-AKT	49
Pituitary adenoma	55- 65	AKT1 & AKT2	50, 51

(HIV) protease inhibitors, HPI's] drugs that inhibit AKT phosphorylation as candidates for not only anti-cancer therapy, but also for developing these agents as radiosensitizers. These compounds have been used as anti-HIV drugs in the clinics for the past decade and their safety profile is well documented in the literature. However, their use in combination with other cytotoxic therapies like radiation therapy (RT) and chemotherapy (CT) is under intense investigation.

The aim of the review was to collect available *in vitro*/*in vivo* data and data from clinical trials related to HIV protease inhibitors as radiosensitizers, and evaluate the role of HPI's, particularly nelfinavir, as a potential candidate drug as a radio sensitizer.

PI3-K/AKT signaling pathway and radiation resistance

Cancer cells have a tendency to acquire resistance to radio/chemotherapy[52-54]. The relevance of the PI3-K/AKT signal-transduction pathway has been shown in radioresistance[52]. One of the factors responsible for resistance to therapy is overexpression/activation of oncogenes (*e.g.* EGFR, RAS) and loss of tumour suppressor gene (*e.g.* PTEN)[55-61]. These molecular alterations ultimately lead to activation of PI3-K/AKT pathway which regulates important mechanisms of cellular radioresistance.

Akt activation and events leading to DNA damage repair: Studies have shown EGFR and RAS activation to be a major contributor to tumour radioresistance which in turn activates the PI3-K/AKT pathway thereby increasing the survival of tumour cells that have been exposed to DNA damaging agents[62,63]. Moreover, selectively blocking this pathway reduces the tumour cell survival after irradiation[62,63]. Cellular radioresistance is linked to the ability of the tumour cells to repair the DNA damage it incurs following exposure to DNA damaging agents. Repair can occur either by homologous recombination (HR) or non-homologous end joining (NHEJ) which is responsible for majority of the double strand DNA break repair. A major protein involved in the NHEJ repair machinery and radiotherapy response is the DNA-dependent protein kinase catalytic subunit (DNA-PKcs)[64]. The Akt has been shown to directly interact with DNA-PKcs through its C-terminal domain[65]. Akt1 and DNA-PKcs form a functional complex after radiation exposure and promotes accumulation of DNA-PKcs and stimulates DNA-PKcs kinase activity at DNA-DSB (double strand break) site for initiating DNA-DSB repair[65-67]. An alternative pathway regulating DNA-DSB repair by Akt is the upregulation of MRE11 expression after Akt activation through Akt/GSK3β (glycogen synthase kinase-3 beta) β-catenin/LEF-1 (lymphoid enhancer binding factor 1) pathway[68]. Another protein complex-MRE11, RAD50 and NBS1 (MRN) complex accumulates at DNA-DSB sites post radiation and acts as a sensor to recruit ATM (ataxia telangectasia mutated) which in turn is activated to phosphorylate MRN complex and a variety of other proteins involved in cell-cycle control and DNA repair[69]. Since targeting Akt leads to downregulation of MRE11 at the transcriptional level, role of Akt1 on DNA repair is ATM dependent. Fraser *et al*[70] have shown that the activation of MRE11-ATM-RNF168 pathway induces Akt phosphorylation thus leading to an Akt-dependent enhanced repair of DNA-DSB. AKT signalling also plays an important role in DNA repair via homologous recombination (HR) pathway. It has been shown that breast cancer patients with HR deficiency have increased phospho AKT levels and similarly tumour formation due to BRCA1 deficiency is reduced by Akt1 depletion[71], while in BRCA1 proficient breast cancer cells HR inhibition due to AKT1 activation is a result of cytoplasmic retention of BRCA1 and RAD51[72]. In HR-deficient cells, Akt1 signalling inhibition of HR is due to impaired Chk1 nuclear localization and subsequent disruption of Chk1-Rad51 interaction[73]. Thus, it is now clear that AKT signalling has contrasting effects on NHEJ and HR pathways. Since DNA-DSB repair is a combination of both NHEJ and HR repair pathways, AKT stimulates repair of DNA-DSB by the NHEJ through activation of DNA-dependent protein kinase catalytic subunit (DNA-PKcs) which is dominant over AKT mediated impairment of DNA-DSB repair in HR-deficient cells.

EGFR signaling by the PI3-K-AKT pathway has been shown to be involved in the regulation of DNA-PKcs and, therefore, DNA repair[64]. Likewise, evidence from *in vitro* studies have shown that targeting of AKT activity by small interfering RNA (siRNA) sensitizes human tumour cells to ionizing radiation[62]. Therefore, EGFR/RAS-activation either by mutation or by receptor tyrosine-kinase activity is a frequent event in human malignancy, suggesting that the PI3-K/AKT-mediated repair of DNA damage might be an important mechanism of intrinsic radioresistance[74].

***Autophagy and AKT signalling*:** Autophagy (or programmed cell death type II) is now considered as an important process in carcinogenesis as well as tumour

cell response to radiation therapy[75,76]. Autophagy is mainly regulated by the mammalian target of rapamycin (mTOR) pathway. Evidence suggests that PI3-K/AKT signaling plays an important role in the regulation of autophagy. Exposure of tumour cells to ionizing radiation induces autophagy. Further, inhibition of autophagy either by autophagy inhibitors[77] or genetic approaches[76] induces radiosensitization. Induction of autophagy through this pathway produces cytotoxic effect on the tumour cell. This is supported by the radiosensitizing effect of AKT inhibition and reduced cell viability in malignant glioma cells U87-MG and U87-MGΔEGFR[78]. The same group showed that AKT inhibition resulted in decreased phosphorylated p70S6 kinase, a downstream target of AKT, and induced autophagy, but not apoptosis. Also, the AKT inhibitor radiosensitized both U87-MG and U87-MGΔEGFR cells by enhancing autophagy. Further studies need to be done to identify the mechanism(s) involved in the cytoprotective effect of radiation-induced autophagy and cytotoxic effect of Akt induced autophagy on post-irradiation survival.

***Tumour cell proliferation*:** The detrimental effect of cellular repopulation for tumour control has been extensively studied in various malignancies[79]. Tumour repopulation is affected by various factors such as cell differentiation status, cell-cycle gene regulation, and micro-environmental factors, including oxygen, neoangiogenesis and nutrient availability. A major mechanism by which cellular proliferation is enhanced in response to ionizing radiation is by induction of EGFR phosphorylation[80]. This EGFR response has been linked to several crucial components of mitogenic or proliferative signaling pathways, a major route being the RAS/RAF/mitogen-activated protein kinase (MAPK) pathway[80]. Studies on PI3-K/AKT have mainly focused on its role in cell survival and progression[81]. Additionally, this PI3-K/AKT also amplifies tumour cell proliferation by signaling the cell cycle machinery as AKT phosphorylation prevents cyclin D1 degradation, which regulates transition of tumour cells from G1 to S phase of cell cycle resulting in radiation resistance[82,83].

***Hypoxia and angiogenesis*:** Solid tumours are known to have an imbalance between oxygen delivery and oxygen consumption, resulting in hypoxia. Tumour hypoxia promotes genetic instability, thus leading the tumour towards a more malignant phenotype by stimulating the invasion of tumour cells and, therefore, metastasis[84]. Furthermore, hypoxia modulates mutations of key regulatory genes that result in overexpression of various protein products of these genes which induce resistance to treatment, resulting in an overall adverse clinical outcome[85,86].

PI3-K/AKT signaling has an important role in this adaptive response of tumour cells to hypoxia. As all these hypoxia-related markers are under the control of AKT, information on AKT-activation status may add significantly to the predictive potential of endogenous tumour markers. Tumour hypoxia results in increased expression of hypoxia-inducible transcription factor-1(HIF-1), which modulates the expression of many genes involved in angiogenesis, *p*H regulation, and glucose metabolism which in turn drive tumour growth and progression. The protein products of these genes, such as vascular endothelial growth factor (VEGF), carbonic anhydrase-IX, the glucose transporters Glut-1 and Glut-3, osteopontin and tyrosine hydroxylase have now been recognized as potential predictive markers for clinical outcome in various tumours[87]. The interaction between hypoxia, angiogenesis and PI3-K/AKT has been shown in various malignancies[88-91] and has been further corroborated by the evidence that downregulation of this signaling pathway by protease inhibitor nelfinavir resulted in decreased expression of HIF-1α and VEGF in response to radiation[92]. Another hypoxia-related product that is under the control of the PI3-K/AKT pathway is osteopontin which is increased in several tumours in response to hypoxia. Hypoxia-induced activation of AKT has been shown to activate an unknown transcriptional factor that triggers osteopontin expression[93,94].

Under hypoxic conditions, VEGF is one of the genes which are activated by HIF-1; while under normoxic conditions it is activated through PI3-K/AKT signalling by either upregulation of EGFR or loss of PTEN[92,95]. VEGF expression plays an important role in neo-angiogenesis by inducing endothelial cell proliferation and vascular permeability crucial for tumour cell proliferation. Prevention of neo-angiogenesis by downregulation of VEGF either directly by the use of VEGF inhibitors such as bevacizumab or indirectly through the use of PI3K/AKT inhibitors or EGFR inhibitors can result in a normalization of the vasculature and improved perfusion leading to a reduction of tumour cell hypoxia[96]. Two distinct pathways (one including HIF-1α translation and the other involving HIF-independent processes) have been recognized as regulators of VEGF expression, both of which involve PI3-K and AKT[92]. Morelli *et al*[97]

observed that VEGF-A blockade, by EGFR inhibition, significantly decreased angiogenesis. A sustained control of tumour cell proliferation and angiogenesis was obtained by the combined blockade of the EGFR pathway in the tumour and the VEGF pathway in endothelial cells. These findings highlight the close relation between EGFR and VEGF inhibition and downstream signal transduction via the PI3-K/AKT pathway. This is corroborated by *in vitro* experiments using PI3-K inhibitor LY294002 which interrupts the PI3-K/AKT pathway resulting in decreased VEGF expression[98].

AKT signalling and glucose metabolism leading to tumour radioresistance: Cancer cells tend to exhibit increased glucose metabolism compared to normal cells leading to excess lactate production by the process of aerobic glycolysis, also called Warburg effect[99-101]. AKT hyperactivation is believed to be associated with increased rates of glucose metabolism observed in tumour cells[102]. This may be through several mechanisms such as, regulation of GLUT-1 on plasma membrane[103], hexokinase expression and mitochondrial protection[104], or Akt may indirectly activate the glycolysis rate-controlling enzyme phosphofructokinase-1 (PFK1) by direct phosphorylation of phosphofructokinase-2 (PFK2)[105], resulting in formation of fructose-2.6-bisphosphate (Fru-1,6-P2), which is a potent allosteric activator of PFK1. *In vitro* study on glioblastoma cell lines showed that AKT activation correlated with increased glycolysis in glioblastoma cells and tumour cell resistance[102]. Therefore, it can be postulated that the increased glycolytic rates observed by Warburg in cancer cells exhibiting mitochondrial respiration malfunction compared to normal cells may involve activation of the Akt pathway. Inhibition of glucose metabolism in cancer cells with AKT pathway inhibitors is assumed to limit glycolysis in the cancer cell and thereby the production of pyruvate and regeneration of NADPH leading to increased levels of hydrogen peroxide and hydroperoxides resulting in preferential cytotoxicity of the cancer cells via oxidative stress. Based on these assumptions, the combination of Akt pathway inhibitors with glycolytic inhibitors and/or manipulations that increase pro-oxidant production should further and preferentially cause cytotoxicity in cancer cells, with minimal to no toxicity to normal cells. Simon *et al*[106] using human head neck squamous cell carcinoma (HNSCC) cell lines (FaDu & cal -27) have shown that inhibition of AKT pathway disrupts glucose metabolism and induces metabolic oxidative stress in cancer cells leading to preferential cytotoxicity. These results indicate that increased Akt pathway signalling may have a significant role in the Warburg effect and this phenomenon should be exploited to selectively target cancer cells for enhancing radio- and chemo-sensitivity in cancer therapy.

Rationale for targeting the AKT pathway for radiosensitization

The P13-K/AKT pathway is a ubiquitous and evolutionary conserved pathway which triggers a cascade of downstream events that regulate various cellular functions namely, cell growth and proliferation, cell survival and motility which drives tumour progression and mediates repair of the damaged DNA resulting in radiation resistance[81,107]. Activation of this pathway and increased intratumoral phosphorylated AKT have been linked to decreased radiation responsiveness in various malignancies[62,89,107].

Clinical evidence of PI3-K/AKT pathway deregulation in various cancers and the identification of downstream kinases involved in mediating the effects of PI3-K/AKT pathway such as the mammalian target of rapamycin (mTOR), pyruvate dehydrogenase kinase 1 (PDK1) and integrin-linked kinase (ILK) provide potential targets for the development of small molecule therapies. Presently, PI3-K/AKT pathway inhibitors are being studied extensively for their radiosensitization properties. Moreover, strong and independent associations have been found between expression of activated AKT (pAKT) and treatment outcome in clinical trials[49,108]. The AKT signal transduction pathway is appealing target for therapeutic intervention, because AKT signalling promotes the three major radioresistance mechanisms (*i.e.* cell survival, tumour cell proliferation and hypoxia)[62,88,92]. Therefore, modulation of AKT signalling pathway may have major implications in the radiotherapeutic management especially in tumours that have activated PI3-K/AKT cascade. Inhibition of the pathway can induce apoptosis or sensitize tumour cells to undergo apoptosis in response to radiation therapy. Extensive *in vitro* and *in vivo* studies have shown that AKT signalling pathway plays an important role in radiation resistance, targeting this pathway to identify drugs that counteract radiation induced cellular defence mechanisms would be logical[92,109-112]. It has been shown that PI3-K/AKT pathway is selectively activated in human cancer cells and sparing the normal cells, suggesting that factors in this cascade are potential molecular target to improve

radiosensitivity[113]. Because of the differential activation of this pathway in tumour cells vs. the normal cells, strategies to block PI3-K/AKT signalling should result in more effective radiation treatment by enhancing the sensitivity of tumour cells to radiation *vis-a-vis* sparing normal tissues surrounding the tumour[109,113]. However, the problem has been to identify inhibitors of this pathway that are suitable for clinical use. For example, *in vitro* studies by Gupta *et al*[113] have shown that LY294002 and wortmannin are potent PI3-K inhibitors with significant radiosensitizing effects but their poor *in vivo* tolerability limits their clinical applications. Currently, the research is being aimed to develop drugs targeting the PI3-K/AKT pathway that are clinically safe. In this context, HIV protease inhibitors have been shown to inhibit AKT phosphorylation and thus radiosensitize tumour cells at concentrations used for anti-HIV treatment. These drugs have been used for over a decade to treat patients with HIV infection and are considered safe for oral use.

HIV protease inhibitors (HPI) as radiosensitizers: mechanism of radiosensitization

The mechanism of radiosensitization is a combination of proteosome inhibition, induction of cell stress, influence on cell signalling cascades, and autophagy[110]. HPIs are selective peptidomimetic, protease inhibitors that bind with high affinity to the active site of HIV protease. The radiosensitizing property of HPIs mainly relates to the inhibition of proteosome which is responsible for degradation of proteins[114]. These compounds inhibit the 20S ribosome which in turn results in endoplasmic reticulum stress triggering the unfolded protein response (UPR) which activates the alpha subunit of eukaryotic translation initiation factor 2 (eIF2α) by phosphorylation. The activation of elf2α increases the production of growth arrest and DNA damage-inducible protein (GADD34) which forms a complex with protein phosphatase 1 and induces the downregulation of Phospho-AKT (Figure)[114]. The AKT2 isoform, regulates the growth of and metabolism of cells by the insulin/insulin like growth factor signalling pathway[115,116]. This explains some of the adverse effects of HIV protease inhibitors including hyperlipidaemia, insulin resistance, peripheral lipoatrophy, central fat accumulation, and hepatic steatosis. It is possible that the insulin resistance caused by nelfinavir could be related to the decrease in Akt phosphorylation[113]. An alternate downstream event of inhibition of proteosome leads to stabilization of IκB cellular inhibitory protein of NF-kappa B[110]. This results in inactivation of NF-Kappa B leading to apoptosis, reduced tumour cell survival and, therefore, enhanced radiosensitivity[117]. Additionally, AKT dephosphorylation also inactivates HIF-1α and VEGF leading to enhanced tumour oxygenation and inhibition of neoangiogenesis[92,118]. This indirectly enhances tumour sensitivity to irradiation (Figure).

Extensive *in vitro* experiments using Western blot assays and clonogenic assays have shown the potential radiosensitive activity of different classes of HPIs in different cancer cell lines (Table II). The results of the *in vitro* experiments were further corroborated in *in vivo* mouse xenograft models using the same class of HPIs[109].

Preclinical evidence has shown that HIV protease inhibitors downregulate AKT at dose range that is clinically used for HIV patients. At this dose range, the safety profile of HPIs has been established clinically as well. The HPIs specifically target the tumour tissue only and this makes them the lead compounds to be used as AKT inhibitors and, therefore, as radiosensitizers. Compared to traditional conventional chemotherapy drugs that are used as radiosensitizers, these drugs can be administered orally with high bioavailability, thereby improving patient compliance.

Nelfinavir - the lead HPI as a radiosensitizer

The radiosensitizing ability of HPIs was first shown in HIV positive patients in whom the peripheral blood leukocytes phospho-AKT levels were downregulated[119]. Patients taking these "active" radiosensitizing protease inhibitors had very low levels of phospho-AKT compared to HIV +ve patients taking either no medications or other antiretroviral regimens[119]. This led to extensive studies (both *in vitro* and *in vivo*) of different classes of HIV protease inhibitors to determine the mechanistic basis of radiation sensitization. Gupta *et al*[109] studied the radiosensitizing ability of five different classes of HPIs (nelfinavir, amprenavir, sequinavir, ritonavir and indinavir) against different cancer cell lines and normal cells (fibroblasts) both *in vitro* as well as *in vivo*. They observed that three of the five HPIs (saquinavir, amprenavir and nelfinavir) showed potent inhibition of 473 serine AKT phosphorylation in the cancer cell lines but not in the normal rat fibroblasts. Nelfinavir, amprenavir and saquinavir were also shown to radiosensitize human umbilical vein endothelial cells (HUVEC) and tumour vascular endothelium along with inhibition of angiogenesis and tumour cell migration[120]. Of the three HPIs, nelfinavir had more profound effect

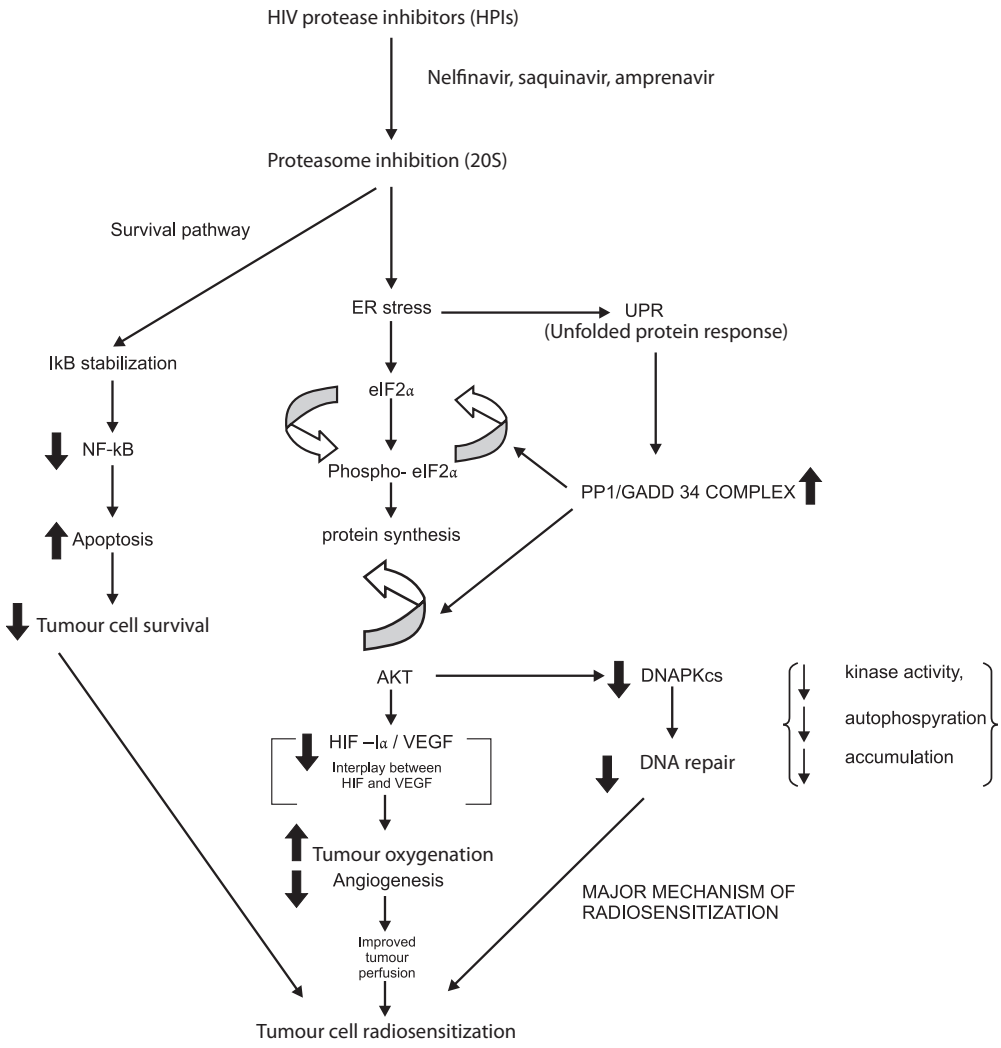

Figure. Mechanisms by which HIV protease inhibitors (HPIs) enhance radiosensitivity. Nelfinavir and other HPIs induce endoplasmic reticulum (ER) stress resulting in unfolded protein response (UPR) which leads to phosphorylation of eukaryotic initiation factor 2 α (eIf2α) leading to global inhibition of protein synthesis and reduced tumour cell survival. A second mechanism is by activation of growth arrest and DNA damage-inducible protein (GADD 34) and protein phosphatase1 (PP1) complex that dephosphorylates phospho-AKT to AKT resulting in decreased DNA replication and increased radiosensitivity. Dephosphorylation of AKT also reduces expression of hypoxia inducible factor (HIF1α) and vascular endothelial growth factor f (VEGF) leading to increased tumour cell oxygenation and decreased angiogenesis which indirectly contributes to enhanced radiosensitivity of the tumour. The third mechanism is by inactivation of nuclear factor Kappa-light-chain-enhancer of activated B cells (NF-kB) which leads to apoptosis and reduced tumour cell survival and thereby indirectly enhancing radiosensitivity. Dephosphorylation of pAKT also activates proapoptotic proteins and inactivates antiapoptotic proteins resulting in activation of apoptotic pathway. Adapted and reproduced from Figure of Ref. 114 with permission from publisher, Taylor and Francis.

on HUVEC and tumour vascular endothelium. *In vitro* pharmacokinetic studies done on SQ20B (head and neck cancer) and T24 (bladder cancer) have shown that low concentration (5 micromol/l) of nelfinavir was enough to downregulate pAKT in comparison to saquinavir and amprenavir (10 micromol/l)[109]. Additionally, nelfinavir was found to be least toxic among all the HPIs, thus making it a lead AKT inhibitor for clinical use as a radiosensitizing agent. The most common side effect of this drug is diarrhoea occurring in 30 per cent patients[121] which is usually mild to moderate and controlled with over the counter antidiarrhoeal drugs. Hyperlipidaemia, hyperglycemia and elevation of transaminases (especially in patients with hepatitis B and C infection due to immune reconstitution) have been reported with long term use of nelfinavir[122]. Table

Table II. Cancer cell line studies using nelfinavir (NFV) as radiosensitizer

Sl. No.	Tumour cell lines	Type of cancer cell line	Mechanism of action of NFV	Reference
1	UMSCC47, UPCI-SCC90 (HPV 16 +)	*SCC of head and neck	Inhibition of AKT activation	111
2	SQ20B	SCC head and neck	Decreased VEGF expression, decreased hypoxic induction of HIF1α via inactivated AKT pathway	109, 113
3	A549	Non-small cell lung cancer	Decreased VEGF expression, decreased hypoxic induction of HIF1α via inactivated Akt pathway	92, 109
4	T98G, LN229, U251, U87MG	Glioblastoma multiforme	Proteasome inhibition, ER stress, and unfolded protein response, Downregulates VEGF and HIF-1 expression	88, 123
5	T24	Bladder cancer	Inhibition of AKT activation	109
6	MIAPACA2	Pancreatic cancer	Inhibition of AKT activation	109
7	LNCaP, PC-3, and DU145 cells	Prostate cancer	Inhibition of proliferation of prostate cancer Cells in conjunction with blockade of signalling by AR, STAT3, and AKT	112
8	GH3, MMQ and AT20	Pitutary adenoma	Inhibiting the PI3-K/AKT/ mTOR pro-survival pathway (downregulation of phospho-S6)	51

SCC, squamous cell carcinoma; STAT, signal transducer and activator of transcription; VEGF, vascular endothelial growth factor; HIF, hypoxia inducible factor; AR, androgen receptor; mTOR, mammalian target of rapamycin; PI3-K, phosphotidylinositol-3 kinase

II summarizes the mechanism of radiosensitization of nelfinavir in different cancer cell lines.

In vivo studies have shown that the oral bioavailability of nelfinavir is 70-80 per cent in fed state. Food increases nelfinavir exposure and decreases nelfinavir pharmacokinetic variability relative to the fasted state. Exposure to nelfinavir is 2-5 fold higher in fed state compared to fasting state. Nelfinavir exposure increases with increasing calorie or fat content of meals. The drug is extensively bound to plasma proteins (>98%) with a plasma half-life of 3.5-5 h. The majority of an oral dose is excreted in the faeces as oxidative metabolites. Only 1-2 per cent of the drug is excreted unchanged through the kidneys.

Clinical trials of nelfinavir as radiosensitizer

With the availability of preclinical data (*in vitro* & *in vivo*) of nelfinavir as a potent radiosensitizing agent, various phase-I and phase-II clinical trials have been initiated. First phase-I clinical trial using nelfinavir was carried out against locally advanced pancreatic cancer. This study showed that the toxicity of nelfinavir along with chemoradiation (radiation dose of 59.4 Gy + gemcitabine and cisplatin) was low with favourable tumour response (metabolic complete response 'CR' in 56% patients)[124]. Another phase-I study of nelfinavir with concurrent chemoradiation (radiation dose of 66.6Gy +cisplatin and etoposide) in stage IIIA/ IIIB non-small cell lung cancer (NSCLC) showed acceptable toxicity and promising activity in patients with locally advanced NSCLC (metabolic CR in 56% patients and partial response in 44%)[125]. Recently, a third phase-I study of nelfinavir in combination with capecitabine in rectal cancer (radiation dose of 50.4 Gy) showed promising results with acceptable toxicity and a pathological complete response of 33 per cent[126]. Till date, only these three studies have reported the results of radiation therapy with concomitant nelfinavir along with conventional chemotherapy as radiosensitizer in clinical settings[124-126]. Both clinical trials have reported grade 3-4 haematologic toxicities attributable to chemotherapy drugs (cisplatin, etoposide and gemcitabine used in these trials). The rectal cancer study had grade-3 lower gastrointestinal (GI) toxicity in the form of diarrhoea. However, all the patients in these three trials could complete their planned treatment. Grade 1 and 2 toxicities were reported in almost all the patients, especially hyperglycaemia, elevated transaminases and lower GI toxicities which were transient and self-limiting. Currently, numerous

Table III. Clinical trials using concurrent nelfinavir and chemotherapy as radiosensitizing agent in HIV seronegative malignant tumours*

Sl. No.	Clinical trial ID	Disease site	Chemotherapy/ radiotherapy	Objective(s) of the study	Type of study	Study status
1	NCT 00704600	Rectal cancer	Pelvic radiotherapy (28x1.8 Gy) and capecitabine 825 mg/m2 BID+ nelfinavir	Drug safety and activity in combination with chemo RT	Phase I/II	Completed, Results of Phase -I published[127]
2	NCT 01068327	Pancreatic cancer	Gemcitabine and 5-fluorouracil and stereotactic radiotherapy+ nelfinavir	Drug safety and dose study with stereotactic RT	Phase I	Recruiting
4	NCT 00791336	Stage III non-small cell lung cancer	Cisplatin, and etoposide + radiotherapy+ nelfinavir	Drug safety and toxicity in combination with concurrent thoracic RT, cisplatin, and etoposide	Phase I/II	Study terminated due to poor enrollment
5	NCT 01086332	Pancreatic cancer	Escalating doses of gemcitabine + radiotherapy+ nelfinavir	1. NFV as radiation sensitizer in combination with gemcitabine: Safety study 2. Surgical resection rate	Phase I/II	Completed, results published[125]
6	NCT 01108666	Stage III non small cell lung cancer (NSCLC)	Nelfinavir + proton beam radiotherapy with concurrent carboplatin/ paclitaxel and cisplatin/etoposide	1. MTD of proton radiotherapy with concurrent cisplatin and etoposide 2. MTD of proton radiotherapy with concurrent carboplatin and paclitaxel 3. MTD of nelfinavir with concurrent chemo-RT 4. Clinical efficacy (metabolic response, sites of recurrence and PFS and OS)	Phase I	Recruiting
7	NCT 00915694	GBM	Nelfinavir + RT+ TMZ in GBM	Drug safety and toxicity in combination with temozolomide	Phase I	Recruiting
8	NCT 00694837	GBM	Nelfinavir + RT+ TMZ in GBM	Drug safety and toxicity studies in combination with radiotherapy	Phase I	Recruiting
9	NCT 01447589	Non-small cell lung cancer (NSCLC)	Only radiotherapy: 66Gy/33 fractions	Drug safety and efficacy study	Phase-I/II	Open but not started recruiting
10	NCT 01485731	Carcinoma cervix (Stage-II-IVA)	Cisplatin + pelvic radiotherapy	1. Drug safety and efficacy study 2. Pharmacokinetics of nelfinavir	Phase-I	Open but not started recruiting

Contd...

Sl. No.	Clinical trial ID	Disease site	Chemotherapy/ radiotherapy	Objective(s) of the study	Type of study	Study status
11	NCT 00589056	Non-small cell lung cancer (NSCLC) stage-III	Concurrent cisplatin + etoposide with nelfinavir and thoracic radiotherapy	1. Drug safety and efficacy study of nelfinavir with concurrent thoracic radiotherapy and cisplatin, and etoposide 2. Response at 3 months 3. Overall survival 4. Expression of molecular markers (total Akt and p-Akt) in primary tumour, lymph nodes and in lymphocytes	Phase-I	Completed, Phase-I results published[126]
12	NCT 01485731	Carcinoma of cervix	Concurrent cisplatin + nelfinavir and pelvic radiotherapy	1. Safety/efficacy study of nelfinavir with concurrent pelvic radiotherapy and cisplatin	Phase-I	Recruiting

*Data from *www.clinicaltrials.gov*; PFS, progression free survival; OS, overall survival; MTD, maximum tolerated dose; GBM, glioblastoma multiforme; TMZ, temozolamide; RT, radiation therapy
Superscript numerals denote reference numbers

clinical trials are in progress to test nelfinavir as a radiosensitizer. The details of the clinical trials are summarized in Table III. Although the present clinical evidence is still immature, the results of these clinical trials are eagerly awaited to see if nelfinavir actually has the potential to be put into clinical use as a radiosensitizer for various cancers.

Conclusion

The role of AKT in cancer has been a subject of discussion over the past decade. It is clear that activation of the AKT pathway is one of the most common molecular alterations in human malignancy conferring to radioresistance thereby providing a strong rationale for targeting the AKT pathway as radiosensitizers. The use of commercially available drugs such as the HPIs is an initial step towards targeting the AKT pathway and these need to be used in clinical radiotherapy trials along with conventional drugs to enhance radiosensitivity of tumours.

Although the complete mechanism of action of HPIs as radiosensitizing agent is not yet completely understood, its broad spectrum of activity, minimal toxicity, and its availability in clinics has made these compounds to be used in cancer therapeutics as a radiosensitizer. Extensive preclinical evidence and ongoing phase-I clinical trials support the use of HPIs with radiation where the effects could be monitored in the patients. Moreover, the tolerability of these compounds has been documented which makes them ideal to be tested in future phase-II and phase-III studies as radiosensitizers.

Conflicts of Interest: None.

References

1. Overgaard J, Hansen HS, Specht L, Overgaard M, Grau C, Andersen E, *et al*. Five compared with six fractions per week of conventional radiotherapy of squamous-cell carcinoma of head and neck: DAHANCA 6 and 7 randomised controlled trial. *Lancet* 2003; *362* : 933-40.
2. Haslett K, Pottgen C, Stuschke M, Faivre-Finn C: Hyperfractionated and accelerated radiotherapy in non-small cell lung cancer. *J Thorac Dis* 2014; *6* : 328-35.
3. Motegi A, Kawashima M, Arahira S, Zenda S, Toshima M, Onozawa M, *et al*. Accelerated radiotherapy for T1 to T2 glottic cancer. *Head Neck* 2015; *37* : 579-84.
4. Gupta T, Kannan S, Ghosh-Laskar S, Agarwal JP. Concomitant chemo-radiotherapy (CT-RT) versus altered fractionation radiotherapy in the radiotherapeutic management of locoregionally advanced head and neck squamous cell carcinoma (HNSCC): an adjusted indirect comparison meta-analysis. *Head Neck* 2015; *37* : 670-6.
5. Chitapanarux I, Tharavichitkul E, Kamnerdsupaphon P, Pukanhapan N, Vongtama R. Randomized phase III

trial of concurrent chemoradiotherapy vs accelerated hyperfractionation radiotherapy in locally advanced head and neck cancer. *J Radiat Res* 2013; *54* : 1110-7.

6. Blanchard P, Baujat B, Holostenco V, Bourredjem A, Baey C, Bourhis J, *et al*; MACH-CH Collaborative Group. Meta-analysis of chemotherapy in head and neck cancer (MACH-NC): a comprehensive analysis by tumour site. *Radiother Oncol* 2011; *100* : 33-40.
7. Pignon JP, le Maitre A, Maillard E, Bourhis J; MACH-CH Collaborative Group. Meta-analysis of chemotherapy in head and neck cancer (MACH-NC): an update on 93 randomised trials and 17,346 patients. *Radiother Oncol* 2009; *92* : 4-14.
8. Green J, Kirwan J, Tierney J, Symonds P, Fresco L, Williams C, *et al*. Concomitant chemotherapy and radiation therapy for cancer of the uterine cervix. *Cochrane Database Syst Rev* 2001 (4): CD002225.
9. Rischin D, Peters L, Fisher R, Macann A, Denham J, Poulsen M, *et al*. Tirapazamine, cisplatin, and radiation versus fluorouracil, cisplatin, and radiation in patients with locally advanced head and neck cancer: a randomized phase II trial of the Trans-Tasman Radiation Oncology Group (TROG 98.02). *J Clin Oncol* 2005; *23* : 79-87.
10. Hassan Metwally MA, Ali R, Kuddu M, Shouman T, Strojan P, Iqbal K, *et al*. IAEA-HypoX. A randomized multicenter study of the hypoxic radiosensitizer nimorazole concomitant with accelerated radiotherapy in head and neck squamous cell carcinoma. *Radiother Oncol* 2015; *116* : 15-20.
11. Overgaard J. Hypoxic modification of radiotherapy in squamous cell carcinoma of the head and neck - a systematic review and meta-analysis. *Radiother Oncol* 2011; *100* : 22-32.
12. Toustrup K, Sorensen BS, Lassen P, Wiuf C, Alsner J, Overgaard J; Danish Head and Neck Cancer Group (DAHANCA). Gene expression classifier predicts for hypoxic modification of radiotherapy with nimorazole in squamous cell carcinomas of the head and neck. *Radiother Oncol* 2012; *102* : 122-9.
13. Bonner JA, Harari PM, Giralt J, Azarnia N, Shin DM, Cohen RB, *et al*. Radiotherapy plus cetuximab for squamous-cell carcinoma of the head and neck. *New Engl J Med* 2006; *354* : 567-78.
14. Heiduschka G, Grah A, Oberndorfer F, Seemann R, Kranz A, Kornek G, *et al*. Significance of p16 expression in head and neck cancer patients treated with radiotherapy and cetuximab. *Strahlenther Onkol* 2014; *190* : 832-8.
15. Shepherd FA, Rodrigues Pereira J, Ciuleanu T, Tan EH, Hirsh V, Thongprasert S, *et al*; National Cancer Institute of Canada Clinical Trials Group. Erlotinib in previously treated non-small-cell lung cancer. *New Engl J Med* 2005; *353* : 123-32.
16. Thatcher N, Chang A, Parikh P, Rodrigues Pereira J, Ciuleanu T, von Pawel J, *et al*. Gefitinib plus best supportive care in previously treated patients with refractory advanced non-small-cell lung cancer: results from a randomised, placebo-controlled, multicentre study (Iressa Survival Evaluation in Lung Cancer). *Lancet* 2005; *366* : 1527-37.
17. Horiike A, Yamamoto N, Tanaka H, Yanagitani N, Kudo K, Ohyanagi F, *et al*. Phase II study of erlotinib for acquired resistance to gefitinib in patients with advanced non-small cell lung cancer. *Anticancer Res* 2014; *34* : 1975-81.
18. Genova C, Rijavec E, Barletta G, Burrafato G, Biello F, Dal Bello MG, *et al*. Afatinib for the treatment of advanced non-small-cell lung cancer. *Expert Opin Pharmacother* 2014; *15* : 889-903.
19. Kabbinavar F, Hurwitz HI, Fehrenbacher L, Meropol NJ, Novotny WF, Lieberman G, *et al*. Phase II, randomized trial comparing bevacizumab plus fluorouracil (FU)/leucovorin (LV) with FU/LV alone in patients with metastatic colorectal cancer. *J Clin Oncol* 2003; *21* : 60-5.
20. Kabbinavar FF, Schulz J, McCleod M, Patel T, Hamm JT, Hecht JR, *et al*. Addition of bevacizumab to bolus fluorouracil and leucovorin in first-line metastatic colorectal cancer: results of a randomized phase II trial. *J Clin Oncol* 2005; *23* : 3697-705.
21. Ponnurangam S, Standing D, Rangarajan P, Subramaniam D. Tandutinib inhibits the Akt/mTOR signaling pathway to inhibit colon cancer growth. *Mol Cancer Ther* 2013; *12* : 598-609.
22. Li HF, Kim JS, Waldman T. Radiation-induced Akt activation modulates radioresistance in human glioblastoma cells. *Radiat Oncol* 2009; *4* : 1-10.
23. Ringel MD, Hayre N, Saito J, Saunier B, Schuppert F, Burch H, *et al*. Overexpression and overactivation of Akt in thyroid carcinoma. *Cancer Res* 2001; *61* : 6105-11.
24. Miyakawa M, Tsushima T, Murakami H, Wakai K, Isozaki O, Takano K. Increased expression of phosphorylated p70S6 kinase and Akt in papillary thyroid cancer tissues. *Endoc J* 2003; *50* : 77-83.
25. Slupianek A, Nieborowska-Skorska M, Hoser G, Morrione A, Majewski M, Xue L, *et al*. Role of phosphatidylinositol 3-kinase-Akt pathway in nucleophosmin/anaplastic lymphoma kinase-mediated lymphomagenesis. *Cancer Res* 2001; *61* : 2194-9.
26. Alkan S, Izban KF. Immunohistochemical localization of phosphorylated AKT in multiple myeloma. *Blood* 2002; *99* : 2278-9.
27. Tanno S, Yanagawa N, Habiro A, Koizumi K, Nakano Y, Osanai M, *et al*. Serine/threonine kinase AKT is frequently activated in human bile duct cancer and is associated with increased radioresistance. *Cancer Res* 2004; *64* : 3486-90.
28. Nam SY, Lee HS, Jung GA, Choi J, Cho SJ, Kim MK, *et al*. Akt/PKB activation in gastric carcinomas correlates with clinicopathologic variables and prognosis. *APMIS* 2003; *111* : 1105-13.
29. Cedres S, Montero MA, Martinez P, Martinez A, Rodriguez-Freixinos V, Torrejon D, *et al*. Exploratory analysis of activation of PTEN-PI3K pathway and downstream proteins in malignant pleural mesothelioma (MPM). *Lung Cancer* 2012; *77* : 192-8.
30. Min YH, Eom JI, Cheong JW, Maeng HO, Kim JY, Jeung HK, *et al*. Constitutive phosphorylation of Akt/PKB protein in acute myeloid leukemia: its significance as a prognostic variable. *Leukemia* 2003; *17* : 995-7.
31. Chakravarti A, Zhai G, Suzuki Y, Sarkesh S, Black PM, Muzikansky A, *et al*. The prognostic significance of phosphatidylinositol 3-kinase pathway activation in human gliomas. *J Clin Oncol* 2004; *22* : 1926-33.

32. Jiang Z, Pore N, Cerniglia GJ, Mick R, Georgescu MM, Bernhard EJ, *et al*. Phosphatase and tensin homologue deficiency in glioblastoma confers resistance to radiation and temozolomide that is reversed by the protease inhibitor nelfinavir. *Cancer Res* 2007; *67* : 4467-73.
33. Blackhall FH, Pintilie M, Michael M, Leighl N, Feld R, Tsao MS, *et al*. Expression and prognostic significance of kit, protein kinase B, and mitogen-activated protein kinase in patients with small cell lung cancer. *Clin Cancer Res* 2003; *9* : 2241-7.
34. Roy HK, Olusola BF, Clemens DL, Karolski WJ, Ratashak A, Lynch HT, *et al*. AKT proto-oncogene overexpression is an early event during sporadic colon carcinogenesis. *Carcinogenesis* 2002; *23* : 201-5.
35. Gupta AK, McKenna WG, Weber CN, Feldman MD, Goldsmith JD, Mick R, *et al*. Local recurrence in head and neck cancer: relationship to radiation resistance and signal transduction. *Clin Cancer Res* 2002; *8* : 885-92.
36. Lim J, Kim JH, Paeng JY, Kim MJ, Hong SD, Lee JI, *et al*. Prognostic value of activated Akt expression in oral squamous cell carcinoma. *J Clin Pathol* 2005; *58* : 1199-205.
37. Balsara BR, Pei J, Mitsuuchi Y, Page R, Klein-Szanto A, Wang H, *et al*. Frequent activation of AKT in non-small cell lung carcinomas and preneoplastic bronchial lesions. *Carcinogenesis* 2004; *25* : 2053-9.
38. Tsao AS, McDonnell T, Lam S, Putnam JB, Bekele N, Hong WK, *et al*. Increased phospho-AKT (Ser(473)) expression in bronchial dysplasia: implications for lung cancer prevention studies. *Cancer Epidemiol Biomarkers Prev* 2003; *12* : 660-4.
39. Altomare DA, Wang HQ, Skele KL, De Rienzo A, Klein-Szanto AJ, Godwin AK, *et al*. AKT and mTOR phosphorylation is frequently detected in ovarian cancer and can be targeted to disrupt ovarian tumor cell growth. *Oncogene* 2004; *23* : 5853-7.
40. Bellacosa A, de Feo D, Godwin AK, Bell DW, Cheng JQ, Altomare DA, *et al*. Molecular alterations of the AKT2 oncogene in ovarian and breast carcinomas. *Int J Cancer J* 1995; *64* : 280-5.
41. Yuan ZQ, Sun M, Feldman RI, Wang G, Ma X, Jiang C, *et al*. Frequent activation of AKT2 and induction of apoptosis by inhibition of phosphoinositide-3-OH kinase/Akt pathway in human ovarian cancer. *Oncogene* 2000; *19* : 2324-30.
42. Cheng JQ, Ruggeri B, Klein WM, Sonoda G, Altomare DA, Watson DK, *et al*. Amplification of AKT2 in human pancreatic cells and inhibition of AKT2 expression and tumorigenicity by antisense RNA. *Proc Natl Acad Sci USA* 1996; *93* : 3636-41.
43. Robertson GP. Functional and therapeutic significance of Akt deregulation in malignant melanoma. *Cancer Metastasis Rev* 2005; *24* : 273-85.
44. Yang Y, Ikezoe T, Takeuchi T, Adachi Y, Ohtsuki Y, Takeuchi S, *et al*. HIV-1 protease inhibitor induces growth arrest and apoptosis of human prostate cancer LNCaP cells *in vitro* and *in vivo* in conjunction with blockade of androgen receptor STAT3 and AKT signaling. *Cancer Sci* 2005; *96* : 425-33.
45. Horiguchi A, Oya M, Uchida A, Marumo K, Murai M. Elevated Akt activation and its impact on clinicopathological features of renal cell carcinoma. *J Urol* 2003; *169* : 710-3.
46. Xu X, Sakon M, Nagano H, Hiraoka N, Yamamoto H, Hayashi N, *et al*. Akt2 expression correlates with prognosis of human hepatocellular carcinoma. *Oncol Rep* 2004; *11* : 25-32.
47. Terakawa N, Kanamori Y, Yoshida S. Loss of PTEN expression followed by Akt phosphorylation is a poor prognostic factor for patients with endometrial cancer. *Endocr-Relat Cancer* 2003; *10* : 203-8.
48. Duensing A, Medeiros F, McConarty B, Joseph NE, Panigrahy D, Singer S, *et al*. Mechanisms of oncogenic KIT signal transduction in primary gastrointestinal stromal tumors (GISTs). *Oncogene* 2004; *23* : 3999-4006.
49. Kim TJ, Lee JW, Song SY, Choi JJ, Choi CH, Kim BG, *et al*. Increased expression of pAKT is associated with radiation resistance in cervical cancer. *Br J Cancer* 2006; *94* : 1678-82.
50. Musat M, Korbonits M, Kola B, Borboli N, Hanson MR, Nanzer AM, *et al*. Enhanced protein kinase B/Akt signalling in pituitary tumours. *Endocri-Relat Cancer* 2005; *12* : 423-33.
51. Zeng J, See AP, Aziz K, Thiyagarajan S, Salih T, Gajula RP, *et al*. Nelfinavir induces radiation sensitization in pituitary adenoma cells. *Cancer Biol Therapy* 2011; *12* : 657-63.
52. Dent P, Yacoub A, Contessa J, Caron R, Amorino G, Valerie K, *et al*. Stress and radiation-induced activation of multiple intracellular signaling pathways. *Radiat Res* 2003; *159* : 283-300.
53. Meads MB, Gatenby RA, Dalton WS. Environment-mediated drug resistance: a major contributor to minimal residual disease. *Nat Rev Cancer* 2009; *9* : 665-74.
54. Fodale V, Pierobon M, Liotta L, Petricoin E. Mechanism of cell adaptation: when and how do cancer cells develop chemoresistance? *Cancer J* 2011; *17* : 89-95.
55. 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* : 863-72.
56. Saki M, Toulany M, Rodemann HP. Acquired resistance to cetuximab is associated with the overexpression of Ras family members and the loss of radiosensitization in head and neck cancer cells. *Radiother Oncol* 2013; *108* : 473-8.
57. 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* : 1506-14.
58. 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* : 220-8.
59. Kim EJ, Jeong JH, Bae S, Kang S, Kim CH, Lim YB. mTOR inhibitors radiosensitize PTEN-deficient non-small-cell lung cancer cells harboring an EGFR activating mutation by inducing autophagy. *J Cell Biochem* 2013; *114* : 1248-56.
60. Christensen M, Najy AJ, Snyder M, Movilla LS, Kim HR. A critical role of the PTEN/PDGF signaling network for the regulation of radiosensitivity in adenocarcinoma of the prostate. *Int J Radiat Oncol Biol Phys* 2014; *88* : 151-8.

61. Rosser CJ, Tanaka M, Pisters LL, Tanaka N, Levy LB, Hoover DC, *et al*. Adenoviral-mediated PTEN transgene expression sensitizes Bcl-2-expressing prostate cancer cells to radiation. *Cancer Gene Ther* 2004; *11* : 273-9.
62. Kim IA, Bae SS, Fernandes A, Wu J, Muschel RJ, McKenna WG, *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.
63. Nijkamp MM, Hoogsteen IJ, Span PN, Takes RP, Lok J, Rijken PF, *et al*. Spatial relationship of phosphorylated epidermal growth factor receptor and activated AKT in head and neck squamous cell carcinoma. *Radiother Oncol* 2011; *101* : 165-70.
64. Toulany M, Kasten-Pisula U, Brammer I, Wang S, Chen J, Dittmann K, *et al*. Blockage of epidermal growth factor receptor-phosphatidylinositol 3-kinase-AKT signaling increases radiosensitivity of K-RAS mutated human tumor cells *in vitro* by affecting DNA repair. *Clin Cancer Res* 2006; *12* : 4119-26.
65. Park J, Feng J, Li Y, Hammarsten O, Brazil DP, Hemmings BA. DNA-dependent protein kinase-mediated phosphorylation of protein kinase B requires a specific recognition sequence in the C-terminal hydrophobic motif. *J Biol Chem* 2009; *284* : 6169-74.
66. Toulany M, Lee KJ, Fattah KR, Lin YF, 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* : 945-57.
67. Bozulic L, Surucu B, Hynx D, Hemmings BA. PKBalpha/Akt1 acts downstream of DNA-PK in the DNA double-strand break response and promotes survival. *Mol Cell* 2008; *30* : 203-13.
68. Deng R, Tang J, Ma JG, Chen SP, Xia LP, Zhou WJ, *et al*. PKB/Akt promotes DSB repair in cancer cells through upregulating Mre11 expression following ionizing radiation. *Oncogene* 2011; *30* : 944-55.
69. Lavin MF. ATM and the Mre11 complex combine to recognize and signal DNA double-strand breaks. *Oncogene* 2007; *26* : 7749-58.
70. Fraser M, Harding SM, Zhao H, Coackley C, Durocher D, Bristow RG. MRE11 promotes AKT phosphorylation in direct response to DNA double-strand breaks. *Cell Cycle* 2011; *10* : 2218-32.
71. Xiang T, Jia Y, Sherris D, Li S, Wang H, Lu D, *et al*. Targeting the Akt/mTOR pathway in Brca1-deficient cancers. *Oncogene* 2011; *30* : 2443-50.
72. Plo I, Laulier C, Gauthier L, Lebrun F, Calvo F, Lopez BS. AKT1 inhibits homologous recombination by inducing cytoplasmic retention of BRCA1 and RAD51. *Cancer Res* 2008; *68* : 9404-12.
73. Jia Y, Song W, Zhang F, Yan J, Yang Q. Akt1 inhibits homologous recombination in Brca1-deficient cells by blocking the Chk1-Rad51 pathway. *Oncogene* 2013; *32* : 1943-9.
74. 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* : 317-28.
75. Apel A, Herr I, Schwarz H, Rodemann HP, Mayer A. Blocked autophagy sensitizes resistant carcinoma cells to radiation therapy. *Cancer Res* 2008; *68* : 1485-94.
76. Rouschop KM, van den Beucken T, Dubois L, Niessen H, Bussink J, Savelkouls K, *et al*. The unfolded protein response protects human tumor cells during hypoxia through regulation of the autophagy genes MAP1LC3B and ATG5. *J Clin Invest* 2010; *120* : 127-41.
77. Chaachouay H, Ohneseit P, Toulany M, Kehlbach R, Multhoff G, Rodemann HP. Autophagy contributes to resistance of tumor cells to ionizing radiation. *Radiother Oncol* 2011; *99* : 287-92.
78. Fujiwara K, Daido S, Yamamoto A, Kobayashi R, Yokoyama T, Aoki H, *et al*. Pivotal role of the cyclin-dependent kinase inhibitor p21WAF1/CIP1 in apoptosis and autophagy. *J Biol Chem* 2008; *283* : 388-97.
79. Kim JJ, Tannock IF. Repopulation of cancer cells during therapy: an important cause of treatment failure. *Nat Rev Cancer* 2005; *5* : 516-25.
80. Schmidt-Ullrich RK, Mikkelsen RB, Dent P, Todd DG, Valerie K, Kavanagh BD, *et al*. Radiation-induced proliferation of the human A431 squamous carcinoma cells is dependent on EGFR tyrosine phosphorylation. *Oncogene* 1997; *15* : 1191-7.
81. Vivanco I, Sawyers CL. The phosphatidylinositol 3-Kinase AKT pathway in human cancer. *Nat Rev Cancer* 2002; *2* : 489-501.
82. Chang F, Lee JT, Navolanic PM, Steelman LS, Shelton JG, Blalock WL, *et al*. Involvement of PI3K/Akt pathway in cell cycle progression, apoptosis, and neoplastic transformation: a target for cancer chemotherapy. *Leukemia* 2003; *17* : 590-603.
83. Liang J, Slingerland JM. Multiple roles of the PI3K/PKB (Akt) pathway in cell cycle progression. *Cell cycle* 2003; *2* : 339-45.
84. Harris AL. Hypoxia - a key regulatory factor in tumour growth. *Nat Rev Cancer* 2002; *2* : 38-47.
85. Koumenis C. ER stress, hypoxia tolerance and tumor progression. *Curr Mol Med* 2006; *6* : 55-69.
86. Bussink J, Kaanders JH, van der Kogel AJ. Tumor hypoxia at the micro-regional level: clinical relevance and predictive value of exogenous and endogenous hypoxic cell markers. *Radiother Oncol* 2003; *67* : 3-15.
87. Lal A, Peters H, St Croix B, Haroon ZA, Dewhirst MW, Strausberg RL, *et al*. Transcriptional response to hypoxia in human tumors. *J Natl Cancer Inst* 2001; *93* : 1337-43.
88. Pore N, Gupta AK, Cerniglia GJ, Maity A. HIV protease inhibitors decrease VEGF/HIF-1alpha expression and angiogenesis in glioblastoma cells. *Neoplasia* 2006; *8* : 889-95.
89. Bussink J, van der Kogel AJ, Kaanders JH. Activation of the PI3-K/AKT pathway and implications for radioresistance mechanisms in head and neck cancer. *Lancet Oncol* 2008; *9* : 288-96.
90. Schuurbiers OC, Kaanders JH, van der Heijden HF, Dekhuijzen RP, Oyen WJ, Bussink J. The PI3-K/AKT-

pathway and radiation resistance mechanisms in non-small cell lung cancer. *J Thorac Oncol* 2009; *4* : 761-7.

91. Karar J, Maity A. PI3K/AKT/mTOR pathway in angiogenesis. *Front Mol Neurosci* 2011; *4* : 51.
92. Pore N, Gupta AK, Cerniglia GJ, Jiang Z, Bernhard EJ, Evans SM, *et al*. Nelfinavir down-regulates hypoxia-inducible factor 1alpha and VEGF expression and increases tumor oxygenation: implications for radiotherapy. *Cancer Res* 2006; *66* : 9252-9.
93. Zhu Y, Denhardt DT, Cao H, Sutphin PD, Koong AC, Giaccia AJ, *et al*. Hypoxia upregulates osteopontin expression in NIH-3T3 cells via a Ras-activated enhancer. *Oncogene* 2005; *24* : 6555-63.
94. Dai J, Peng L, Fan K, Wang H, Wei R, Ji G, *et al*. Osteopontin induces angiogenesis through activation of PI3K/AKT and ERK1/2 in endothelial cells. *Oncogene* 2009; *28* : 3412-22.
95. Fukumura D, Xu L, Chen Y, Gohongi T, Seed B, Jain RK. Hypoxia and acidosis independently up-regulate vascular endothelial growth factor transcription in brain tumors *in vivo*. *Cancer Res* 2001; *61* : 6020-4.
96. Fokas E, Im JH, Hill S, Yameen S, Stratford M, Beech J, *et al*. Dual inhibition of the PI3K/mTOR pathway increases tumor radiosensitivity by normalizing tumor vasculature. *Cancer Res* 2012; *72* : 239-48.
97. Morelli MP, Cascone T, Troiani T, Tuccillo C, Bianco R, Normanno N, *et al*. Anti-tumor activity of the combination of cetuximab, an anti-EGFR blocking monoclonal antibody and ZD6474, an inhibitor of VEGFR and EGFR tyrosine kinases. *J Cell Physiol* 2006; *208* : 344-53.
98. Mabeta P. Inhibition of phosphoinositide 3-kinase is associated with reduced angiogenesis and an altered expression of angiogenic markers in endothelioma cells. *Biomed Pharmacother* 2014; *68* : 611-7.
99. Warburg O. On the origin of cancer cells. *Science* 1956; *123* : 309-14.
100. Weber G. Enzymology of cancer cells (second of two parts). *New Engl J Med* 1977; *296* : 541-51.
101. Weber G. Enzymology of cancer cells (first of two parts). *New Engl J Med* 1977; *296* : 486-92.
102. Elstrom RL, Bauer DE, Buzzai M, Karnauskas R, Harris MH, Plas DR, *et al*. Akt stimulates aerobic glycolysis in cancer cells. *Cancer Res* 2004; *64* : 3892-9.
103. Kim DI, Lim SK, Park MJ, Han HJ, Kim GY, Park SH. The involvement of phosphatidylinositol 3-kinase /Akt signaling in high glucose-induced downregulation of GLUT-1 expression in ARPE cells. *Life Sci* 2007; *80* : 626-32.
104. Miyamoto S, Murphy AN, Brown JH. Akt mediates mitochondrial protection in cardiomyocytes through phosphorylation of mitochondrial hexokinase-II. *Cell Death Differ* 2008; *15* : 521-9.
105. Deprez J, Vertommen D, Alessi DR, Hue L, Rider MH. Phosphorylation and activation of heart 6-phosphofructo-2-kinase by protein kinase B and other protein kinases of the insulin signaling cascades. *J Biol Chem* 1997; *272* : 17269-75.
106. Simons AL, Orcutt KP, Madsen JM, Scarbrough PM, Spitz DR. The role of AKt pathway signalling in glucose metabolism and metabolic oxidative stress. In: Spitz DR, Dornfeld KJ, Krishnan K, Gius D, editors. *Oxidative stress in cancer biology and therapy.* New York: Springer Science & Business Media; 2012. p. 21-46.
107. Fresno Vara JA, Casado E, de Castro J, Cejas P, Belda-Iniesta C, Gonzalez-Baron M. PI3K/Akt signalling pathway and cancer. *Cancer Treat Rev* 2004; *30* : 193-204.
108. Ocana A, Vera-Badillo F, Al-Mubarak M, Templeton AJ, Corrales-Sanchez V, Diez-Gonzalez L, *et al*. Activation of the PI3K/mTOR/AKT pathway and survival in solid tumors: systematic review and meta-analysis. *PLoS One* 2014; *9* : e95219.
109. Gupta AK, Cerniglia GJ, Mick R, McKenna WG, Muschel RJ. HIV protease inhibitors block Akt signaling and radiosensitize tumor cells both *in vitro* and *in vivo*. *Cancer Res* 2005; *65* : 8256-65.
110. Russo SM, Tepper JE, Baldwin AS Jr, Liu R, Adams J, Elliott P, *et al*. Enhancement of radiosensitivity by proteasome inhibition: implications for a role of NF-kappaB. *Int J Radiat Oncol, Biol, Phy* 2001; *50* : 183-93.
111. Gupta AK, Lee JH, Wilke WW, Quon H, Smith G, Maity A, *et al*. Radiation response in two HPV-infected head-and-neck cancer cell lines in comparison to a non-HPV-infected cell line and relationship to signaling through AKT. *Int J Radiat Oncol Bio Phy* 2009; *74* : 928-33.
112. Pajonk F, Himmelsbach J, Riess K, Sommer A, McBride WH. The human immunodeficiency virus (HIV)-1 protease inhibitor saquinavir inhibits proteasome function and causes apoptosis and radiosensitization in non-HIV-associated human cancer cells. *Cancer Res* 2002; *62* : 5230-5.
113. Gupta AK, Li B, Cerniglia GJ, Ahmed MS, Hahn SM, Maity A. The HIV protease inhibitor nelfinavir downregulates Akt phosphorylation by inhibiting proteasomal activity and inducing the unfolded protein response. *Neoplasia* 2007; *9* : 271-8.
114. Bernhard EJ, Brunner TB. Progress towards the use of HIV protease inhibitors in cancer therapy. *Cancer Biol Ther* 2008; *7* : 636-7.
115. Bae SS, Cho H, Mu J, Birnbaum MJ. Isoform-specific regulation of insulin-dependent glucose uptake by Akt/protein kinase B. *J Biol Chem* 2003; *278* : 49530-6.
116. Cross DA, Watt PW, Shaw M, van der Kaay J, Downes CP, Holder JC, *et al*. Insulin activates protein kinase B, inhibits glycogen synthase kinase-3 and activates glycogen synthase by rapamycin-insensitive pathways in skeletal muscle and adipose tissue. *FEBS Lett* 1997; *406* : 211-5.
117. Kariya R, Taura M, Suzu S, Kai H, Katano H, Okada S. HIV protease inhibitor Lopinavir induces apoptosis of primary effusion lymphoma cells via suppression of NF-kappaB pathway. *Cancer Lett* 2014; *342* : 52-9.
118. Birle DC, Hedley DW. Suppression of the hypoxia-inducible factor-1 response in cervical carcinoma xenografts by proteasome inhibitors. *Cancer Res* 2007; *67* : 1735-43.
119. Plastaras JP, Vapiwala N, Ahmed MS, Gudonis D, Cerniglia GJ, Feldman MD, *et al*. Validation and toxicity of PI3K/Akt pathway inhibition by HIV protease inhibitors in humans. *Cancer Biol Ther* 2008; *7* : 628-35.

120. Cuneo KC, Tu T, Geng L, Fu A, Hallahan DE, Willey CD. HIV protease inhibitors enhance the efficacy of irradiation. *Cancer Res* 2007; *67* : 4886-93.

121. Tebas P, Powderly WG. Nelfinavir mesylate. *Expert Opin Pharmacother* 2000; *1* : 1429-40.

122. Ofotokun I, Smithson SE, Lu C, Easley KA, Lennox JL. Liver enzymes elevation and immune reconstitution among treatment-naive HIV-infected patients instituting antiretroviral therapy. *Am J Med Sci* 2007; *334* : 334-41.

123. Pyrko P, Kardosh A, Wang W, Xiong W, Schonthal AH, Chen TC. HIV-1 protease inhibitors nelfinavir and atazanavir induce malignant glioma death by triggering endoplasmic reticulum stress. *Cancer Res* 2007; *67* : 10920-8.

124. Brunner TB, Geiger M, Grabenbauer GG, Lang-Welzenbach M, Mantoni TS, Cavallaro A, *et al*. Phase I trial of the human immunodeficiency virus protease inhibitor nelfinavir and chemoradiation for locally advanced pancreatic cancer. *J Clin Oncol* 2008; *26* : 2699-706.

125. Rengan R, Mick R, Pryma D, Rosen MA, Lin LL, Maity AM, *et al*. A phase I trial of the HIV protease inhibitor nelfinavir with concurrent chemoradiotherapy for unresectable stage IIIA/IIIB non-small cell lung cancer: a report of toxicities and clinical response. *J Thorac Oncol* 2012; *7* : 709-15.

126. Buijsen J, Lammering G, Jansen RL, Beets GL, Wals J, Sosef M, *et al*. Phase I trial of the combination of the Akt inhibitor nelfinavir and chemoradiation for locally advanced rectal cancer. *Radiother Oncol* 2013; *107* : 184-8.

Reprint requests: Dr Jayant S. Goda, Department of Radiation Oncology, Advance Centre for Treatment Research & Education in Cancer, Tata Memorial Centre, Kharghar, Navi Mumbai 410 210, Maharashtra, India
e-mail: godajayantsastri@gmail.com, jgoda@actrec.gov.in