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Strategies for optimizing the response of cancer and normal tissues to radiation

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Abstract

Approximately 50% of all patients with cancer receive radiation therapy at some point during the course of their treatment, and the majority of these patients are treated with curative intent. Despite recent advances in the planning of radiation treatment and the delivery of image-guided radiation therapy, acute toxicity and potential long-term side effects often limit the ability to deliver a sufficient dose of radiation to control tumours locally. In the past two decades, a better understanding of the hallmarks of cancer and the discovery of specific signalling pathways by which cells respond to radiation have provided new opportunities to design molecularly targeted therapies to increase the therapeutic window of radiation therapy. Here, we review efforts to develop approaches that could improve outcomes with radiation therapy by increasing the probability of tumour cure or by decreasing normal tissue toxicity.

Ionizing radiation is a commonly used modality for treating cancers (BOX 1). The majority of patients are treated with external beam radiation therapy, in which a radiation source external to the patient generates ionizing radiation that is directed towards the tumour. Modern radiation therapy is delivered mainly via linear accelerators, which generate high-energy X-rays that can be collimated to selectively shape the treatment field. Intensity modulated radiation therapy (IMRT) uses non-uniform, computer-optimized radiation fields to deliver a high dose of radiation to the tumour while limiting the radiation to normal tissues[1]. With IMRT, the high-dose region conforms better to the tumour, but a larger volume of normal tissue is exposed to low-dose radiation. The long-term effects of this radiation on normal tissues are not known.

Patients are typically treated with small 1.8–2 Gy fractions over the course of 4–8 weeks to limit toxicity to normal tissues. However, advances in treatment planning and delivery have made it possible to safely deliver a small number of high doses (15–20 Gy) to tumours. This treatment modality has been termed `stereotactic body radiation therapy' or radiosurgery. Stereotactic body radiation therapy, which is currently being used clinically for some early-stage cancers and oligom etastatic disease, may be more effective than standard radiation therapy for some cancers[2]. Although normal tissue toxicity limits the use of stereotactic body radiation therapy in certain anatomical locations[3–5], it has been successfully utilized for many cancer types including non-small-cell lung cancer, prostate cancer, renal cell carcinoma and hepatocellular carcinoma[6–9].

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An emerging technique in radiation oncology is the use of high-energy charged particles to treat tumours[10]. Particle therapy offers a physical advantage over X-ray irradiation[11]. Unlike X-rays, which deposit radiation distal to the tumour target as they exit the patient, charged particles stop abruptly within the tissue and deposit the majority of their energy within a small area called the Bragg peak. This dose profile delivers radiation to the tumour while sparing normal tissues from exit irradiation. This may be especially useful for treating tumours that are adjacent to dose-limiting structures, such as the brainstem, or for treating children with cancer who may be at a relatively high risk of developing radiation-induced cancers. Protons are the most commonly used particle therapy[11]. Although protons are approximately equivalent to X-rays in terms of biological effectiveness, they have a Bragg peak that offers improved sparing of normal tissues. Protons are currently utilized for a broad range of tumours, including paediatric tumours, uveal melanomas, skull base tumours and prostate tumours[12]. Recently, a retrospective study of SEER (surveillance, epidemiology and end results) Medicare-linked data suggested that there was an increased incidence of gastrointestinal side effects in patients who were treated with protons[13]. A randomized clinical trial at Massachusetts General Hospital, Boston, USA, and the University of Pennsylvania, Philadelphia, USA, is currently underway to compare the effectiveness of protons and IMRT for the treatment of prostate cancer.

Carbon ions, which are used to treat patients with cancer in Japan and Germany, are also charged and therefore deposit energy with a Bragg peak. However, these larger particles cause concentrated damage that is more lethal to irradiated cells than the damage inflicted by X-rays or protons. Thus, for a given dose, carbon ions have a higher relative biological effectiveness (RBE). In addition, the cellular damage caused by carbon ions may be less dependent on oxygen to stabilize free radicals within cells. As a result, the oxygen enhancement ratio (OER) for heavy particles is lower than for X-rays.

In contrast to external beam radiation therapy, brachytherapy involves the implantation of a radiation source temporarily or permanently into the tumour site. Because the radiation exposure decreases with the square of the distance from the source, brachytherapy is a highly conformal therapy. Therefore, this approach may be particularly useful in com bination with radiosensitizing drugs because the entrance and exit dose (to normal tissues) associated with external beam radiation therapy is eliminated. Brachytherapy has been commonly used to treat prostate, breast and gynaecological cancers. Recent changes in source availability and the implementation ol remote afterloaders have resulted in the introduction of high-dose-rate brachytherapy into routine clinical practice at many institutions[14]. Unlike traditional low- to medium -dose-rate brachytherapy, which is delivered via perm anent implants, high-dose-rate systems utilize sources with dose rates that are similar to linear accelerators. As a result, the sources are implanted temporarily and the doses are often fractionated. New approaches to deliver radiation selectively to tumours via isotope-conjugated antibodies or nanoparticles are under development.

Advances in all aspects of radiation oncology are making it more feasible to com bine radiation treatment with targeted drugs. Limiting toxicity to normal tissues starts with treatment planning and optimized dose distributions[15]. Imaging tumours before, during and after radiation delivery makes it possible to make adjustments to account for changes in tum our position, size and shape[16]. Precise patient immobilization and localization on the treatment couch, as well as on-board X-ray, ultrasound and infrared imaging to ensure patient positioning, limit treatment errors and allow radiation to be delivered with smaller margins[17], As the doses delivered to normal tissues are limited, radiation oncologists can focus more on sensitizing tumours than on sparing normal tissues.

History of radiation modulators

The potential for concurrent therapies to enhance the local control of tum our recurrance has long been recognized and numerous approaches have been attempted. Initial experiments in the 1960s and prospective trials in the 1970s investigated sequential and concurrent chemotherapy and radiation with the hypothesis that chem otherapy-mediated inhibition of DNA repair mechanisms would lead to synergistic effects[18]. Since then, continued experiments have demonstrated that drugs such as cisplatin, 5-fluorouracil and mitomycin C radiosensitize tumours including those from head and neck cancers, lung cancers and gastrointestinal cancers[19]. Although these concurrent chemotherapies increase the rates of local control and, in some cases, overall survival, because they are not specific for tumour cells they also increase radiation toxicity to normal tissues[20]. Improved radiosensitizers will therefore need to selectively increase tum our cell killing and local control while minimally affecting normal tissues.

Hypoxia: an example of selective tumour targeting

Historically, tumour hypoxia has been one o f the most frequently targeted characteristics of tumours to improve the efficacy of radiation. Unlike most normal tissues, tumours experience considerable hypoxia owing to fluctuations in blood flow and increased metabolicdemand[21]. Low oxygen availability decreases the efficacy of radiation and adversely affects the prognosis of patients with cancer[22,23]. Hypoxic sensitizers have long been recognized as potential radiosensitizers[24], and numerous attempts have been made to decrease the negative effect of hypoxia on the outcomes of radiation therapy.

Generally, hypoxic drugs can be divided into three categories: drugs that increase delivery of oxygen to tumours; hypoxic cell radiosensitizers; and direct hypoxic cell cytotoxins[25]. Approaches to increase oxygen delivery to tumours include breathing oxygen under nonnobaric and hyperbaric pressure, blood transfusions, nicotinamide administration and the use of erythropoietin. Although there is some evidence that these approaches may improve local control, additional studies are needed to justify the implementation of cumbersome treatment techniques — such as hyperbaric oxygen administration — into clinical practice[26]. Notably, combining erythropoietin treatment with radiation therapy for head and neck cancers led to significantly worse outcomes in patients, presumably because the erythropoietin receptor is also expressed on squamous cell carcinoma tumour cells[27],[28]. Therefore, in addition to promoting the production of red blood cells, erythropoietin may cause proliferation of head and neck cancer cells and/or protect them from cell death.

The nitroimidazoles are a family of electron-affinic drugs that mimic the effect of oxygen by reacting with DNA free radicals, and this sensitizes hypoxic cells to radiation. Nimorazole has been shown to improve the effect of radiation therapy on supraglottic and pharynx tumours in a Danish head and neck cancer study[29] and is commonly used in clinics in Denmark. However, another nitroimidazole — etanidazole — was tested in a Radiation Therapy Oncology Group (RTOG) trial but it did not significantly improve outcomes[30]. As a result, the use of nitroimidazoles is not common in clinical practice in the United States.

A theoretical study suggested that hypoxic cell cytotoxins might be the best way to target hypoxic cells during radiation therapy[31]. Tirapazamine showed promise in early clinical trials, but no therapeutic benefit was observed in a recent Phase III clinical trial of unselected patients with head and neck cancers[32]. The idea that tirapazamine may be more effective in patients with hypoxic tumours led to attempts to retrospectively identify biomarkers of patients with hypoxic tumours. A subgroup of patients with elevated plasma levels of hepatocyte growth factor (HGF) and interleukin-8 (IL-8) seemed to benefit more from tirapazamine[33], but prospective studies will be required to determine whether plasma

biomarkers or functional imaging[34] can be used to identify patients with hypoxic tumours that may benefit from hypoxic cell cytotoxins.

Numerous clinical trials have been completed to evaluate the effect of hypoxia modification on treatment outcome. Many of these trials have been inconclusive because they were small and underpowered. In a systematic review incorporating all of these trials, modification of hypoxia was shown to significantly improve locoregional control and overall survival without affecting radiation-related complications[25]. Therefore, further work to identify biomarkers of hypoxia will be important to enrich for patients who may benefit from hypoxia modification in future clinical trials of radiation therapy.

Amifostine: a clinical success story

Although numerous drugs that modify radiation response have been developed and clinically tested, amifostine is one of the few modifiers of radiation response that has received approval from the US Food and Drug Administration. This thiol drug acts as a free radical scavenger and radio-protector[35]. Amifostine concentrates more rapidly in normal tissues than in tumours[36], and is not taken up by cells until it is dephosphorylated by alkaline phosphatase[37]. As a result, amifostine preferentially protects normal tissues — but not tumours — from radiation damage[38].

Amifostine has been tested in Phase III clinical trials for head and neck cancer, non-small-cell lung cancer and pelvic malignancies[39–41]. Numerous randomized controlled studies have suggested that amifostine may protect against radiation-induced toxicity in patients with head and neck cancer[35]; this has led the American Society of Clinical Oncology to recommend that amifostine be considered for the prevention of xerostomia during fractionated radiotherapy. However, amifostine has not been recommended for the prevention of mucositis during concurrent platinum-based chemoradiotherapy for head and neck cancer[42]. As concurrent chemoradiotherapy is the standard of treatment for this type of cancer, this has limited the clinical application of amifostine as a radioprotector. However, amifostine has also been shown to decrease the incidence of nephrotoxicity[43], ototoxicity[44] and neurotoxicity[45] in patients receiving cisplatin-based chemotherapy regimens in the absence of radiation therapy.

Limitations of current radiation modulators

To date, one of the major factors limiting the implementation of radiosensitizers and radioprotectors in clinical care has been the nonspecific mechanism of action of many radiation modulators. Drugs that affect radiosensitivity by targeting key cell survival pathways are likely to affect both tumours and normal tissues. Thus, selectivity is an important goal for designing improved radiation modulators. As our knowledge of the genes that are altered in cancer increases and as we gain further insight into the signalling pathways that mediate the DNA damage response after radiation in tumour and normal tissues, it may become more feasible to select drug targets that regulate radiosensitivity specifically in tumour cells or normal cells and tissues. However, the response of different cell types to radiation varies[46]. Therefore, a target of radiosensitivity that is identified in a certain tumour type may not translate to all clinical situations. Tumours arising in different tissues show varied responses to radiation[47,48], and this should be considered when testing radiation modulators in the clinic.

Most radiation modulators are initially developed in preclinical models. However, not all preclinical models recapitulate human disease to the same extent[49]. It is important to account for the limitations of each preclinical model when therapies are translated from cells to animals and ultimately to patients. For example, the tumour microenvironment has been

shown to contribute to tumour initiation, progression and response to therapy[50]. The tumour microenvironment can also affect the response of tumours to radiation[51], and the immune system may be important for clearing tumours following radiation therapy[52–54]. Thus, cell culture models, xenografts established in immunocompromised mice and even syngeneic implantation of tumours may not accurately capture all of the important components of human tumours that regulate the response to radiation.

A solution may be to test radiation modulators in genetically engineered mouse models (GEMMs), in which primary cancers develop in the native tumour microenvironment in immunocompetent mice[55]. Numerous studies have suggested that GEMMs may more faithfully recapitulate the tumour stroma and microenvironment of human cancer than xenograft models[56–58]. Importantly, GEMMs have been shown to closely model the response of human cancers to systemic therapy in clinical trials[59–60]. However, even with GEMMs the limitations of testing drugs on mouse tumours must be taken into account. The pharmacology of drugs and the role of therapeutic targets may not be conserved across species. Nevertheless, as primary tumour models improve, it is likely that they will improve the translation of promising preclinical data on radiation modulators into successful clinical trials.

New insights into tumour biology

Tumour heterogeneity and cancer stem cells

Recent discoveries in cancer biology have suggested new mechanisms by which tumours recur after radiation therapy. Tumour heterogeneity has long been recognized as a mechanism by which tumours evade radiation therapy[61]. Heterogeneity arising from differences in the type of cancer (that is, sarcoma, carcinoma or glioma, and so on) can alter the response ol individual tumours to radiation therapy[47,48]. Even among tumours of the same histological subtype, the radiation response can vary substantially from patient to patient. Such differences between patients may be due to the mutations within each tumour as the tumour genotype may influence the response to radiation therapy and the effectiveness of radiosensitizers[62–64]. This observation should be taken into consideration when radiation modulators are tested in heterogeneous patient and tumour populations. Even at the individual tumour level, heterogeneity can be quite extensive. Single-cell sequencing has demonstrated that a given tumour contains many distinct clones with diverse mutations[65]. Each of these mutations has the potential to modify responsiveness to radiation, so it is possible that multiple radiation modulators may be necessary in order to sensitize an entire tumour to radiation therapy.

Within a tumour, individual cells have varying abilities to contribute to tumour regrowth following radiation therapy. Indeed, lineage-tracing experiments suggest that a small subset of tumour cells drives tumour growth and regrowth following therapy[66–68]. These tumour-propagating cells, or cancer stem cells, which have been identified in several different types of tumours, seem to be maintained in a stem-cell-like state by their niche within tumours[69]. Although these cells must be eliminated for a cancer to be cured, accumulating evidence suggests that cancer stem cells may be inherently radio-resistant[70–72]. Like haematopoietic stem cells[73], cancer stem cells may reside in hypoxic niches. Moreover, these cells are generally quiescent and may show increased activation of the DNA damage response[74]. However, by understanding the unique properties of these cells, it may be possible to design drugs that sensitize cancer stem cells to radiation therapy. For example, drugs that disrupt the stem cell niche or force these cells out of quiescence and into the cell cycle have the potential to act as radiosensitizers[75].

Synthetic lethality

Targeting the pathways that are required for cellular survivalis likely to be toxic to normal tissues. However, many important pathways — including the DNA damage response pathway — exhibit redundancy in normal cells. Growing evidence suggests that many tumours have mutations that cause the loss of a signalling pathway, which may remove this redundancy. As a result, targeting the remaining pathway in the tumour cell can induce synthetic lethality (fig.1). Because the normal cells retain the redundant signalling pathway, this approach can provide a therapeutic window to kill cancer cells while sparing normal tissues[76]. As DNA is the main target for radiation-induced cell killing[77], and there is considerable redundancy in the ability of cells to repair DNA damage[78], targeting DNA damage response pathways is a promising approach for the selective radiosensitization of tumour cells[79].

The application of poly(ADP-ribose) polymerase (PARP) inhibitors to tumours with BRCA1 (breast cancer susceptibility 1) and BRCA2 mutations demonstrates the potential of synthetic lethality in selectively targeting cancer cells. PARP1 has an important role in base excision repair of single-strand DNA breaks[80]. PARP inhibition may increase the number of endogenous and radiation-induced double-strand DNA breaks owing to the impaired repair of single-strand breaks[81]. In normal cells, these double-strand breaks can be repaired via homologous recombination and non-homologous end joining. However, many familial breast cancers are due to mutations in the BRCA1 or BRCA2 genes, which are required for the efficient repair of double-strand DNA breaks by homologous recombination[82]. Therefore, many BRCA1- and BRCA2-mutant breast cancers are profoundly sensitive to PARP inhibition[83–85], which appears to be due to defective homologous recombination specifically in the tumour cells[86]. As a result, PARP inhibition may also be useful in tumours with other mutations associated with homologous recombination[87,88]. There is evidence that other mutations in the DNA damage response pathways can also sensitize cells to synthetic lethality. For example, cells with a mutated Fanconi anaemia pathway have increased sensitivity to ataxia telangiectasia mutated (ATM) inhibition[89].

As the harsh tumour microenvironment can present unique challenges for cellular survival, drugs could also trigger synthetic lethality by targeting the pathways used by cells to withstand the stresses found in the tumour microenvironment, such as hypoxia, oxidative stress or cell–cell contact abnormalities. Tumour cells exposed to these environmental stresses, but not normal cells (which exist in a less harsh microenvironment), should be preferentially killed by inhibitors that block stress survival signalling pathways. These examples demonstrate the feasibility of targeting the loss of pathway redundancy to induce synthetic lethality and selectively target cancer cells.

Promising radiosensitization targets

Numerous approaches to radiosensitize tumours have shown promise using in vitro assays. Before these therapies can move into the clinic, the safety and efficacy of these drugs needs to be shown using relevant in vivo models. Targets for radiosensitization can be grouped into two broad categories: targets within the tumour cell, such as phosphoinositide 3-kinase (PI3K)-like kinases (PI3KKs) and survival pathways; and targets within the tumour microenvironment.

PI3K-like kinases

The PI3KK family contains several serine/threonine kinases with highly conserved catalytic domains that have homology to PI3K[90]. This includes mammalian target of rapamycin (mTOR), DNA-dependent protein kinase catalytic subunit (DNA-PK_{CS}), ATM and ATR (ataxia telangiectasia and RAD3 related) (fig. 2). Following radiation, these proteins have

crucial roles in responding to DNA damage, orchestrating DNA repair and promoting cellular survival. Selective inhibition of ATM, the gene that is mutated in the radiation hypersensitivity syndrome ataxia telangiectasia, has been shown to sensitize human tumour cell lines to radiation in vitro[91,92], Importantly, ATM loss sensitizes both p53-null and wild-type tissues[93], and cancer stem cells may be particularly sensitive to ATM inhibition[94]. Small-molecule inhibitors that are designed to target one member of the PI3KK family may also inhibit other family members, thereby leading to synergistic radiosensitization (fig. 2). Although inhibition of some PI3KK family members in normal tissues can be tolerated, inhibition of all the Pl3KKs may cause toxicity in normal cells. For example, cells lacking ATR are generally non-viable[95,96] because ATR is critical for resolving stalled DNA replication forks to allow efficient progression through the S phase of the cell cycle[97]. However, cells that are under oncogene-induced replicative stress may be more sensitive to ATR inhibition than normal cells[98]. It was shown that a small-molecule ATR inhibitor in combination with radiotherapy prolonged the growth delay of pancreatic cancer xenografts compared to radiation therapy alone, without causing toxicity to normal tissues$_{99}$. Nevertheless, to minimize toxicity to normal tissues, it will probably be important to design small molecules that inhibit some — but not all — members ol the PI3KK family.

NVP-BEZ235 was designed as a combined PI3K–mTOR inhibitor and demonstrated potent anticancer activity as a monotherapeutic agent[100]. The drug is currently in Phase II clinical trials for advanced solid tumours and has shown efficacy as a radiosensitizer in preclinical models[101]. Recent studies have demonstrated that its radiosensitizing effect is partially mediated via inhibition of the DNA damage response proteins ATM and DNA-PK$_{CS}$ (ref. 102). However, there is evidence that PI3K activation alone can cause resistance to radiation[103]. Delivery of NVP-BEZ235 before irradiation may provide additional benefit owing to normalization of the tumour vasculature[104]. However, NVP-REZ235 may be a nonspecific inhibitor of the PI3KK family because it has also been shown to inhibit ATR[98]; this may limit its utility because ATR inhibition is associated with toxicity.

Survival pathways

Several survival pathways have been shown to be activated following ionizing radiation, including PI3K, mitogen-activated protein kinase (MAPK), nuclear factor-κB (NF-κB) and transforming growth factor-β (TGFβ) pathways[105,106]. In addition to regulating cell death, many of these pathways can affect the tumour microenvironment, and activate DNA damage repair[104,107–109]. As a result, inhibition of these pathways has the potential to increase the radiosensitivity of cancer cells through multiple mechanisms. However, inhibition of cellular survival could affect the radiosensitivity of normal tissues as well, thus decreasing the therapeutic index of radiation.

The PI3K–AKT pathway is activated by various mutations that are commonly found in cancer, including receptor tyrosine kinase activations, activating mutations in PI3K, loss of the tumour suppressor phosphatase and tensin homolog (PTEN) and activating RAS mutations[110]. Importantly, activation of the P13K–AKT pathway contributes to resistance to radiation[111,112]. As a result, selective inhibitors of this pathway increase the radiosensitivity of cancer cells, and many drugs targeting this pathway are currently in preclinical development or in clinical trials[113]. The specific activating mutation in tumours needs to be taken into account when targeting the PI3K–AKT signalling pathway, as drugs that block signalling upstream of the mutant protein may not be effective.

For example, epidermal growth factor receptor (EGFR) is a tyrosine kinase that signals upstream of RAS and PI3K. EGFR is commonly overexpressed in squamous cell carcinomas of the head and neck. The EGFR-specitic antibody cetuximab (Erbitux; Bristol-Myers Squibb/Lilly) significantly improved local regional control and overall survival by

radiotherapy in a Phase III clinical trial of patients with head and neck eancers[114,115] However, the inclusion of EGFR inhibitors in chemotherapy for patients with KRAS-mutant non-small-cell lung cancers actually led to worse clinical outcomes in comparison with patients who were treated with chemotherapy alone[116]. Similarly, patients with colon cancers only respond favourably to cetuximab if their tumours do not contain KRAS mutations[117].

The intracellular kinases extracellular signal-regulated kinase 1 (ERK1) and ERK2 are activated downstream of RAS signalling following the exposure of cells to radiation[118]. As ERK signalling can promote growth and survival, several studies have attempted to inhibit ERK to enhance radiation-induced cell killing. Interestingly, the effect of inhibiting ERK1 or EREC2 signalling depends on thecell type and timingofinhibition[105]. Although it has the potential to radiosensitize tumour cells, some cells are not affected by ERK inhibition and others actually seem to be protected from radiation following ERK inhibition.

As many tumours develop resistance to apoptosis during cancer progression, programmed cell death has been shown to have only a modest role in the treatment response of most solid tumours to radiation therapy[119]. However, antagonism of anti-apoptotic proteins may reactivate cellular apoptosis m achinery and sensitize tumours to radiation-induced apoptosis[120]. As with PI3KK inhibitors, combined inhibition of several anti-apoptotic proteins may be more effective at radiosensitizing tumour cells. ART-737, a combined inhibitor of B cell lymphoma 2 (BCL-2), BCL-2-like protein 1 (BCL-2L1) and BCL2-L2, synergized with radiation to kill cancer cells in xenograft tum ours[121]. Phase I studies of ABT-263, an analogue of ABT-737 with improved oral availability, demonstrated safety and encouraging efficacy in small-cell lung cancer and lymphoid malignancies[122–124].

Another anti-apoptotic protein, myeloid cell leukaemia sequence 1 protein (MCL1), has been shown to block radiation-induced apoptosis in some cell types and may be more important than BCL-2 for sensitizing haematopoietic tumours, as many haematopoietic cells require MCL1 for survival[125–126]. Antisense nucleotides targeting MCL1 synergize with radiation to increase cell death[127], and the combination of MCL1 inhibition together with ABT-737 potentiates tum our cell a poptosis in vtro[128]. In addition, AT-101, a BH3 mimetic that targets MCL1 and other BCL-2 proteins, radiosensitized small-cell lung cancer cell lines in vitro[129] and enhanced growth delay after radiation therapy in a xenograft model of prostate cancer[130]. Similarly, the pan-BCL-2 inhibitor obatoclax increased radiation-induced apoptosis in breast cancer cell lines with elevated expression of BCL-2 family mRNA[131].

Approximately half of all human cancers have alterations in the TP53 gene, resulting in loss or inactivation of tumour suppressor p53 protein[112]. In tumours expressing wiki-type TP53, p53 function is generally inhibited through other mutations in the p53 pathway. HDM2 (known as MDM2 in mice) is an E3 ubiquitin ligase that binds directly to p53, blocking its transcriptional activity and leading to its degradation[153]. As HDM2 tightly regulates p53 activity, small-molecule HDM2 inhibitors have been designed to reactivate the p53 pathway in cancer cells expressing wild-type p53 (ref. 134). This approach could be used to reactivate p53-mediated cell death pathways, which can be triggered by radiation.

Checkpoint inhibitors

The activation of cell cycle checkpoints is an important consequence of the DNA damage response because cell cycle arrest may prevent mitosis with double-strand breaks, which can lead to the loss of critical chromosomal material in a process termed mitotic catastrophe[135]. In vitro, abrogation of a single cell cycle checkpoint does not alter cellular radiosensitivity — as measured by clonogenic survival — after radiation. For example, the radio sensitivity of BRCA1-deficient cancer cells, which are incapable of triggering an S phase checkpoint,

can be rescued with a mutant BRCA1 that is incapable of triggering the S phase checkpoint[136]. This suggests that BRCA1-mediated radiosensitivity is not due to loss of the S phase checkpoint. Complementary experiments have dem onstrated that cells that have mutant BRCA1 and are incapable of triggering the early G2/M checkpoint have the same radiosensitivity as wild-type cells, as measured by clonogenic survival[137]. These experiments suggest that loss of BRCA does not increase radiosensitivity through loss of cell cycle checkpoints but instead through other mechanisms such as impaired DNA damage repair.

The cyclin-dependent kinase inhibitor p21 (also known as CDKNlA) is required for the radiation-induced G1 cell cycle checkpoint and, at least in some cells, also for a sustained G2 checkpoint[138]. Interestingly, loss of p21 does not affect the clonogenic survival of cells irradiated in vitro. However, when these same cells are grown as xenografts, tumours lacking p21 are sensitized to radiation therapy in in vivo[139,140]. Similarly, the deletion of p21 sensitizes mice to radiation injury of some normal tissues. For example, mice lacking p21 are sensitized to the radiation-induced gastrointestinal syndrome[141–145] and to radiation-induced cardiac, injury[144]. Taken together, these studies underscore the importance of studying radiation responses using both in vitro and in vivo systems.

WEE1 is a protein kinase that prevents cells from entering mitosis following radiation by phosphorylating cyclin-dependent kinase 1 (CDK1)[145]. Several studies have shown that inhibiting WEE1 increases mitotic catastrophe following radiation and can sensitize tumour cells to radiation in vitro and in vivo[146–148]. However, these inhibitors also target other kinases, which could affect cellular radiosensitivity[149].

Simultaneously targeting several cell cycle checkpoints may increase radiosensitivity. For example, it is possible that combined loss of the S and G2 checkpoints may cause premature mitotic entry[150]. The dual checkpoint kinase 1 (CHK1) and CHK2 inhibitor AZD7762 has been shown to radiosensitize human cancer cells and xenografts[151]. However, this may be due to its direct effects on homologous recombination[152]. In addition, inhibitors ot ATM and downstream targets such as cell division cycle 25 (CDC25) may potentiate radiosensitivity in part by targeting cell cycle checkpoints[153].

Tumour microenvironment

Most attempts to sensitize tumours by manipulating the tumour microenvironment have focused on the tumour vasculature, as well as on the immune system (fig. 3). The vasculature is essential for tumour growth, and numerous groups have demonstrated that it is structurally and functionally abnormal in tumours[154]. Despite extensive evidence regarding the contribution of the vasculature to tumour development, the amount of vascular disruption following radiation therapy and the contribution of vascular collapse to tumour cure by radiation therapy remains controversial[155].

Detailed experiments using syngeneic tumours have suggested that microvascular collapse owing to endothelial cell apoptosis may contribute to the response of tumours to radiation therapy[156–158]. Unlike typical radiation-induced cell death, which is dependent on DNA damage, endothelial cell apoptosis has been reported to be triggered by membrane damage, which leads to ceramide-mediated apoptosis[159]. Interestingly, endothelial cells may be particularly sensitive at higher doses[160], so this mechanism ol endothelial cell death may be most pertinent to hypofractionaled regimens in stereotactic body radiation therapy. Although other groups have suggested that endothelial cells do not contribute to tumour cure[162–163], drugs that trigger endothelial cell apoptosis could potentially increase the radiosensitivity of tumour tissues.

Regardless of the amount ol vascular damage caused by radiation, vascular function must be re-established for a tumour to regrow following radiation therapy[164]. Endothelial cells may be established through the local sprouting of existing blood vessels or by the recruitment of vascular progenitor cells from outside the irradiated field[165]. Although several studies have reported that endothelial progenitor cells from the bone marrow contribute to the formation of the tumour vasculature[166–168], other studies have emphasized that bone marrow-derived cells do not contribute to the formation of the vascular endothelium[169]. Similarly, the contribution of each of these mechanisms following radiation remains controversial[170], with no studies definitively demonstrating that vascular progenitor cells contribute to vessel formation after radiation, and one study emphasizing that bone marrow-derived cells do not contribute to revascularization following radiation[171].

Several studies have suggested that myeloid cell recruitment after radiation contributes to vascular regrowth[171–173]. The recruitment of myeloid cells appears to be dependent on stromal cell-derived factor 1 (SDF1; also known as CXCL12)-CXC-chemokine receptor 4 (CXCR4) signalling in many tumours[171,172,174], and inhibition of this signalling axis with AMD3100 represents a promising therapeutic target post-irradiation[175]. Notch signalling also regulates tumour angiogenesis, and an antibody against a Notch ligand — delta-like ligand 4 (DLL4) — impaired tumour regrowth by promoting non-functional tumour angiogenesis[176].

Vascular normalization via anti-VEGF (vascular endothelial growth factor) therapies has long been championed as a mechanism for enhancing the response of tumours to radiation[154]. Preclinical experiments have demonstrated that anti-VEGF therapies can enhance the response of tumours to radiation in animal models[177]. Notably, there may be a tumour oxygenation window after anti-angiogenesis therapy, during which radiation therapy will be most effective[178]. However, clinical studies have not compared different treatment schedules, which may be important for therapies that normalize the vasculature to have maximum efficacy[179].

Anti-VEGF therapies have measurable effects on human tumours[180] and are currently being investigated in combination with radiation therapy in clinical trials. A Phase II trial of bevacizumab, radiation therapy and fluorouracil in rectal cancer demonstrated promising efficacy[181]. More recently, a Phase II study combining bevacizumab with radiation therapy and temozolomide for newly diagnosed glioblastoma demonstrated improved progression-free survival. However, overall survival was not improved[182]. Of note, small molecules can be designed to target both VEGF signalling and cell survival pathways. For example, vandetanib is a dual VEGF receptor 2 (VEGFR2) and EGFR inhibitor that is currently in Phase II clinical trials for glioblastoma[183].

As described above, TP53 encodes a transcription factor — p53 — that responds to ionizing radiation by initiating a spectrum of cell-type specific responses, including cell cycle arrest, senescence, apoptosis and DNA damage repair[184]. As a result of these functions, p53 is a key molecule for determining cellular responses to ionizing radiation[185]. The function of p53 is complex and tissue-dependent[186,187] (fig. 4). Although p53 protects the gut and cardiac endothelial cells from radiation[141,142,144], it can trigger apoptosis in haematopoietic and lymphoid tissues[188,189]. As TP53 is one of the most commonly mutated genes in cancer[190], it may be possible to inhibit p53 in tumour stromal cells without affecting the radiation response of tumour parenchymal cells, which already express mutant p53. For example, one study reported that p53 inhibition in the tumour stroma increases the sensitivity of transplanted syngeneic tumours to radiation, which is probably due to the increased sensitivity of endothelial cells to radiation[191].

Immunomodulation is an emerging approach for increasing the probability of tumour cure following radiation therapy[192]. Accumulating evidence suggests that the immune system is capable of recognizing tumours, impacting on cancer development[193–195]. As tumours develop in immunocompetent animals, they evolve to avoid cell death from immunosurveillance[196]. However, radiation treatment may present a unique opportunity to prime the immune system against tumours. There have been anecdotal reports that local irradiation of a tumour can reduce tumour growth outside the field of radiation. This abscopal effect is rare but it has been reported for many malignancies[197–201]. The immune system seems to be the main driver of this effect, as the radiation of primary tumours in combination with a growth factor that stimulates dendritic cell production has been shown to result in the regression of non-irradiated tumours[202]. Importantly, this effect was shown to be dependent on T cells.

Immunological mechanisms can also contribute to the regression of primary tumours after radiation therapy. It is well established that radiation increases the release of pro-inflammatory cytokines[203–205] and tumour-associated antigens[206,207]. These immune cues may trigger the immune system to eliminate surviving cancer cells following radiation therapy. Indeed, it has been demonstrated that tumour-antigen-specific immune responses develop during radiation treatment[208]. In addition, CD8 T cell depletion largely diminishes the therapeutic effect of radiation in mouse tumour models[54]. Notably, fractionated radiotherapy was less effective than hypofractionated radiotherapy in mice with T cells, but not in mice that were deficient in recombination activating gene (Rag) — Rag-knockout mice; this suggests that stereotactic body radiation therapy may be more effective at stimulating immune responses than standard fractitonated radiotherapy.

Many tumours have been shown to recruit regulatory T cells to inhibit the development of pro-inflammatory tumour immune responses[209]. This subset of T cells may be an important therapeutic target because CD25- and cytotoxic lymphocyte antigen 4 (CTLA4)-specific antibodies, which target regulatory T cells, enhance the efficacy of radiation therapy[210,211]. Therefore, combining immunomodulators with radiation treatment that is optimized to prime the immune system represents a promising approach to improve local control after radiation therapy and potentially affect the development of cancer at distant sites.

Radiation protectors and mitigators

Radiation causes both acute toxicity (occurring within days to weeks after radiation) and late toxicity (occurring months to years after exposure) to normal tissues, which often limits the dose of radiation that can be delivered to tumours. Thus, the use of radiation protectors to selectively protect normal tissues (fig. 5) is a complementary approach to sensitizing tumours to radiation, allowing more cancers to be cured by exposing them to higher doses of radiation. In addition to the clinical setting of treating patients with cancer, radioprotectors could be useful as countermeasures against radiological attacks and in other pathophysiological settings to protect against damage to normal tissues. For example, during reperfusion injury after ischaemia, reactive oxygen species (ROS) are generated that damage cells and activate cell death pathways via mechanisms that are similar to ionizing radiation[212]. Thus, radioprotectors may prove to be useful in protecting normal tissues from insults other than radiation therapy.

When describing radiation countermeasures, a US National Cancer Institute Workshop recom mended distinguishing among radiation protectors, radiation mitigators and treatments for radiation injury[213]. These terms are defined by the timing of drug administration in relation to the exposure to radiation and the development of symptoms from radiation injury. Protectors are given before exposure to radiation, mitigators are given

during or after exposure (but before the appearance of symptoms) and treatments are administered after symptoms from radiation injury develop. Although all of these drug classes would potentially be useful for treating patients in the clinic, the US government is actively seeking mitigators — which are effective when administered more than 24 hours after exposure to radiation — for the national stockpile for use in the event of a nuclear or radiological attack. Cellular responses to radiation begin almost instantaneously after exposure to radiation[214], and radiosensitive organs such as the bone marrow and the gastrointestinal tract undergo a significant amount of cell death within the first 24 hours. Therefore, in order for mitigators to be effective when administered after this time point, they will probably need to promote the regeneration of surviving cells or prevent the life-threatening sequelae of cell loss after the first 24 hours.

The ability of stem cells and oligopotent progenitors to maintain tissue function by replacing damaged cells determines the onset and severity of the effects of radiation[215], so strategies to mitigate normal tissue injury must increase the survival and functionality of these cells. Increasing evidence suggests that stem and progenitor cell populations respond differently to DNA damage than more differentiated cells[216]. Thus, mitigation strategies that alter the homeostasis of tissue-specific stem cells and progenitor cells could enhance their fitness and regenerative capacity.

Antioxidants

In addition to amifostine, several other antioxidants have been used to protect cells and tissues from radiation injury. The nitroxides are recycling anti-oxidants that protect cells exposed to oxidative stress[217]. A Phase I clinical trial in patients undergoing whole-brain radiotherapy suggested that one nitroxide, tempo, may be effective at preventing radiation-induced alopecia[218]. Antioxidant vitamins such as α-tocopherol and β-carotene have also been suggested to be effective at reducing xerostomia, mucositis, pulmonary fibrosis, cystitis and alopecia during radiation therapy[219]. However, antioxidant vitamins taken during radiation therapy have also been associated with decreased tum our control, which limits their clinical use[220,221].

In addition to the rapid production o f ROS caused by radiation, cells can exhibit chronic increases in ROS levels following exposure to radiation[222]. There is evidence that elevated levels of ROS, which may be a by-product of inflammatory cytokines such as TGFβ, can lead to persistent tissue damage several months after irradiation[223–225]. Inducing superoxide dismutase (SOD) expression seems to protect against ROS-induced damage[226]. Indeed, SOD can mitigate radiation-induced fibrosis in a porcine model[227] and in humans[228]. Similar results have been observed with a combination of tocopherol and pentoxifyl line (a vasodilator and anti-inflammatory drug), which suggests that this combination may work in part through reducing ROS[229,230].

p53 modulation

In contrast to the radiosensitizing effect of inhibiting p53 in endothelial cells, p53 inhibition has been proposal as a mechanism for protecting other normal tissues such as haematopoietic cells against radiation injury[185]. The development of pifithrin-α, a small-molecule inhibitor of p53, has led to accumulating evidence that p53 inhibition can protect some normal tissues from radiation-induced apoptosis without affecting tumour radionsensitivity[231]. As p53 is induced by DNA damage in part via ribosomal protein L26 (RPL26)-mediated increases in TP53 niRNA translation[252], oligonucleotides binding to TP53 mRNA to blunt p53 induction after radiation could potentially be used as therapeutics to block the p53-mediated response to radiation[233].

Importantly, the p53-mediated response to DNA damage does not seem to contribute to tumour suppression[234–236]. Thus, transient inhibition of p53 during radiation therapy may protect some normal tissues from radiation without increasing the probability of radiation-induced tumorigenesis. Moreover, the transcriptional programmes involved in acute DNA damage responses and tumour suppression can be separated, which indicates that the acute p53 response can be targeted selectively without affecting tumour suppression[235,236]. Despite the evidence supporting the safe use of p53 inhibitors in animal models, to our knowledge pifithrin-α and other drugs targeting p53 have not yet been tested in the clinic.

Hormones, cytokines and cell signalling pathways

Several hormones and cytokines have been utilized to support cellular survival and promote the proliferation of normal tissue stem cells before, during and after radiation exposure. Care must be taken when using hormones and cytokines during cancer therapy because tumours commonly express the receptors for cytokines and growth factors. Melatonin is a hormone that increases the expression of antioxidant enzymes within cells[237]. Owing to its additional antitumour effects[238], it has been tested as a neurological protector in patients with brain metastases. However, a Phase II clinical trial of melatonin during brain radiation therapy showed no difference in survival time or neurological function[239].

Various growth factors have been utilized to directly affect stem cell survival and recovery. It has been established that G-CSF (granulocyte colony-stimulating factor) promotes bone marrow recovery and can reduce the lethality of total-body radiation exposure when administered to non-human primates 6 hours after exposure to radiation[240]. Notably, this approach was not effective when administered to mice 24 hours after radiation[241]. Keratinocyte growth factor (KGF) stimulates DNA repair, cell proliferation, survival, differentiation and a reduction in levels of ROS, making it potentially useful as a radiation mitigator[242]. KGF has been shown to prevent radiation-induced xerostomia[243] and mucositis[244] in animal models. A recombinant human KGF, palifermin, is currently in clinical trials for patients with head and neck cancer. Phase II clinical trials of palifermin in patients receiving hyperfractionated radiotherapy showed a lower incidence and shorter duration of mucositis[245].

In addition to triggering microvascular collapse in tumours after radiation, ceramide-mediated endothelial cell apoptosis has been suggested to mediate acute radiation-induced gastrointestinal syndrome[246]. However, other groups have not observed similar levels of endothelial cell apoptosis and have suggested that intestinal crypt stem cells are the main target of radiation-induced gastrointestinal syndrome[141,247] Basic fibroblast growth factor (FGF) inhibits endothelial cell apoptosis and protects the gastrointestinal tract from radiation injury[246,248]. In addition, a ceramide-targeting antibody to prevent ceramide platform formation in endothelial cells has been reported to protect against gastrointestinal syndrome[249]. Another group has shown that a peptide derived from the receptor binding domain of FGF2, given to mice 4 hours after a lethal dose of subtotal-body irradiation, increased survival by preventing gastrointestinal syndrome[250]. However, the mechanism of mitigation may not be through the inhibition of apoptosis, because radiation-induced apoptosis has already started to peak 4 hours after exposure to radiation[246].

NF-κB signalling appears to be important for intestinal crypt survival following exposure to radiation[251]. It was shown that the Toll-like receptor 5 agonist CBLB502 can activate NF-κB and protect mice and rhesus monkeys from acute radiation syndrome when given before or after lethal total-body irradiation[252].

Many of the late effects of radiation result from normal tissue fibrosis. Because TGFβ drives smooth muscle cell proliferation and collagen production in fibroblasts, which can lead to

fibrosis[253], TGFβ inhibition may protect or mitigate against late-developing tissue injury. Experimen is in animal models have shown that blockade of TGFβ signalling decreases fibrosis in the lung[254,255] and intestine[256] following exposure to radiation.

Anti-inflammatory drugs

The most efficacious drugs for mitigating toxicity to normal tissues are anti-inflammatory drugs. Many different inflammatory pathways have been targeted with the same goal of reducing the autoinflammatory responses and vascular damage that can lead to catastrophic late radiation effects. Directly inhibiting coagulation can block microvascular damage and reduce radiation-induced nephropathy[257] as well, is small intestine toxicity[258,259] and nervous system injury[260] in animal models.

Activated protein C is a potent anticoagulant and cytoprotectant that inhibits blood clotting (through the proteolysis of factors V and VII), promotes fibrinolysis and exerts potent anti-inflammatory and cytoprotective effects on endothelial cells, neurons and innate immune cell populations[261]. It has shown considerable promise as a radiation mitigator. Systemic administration of activated protein C as late as 24 hours after exposure to radiation mitigated radiation-induced mortality in mice after total-body irradiation[262].

Statins are HMG-CoA reductase inhibitors that were originally developed as lipid-lowering agents. However, they have also demonstrated potent anti-inflammatory and antithrom botic properties[263]. Statins have shown potential as mitigators of late radiation damage by decreasing lung fibrosis and increasing survival when started 8 weeks post-irradiation[264]. In addition, statins can ameliorate delayed radiation-induced damage in the intestine when started 2 weeks before radiation[265]. They also have potential in radiation oncology, as they are relatively safe and can mitigate radiation-induced enteropathy when given 14 days alter radiation without allecting the efficacy of radiation on tumours[266].

The peroxisome proliferator-activated receptor (PPAR) inhibitors are another family of drugs that are currently in clinical use and may be useful in protecting against normal tissue injury after radiation therapy. PPAR inhibitors are currently used to treat diabetes but they can inhibit pro-inflammatory responses in several cell types[267]. One PPAR inhibitor, fenofibrate (Tricor; Abbott), was shown to improve neurogenesis in mice after whole-brain irradiation[268], which indicates that PPAR inhibitors could decrease neurological side effects in patients receiving irradiation for brain tumours.

Angiotensin-converting enzyme inhibitors have been shown to mitigate lung[269], renal[270] and neurological injuries[271] following irradiation, presumably by protecting the vasculature. However, the mechanism of action is unclear because there is no substantial evidence that the renin–angiotensin system is activated by radiation[272].

Stem cell therapies

As damage to stem cells seems to be a major cause of normal tissue injury following exposure to radiation, stem cell therapy could potentially alleviate many of the adverse effects of radiation on normal tissues[215]. Tissue-specific stem cells have been identified in many tissues[215], but very few stem cells have been demonstrated to reconstitute normal tissues when transplanted after radiation. Bone marrow transplantation has been well established as a mitigation strategy for haematopoietic syndrome and is recommended by the Strategic National Stockpile (SNS) Radiation Working Group if sufficient resources are available[273]. However, the salivary gland is the only solid tissue for which adult stem cell transplantation has been demonstrated to contribute to tissue function after radiation[274].

Bone marrow-derived stem cells may contribute to the recovery of several organs outside the bone marrow[275]. These cells can be stimulated by G-CSF to reduce radiation damage in salivary glands[276]. Stromal stem cells in the bone marrow, referred to as mesenchymal stem cells, contribute to the haematopoietic stem cell niche and can differentiate into multiple lineages in vitro[277]. These cells may contribute to the fibrotic response of irradiated tissues[278] but they may also help to repair radiation damage in the oesophagus[278].

Finally, induced pluripotent stem cells can differentiate into all cell lineages in a living organism[239] and have tremendous potential for regenerative medicine in situations of normal tissue damage. However, these cells have not been used successfully in the clinic.

Although a considerable amount of effort will be required to translate the use of stem cells to the clinic, the potential of these cells to repopulate and restore function within normal tissues makes them promising future therapeutics for mitigating radiation injury.

Conclusion

Tremendous progress has been made towards understanding the hallmarks of cancer. Likewise, elucidating the mechanisms through which tum ours evade radiation therapy has led to the development of a new generation of targeted radiosensitizers and radioprotectors that have the potential to selectively in crease the killing of tumour cells during radiation therapy. Although only a few of these approaches have been successfully translated to the clinic, promising approaches to improve local control with radiation therapy in the clinic include triggering synthetic lethality, targeting cancer stem cells and inhibiting multiple kinases that mediate the DNA damage response. Although tumours are heterogeneous and may contain regions of hypoxia or harbour tumour cells with mutations that promote resistance to radiation therapy, this heterogeneity offers opportunities to use drugs that target hypoxic cells or specific mutant proteins to sensitize tumours to radiation therapy. Furthermore, with a greater understanding of the mechanisms by which the microenvironment, and particularly the immune system, affects tumour cure by radiation therapy, there will be new opportunities for improving tumour eradication by manipulating the tumour microenvironment with targeted drugs that block tumour recurrence, by inhibiting angiogenesis or by triggering the immune response to eliminate surviving cancer cells. As radiation therapy is utilized to treat over half of all patients with cancer, translating our growing understanding of cancer and radiation biology into effective radiosensitizers of tumours or radioprotectors of normal tissues has the potential to improve outcomes for many patients with cancer.

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Glossary

Hoematopoietic	syndrme Acute radiation toxicity caused by bone marrow failure that occurs within a month after whole-body exposure to radiation.

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Box 1 | Using radiation to treat tumours

Radiation therapy is ore of the most commonly used anticancer therapies in the clinic. Although radiation can be used to palliate cancer symptoms such as pain, the majority of patients are treated with the intent to cure their tumour. Radiation therapy uses high-energy ionizing radiation to kill cancer cells by damaging their DNA. As normal cells are also affected by radiation, radiation oncologists deliver radiation to the tumour area while limiting the dose of radiation to surrounding normal tissues as much as possible. For many cancers, such as breast cancer, radiation is used before or after surgery to increase the probability of local control. For a subset of these patients, adjuvant radiation therapy increases the probability of a cure. For other cancers, such as squamous cell carcinoma of the head and neck, definitive radiation therapy is used to achieve local control. The addition of concurrent chemotherapy or biologically targeted agents such as the epidermal growth factor receptor (EGFR) inhibitor cetuximab (Erbitux; Bristol-Myers Squibb/Lilly) can increase local control.

Before delivering radiation therapy, radiation oncologists develop an individualized treatment plan for each patient. This process begins with a simulation where the patient is immobilized in the treatment position and a computed tomography scan is acquired for treatment planning. As small changes in body position can affect the total dose delivered to the tumour and normal tissues, skin marks, moulds and other devices are used during simulation to ensure that the patientis in the same position for daily treatment. Following simulation, the radiation on cologist uses all of the imaging and clinical data available to determine the area that needs to be treated, the total dose that will be delivered and how much radiation can be safely delivered to surrounding normal tissues. Based on these constraints, the radiation on cology team uses detailed computer modelling to design the treatment plan for the patient.

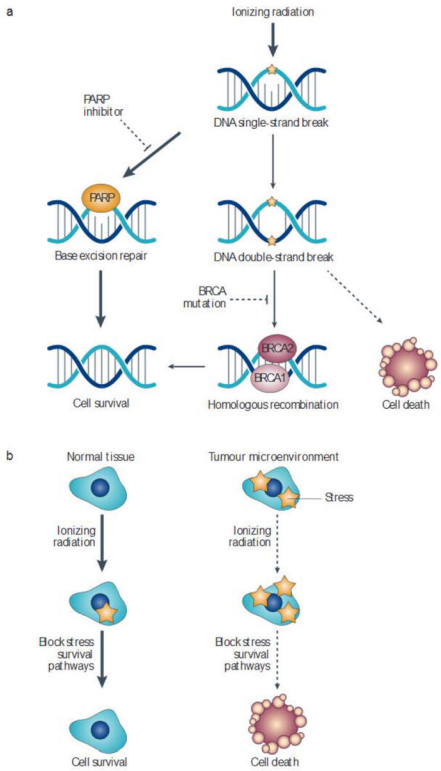

Figure 1. Selectively targeting tumour cells through synthetic lethality

a | Ionizing radiation causes single-strand and double-strand DNA breaks. Some single-strand DNA breaks can be repaired by base excision repair. Poly(ADP-ribose) polymerase (PARP) inhibitors block base excision repair, causing some single-strend breaks to become double-strand breaks. In normal cells, **BRCA** (breast cancer susceptibility)-mediated homologous recombination can repair these breaks However, cancer cells with mutations in **BRCA1** or **BRCA2** are unable to repair this damage, allowing PARP inhibitors to radiosensitize these cellsvia synthetic lethality. **b** | Similarly synthetic lethality could potentially be used to sensitize cellsin the harsh tumour microenvironment Drugs that block stress survivel signalling pathways should preferentially kill tumour cells that are exposed to stresses such as hypoxia, oxidative stress or cell-cell contact abnormalities (right side) but not normal cells, which exist in a less harsh microenvironment (left side). These drugs have the potential to kill tumour cells in the absence of radiation, but radiation provides an additional stress that could enhance the probability of tumour eradication.

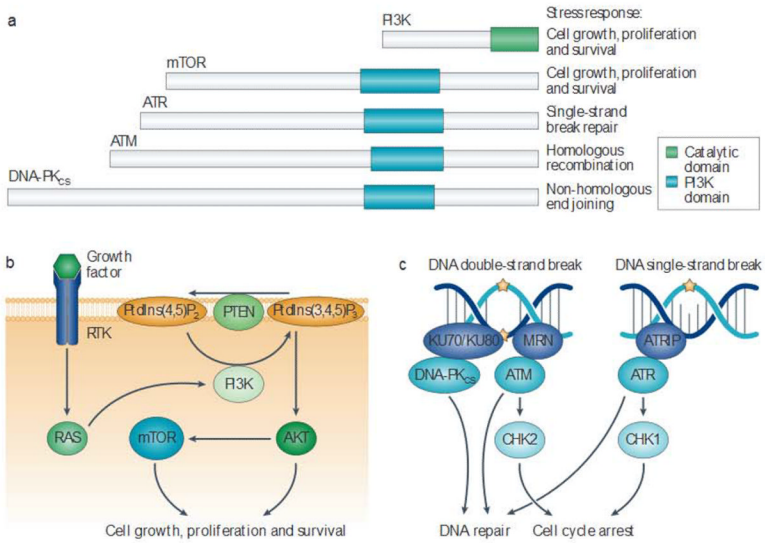

Figure 2. Inhibiting P13 Kand the P13 K-like protein kinase family

a | The phosphoinositide 3-kinase (H3K)-like protein kinase family includes several cellular kinases with catalytic domains that are homologous to PI3K(these are known as PI3K domains) This protein family coordinates diverse cellular responses that are crucial for the response to ionizing radiation. **b** | PI3K and mammalian target of rapamyc in (mTOR) are activated by numerous growth factors to stimulate cellular growth, survival and proliferation. Growth factors bind to receptor tyrosine kinases. Leading to downstream activation of PI3K by the RAS family of small GTFases. Active PI3K phosphorylates phosphatidylinositol-4,5-bisphophate (Ptdlns(4,5)P_2) in the cellular membrane, converting it to the second messenger phosphatidylinositol-3,4,5-trisphophate (Ptdlns(3,4,5)P_3). Ptdlns(3,4,5)P_3 can activate the protein kinase AKT, leading to increased cell growth, proliferation and survivalby activating several proteins including mTOR. **c** | DNA-dependent protein kinase catalytic subunit (DNA-PKC_{CS}), ataxia telangiectasia mutated (ATM) and ATR (ataxia telangiectasia and RAD3-related) respond to DNA damage by activating cell cycle arrest and engaging distinct DNA repair programmes. Because the catalytic domain is conserved across these diverse kinases, it is possible that a single drug could target several family members, thereby inhibiting some or all of these DNA repair and cell survival pathways. KU70 and/or KU80, the MRN complex(composed of MREll, RAD50 and Nijmegen breakage syndrome protein 1 (NBSl)) and ATR-interacting protein (ATRIP)sense DNA damage and promote the activation of DNA-PK_{CS}, ATM and ATR. respectively. These kinases then phosphorylate several target sinside the cell, including checkpoint kinase | (CHK1) and CHK2.PTEN, phosphat ase and tensin homolog.

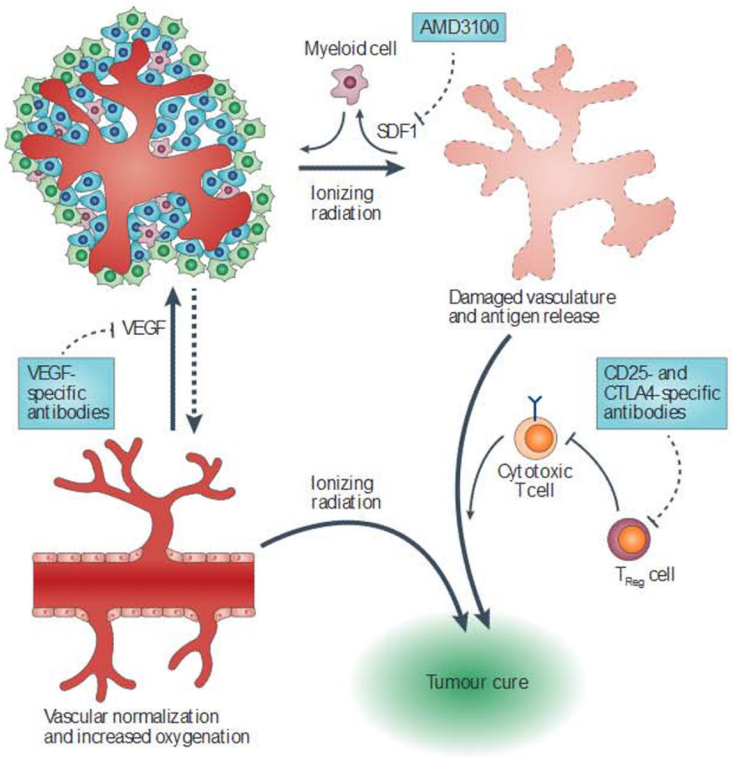

Figure 3. Enhancing tumour cure by modulating the tumour microenvironment

The tumour microenvironment can regulate the response of tumours to radiation by altering tumour oxygenation and vascular rebuilding, and by regulating the clearance of surviving cells by the immune system. Targeted drugs can alter the interaction between tumour cells and their microenvironment, which may enhance tumour cell killing and increase the probability of a cure. Anti-angiogenic therapies such as blockade of vascular endothelial growth factor (VEGF) can cause vascular normalization, leading to a window of increased tumour oxygenation. Myeloid cells are recruited to inradiated tumours by cytokines suchasstromal cell-derived factor 1 (SDF1). Blocking their recruitment may impair vascular regrowth. Irradiated tumours release antigens that can be recognized by the immune system, leading to the destruction of tumour cells by cytotoxic T cells. Regulatory T(T_{Reg}) cells can abrogate this response,so targeting these cells with CD25- and cytotoxic T lymphocyte antigen 4 (CTLA4)-specific antibodies may enhance tumour cure by radiation.

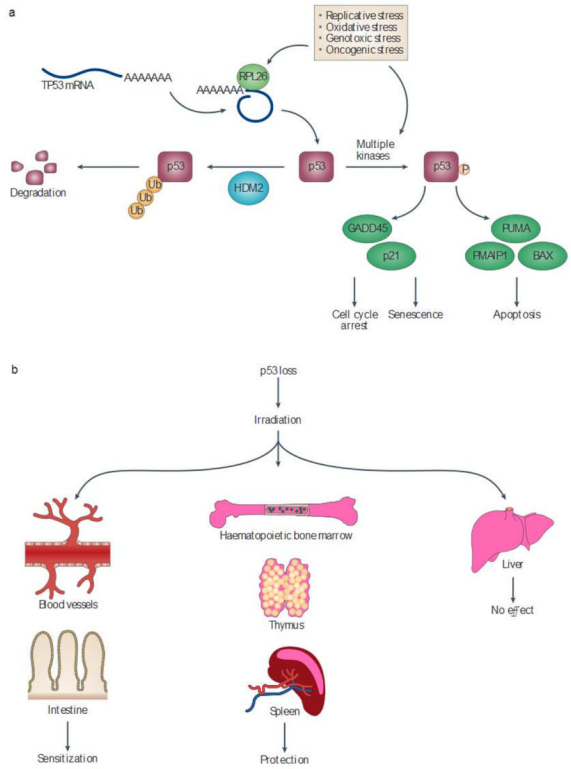

Figure 4. The tissue-dependent role of p53 in the response of cells to radiation

a | In unstressed cells, HDM2 ubiquitylates the tumour suppressor p53, leading to its degradation. Numerous cellular stresses increase the translation of TP53 mRNA and the phosphorylation of p53 protein, increasing its stability. p53 acts predominantly as a transcription factor that upregulates the expression of target genes to arrest cell growth or lead to cellular senescence and apoptosis, depending on the cellular context. Ribosomal protein |26 (RPL26) binds to TP53 mRNA and increases its translation. The p53 transcriptional targets GADD45 (growth arrest and DNA-damage-inducible protein 45) and p21 lead to cell cycle arrest, whereas BCL-2-associated X protein (BAX) and the BH3-only proteins PUMA (p53-upregulated modulator of apoptosis) and PMAIP1 (PMA-induced protein 1) can trigger apoptosis. **b** | The consequence Of p53 loss on the cellular radiation response varies depending on the type of tissue As a result, p53 inhibitors may be useful as both radiosensitizers and radioprotectors.

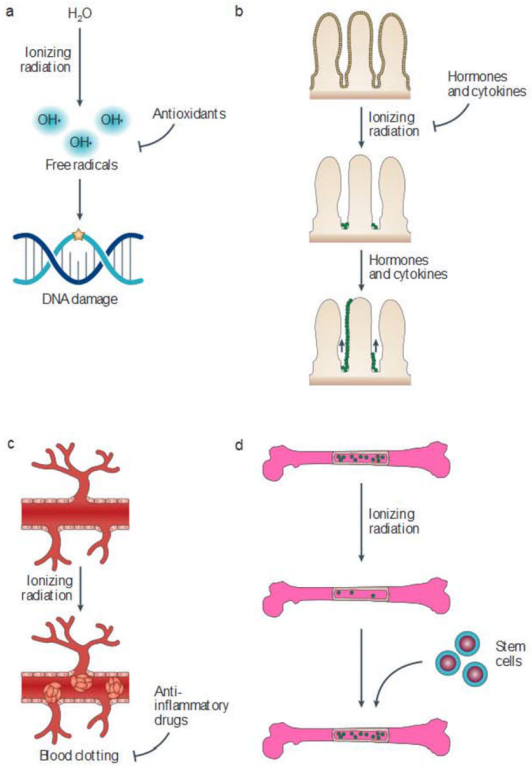

Figure 5. Strategies to protect and mitigate normal tissues from radiation damage

a |Antioxidants can scavenge free radicals that damage DNA **b** | Hormones and cytokines can be administered before or shortly after radiation to enhance cellular survival and facilitate tissue repopulation. **c** | Anti-inflammatory drugs can be used to reduce autoinflammatory responses and vascular damage that results in late radiation effects, **d** | Stem cell scan be used to reconstitute tissues that have been damaged by radiation. Amifostine, an antioxidant, is approved by the US Food and Drug Administration for use in combination with radiation therapy, and paliformin (a recombinant form of keratinocyte growth factor) has been approved for preventing mucositis from total-body irradiation. Several other hormones, cytokines and anti-inflammatory drugs have shown promise as mitigators of radiation injury in animal models. Although bone marrow transplantation has been established as a mitigator of the haematopoietic syndrome, substantial work will be necessary to enable the use of stem cells to regenerate solid tissues following exposure to radiation.