Expert Reviews in Molecular Medicine

cambridge.org/erm

Review

Cite this article: Li C, Wiseman L, Okoh E, Lind M, Roy R, Beavis AW, Pires IM (2022). Exploring hypoxic biology to improve radiotherapy outcomes. *Expert Reviews in Molecular Medicine* **24**, e21, 1–18. https://doi.org/10.1017/erm.2022.14

Received: 18 January 2022
Revised: 7 April 2022
Accepted: 12 April 2022

Key words:
DNA damage response; HAPs; HIF; hypoxia; radioresistance; radiotherapy; targeted therapy

Author for correspondence:
Isabel M. Pires,
E-mail: i.pires@hull.ac.uk

CAMBRIDGE UNIVERSITY PRESS

Exploring hypoxic biology to improve radiotherapy outcomes

Chun Li[1], Lucy Wiseman[1], Ene Okoh[1], Michael Lind[2,3], Rajarshi Roy[2], Andrew W. Beavis[3,4,5] and Isabel M. Pires[1]

[1]Hypoxia and Tumour Microenvironment Lab, Department of Biomedical Sciences, Faculty of Health Sciences, University of Hull, Hull, UK; [2]Queen's Centre for Oncology and Haematology, Castle Hill Hospital, Castle Rd, Cottingham HU16 5JQ, UK; [3]Faculty of Health Sciences, University of Hull, Cottingham Road, Hull, HU16 7RX, UK; [4]Department of Medical Physics, Queen's Centre for Oncology, Hull University Teaching Hospitals NHS Trust, Cottingham, HU16 5JQ, UK and [5]Faculty of Health and Well Being, Sheffield-Hallam University, Collegiate Crescent, Sheffield, S10 2BP, UK

Abstract

Ionising radiotherapy is a well-established, effective cancer treatment modality, whose efficacy has improved with the application of newer technological modalities. However, patient outcomes are governed and potentially limited by aspects of tumour biology that are associated with radioresistance. Patients also still endure treatment-associated toxicities owed to the action of ionising radiation in normoxic tissue adjacent to the tumour mass. Tumour hypoxia is recognised as a key component of the tumour microenvironment and is well established as leading to therapy resistance and poor prognosis. In this review, we outline the current understanding of hypoxia-mediated radiotherapy resistance, before exploring targeting tumour hypoxia for radiotherapy sensitisation to improve treatment outcomes and increase the therapeutic window. This includes increasing oxygen availability in solid tumours, the use of hypoxia-activated prodrugs, targeting of hypoxia-regulated or associated signalling pathways, as well as the use of high-LET radiotherapy modalities. Ultimately, targeting hypoxic radiobiology combined with precise radiotherapy delivery modalities and modelling should be associated with improvement to patient outcomes.

Introduction

Ionising radiation is a type of high-energy electromagnetic wave that releases electrons from atoms and molecules generating highly reactive free radicals which can damage genomic DNA and result in cell death (Ref. 1). Radiotherapy is one of the primary therapeutic strategies for many cancer types, either alone or in combination with surgery, chemotherapy, targeted therapy and/or immunotherapy (Ref. 2). For example, the standard treatment of nasopharyngeal carcinoma is radiotherapy, and early-stage laryngeal cancer patients are treated with radiotherapy as a primary therapy, with advanced laryngeal cancers also sensitive to chemoradiation therapy (CRT) (Refs 3, 4). More recently, radiotherapy has been explored with newer cancer treatment modalities, such as with immunotherapeutic agent pembrolizumab, which significantly increased responses in patients with metastatic non-small-cell lung cancer (NSCLC) (Ref. 5).

Unfortunately, treatment resistance leads to poor outcomes for some patients. A key aspect of tumour biology that affects ionising radiotherapy efficacy is the tumour microenvironment, in particular tumour hypoxia, as the cellular responses to ionising radiation are dependent on how well oxygenated a tissue is (Ref. 6). In fact, threefold higher radiation doses are required in hypoxic conditions to achieve the same impact as in normoxic conditions, a factor noted as the oxygen enhancement ratio (OER) (Ref. 7). Elevated hypoxic content in tumours has therefore been shown to be a factor of poor prognosis and therapy resistance in many tumour types (Refs 8–10). The oxygen levels at which significant radioresistance is observed (<0.13% O_2) are also known as radiobiological hypoxia (Ref. 11). Hypoxia is therefore considered a significant challenge to ionising radiotherapy efficiency, so there is an expanding field of study looking at exploring strategies to radiosensitise hypoxic cells. This involves strategies such as increasing oxygen availability, hypoxia-activated prodrugs (HAPs) or targeted therapies for hypoxia-regulated signalling. Interestingly, high-LET (linear energy transfer) radiotherapy modalities have been shown to be less dependent on oxygen levels than low-LET ionising radiation (Refs 7, 12, 13).

The aim of this review is to discuss how hypoxic biology impacts radiotherapy response, how hypoxic radiobiology can be explored therapeutically to avoid radiotherapy resistance and how high-LET modalities might be an alternative approach to hypoxia-induced ionising radiation resistance.

Hypoxia-mediated radiotherapy resistance

An overview of tumour hypoxia

In normal tissue the oxygen supply matches the metabolic requirements of the cells, whereas in tumour tissue oxygen consumption increases significantly and exceeds the supply, resulting

in a drop of normal oxygen levels (pO_2) from about 20–80 mmHg to hypoxic levels <5 mmHg, or even levels which can cause increased radioresistance (<1–10 mmHg or 0.13–1.3% O_2) (Ref. 14). In particular, oxygen tensions of lower than 1 mmHg (<0.13% O_2) are associated with significant radiotherapy resistance and are therefore called radiobiological hypoxia (Ref. 11).

Chronic hypoxia is caused by the long-term oxygen depletion, which can be derived from increased distance from blood vessels to the tissue, as well as permanent limitations in oxygen diffusion (Ref. 15). Acute hypoxia occurs when a temporary disruption of blood flow to the tumour mass occurs because of the severely abnormal changes in the structure and function of tumour vasculatures, producing oxygen fluctuation in the tumour microenvironment (Ref. 16). Because of this, solid tumours contain regions of cycling, or intermittent, hypoxia. The levels of hypoxia and proportion of the tumour that is hypoxic vary significantly due to the disorganised vessels with intermittent blood flow, which generate cyclic changes of oxygen concentrations, resulting in a dynamic microenvironment between hypoxic and reoxygenated states (Ref. 17).

Hypoxic adaptation is underpinned by dramatic changes in gene expression patterns, and these are primarily regulated by the hypoxia-inducible factors (HIFs) (Ref. 18). HIFs can transactivate the expression of genes involved in key tumour-promoting hallmarks, such as tumour angiogenesis, energy metabolism adaptation, cell death and autophagy, cell cycle regulation, metastatic spread (including the epithelial-mesenchymal transition), and both chemo- and radio-therapy resistance (Ref. 19) (Fig. 1). HIF consists of an oxygen-sensitive α subunit (HIF-α, which includes three isoforms: HIF-1α, HIF-2α and HIF-3α), and a constitutively expressed β subunit (HIF1-β). Under normoxic conditions, HIF-α is hydroxylated by both prolyl hydroxylases (PHDs) and factor inhibiting HIF (Ref. 20). Proline hydroxylation within HIF-α's oxygen-dependent degradation domain by PHDs allows HIF-α to be recognised and bound by the von Hippel-Lindau E3 ligase, resulting poly-ubiquitination and subsequent degradation by the proteasome (Ref. 21). However, under hypoxic conditions the PHDs are inhibited due to the lack of oxygen as a co-factor, leading to the rapid stabilisation of HIF-α protein levels and increased interaction with its co-activators p300 and CREB binding protein (Ref. 22). HIF-α then heterodimerises with HIF1-β, and the heterodimeric transcription factor then binds to hypoxia response elements located in target gene promoters and transactivates these targeted genes (Fig. 1) (Ref. 23).

Hypoxia-mediated radiotherapy resistance

There are primarily two aspects by which hypoxia leads to radiotherapy resistance based on the mechanism of action of ionising radiation. As stated by the oxygen fixation hypothesis, during treatment with ionising radiation DNA radicals are formed either by direct ionisation or indirectly by interaction with free radicals generated by water radiolysis (Ref. 24). Molecular oxygen rapidly interacts with these indirect radiation-induced DNA radicals leading to the production of single-strand breaks and oxidised bases, which can be resolved into lethal double-strand breaks (DSBs), leading to cell death (Ref. 25). Therefore, in the absence of sufficient oxygen this process is inhibited, and the amount of DNA damage produced by radiation and its impact on cell viability is reduced. Other mechanisms by which hypoxic biology decreases ionising radiation efficacy include changes in reactive oxygen species (ROS) levels, inflammation signalling and HIF-regulated signalling such as induction of angiogenesis and other tumour promoting pathways (Fig. 1) (Ref. 26). HIF-1α and HIF-2α expression have been shown to have poor prognostic value for response to radiotherapy or CRT (Refs 27, 28). Counterintuitively, HIF-1α levels have been shown to increase after ionising radiation treatment through a variety of molecular mechanisms (Ref. 29). Importantly, hypoxia can also drive increased genomic instability phenotypes through the clonal loss of tumour suppressor p53, repression of the expression of other tumour suppressive factors such as E2F1 as well as key players of DNA repair pathways such as DSB repair (homologous recombination (HR)) and mismatch repair, such as RAD51, BRCA1, MLH1, amongst others (Refs 30–32). It is important to note that these latter resistance mechanisms are characteristic of, but not exclusive to, radiobiological hypoxia and are associated with activation of DNA damage response (DDR) signalling and DNA replication downregulation through decreased nucleotide signalling (Refs 11, 33–35).

Increasing sensitisation to ionising radiation via increased oxygen availability

There are several approaches to target hypoxia-mediated radioresistance, and one of the longest established one is the direct or indirect modulation of oxygen levels in the tumour tissue to reduce hypoxic content and increase radiosensitisation. These utilise three main broad approaches: increasing oxygen diffusion to the tissue, reducing oxygen consumption or using oxygen-mimetic molecules.

Increased oxygen diffusion

Hyperbaric oxygen (HBO) therapy has been used as a treatment for late radiation tissue injury by increasing the availability of oxygen in plasma, which improves oxygen tissue availability (Ref. 36). A meta-analysis of several clinical trials to investigate the effect of HBO as radiosensitisers in patients with squamous cell carcinoma of head and neck showed a significant improvement in overall radiation treatment response, as well as metastasis reduction (Ref. 37). Radiotherapy after HBO breathing was found to be radiosensitised in a study using experimental models (Ref. 38). However, this technique is not cost-effective for broad clinical use in later study (Ref. 37).

A phase II clinical trial investigated the effect of the combination of nicotinamide and carbogen (CON) on radiotherapy outcome for patients with advanced bladder carcinoma (Ref. 39). Nicotinamide is a vitamin modified to enhance blood flow in the tumours and administered 2 h before radiotherapy while carbogen refers to a gaseous mixture of 2% carbon dioxide and 98% oxygen inhalant (Ref. 40). This study demonstrated improvement in overall response of 50% for those administered with the CON combination therapy, whilst radiotherapy alone only had a 38% overall response (Ref. 39). A report from a phase III trial for laryngeal cancer also reported positive outcome of accelerated radiotherapy combined with carbon inhalation and nicotinamide compared to radiotherapy alone with a 93% control rate seen in patients with hypoxic tumours treated with the combination therapy (Ref. 41).

Other approaches that enhance oxygen diffusion for reversing tumour hypoxia and improve radiotherapy are also under investigation. Trans sodium crocetinate (TSC) causes physical changes in blood plasma which results in rapid oxygen diffusion from the cell wall to the vascular wall (Ref. 42). TSC was combined with temozolomide and radiotherapy on glioma cells and magnetic resonance imaging (MRI) imaging obtained before and after treatment showed a significant reduction in tumour size when compared with those treated with temozolomide and radiotherapy alone (Ref. 43). TSC is being developed as a radiosensitiser for improving radiotherapy outcome in glioblastoma multiforme (GBM), pancreatic cancer and brain metastases after a successful phase II clinical trial was completed (Ref. 42).

https://doi.org/10.1017/erm.2022.14 Published online by Cambridge University Press

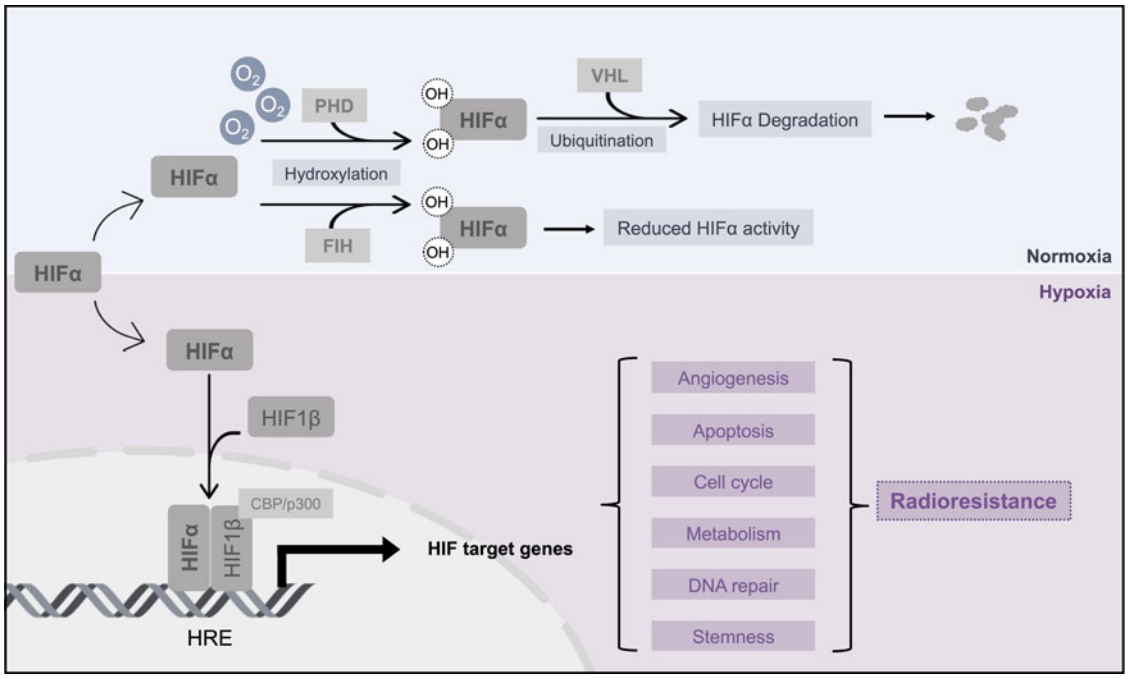

Fig. 1. Mechanisms for HIF-α-mediated radiotherapy resistance. This schematic illustrates the key mechanisms for HIF stabilisation in hypoxic conditions, and highlights key pathways up-regulated by HIF that contribute to hypoxia-mediated radiotherapy resistance. HIF, hypoxia-inducible factor; PHD, prolyl hydroxylases; FIH, factor-inhibiting HIF; VHL, von Hippel–Lindau; OH, hydroxyl groups; CBP, CREB binding protein; HRE, hypoxia response elements.

Oxygen transport agents are also being explored to meet the challenges of hypoxia to radiotherapy. Preclinical investigation of liposome-encapsulated haemoglobin was shown to effectively reverse hypoxia in tumours (Ref. 44). Specifically, the results showed a remarkable reduction of HIF-1α and improved radiation therapy outcome, as tumour growth was significantly inhibited (Ref. 44). OMX is a recent oxygen carrier developed to target hypoxia and improve radiotherapy (Ref. 45). Preclinical studies showed OMX reduced hypoxia significantly, enhancing T-cell localisation, and increasing CD8 accumulation and other cytotoxic activity previously impaired by tumour hypoxia (Ref. 45). Fluorocarbon-based agents, through their gas-dissolving and chemically inert properties, can carry and diffuse oxygen at high concentrations (Ref. 46). A phase II clinical trial (NCT03862430) in GBM, evaluating the combination of radiotherapy with NVX-108, a dodecafluoropentane-based perfluorocarbon emulsion, is currently recruiting (Ref. 47).

Decreased oxygen consumption

As well as increased oxygen delivery, suppressors of oxygen consumption have also been explored as radiosensitiser agents.

Nitric oxide (NO) is a free radical that plays a vital role as a vasodilator, as well as inhibitor of tissue oxygen consumption (Ref. 48). The mechanism of NO in radiosensitisation is similar to those of oxygen-induced oxidative stress by stabilising radiation-induced DNA damage via the nitrosative stress pathways (Ref. 49). The radiosensitising effect of NO has been shown both *in vitro* and in patients, including a phase II study indicating that NO can palliate hypoxia-induced progression in prostate cancer (Ref. 50).

More recently, the anti-microbial agent atovaquone was found to rapidly decrease hypoxic content of tumours, and was identified as a suppressor of oxygen consumption through a high-throughput analysis of FDA-approved drugs (Ref. 51). One clinical study found that atovaquone can increase tumour oxygenation and suppress hypoxic gene expression, therefore improve treatment outcomes for NSCLC patients (Ref. 52).

Finally, papaverine, another FDA-approved agent, has also been shown as an ideal agent for radiosensitisation of hypoxic tumours as it reduces mitochondrial oxygen consumption (Ref. 53). This anti-spasmodic drug was shown to increase oxygenation in tumour and enhanced radiation response directly by inhibiting mitochondrial metabolism with fewer side effects, which makes it a potential clinical radiosensitiser (Refs 53, 54).

Oxygen mimetics as radiosensitisers

Oxygen mimetics, which are compounds developed with chemical properties of molecular oxygen with a better diffusion ability to low oxygen tissues, have also been explored for their radiosensitising properties (Ref. 55). These include compounds such as misonidazole and nimorazole, which have been developed to mimic oxygen by promoting fixation of free radical damage during radiation (Ref. 55). The use of misonidazole was halted at trial in combination with radiotherapy for treatment of inoperable squamous cell carcinoma of lung cancer due to its high toxicity, and a similar effect was observed in an investigation for treatment of advanced uterine carcinoma (Refs 56, 57). Finally, the NIMRAD phase III trial explored the use of nimorazole in combination with intensity-modulated radiotherapy (IMRT) in head and neck squamous cell carcinoma (HNSCC) (Ref. 58) and has been approved by the Centre for Clinical Practice (Ref. 59).

Hypoxia-activated prodrugs as radiosensitisers

HAPs are compounds with high specificity for hypoxic tumours, as these are genotoxic compounds which are inactive in the presence of oxygen but are selectively activated under hypoxic conditions, and therefore can accurately target regions of tumour hypoxia (Ref. 55). These HAPs have been identified and grouped into five main types: nitro compounds, aromatic N-oxides, aliphatic N-oxides, quinones and molecularly targeted HAPs (Ref. 60). Nitro compounds-based HAPs include Metronidazole, PR-104A and TH-302, etc. The most representative

https://doi.org/10.1017/erm.2022.14 Published online by Cambridge University Press

N-oxide-based HAPs are Tirapazamine (TPZ), AQ4N and SN30000. Quinone-based HAPs, such as EO9 (Apaziquone), and Mitomycin C (MMC) are the earliest developed HAPs (Ref. 61). Despite promising preclinical data of classical HAPs, limited clinical therapeutic efficacy has been shown in several HAPs, which led to the development of novel molecularly targeted HAPs in recent years, including CH-01 (hypoxia-activated Chk1/Aurora A inhibitor), TH-4000 (hypoxia-activated tyrosine kinase inhibitor) and CH-03 (hypoxia-activated KDAC inhibitor) (Refs 62–64). However, none of these have yet been evaluated in combination with radiotherapy. Details of HAPs being investigated in clinical trials as possible radiosensitisers of hypoxic cells are summarised in Table 1, with some examples detailed below.

Evofosfamide (TH-302)

TH-302 is an inactive compound of bromo-isophosphoramide which is released in hypoxic conditions and leads to alkylation of DNA (Ref. 99). Interestingly, it has been shown that TH-302 in combination with radiotherapy enhances therapeutic outcomes (Ref. 100). A further study also found that TH-302 has radiosensitising effects when administered in combination with a VEGF-A inhibitor in preclinical models of sarcoma, increasing DNA damage and apoptosis and decreasing HIF-1α activity (Ref. 101). Further studies combining TH-302 and radiotherapy *in vivo* and *in vitro* reported a mild effect of treatment with TH-302 and a significant increase of apoptosis in hypoxic cells (Ref. 102). However, a phase III clinical trial of TH-302 reported non-significant benefits and high toxicity, and therefore it has not been adopted clinically (Ref. 103). There was a phase I clinical trial using TH-302 with chemoradiotherapy in oesophageal cancer (NCT02598687) (Ref. 104), however it was withdrawn as phase II/III trials did not meet their primary endpoint, so further development and testing of TH-302 is uncertain.

Tirapazamine

TPZ is an aromatic N-oxide which was first evaluated in 1986 and has been studied for its greater toxicity in anoxia when compared with aerobic conditions *in vitro* (Ref. 105). TPZ specificity for hypoxic cells initially showed positive results in improving radiotherapy outcomes by using gene-directed enzyme prodrug therapy (GDEPT) in which hypoxia is a trigger for both enzyme expression and drug metabolism (Ref. 106). Preclinical studies in the early 90s had shown great promise. For example, a phase I clinical trial of TPZ in combination with cisplatin and radiotherapy in small-cell lung cancer leading to improved survival rate among patients, and a phase II clinical trial carried out on patients with locally advanced head and neck cancer reporting improved 3-year survival (Refs 82, 107, 108). Unfortunately, a later phase III clinical trial in locally advanced head and neck cancer showed no significant increase of patient survival (Ref. 109).

AQ4N

Banoxantrone (AQ4N) is a bioreductive HAP, which is bioreduced in hypoxic cells by cytochrome P450s to the cytotoxin AQ4 (Ref. 110). Study found that AQ4N can selectively kill hypoxic cells via an inducible nitric oxide synthase-dependent mechanism when used in combination with radiation (Ref. 111). Moreover, the use of AQ4N combined with radiotherapy and Temozolomide in glioblastoma entered a phase II clinical trial (NCT00394628), but no results have been published to date (Ref. 112).

Mitomycin C

MMC is also a HAP that generates DNA-damaging species via DNA cross-linking and has been shown to enhance toxicity against hypoxic compared to normoxic cells (Ref. 60). Preclinical study revealed that MMC could enhance radio response and modulate hypoxic tumour microenvironment in combination with radiotherapy in rectal cancer (Ref. 113). Clinical trials that used MMC combination with radiation are listed in Table 1. Combined therapy including 5-fluorouracil, MMC and radiation has become current standard treatments of anal cancers and bladder cancers. RTOG-87-04 study phase III randomised trial suggested that despite greater toxicity of MMC, the use of MMC can be beneficial, especially for those patients with large primary tumours (Ref. 114). Long-term update of US GI intergroup RTOG 98-11 phase III trial compares CRT, replacing MMC with cisplatin due to the toxicity of MMC. However, cisplatin-based therapy failed to improve disease-free-survival compared with mitomycin-based therapy, therefore suggested RT + FU5/MMC remains the preferred standard of care of anal cancers (Refs 92, 93).

Targeting of hypoxia-mediated signalling reprogramming as radiosensitising strategies

Targeting hypoxia-regulated signalling including and beyond direct HIF targeting in cancer has been explored as a therapeutic approach to reduce its tumour-promoting characteristics, and below we explore how targeting various hypoxia-regulated pathways can lead to improvement in radiotherapy responses (Fig. 2).

HIF inhibition as a radiosensitiser strategy

As mentioned earlier, HIF is a critical factor in adaptation to the hypoxic microenvironment and is therefore an obvious molecular target to overcome radioresistance of hypoxic tumour cells (Ref. 115). Several compounds have been studied as inhibitors of HIF-α transcription, translation, and protein stabilisation (Ref. 116). Of these, some, such as SN-38 (the active metabolite of irinotecan), alongside its well-established radiosensitiser effect as a topoisomerase I inhibitor, can also lead to increased radiosensitivity through inhibiting radiation-induced HIF-1α in colorectal cancer (Ref. 117). T-type Ca^{2+} channel blockers, such as Mibefradil, which can block HIF-1 activation by reducing mitochondrial ROS production and increase HIF-1α protein hydroxylation and degradation (Ref. 118), have also been studied in a clinical trial using Mibefradil with hypofractionated irradiation in recurrent GBM (Ref. 119), with results suggesting that Mibefradil can be safely co-administered with RT. STAT3 plays an important role in the response of tumour cells to radiotherapy, and STAT3 inhibitors NSC74859 and Stattic have been found to increase radiosensitivity by downregulating HIF-1α expression in oesophageal cancer (Refs 120–122). YC-1, a NO-independent activator of soluble guanylyl cyclase, was shown to enhance radiosensitivity across different types of cancer cells by inducing HIF-1α protein degradation and hence inhibition of HIF-1α function (Refs 123–125). More recently, other novel small-molecule inhibitors of HIF have been investigated. PX-478 decreases HIF-1α levels by inhibiting HIF-1α translation, as well as inhibiting de-ubiquitination leading to HIF-1α protein degradation (Ref. 116). Palayoor *et al.* have shown a potential role for PX-478 as a clinical radiation enhancer in prostate carcinoma cells (Ref. 126). HIF-2α inhibitors, including PT2399, PT2977 and PT2385, are also showing promise as single agents in clear cell renal cell carcinoma in phase II clinical trials, but their combination with radiotherapy is not yet explored (Refs 127–129).

https://doi.org/10.1017/erm.2022.14 Published online by Cambridge University Press

Table 1. Clinical trials evaluating combination of HAPs with radiotherapy

Drug name	Cancer type	ClinicalTrials.gov identifier	Clinical trial status (recruiting/active/completed)	References
Nitro compounds				
Metronidazole	Cervical cancer	NCT01937650	Phase II/III	(Ref. 65)
Misonidazole	Head and neck cancer	NCT00606294	Not applicable	(Ref. 66)
Pimonidazole	Oral tongue cancer	NCT03181035	Phase I/II	(Ref. 67)
Pimonidazole	Rectal cancer	NCT02157246	Not applicable	(Ref. 68)
Etanidazole	Breast cancer brain metastasis	NCT01985971	Not applicable	(Ref. 69)
Nimorazole	HNSCC	NCT01950689	Phase III	(Ref. 70)
Nimorazole	HNSCC	NCT02661152	Phase III	(Ref. 71)
Nimorazole	HNSCC	NCT01880359	Phase III	(Ref. 72)
Nimorazole	HNSCC	NCT01733823	Phase I/II	(Ref. 73)
Nimorazole	OSCC	NCT04124198	Not applicable	(Ref. 74)
N-Oxides				
Tirapazamine	SCCHN	NCT00002774	Phase II	(Ref. 75)
Tirapazamine	Lung cancer	NCT00066742	Phase II	(Ref. 76)
Tirapazamine	Lung cancer	NCT00033410	Phase I	(Ref. 77)
Tirapazamine	Head and neck cancer	NCT00094081	Phase III	(Ref. 78)
Tirapazamine	Cervical cancer	NCT00262821	Phase III	(Ref. 79)
Tirapazamine	Cervical cancer	NCT00098995	Phase I	(Ref. 80)
Tirapazamine	HNSCC	NCT00174837	Phase III	(Ref. 81)
Tirapazamine	Lung cancer	NCT00006487	Phase I	(Ref. 82)
AQ4N	Glioblastoma multiforme	NCT00394628	Phase I/II	(Ref. 83)
Quinones				
Mitomycin	Nasopharyngeal carcinoma	NCT00201396	Phase III	(Ref. 84)
Mitomycin	Pulmonary neoplasm	NCT00128037	Phase II	(Ref. 85)
Mitomycin	Bladder cancer	NCT00002490	Phase III	(Ref. 86)
Mitomycin	Bladder cancer	NCT00024349	Phase III	(Refs 87, 88)
Mitomycin	Bladder cancer	NCT00981656	Phase II	(Ref. 89)
Mitomycin	Head and neck cancer	NCT00002507	Phase III	(Ref. 90)
Mitomycin	Anal cancer	NCT00025090	Phase III	(Ref. 91)
Mitomycin	Anal cancer	NCT00003596	Phase III	(Refs 92, 93)
Mitomycin	Anal cancer	NCT01621217	Phase I	(Ref. 94)
Mitomycin	Anal cancer	NCT01941966	Phase II	(Ref. 95)
Mitomycin	Anal cancer	NCT02701088	Phase II	(Ref. 96)
Mitomycin	Anal cancer	NCT00423293	Phase II	(Ref. 97)
Porfiromycin	Head and neck cancer	NCT00003328	Phase III	(Ref. 98)
Porfiromycin	Head and neck cancer	NCT00002507	Phase III	(Ref. 90)

HNSCC, head and neck squamous cell carcinoma; OSCC, oropharyngeal squamous cell carcinoma; SCCHN, squamous neck carcinoma of the head and neck cancer.

Targeting DNA damage response

Hypoxia can drive cancer progression and lead to radioresistance through its impact on genomic integrity by inhibiting DNA repair pathways (Ref. 130). As outlined previously radiation kills cancer cells by damaging their DNA. DNA repair dysregulation provides a promising opportunity to exploit this key vulnerability for overcoming radioresistance, specifically through targeting DSBs repair pathways (Ref. 131). This is linked with the concept of 'synthetic lethality', which occurs when functional defects of complementary pathways can result in cell death, whereas the perturbation of either pathway does not impact cell survival. Targeting one of the pathways using small molecule inhibitors in cells with a pre-existing defect in the complementary pathway (e.g., use of PARP inhibitors (PARPi) in tumours defective for BRCA1/2) can be very effective, so other such pathway combinations have been explored (Refs 132–134). One of these is hypoxia-mediated repression of DNA repair in 'contextual synthetic lethality' approaches, for example, through combination with PARPi (Ref. 135). Finally, targeting of DDR key factors in combination with radiotherapy has shown a lot of potential for overcoming

https://doi.org/10.1017/erm.2022.14 Published online by Cambridge University Press

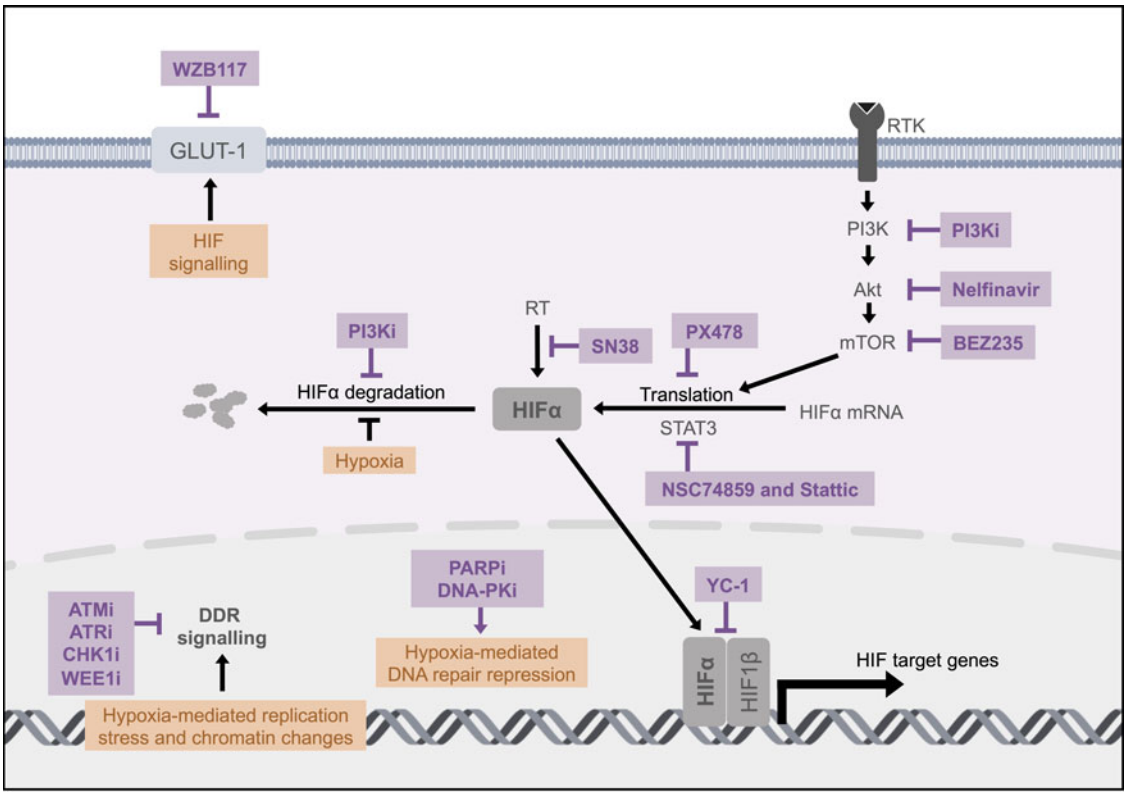

Fig. 2. Targeting of hypoxia-mediated signalling reprogramming as radiosensitising strategies. This schematic indicates the key hypoxia-regulated or associated signalling pathways targeted in radiosensitising approaches, as detailed in section 'Targeting of hypoxia-mediated signalling reprogramming as radiosensitising strategies'. RT, radiotherapy; HIF, hypoxia-inducible factor; RTK, receptor tyrosine kinases; DDR, DNA damage response; GLUT-1, glucose transporter 1; ATM, ataxia telangiectasia mutated; ATR, ataxia telangiectasia and Rad3 related; CHK1, checkpoint kinase 1; PARP1, poly(ADP-ribose) polymerase.

hypoxic radioresistance (Ref. 136). Details of DDR inhibitors investigated in clinical trials as possible radiosensitisers are summarised in Table 2, and examples of these strategies are detailed below.

PARP1 inhibitors

PARPi, which can effectively prevent the repair of damaged DNA by blocking PARP enzyme activity and PARylation reactions, are the first clinically approved drugs based on the principle of synthetic lethality (Ref. 174). BRCA1/2 are major components of the HR pathway for DSB repair, and deficiency in BRCA1/2 genes leads to high susceptibility for breast and ovarian cancer (Ref. 175). HR deficiency due to BRCA1/2 mutations leads to an exquisite sensitivity to PARPi through synthetic lethality between these two pathways, a phenomenon also described as BRCAness (Ref. 176). Many clinical trials have been carried out in various BRCA-mutated tumours that have evaluated the benefits with the treatments of PARPi both as single agents and in combination with radiotherapy (Ref. 177). Importantly, a study from 2010 reported that HR-defective hypoxic cells selectively died because of microenvironment-mediated 'contextual synthetic lethality', where hypoxia-mediated repression of HR represented a BRCAness-like phenotype, and also enhanced sensitivity to ionising radiation (Ref. 135). Other studies have also shown that the combination of PARP1 inhibitor Olaparib with radiotherapy led to radiosensitising effects in hypoxia in NSCLC through this contextual synthetic lethality effect (Ref. 178). Moreover, PARPi also improves the radiotherapy responses, as well as the efficacy of some chemotherapeutic agents, targeted therapy and immunotherapy (Ref. 179). This has led to a significant number of clinical trials focused on the combination with PARP1i and radiation to improve the response to radiotherapy (Table 2).

DNA-PK inhibitors

DNA DSBs generated by ionising radiation can also be repaired through non-homologous end joining), a more error-prone repair pathway than HR (Ref. 180). The KU heterodimers (KU70 and KU80) recognise the DNA DSBs, then activate and recruit DNA-PKcs to the DNA break sites. This complex formed at the DSBs consisting of DNA, Ku70/80 and DNA-PKcs is referred to as DNA-PK (Ref. 181). The expression and activity of DNA-PK in cancers is correlated with the response to anticancer therapy, including radiotherapy (Ref. 182). A study showed that DNA-PKcs inhibition led to increased sensitivity of gastric cancer cells to ionising radiation (Ref. 183). Moreover, another study also found that DNA-PK inhibitor NU5455 may preferentially sensitise chronically hypoxic tumour cells to radiotherapy *in vivo* (Ref. 184). Another study showed that DNA-PKcs inhibition potentially overcome hypoxia-induced radioresistance in NSCLC by the combination of ionising radiation treatment with the DNA-PK inhibitor M3814 (Ref. 185). To our knowledge, M3814 is the only DNA-PK inhibitor currently in clinical development (see Table 2).

ATM/ATR inhibitors

Ataxia-telangiectasia mutated (ATM) is one of the central kinases of the DDR and has a critical role in cancer suppression and DNA DSBs repair (Ref. 186). Like ATM, ataxia telangiectasia and Rad3 related (ATR) is also a central kinase involved in the DDR (Ref. 187). Inhibition of ATM or ATR has been shown to sensitise the cancer cells to radiation treatments. Moreover, ATR and ATM have a role to play in hypoxia/re-oxygenation (Refs 188–190), which led to the exploration of ATM/ATR inhibitor treatment in overcoming hypoxia-mediated radioresistance in cancer. Inhibition of ATM or ATR has been shown to be potential radiosensitisers under hypoxic condition in several studies. One study

https://doi.org/10.1017/erm.2022.14 Published online by Cambridge University Press

Table 2. Clinical trials evaluating the combination of DDR inhibitors and radiotherapy

Drug name	Cancer type	ClinicalTrials.gov identifier	Clinical trial status (recruiting/active/ completed)	Strategies for combination with radiotherapy	References
PARP-1 inhibitors					
Olaparib	Inflammatory breast carcinoma	NCT03598257	Phase II	Radiation	(Ref. 137)
Olaparib	TNBC	NCT03109080	Phase I	Radiation	(Ref. 138)
Olaparib	GBM	NCT03212742	Phase I/IIa	IMRT, TMZ	(Ref. 139)
Olaparib	NSCLC, breast cancer, HNSCC	NCT01562210, NCT02227082, NCT02229656	Phase I	Radiation, cisplatin	(Ref. 140)
Olaparib	NSCLC	NCT04380636	Phase III	Radiation, etoposide, carboplatin, cisplatin, Paclitaxel, pemetrexed, durvalumab	(Ref. 141)
Olaparib	Head and neck cancer	NCT02308072	Phase I	IMRT, cisplatin	(Ref. 142)
Olaparib	Prostate cancer	NCT03317392	Phase I/II	Radium Ra 223 dichloride	(Ref. 143)
Olaparib	Extensive-stage small-cell lung cancer	NCT04728230	Phase I/II	Radiation, carboplatin durvalumab, etoposide	(Ref. 144)
Veliparib	Peritoneal carcinomatosis	NCT01264432	Phase I	LDFWAR	(Ref. 145)
Veliparib	Brain metastases from NSCLC	NCT01657799	phase II	WBRT, placebo	(Ref. 146)
Veliparib	NSCLC	NCT02412371	Phase I	Radiation, carboplatin, paclitaxel	(Ref. 147)
Veliparib	Rectal cancer	NCT01589419	phase I	Radiation, capecitabine	(Ref. 148)
Veliparib	Head and neck cancer	NCT01711541	Phase I/II	Radiation, cisplatin, carboplatin, fluorouracil, hydroxyurea	(Ref. 149)
Veliparib	Cancer patients with brain metastases	NCT00649207	Phase I	WBRT	(Ref. 150)
Veliparib	Pancreatic cancer	NCT01908478	Phase I	Radiation, gemcitabine	(Ref. 151)
Veliparib	Breast cancer	NCT01477489	Phase I	Radiation	(Ref. 152)
Veliparib	GBM	NCT01514201	Phase I/II	3D CRT, TMZ	(Ref. 153)
Veliparib	GBM	NCT03581292	Phase II	Radiation, TMZ	
Veliparib	Lung adenocarcinoma	NCT01386385	Phase I/II	3D CRT, carboplatin, paclitaxel	(Ref. 154)
Niraparib	Prostate cancer	NCT04194554	Phase I	SBRT, leuprolide, abiraterone acetate	(Ref. 155)
Niraparib	Metastatic invasive carcinoma of the cervix	NCT03644342	Phase I/II	Radiation	(Ref. 156)
Niraparib	TNBC	NCT03945721	Phase I	Radiation	(Ref. 157)
Niraparib	Breast cancer	NCT04837209	Phase II	Radiation, dostarlimab	
DNA PK inhibitors					
M3814	Advanced solid tumours	NCT02516813	Phase I	Radiation, cisplatin	(Ref. 158)
M3814	Rectal cancer	NCT03770689	Phase I/II	Radiation, capecitabine, placebo	(Ref. 159)
M3814	Solid tumours	NCT03724890	Phase I	Radiation, avelumab	(Ref. 160)
M3814	GBM	NCT04555577	Phase I	Radiation, TMZ	(Ref. 161)
M3814	HNSCC	NCT04533750	Phase I	Radiation	(Ref. 162)
ATM/ATR inhibitors					
AZD1390	Brain cancer	NCT03423628	Phase I	Radiation	(Ref. 163)
AZD6738	Solid tumours	NCT02223923	Phase I	Radiation	(Ref. 164)
VX-970	HNSCC	NCT02567422	Phase I	Radiation, cisplatin	(Ref. 165)
VX-970	NSCLC brain metastases	NCT02589522	Phase I	WBRT	(Ref. 166)
VX-970	Oesophageal adenocarcinoma Squamous cell carcinoma Solid tumour	NCT03641547	Phase I	Radiation, cisplatin, capecitabine	(Ref. 167)

(*Continued*)

https://doi.org/10.1017/erm.2022.14 Published online by Cambridge University Press

Table 2. (*Continued.*)

Drug name	Cancer type	ClinicalTrials.gov identifier	Clinical trial status (recruiting/active/ completed)	Strategies for combination with radiotherapy	References
Elimusertib	Head and neck cancer	NCT04576091	Phase I	Radiation	(Ref. 168)
WEE1 inhibitors					
AZD1775	Head and neck cancer	ISRCTN76291951 NCT03028766	Phase I	Radiation, cisplatin	(Ref. 169)
AZD1775	Adenocarcinoma of the pancreas	NCT02037230	Phase I/II	Radiation, gemcitabine	(Ref. 170)
AZD1775	Cervical, upper vaginal and uterine cancers	NCT03345784	Phase I	Radiation, cisplatin, adavosertib	(Ref. 171)
AZD1775	Cervical cancer	NCT01958658	Phase I	Radiation, cisplatin	(Ref. 171)
AZD1775	Head and neck cancer	NCT02585973	Phase I	Radiation, cisplatin	(Ref. 172)
AZD1775	GBM	NCT01849146, NCT01922076	Phase I	Radiation, TMZ	(Ref. 173)

TNBC, triple negative breast cancer; GBM, glioblastoma; NSCLC, non-small-cell lung cancer; HNSCC, head and neck squamous cell carcinoma; IMRT, intensity-modulated radiotherapy; TMZ, temozolomide; LDFWAR, low-dose fractionated whole abdominal radiation; WBRT, whole brain radiation therapy; 3D CRT, 3-dimensional conformal radiation therapy; SBRT, stereotactic body radiotherapy.

found ATM inhibition can increase the radiosensitising effect under hypoxic conditions in NSCLC (Ref. 185). ATR inhibitor VE-821 has reported to increase sensitivity of pancreatic cancer cells to radiation and chemotherapy in pancreatic cancer under both normoxic and hypoxic conditions (Refs 188, 191). Another ATR inhibitor from the same chemical series as VE-821, Berzosertib (formerly VE-822, M6620 and VX-970), has also been shown to sensitise response to chemo/radiotherapy, which could improve the treatment efficacy in oesophageal cancer (Ref. 192). Clinical trials regarding combination of ATM or ATR inhibitors with radiation are ongoing, such as ATM inhibitors AZD1390 and AZD6738, and ATR inhibitor VX-970 (Table 2). ATM and ATR target kinases CHK1 and CHK2 also represent attractive targets to be combined with established cancer therapies, including radiotherapy, but to date only CHK1 inhibitor Prexasertib/LY2606368 combined with radiation has entered clinical trial and suggest that this combination therapy may increase clinical benefit (Refs 193, 194).

WEE1 kinase inhibitor

WEE1 kinase is a key regulator of the G2/M phase transition that allows DNA repair before mitotic entry (Ref. 195). Amongst several WEE1 inhibitors evaluated in combination with radiotherapy (Table 2), combination of AZD1775 and ionising radiation has shown significantly increased apoptosis in cervical cancer cells (Ref. 196). Another study also highlighted the radiosensitised effect of WEE1 kinase inhibitor AZD1775 through inducing replication stress in hepatocellular carcinoma (Ref. 197). Furthermore, another study investigated the impact of WEE1 inhibition using the MK-1775 on hypoxic cells in combination with radiation, showing MK-1775 sensitised radiation under normoxia, but not hypoxic conditions (Ref. 198).

Targeting cell metabolism

There are an increasing number of studies that conclude that metabolic alterations in cancer are one of the major reasons contributing to radioresistance (Ref. 199). The PI3K/AKT/mTOR is a key signalling pathway that can stimulate glucose uptake, therefore controlling cell metabolism in cancer cells. The PI3K/AKT/mTOR pathway is involved in hypoxia-ischemia signalling, and HIF-1α is regulated by PI3K/Akt signalling pathway (Ref. 200). PI3K inhibition by LY294002 radiosensitises human cervical cancer cell lines (Ref. 201). Studies have also found that PI3K/Akt/mTOR pathway inhibitors (BEZ235 or PI103) enhance radiosensitivity in radioresistant tumour cells such as prostate cancer cells (Ref. 202). A dual PI3K and mTOR inhibition NVP-BEZ235 has been shown to significantly reduce tumour hypoxia by normalising tumour vasculature (Ref. 203). PI3K/mTOR inhibitors BEZ235 and BKM120 were shown to significantly reduce oxygen consumption in cancer cell lines, with associated reduced mitochondrial respiration (Ref. 204). Several clinical studies have now evaluated the efficacy of PI3K/Akt/mTOR inhibitors in combination with radiotherapy, and these are summarised in Table 3. Nelfinavir, which is AKT phosphorylation inhibitor, has entered clinical trial phase III in cervical cancer (Ref. 205). Another study using Nelfinavir with concurrent CT-RT is associated with acceptable toxicity. Moreover, the results from metabolic response and tumour response suggested the benefit of Nelfinavir is promising in stage IIIA/IIIB NSCLC (Ref. 206).

Glucose transporter 1 (GLUT1) is an essential factor for glucose metabolism and is also a canonical HIF target gene (Ref. 225). Studies found increased GLUT1 levels in radioresistant tumour cells, which indicates that GLUT1 expression may be used as an indicator of the sensitivity to radiation and prognosis of radiotherapy (Refs 226–228). Targeting GLUT1 and related signalling pathways may therefore represent an effective way to improve radiotherapy efficacy. A small molecule inhibitor of GLUT1, WZB117, can increase the sensitivity of radiation in breast cancer cells (Ref. 229). Another study found that modulating the glucose metabolism sensitised glioblastoma cells to ionising radiation (Ref. 230). However, there are no GLUT1 inhibitors combined with radiation entered in clinic trails yet.

Combined immunotherapy

During radiotherapy treatment, radiation not only damages cancer cells directly, but also activates an immune response (Ref. 231). Meanwhile, hypoxia also plays a pivotal role in the regulation of immunosuppressive molecules and participates in the activation of immunosuppressive cells (Ref. 232). For example, IL10 and TGFβ are increased under hypoxia, which induces the differentiation of tumour-associated macrophages into M2 macrophages and therefore activates immune-suppressive

https://doi.org/10.1017/erm.2022.14 Published online by Cambridge University Press

Table 3. Clinical trials evaluating the combination of PI3K/AKT/mTOR inhibitors and radiotherapy

Drug name	Cancer types	ClinicalTrials.gov identifier	Clinical trial status (recruiting/active/ completed)	Combination strategy	References
GDC-0084	Brain metastases leptomeningeal metastasis	NCT04192981	Phase I	WBRT	(Ref. 207)
GDC-0084	Brain and central nervous system tumours	NCT03696355	Phase I	Radiation	(Ref. 208)
GDC-0084	Glioma	NCT05009992	Phase II	Radiation, ONC201, panobinostat	(Ref. 209)
BKM120	NSCLC	NCT02128724	Phase I	Radiation	(Ref. 203)
BKM120	HNSCC	NCT02113878	Phase I	IMRT, cisplatin	(Ref. 210)
Nelfinavir	Cervical cancer	NCT03256916	Phase III	Radiation, cisplatin	(Ref. 205)
Nelfinavir	Locally advanced pancreatic cancer	NCT03256916	Phase I	Radiation, cisplatin, gemcitabine	(Ref. 211)
Nelfinavir	NSCLC	NCT03256916	Phase I	Chemoradiotherapy	(Ref. 206)
Nelfinavir	locally advanced rectal cancer	NCT03256916	Phase I	Chemoradiotherapy	(Ref. 212)
Nelfinavir	Cervical cancer	NCT01485731	Phase I	Radiation, cisplatin	(Ref. 213)
Nelfinavir	GMB	NCT00694837	Phase I	Radiation	(Ref. 214)
Nelfinavir	Oligometastases	NCT01728779	Phase II	SBRT	(Ref. 215)
Nelfinavir	Pancreatic cancer	NCT01068327	Phase I	Radiation	(Ref. 216)
BYL719	HNSCC	NCT02537223	Phase I	IMRT, cisplatin	(Ref. 217)
XL765	GMB	NCT00704080	Phase I	Radiation, TMZ	(Ref. 218)
Alpelisib	Meningioma	NCT03631953	Phase I	MRI, trametinib	(Ref. 219)
Everolimus	Cervical cancer	NCT01217177	Phase I	Radiation	(Ref. 220)
Everolimus	Prostate cancer	NCT01548807	Phase I	Radiation	(Ref. 221)
Rapamycin	Rectum cancer	NCT00409994	Phase I/II	Radiation	(Ref. 222)
Temsirolimus	NSCLC	NCT00796796	Phase I	Radiation	(Refs 223, 224)

GBM, glioblastoma; NSCLC, non-small-cell lung cancer; HNSCC, head and neck squamous cell carcinoma; TMZ, temozolomide; WBRT, whole brain radiation therapy; SBRT, stereotactic body radiotherapy; IMRT, intensity-modulated radiotherapy.

activities (Ref. 233). Hypoxia also regulates the differentiation and activation of dendritic cells (Ref. 234). On the other hand, hypoxia activates immunosuppressive cells, such as myeloid-derived suppressor cells, regulatory T cells and decreased infiltration and activation of CTCs, which suggests that targeting HIF in the immune system could be beneficial for anti-tumour immune responses (Ref. 235).

Radiotherapy has both pro-immunogenic and immunosuppressive effects on immune response in various levels. This includes the induction of immunogenic cell death, promoting the recruitment and function of T cells within the tumour microenvironment, and improving the recognition and killing of cancer cells by CD8+ CTLs (Ref. 236). This is key to the synergistic effect of radiation with immune checkpoint inhibitors, antibodies targeting inhibitory receptors on T cells, including cytotoxic T lymphocyte antigen-4 (CTLA-4) and programmed death-1 (PD-1), and has become an optimal partner for immune check point inhibitors (Ref. 237). In fact, several completed clinical trials evaluated the efficacy of combining immunotherapy approaches using immune checkpoint inhibitors with radiotherapy, and the completed clinical trials are summarised in Table 4.

Furthermore, studies also found that immunosuppressive macrophages were recruited by radiation, which induced upregulation of CSF-1. Depletion of these macrophages by using anti-CSF antibody (aCSF) significantly delays tumour regrowth following radiation. Moreover, the addition of an anti-PD-L1 antibody to aCSF resulted in improved tumour suppression and even regression in a highly resistant murine pancreatic cancer model (Ref. 265); therefore, macrophage depletion may play a role in immune checkpoint blockade-resistant tumours. Ultimately, as suggested by Eckert *et al.*, as hypoxia mediates radioresistance and immune escape, the combination of immune checkpoint inhibition and radiotherapy might be a promising strategy to improve outcome in tumours with high hypoxic content (Ref. 266).

IMRT combination with radiosensitiser approaches

IMRT is a radiotherapy modality that delivers highly conformal dose distributions (Ref. 267). It is designed by inverse optimisation algorithms, with the following inputs: the dose required to the 'tumour' to gain control of the disease; and constraints or dose limitations for proximal tissues and 'organs at risk'. The optimisation process is controlled by cost functions, these essentially compare dose distributions achieved by a set of x-ray beams, to the desired outcome; they then guide modulation of each beam in a systematic manner until a solution close to that originally specified is obtained. In simple terms the described process results in a set of beams, each consisting of a number of segments whose individual dose patterns superpose to create exquisite dose distributions that acknowledge the 3D nature of tumours and the discrete hypoxic and normoxic regions present in tumour masses (Ref. 268). Commonly, the degrees of freedom available to the

https://doi.org/10.1017/erm.2022.14 Published online by Cambridge University Press

Table 4. Clinical trials evaluating the combination of immunotherapy therapeutics and radiotherapy

Drug name	Cancer types	ClinicalTrials.gov identifier	Clinical trial (completed)	Combination with RT	References
Anti-PD-1/PD-L1					
SHR-1210	Oesophageal cancer	NCT03187314	Phase I	Radiation	(Ref. 238)
SHR-1210	Oesophageal cancer	NCT03222440	Not applicable	Radiation	(Ref. 239)
Nivolumab	NSCLC	NCT02434081	Phase II	Radiation	(Ref. 240)
Nivolumab	Small-cell lung cancer	NCT03325816	Phase I/II	Radiation	(Ref. 241)
Nivolumab	Hepatocellular carcinoma	NCT03380130	Phase II	SIRT	(Ref. 242)
Nivolumab	Lung cancer	NCT03044626	Phase II	Radiation	(Ref. 243)
Pembrolizumab	Renal cell carcinoma	NCT02855203	Phase I/II	SABR	(Ref. 244)
Pembrolizumab	Head and neck cancer	NCT02759575	Phase I/II	Radiation, cisplatin	(Ref. 245)
Pembrolizumab	Follicular lymphoma	NCT02677155	Phase II	Radiation	(Ref. 246)
Pembrolizumab	Metastatic cancers	NCT02303990	Phase I	Radiation	(Ref. 247)
Pembrolizumab	Oligometastatic breast neoplasia	NCT02303366	Phase I	SABR	(Ref. 248)
Pembrolizumab	Oesophageal cancer	NCT02642809	Phase I	Radiation	(Ref. 249)
Pembrolizumab	Renal cell cancer	NCT02599779	Phase II	SBRT	(Ref. 250)
Nivolumab/ Pembrolizumab	Lung cancer	NCT03224871	Phase I	Radiation, intralesional IL-2	(Ref. 251)
AMP-224	Colorectal cancer	NCT02298946	Phase I	SBRT, cyclophosphamide	(Ref. 252)
Avelumab	NSCLC	NCT03158883	Phase I	SABR	(Ref. 253)
Avelumab	GBM	NCT02968940	Phase II	HFRT	(Ref. 254)
Cemiplimab	Advanced malignancies	NCT02383212	Phase I	Radiation	(Refs 255, 256)
anti-CTLA-4					
Ipilimumab	NSCLC	NCT02221739	Phase I/II	Radiation	(Ref. 257)
Ipilimumab	Lymphoma	NCT02254772	Phase I/II	Radiation, SD-101	(Ref. 258)
Ipilimumab	Melanoma	NCT01449279	Phase II	Radiation	(Ref. 259)
Ipilimumab	Melanoma	NCT02406183	Phase I	SBRT	(Ref. 260)
Ipilimumab	Melanoma, brain metastases	NCT02115139	Phase II	Radiation	(Ref. 261)
Ipilimumab	Cervical cancer	NCT01711515	Phase I	Radiation, cisplatin	(Ref. 262)
Tremelimumab	Pancreatic cancer	NCT02311361	Phase I/II	SBRT, durvalumab	(Ref. 263)
Tremelimumab	Recurrent small cell lung carcinoma	NCT02701400	Phase II	SBRT, durvalumab	(Ref. 264)

GBM, glioblastoma; NSCLC, non-small-cell lung cancer; IMRT, intensity-modulated radiotherapy; TMZ, temozolomide; LDFWAR, low-dose fractionated whole abdominal radiation; WBRT, whole brain radiation therapy; 3D CRT, 3-dimensional conformal radiation therapy; SBRT, stereotactic body radiotherapy, SIRT, selective internal radiation therapy; SABR, stereotactic ablative radiotherapy; SBRT, stereotactic body radiation therapy; HFRT, hypofractionated radiation therapy.

optimiser is increased by using arc-based treatment beams rather than a discrete set of fixed directions. IMRT has had a clear impact on the success of modern radiotherapy strategies. However, given it typically is implemented with high-energy x-rays which are low-LET radiation, further developments considering strategy modification related to hypoxia management may be limited, see section 'High-LET modalities as alternatives to oxygen-dependent low-LET ionising radiation'.

Combining precise delivery via IMRT with radiosensitiser approaches such as DDR inhibitors (section 'Targeting DNA damage response') and immunotherapy (section 'Combined immunotherapy') has the potential to improve patient outcomes. Furthermore, nanotechnology has potential to provide a new dimension to this strategy with metallic nanomaterials being developed as possible hypoxic radiosensitisers (Ref. 269). Gold nanoparticles (GNPs), for example, are gaining attention due to gold's ability to readily donate electrons and thereby promote the production of ROS, even in low oxygen environments. In a study of colon cancer, CT26 cells were incubated in hypoxia both with and without GNPs prior to radiotherapy application. Significantly improved responses were observed in the GNP group, suggesting dual IMRT-GNP therapeutics could improve the relative biological effectiveness (RBE) and OER of low-LET modalities compared to x-ray application alone (Ref. 270).

High-LET modalities as alternatives to oxygen-dependent low-LET ionising radiation

LET is the energy loss of a radioactive particle per unit of distance travelled and in radiotherapy, a measure of the amount of energy

https://doi.org/10.1017/erm.2022.14 Published online by Cambridge University Press

transferred from the radiation source to the patient. High-LET radiation sources include alpha particles, with high mass and positive charge, and low-energy neutrons which have no charge and are approximately ¼ mass of an alpha particle (Ref. 271). Low-LET radiation sources, most commonly x-rays or gamma-rays, are photons having no mass or charge and wavelengths below 10^{-8} m (Ref. 272). High-LET particles deposit their energy within a short distance from the radiation source, following a discrete pathway and causing significant cellular disruption localised to a smaller area close to the target (Ref. 273). Low-LET waves however penetrate tissues more readily and are widely scattered as they transverse through the patient, causing less intense damage to a larger area of tissue (Ref. 273).

The radiobiology of high-LET RT modalities

Tumours with oxygen-deficient areas experience increased radioresistance termed the OER, a comparison of the dose of radiation needed to cause the same damage in normoxic verses hypoxic tissue environments. Experimentally, the OER is inversely proportional to LET suggesting a potential clinical advantage of high-LET radiotherapy compared to low-LET irradiation (Ref. 274). RBE (relative biological effectiveness) is a comparison of biological efficacy of one type of ionising radiation compared to another (such as DNA damage and apoptosis levels), and indicates the dose of different ionisation sources that are needed to produce the same biological effect (Ref. 272). High LET radiation has an increased biological effectiveness compared to photons of low LET, causing more extensive and clustered DNA damage (Ref. 275). Specifically, application of high-LET radiotherapy causes closely interspaced DSBs leading to high local concentrations of repair proteins and perturbed DNA damage owed to its discrete pattern of energy deposition compared to low-LET X-ray irradiation (Fig. 3) (Ref. 276). Contemporary proton particle therapy utilises scanning beam technology which facilitates intensity modulated proton therapy, wherein the benefits afforded by intensity modulation and high-LET delivery are combined (Ref. 277).

FLASH

FLASH radiotherapy is a treatment method that decreases the damage caused to the normal tissue (tissue sparing) whilst maintaining a tumour response compared with conventional low dose rate radiotherapy (Refs 280, 281). The FLASH technique involves application of a single, ultra-high dose of radiation over a short time period. When compared to conventional radiotherapy *in vitro*, FLASH radiotherapy caused significantly less DNA damage to normal tissue than conventional radiation. The mechanisms underpinning the tissue sparing effect of FLASH are hypothesised to be diverse, including rapid radiochemical depletion of oxygen leading to transient hypoxia in normal tissue, radical interaction or inhibition of activation of genes that drive inflammation and proliferation of tumours (Ref. 282). In the oxygen depletion/transient hypoxia hypothesis, normal tissue with physiological oxygen levels would experience rapid oxygen depletion after FLASH, leading to transient radioresistance which would in turn would lead to decreased damage and ultimately a tissue sparing effect. Further investigation on post irradiation effect showed that FLASH halted repopulation, whilst significantly reducing radio-induced senescence (Ref. 283). Importantly, FLASH radiotherapy has increased RBE when delivered in high-LET modalities harnessing a proton beam radiation source compared to low-LET x-ray sources (Ref. 284). Experiments to validate its efficacy in hypoxia however suggest FLASH radiotherapy has a high OER *in vitro*, with tissue oxygen concentrations above 4.4% needed for the technique to match the efficacy of conventional RT as hypoxic regions lack the oxygen availability to support the rapid oxygen consumption occurring in local tissues during FLASH therapy (Ref. 285). The mechanism and biological nature of the FLASH effect is complex, but it is expected this will be an area of increased interest in the radiobiology field.

Dose painting

Positron emission tomography (PET) and MRI are functional, non-invasive imaging modalities utilised to identify hypoxic tissue regions in patient tumours (Ref. 286). Such imaging allows clinicians to define areas likely to be resistant to radiotherapy, such as areas of tumour hypoxia. Therefore, strategic delivery of higher ionising doses to hypoxic areas while reducing the dose delivered to more oxygenated regions thereby limiting dose-related side effects, a process also known as dose painting (Refs 287, 288).

In a study of 12 patients with locally advanced HNSCC, hypoxia-specific tracer ^{18}F-Fluoroazomycin arabinoside was harnessed alongside PET technology to assess the capabilities of hypoxia-guided dose painting. FAZA accumulation successfully identified hypoxic voxels in 80% of the cohort, while hypoxic volume made up to 54% of the patients' total tumour masses. Subsequently, 86 Gy doses were delivered to hypoxic voxels while a 70 Gy mean dose was administered across other regions and results revealed that dose escalation had no impact on adjoining healthy tissues (Ref. 282). Another dose painting study involving 10 HNSCC patients harnessed hypoxic tracer ^{18}F-Fluoromisonidazole in combination with PET to identify and image chronic hypoxic voxels. Post imaging, one sub-group received 35 fraction schedules of 2 Gy irradiation (70 Gy total) homogenously while a second sub-group received an escalated dose of 2.28 Gy to hypoxic regions (79.8 Gy total). Comparison of the two treatment plans demonstrated dose escalation to hypoxic regions can be delivered safely and efficaciously, without any increased delivery to at-risk organs (Ref. 283). Therefore, the literature suggests that combining dose-painting methodologies with high-LET radiation could therefore increase the benefit of hypoxia mapping as patients could benefit from the improved OER and RBE that high-LET therapies provide, accompanied by increased precision of application, allowing potent radiation doses to be delivered with minimal damage to healthy cells. However, a caveat of this approach is that it is based on a plan prior to treatment. A course of radiotherapy is delivered over a period of 1 and 7 weeks and oxygen level distribution can change in response to the treatment, thus impacting on the efficacy of this approach.

Concluding thoughts and future directions

Radiotherapy remains one of the most effective non-invasive treatments for solid tumours, but the impact of tumour biology on response of tumour cells to radiation remains a fundamental limitation to what radiotherapy can ultimately achieve. Challenges associated with radiotherapy response include inherent radioresistance of cancer cells, lack of discrimination between normal tissue and tumour cells, and, pertinent to this review, tumour hypoxia-mediated radioresistance. State-of-the-art dual treatment modalities for cancer patients have previously relied upon radiotherapy accompanied by surgery, chemotherapy and more recently, immunotherapy. However, these combinations have been unable to abolish treatment-resistant hypoxic regions often resulting in poor survival rates and disease recurrence. Furthermore, radiotherapy technology (instrumentation and software) and delivery has improved significantly over last 15 years,

https://doi.org/10.1017/erm.2022.14 Published online by Cambridge University Press

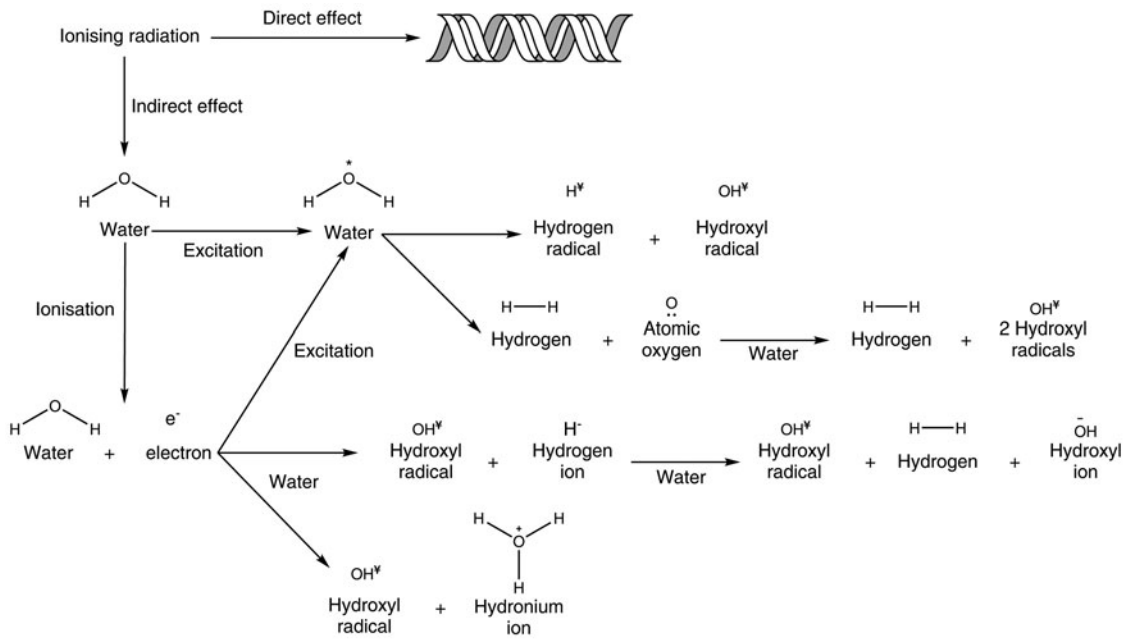

Fig. 3. Water radiolysis in high vs low LET radiation. Water radiolysis, propagated by ionising radiation, can follow numerable reaction pathways resulting in a snowballing mechanism that produces numerable ROS (oxygen containing radicals) that damage DNA, known as the indirect effect. Additionally, radiation treatment can damage DNA through impact alone and subsequently damage molecular structure, known as the direct effect. Application of high-LET radiotherapy sources induce a larger degree of the direct effect compared to low-LET sources, owed to the particles high mass and charge, while low-LET modalities rely more on the presence of sufficient oxygen to be efficacious. Figure created in ChemDraw 20.1.1 and adapted from (Refs 278, 279).

but has potentially encountered an era of diminishing returns, where increased accuracy in radiotherapy delivery may not substantially improve outcomes alone.

We suggest that hypoxia targeting in radiotherapy treatment strategies should encapsulate the mainstream treatment strategy for cancer, especially solid tumours, with experimental and clinical evidence suggesting some of these strategies even carry the benefit of reduced off-target effects. Of particular interest are treatment plans that strategically exploit the hypoxic tumour microenvironment by targeting hypoxia-mediated radioresistance signalling, such as HIF inhibition and targeting DDR, as well as employment of HAPs. However, further studies using accurate evaluation of hypoxic content of tumours are needed to validate their efficacy in combination with radiotherapy and advance such strategies towards the clinic.

Clinical validation of existing hypoxia-targeted radiosensitisers should therefore continue to be a priority area in radiotherapy research, alongside prioritising treatment metrics that include hypoxic indices of tumours, capitalising on the disease-specific, druggable targets in the hypoxic microenvironment. This should include evaluating combination approaches of radiotherapy with relevant hypoxia signalling targeting small molecule inhibitors (such as HIF and DDR inhibitors) as well as immunotherapy. These strategies should be also combined with current radiotherapy delivery modalities, including developing the use of hypoxia content scores in increasing the effectiveness of fractionated radiotherapy strategies using machine-learning in *in silico* modelling. It will also involve a shift towards high-LET radiotherapeutics over low-LET options to provide relatively immediate benefits to the cancer patient group.

Ultimately, the use of these various strategies targeting hypoxic radiobiology, combined with cutting-edge precise radiotherapy delivery and modelling, should lead to improvement in patient outcomes.

Acknowledgements. The authors would like to acknowledge all the studies were not able to include due to space limitations.

Financial support. CL and IMP are funded by a University of Hull studentship part of the Adaptive Radiotherapy PhD cluster, which is a collaborative project with the NHS Trust, led by IMP (University of Hull) and AWB (Hull University Teaching Hospitals NHS Trust). IMP, LW and RR are funded by a Bowel Research UK PhD studentship (reference 000100009).

Conflict of interest. None.

References

1. **Reisz JA *et al.*** (2014) Effects of ionizing radiation on biological molecules – mechanisms of damage and emerging methods of detection. *Antioxidants & Redox Signaling* **21**, 260–292.
2. **Bahadoer RR *et al.*** (2021) Short-course radiotherapy followed by chemotherapy before total mesorectal excision (TME) versus preoperative chemoradiotherapy, TME, and optional adjuvant chemotherapy in locally advanced rectal cancer (RAPIDO): a randomised, open-label, phase 3 trial. *The Lancet Oncology* **22**, 29–42.
3. **Nishimura H *et al.*** (2012) Radiotherapy for stage I or II hypopharyngeal carcinoma. *Journal of Radiation Research* **53**, 892–899.
4. **Anderson G *et al.*** (2021) An updated review on head and neck cancer treatment with radiation therapy. *Cancers* **13**, 4912.
5. **Theelen W *et al.*** (2021) Pembrolizumab with or without radiotherapy for metastatic non-small-cell lung cancer: a pooled analysis of two randomised trials. *The Lancet. Respiratory Medicine* **9**, 467–475.
6. **Gray LH *et al.*** (1959) The influence of oxygen and peroxides on the response of mammalian cells and tissues to ionizing radiations. *Prog Nucl Energy* 6 *Biological Sciences* **2**, 69–81.
7. **Goodhead DT** (1999) Mechanisms for the biological effectiveness of high-LET radiations. *Journal of Radiation Research* **40**(suppl), 1–13.
8. **Brizel DM *et al.*** (1996) Radiation therapy and hyperthermia improve the oxygenation of human soft tissue sarcomas. *Cancer Research* **56**, 5347–5350.
9. **Nordsmark M *et al.*** (2005) Prognostic value of tumor oxygenation in 397 head and neck tumors after primary radiation therapy. An international multi-center study. *Radiotherapy & Oncology* **77**, 18–24.
10. **Nordsmark M *et al.*** (2006) The prognostic value of pimonidazole and tumour pO2 in human cervix carcinomas after radiation therapy: a prospective international multi-center study. *Radiotherapy & Oncology* **80**, 123–131.

https://doi.org/10.1017/erm.2022.14 Published online by Cambridge University Press

11. **Hammond EM *et al.*** (2014) The meaning, measurement and modification of hypoxia in the laboratory and the clinic. *Clinical Oncology* **26**, 277–288.
12. **Bassler N *et al.*** (2014) LET-painting increases tumour control probability in hypoxic tumours. *Acta Oncologica* **53**, 25–32.
13. **Tinganelli W *et al.*** (2015) Kill-painting of hypoxic tumours in charged particle therapy. *Scientific Reports* **5**, 17016.
14. **Hill RP *et al.*** (2015) Hypoxia and predicting radiation response. *Seminars in Radiation Oncology* **25**, 260–272.
15. **Vaupel P and Mayer A** (2017) Tumor oxygenation status: facts and fallacies. *Advances in Experimental Medicine and Biology* **977**, 91–99.
16. **Saxena K and Jolly MK** (2019) Acute vs. chronic vs. cyclic hypoxia: their differential dynamics, molecular mechanisms, and effects on tumor progression. *Biomolecules* **9**, 339.
17. **Bader SB, Dewhirst MW and Hammond EM** (2020) Cyclic hypoxia: an update on its characteristics, methods to measure it and biological implications in cancer. *Cancers* **13**, 23.
18. **Majmundar AJ, Wong WJ and Simon MC** (2010) Hypoxia-inducible factors and the response to hypoxic stress. *Molecular Cell* **40**, 294–309.
19. **Kabakov AE and Yakimova AO** (2021) Hypoxia-induced cancer cell responses driving radioresistance of hypoxic tumors: approaches to targeting and radiosensitizing. *Cancers* **13**, 1102.
20. **Fong GH and Takeda K** (2008) Role and regulation of prolyl hydroxylase domain proteins. *Cell Death & Differentiation* **15**, 635–641.
21. **Kubaichuk K and Kietzmann T** (2019) Involvement of E3 ligases and deubiquitinases in the control of HIF-α subunit abundance. *Cells* **8**, 598.
22. **Dames SA *et al.*** (2002) Structural basis for Hif-1 alpha/CBP recognition in the cellular hypoxic response. *Proceedings of the National Academy of Sciences of the USA* **99**, 5271–5276.
23. **Masoud GN and Li W** (2015) HIF-1α pathway: role, regulation and intervention for cancer therapy. *Acta Pharmaceutica Sinica B* **5**, 378–389.
24. **Wang H *et al.*** (2019) Hypoxic radioresistance: can ROS be the key to overcome it? *Cancers* **11**, 112.
25. **Lomax ME, Folkes LK and O'Neill P** (2013) Biological consequences of radiation-induced DNA damage: relevance to radiotherapy. *Clinical Oncology* **25**, 578–585.
26. **Brown JM** (2020) Radiation damage to tumor vasculature initiates a program that promotes tumor recurrences. *International Journal of Radiation Oncology Biology Physics* **108**, 734–744.
27. **Aebersold DM *et al.*** (2001) Expression of hypoxia-inducible factor-1 alpha: a novel predictive and prognostic parameter in the radiotherapy of oropharyngeal cancer. *Cancer Research* **61**, 2911–2916.
28. **Koukourakis MI *et al.*** (2002) Hypoxia-inducible factor (HIF1A and HIF2A), angiogenesis, and chemoradiotherapy outcome of squamous cell head-and-neck cancer. *International Journal of Radiation Oncology Biology Physics* **53**, 1192–1202.
29. **Dewhirst MW *et al.*** (2007) Exploring the role of HIF-1 in early angiogenesis and response to radiotherapy. *Radiotherapy & Oncology* **83**, 249–255.
30. **Chan N *et al.*** (2008) Chronic hypoxia decreases synthesis of homologous recombination proteins to offset chemoresistance and radioresistance. *Cancer Research* **68**, 605–614.
31. **Hassan Venkatesh G *et al.*** (2020) Hypoxia increases mutational load of breast cancer cells through frameshift mutations. *Oncoimmunology* **9**, 1750750.
32. **Lu Y *et al.*** (2014) Silencing of the DNA mismatch repair gene MLH1 induced by hypoxic stress in a pathway dependent on the histone demethylase LSD1. *Cell Reports* **8**, 501–513.
33. **Pires IM *et al.*** (2010) Effects of acute versus chronic hypoxia on DNA damage responses and genomic instability. *Cancer Research* **70**, 925–935.
34. **Olcina MM *et al.*** (2013) Replication stress and chromatin context link ATM activation to a role in DNA replication. *Molecular Cell* **52**, 758–766.
35. **Foskolou IP *et al.*** (2017) Ribonucleotide reductase requires subunit switching in hypoxia to maintain DNA replication. *Molecular Cell* **66**, 206–220. e209.
36. **Bennett MH *et al.*** (2016) Hyperbaric oxygen therapy for late radiation tissue injury. *The Cochrane Database of Systematic Reviews* **4**, Cd005005.
37. **Overgaard J** (2011) Hypoxic modification of radiotherapy in squamous cell carcinoma of the head and neck – a systematic review and meta-analysis. *Radiotherapy & Oncology* **100**, 22–32.
38. **Kunugita N *et al.*** (2001) Radiotherapy after hyperbaric oxygenation improves radioresponse in experimental tumor models. *Cancer Letters* **164**, 149–154.
39. **Hoskin PJ *et al.*** (2010) Radiotherapy with concurrent carbogen and nicotinamide in bladder carcinoma. *Journal of Clinical Oncology* **28**, 4912–4918.
40. **Tharmalingham H and Hoskin P** (2019) Clinical trials targeting hypoxia. *The British Journal of Radiology* **92**, 20170966.
41. **Janssens GO *et al.*** (2012) Accelerated radiotherapy with carbogen and nicotinamide for laryngeal cancer: results of a phase III randomized trial. *Journal of Clinical Oncology* **30**, 1777–1783.
42. **Gainer JL *et al.*** (2017) Trans sodium crocetinate with temozolomide and radiation therapy for glioblastoma multiforme. *Journal of Neurosurgery* **126**, 460–466.
43. **Sheehan J *et al.*** (2010) Trans-sodium crocetinate enhancing survival and glioma response on magnetic resonance imaging to radiation and temozolomide. *Journal of Neurosurgery* **113**, 234–239.
44. **Murayama C *et al.*** (2012) Liposome-encapsulated hemoglobin ameliorates tumor hypoxia and enhances radiation therapy to suppress tumor growth in mice. *Artificial Organs* **36**, 170–177.
45. **Le Moan N *et al.*** (2017) Abstract 4686: Omx a hypoxia modulator reverses the immunosuppressive glioblastoma microenvironment by stimulating T cell infiltration and activation that results in increased number of long-term survivors. *Cancer Research* **77**, 4686.
46. **Johnson JLH** (2017) Oxygen carriers: are they enough for cellular support? In Lapchak PA and Zhang JH (eds), *Neuroprotective Therapy for Stroke and Ischemic Disease*. Cham: Springer International Publishing, pp. 621–640.
47. **NuvOx LLC** (2019) NVX-108 combined with radiation & TMZ, and during maintenance phase for newly-diagnosed glioblastoma multiform. Available at https://clinicaltrials.gov/ct2/show/NCT03862430.
48. **Farah C, Michel LYM and Balligand JL** (2018) Nitric oxide signalling in cardiovascular health and disease. *Nature Reviews Cardiology* **15**, 292–316.
49. **Scicinski J *et al.*** (2015) No to cancer: the complex and multifaceted role of nitric oxide and the epigenetic nitric oxide donor, RRx-001. *Redox Biology* **6**, 1–8.
50. **Wardman P *et al.*** (2007) Radiosensitization by nitric oxide at low radiation doses. *Radiation Research* **167**, 475–484.
51. **Ashton TM *et al.*** (2016) The anti-malarial atovaquone increases radiosensitivity by alleviating tumour hypoxia. *Nature Communications* **7**, 12308.
52. **Skwarski M *et al.*** (2021) Mitochondrial inhibitor atovaquone increases tumor oxygenation and inhibits hypoxic gene expression in patients with non-small cell lung cancer. *Clinical Cancer Research* **27**, 2459–2469.
53. **Benej M *et al.*** (2018) Papaverine and its derivatives radiosensitize solid tumors by inhibiting mitochondrial metabolism. *Proceedings of the National Academy of Sciences of the USA* **115**, 10756–10761.
54. **Benej M *et al.*** (2021) Pharmacological regulation of tumor hypoxia in model murine tumors and spontaneous canine tumors. *Cancers* **13**, 1696.
55. **Coates JT, Skwarski M and Higgins GS** (2019) Targeting tumour hypoxia: shifting focus from oxygen supply to demand. *The British Journal of Radiology* **92**, 20170843.
56. **Overgaard J *et al.*** (1989) Misonidazole combined with radiotherapy in the treatment of carcinoma of the uterine cervix. *International Journal of Radiation Oncology, Biology, Physics* **16**, 1069–1072.
57. **Panduro J *et al.*** (1983) Misonidazole combined with radiotherapy in the treatment of inoperable squamous cell carcinoma of the lung a double-blind randomized trial. *Cancer* **52**, 20–24.
58. **Thomson D *et al.*** (2014) NIMRAD – a phase III trial to investigate the use of nimorazole hypoxia modification with intensity-modulated radiotherapy in head and neck cancer. *Clinical Oncology* **26**, 344–347.
59. **Guidelines CFCP and Cancer** (2020) Treatment with the hypoxic radiosensitizer Nimorazole in squamous cell carcinoma of the head and neck. *January 14th 2021 (Center for Clinical Practice Guidelines|Cancer)*.
60. **Mistry IN *et al.*** (2017) Clinical advances of hypoxia-activated prodrugs in combination with radiation therapy. *International Journal of Radiation Oncology, Biology, Physics* **98**, 1183–1196.
61. **Zeng Y *et al.*** (2018) Hypoxia-activated prodrugs and redox-responsive nanocarriers. *International Journal of Nanomedicine* **13**, 6551–6574.
62. **Cazares-Korner C *et al.*** (2013) CH-01 is a hypoxia-activated prodrug that sensitizes cells to hypoxia/reoxygenation through inhibition of Chk1 and Aurora A. *ACS Chemical Biology* **8**, 1451–1459.
63. **Skwarska A *et al.*** (2021) Development and pre-clinical testing of a novel hypoxia-activated KDAC inhibitor. *Cell Chemical Biology* **28**, 1258–1270. e1213.

https://doi.org/10.1017/erm.2022.14 Published online by Cambridge University Press

64. **Patterson AV *et al.*** (2007) Mechanism of action and preclinical antitumor activity of the novel hypoxia-activated DNA cross-linking agent PR-104. *Clinical Cancer Research* **13**, 3922–3932.
65. **Kibuuka P** (2013) Radiotherapy + metronidazole vs radiotherapy alone in improving treatment outcomes in advanced cervical cancer in Uganda. Available at https://clinicaltrials.gov/ct2/show/NCT01937650.
66. **Riaz N *et al.*** (2017) A personalized approach using hypoxia resolution to guide curative-intent radiation dose-reduction to 30 Gy: a novel de-escalation paradigm for HPV-associated oropharynx cancers (OPC). *Journal of Clinical Oncology* **35**, 6076.
67. **Poon I** (2017) Correlation of FAZA PET hypoxia imaging to 3D histology in oral tongue cancer (FAITH). Available at https://clinicaltrials.gov/ct2/show/NCT03181035.
68. **Maughan T** (2017) RHYTHM-I: investigating hypoxia in rectal tumours (RHYTHM-I). Available at https://clinicaltrials.gov/ct2/show/NCT02157246.
69. **Lin L** (2013) F18 EF5 PET/CT imaging in patients with brain metastases from breast cancer. Available at https://clinicaltrials.gov/ct2/show/NCT01985971.
70. **Thomson D** (2013) NIMRAD (a randomised placebo-controlled trial of synchronous NIMorazole vsus RADiotherapy alone in patients with locally advanced head and neck squamous cell carcinoma not suitable for synchronous chemotherapy or cetuximab) (NIMRAD). Available at https://clinicaltrials.gov/ct2/show/NCT01950689.
71. **Overgaard J** (2021) DAHANCA 30: a randomized non-inferiority trial of hypoxia-profile guided hypoxic modification of radiotherapy of HNSCC. Available at https://clinicaltrials.gov/ct2/show/NCT02661152.
72. **Jens Overgaard VG** (2020) AF CRT +/– nimorazole in HNSCC. Available at https://clinicaltrials.gov/ct2/show/NCT01880359.
73. **Overgaard J** (2017) DAHANCA 28A: phase I/II study on accelerated, hyperfractionated radiotherapy with concomitant cisplatin and nimorazole. Available at https://clinicaltrials.gov/ct2/show/NCT01733823.
74. **Rubek N and Buchwald CV** (2019) Quality of life after primary TORS vs IMRT for patients with early-stage oropharyngeal squamous cell carcinoma (QoLATI). Available at https://clinicaltrials.gov/ct2/show/NCT04124198.
75. **Le QT *et al.*** (2006) Mature results from a randomized phase II trial of cisplatin plus 5-fluorouracil and radiotherapy with or without tirapazamine in patients with resectable stage IV head and neck squamous cell carcinomas. *Cancer* **106**, 1940–1949.
76. **Le Q-T** (2014) Tirapazamine combined with chemo and RT in limited-stage small cell lung cancer, https://clinicaltrials.gov/ct2/show/NCT00066742.
77. **Lau DH** (2009) Chemotherapy, tirapazamine, and radiation therapy in treating patients with non-small cell lung cancer, https://clinicaltrials.gov/ct2/show/NCT00033410.
78. **Ringash J *et al.*** (2017) Effect of p16 status on the quality-of-life experience during chemoradiation for locally advanced oropharyngeal cancer: a substudy of randomized trial Trans-Tasman Radiation Oncology Group (TROG) 02.02 (HeadSTART). *International Journal of Radiation Oncology Biology Physics* **97**, 678–686.
79. **DiSilvestro PA *et al.*** (2014) Phase III randomized trial of weekly cisplatin and irradiation versus cisplatin and tirapazamine and irradiation in stages IB2, IIA, IIB, IIIB, and IVA cervical carcinoma limited to the pelvis: a Gynecologic Oncology Group study. *Journal of Clinical Oncology* **32**, 458–464.
80. **Rischin D** (2013) Tirapazamine, cisplatin, and radiation therapy in treating patients with stage IB, stage II, stage III, or stage IVA cervical cancer. Available at https://clinicaltrials.gov/ct2/show/NCT00098995.
81. **Sanofi** (2016) TRACE: tirapazamine-radiation and cisplatin evaluation. Available at https://clinicaltrials.gov/ct2/show/NCT00174837.
82. **Le QT *et al.*** (2004) Phase I study of tirapazamine plus cisplatin/etoposide and concurrent thoracic radiotherapy in limited-stage small cell lung cancer (S0004): a Southwest Oncology Group study. *Clinical Cancer Research* **10**, 5418–5424.
83. **Novacea** (2007) AQ4N in combination with radiotherapy and temozolomide in subjects with newly diagnosed glioblastoma multiforme. Available at https://clinicaltrials.gov/ct2/show/NCT00394628.
84. **Hong RL *et al.*** (2018) Final results of a randomized phase III trial of induction chemotherapy followed by concurrent chemoradiotherapy versus concurrent chemoradiotherapy alone in patients with stage IVA and IVB nasopharyngeal carcinoma-Taiwan Cooperative Oncology Group (TCOG) 1303 Study. *Annals of Oncology* **29**, 1972–1979.
85. **Japan Clinical Oncology Group** (2016) Trial of pre-operative chemoradiotherapy followed by surgical resection in pancoast tumors (JCOG 9806). Available at https://clinicaltrials.gov/ct2/show/NCT00128037.
86. **Harland SJ** (2013) Radiation therapy, chemotherapy, or observation in treating patients with bladder cancer. Available at https://clinicaltrials.gov/ct2/show/NCT00002490.
87. **James ND *et al.*** (2012) Radiotherapy with or without chemotherapy in muscle-invasive bladder cancer. *New England Journal of Medicine* **366**, 1477–1488.
88. **Choudhury A *et al.*** (2021) Hypofractionated radiotherapy in locally advanced bladder cancer: an individual patient data meta-analysis of the BC2001 and BCON trials. *The Lancet Oncology* **22**, 246–255.
89. **Shipley WU** (2009) Radiation therapy and chemotherapy in treating patients with stage I bladder cancer. Available at https://clinicaltrials.gov/ct2/show/NCT00981656.
90. **Fischer JJ** (2013) Radiation therapy and chemotherapy in treating patients with head and neck cancer. Available at https://clinicaltrials.gov/ct2/show/NCT00002507.
91. **James RD** (2013) Radiation therapy plus fluorouracil with or without additional chemotherapy in treating patients with primary anal cancer. Available at https://clinicaltrials.gov/ct2/show/NCT00025090.
92. **Gunderson LL *et al.*** (2012) Long-term update of US GI intergroup RTOG 98-11 phase III trial for anal carcinoma: survival, relapse, and colostomy failure with concurrent chemoradiation involving fluorouracil/mitomycin versus fluorouracil/cisplatin. *Journal of Clinical Oncology* **30**, 4344–4351.
93. **Ajani JA *et al.*** (2008) Fluorouracil, mitomycin, and radiotherapy vs fluorouracil, cisplatin, and radiotherapy for carcinoma of the anal canal: a randomized controlled trial. *Jama* **299**, 1914–1921.
94. **Leon O *et al.*** (2015) Phase I study of cetuximab in combination with 5-fluorouracil, mitomycin C and radiotherapy in patients with locally advanced anal cancer. *European Journal of Cancer* **51**, 2740–2746.
95. **Hoff PM** (2014) A study of substitution of 5-FU (fluorouracil) by capecitabine in scheme of chemo-radiotherapy in patients with squamous cell carcinoma of the anal canal. Available at https://clinicaltrials.gov/ct2/show/NCT01941966.
96. **Florescu C *et al.*** (2021) Interim analysis of a phase II study of simultaneously integrated boost intensity modulated radiation therapy (SIB-IMRT) in combination with 5-FU and mitomycin-C among patients with locally advanced anal canal cancer. *Journal of Clinical Oncology* **39**, e15501.
97. **Kachnic LA** (2013) Intensity-modulated radiation therapy, fluorouracil, and mitomycin C in treating patients with invasive anal cancer. Available at https://clinicaltrials.gov/ct2/show/NCT00423293.
98. **Glassman PM** (2010) Radiation therapy plus porfiromycin in treating patients with stage III or stage IV head and neck cancer. Available at https://clinicaltrials.gov/ct2/show/NCT00003328.
99. **Meng F *et al.*** (2012) Molecular and cellular pharmacology of the hypoxia-activated prodrug TH-302. *Molecular Cancer Therapeutics* **11**, 740–751.
100. **Peeters SG *et al.*** (2015) TH-302 in combination with radiotherapy enhances the therapeutic outcome and is associated with pretreatment [18F]HX4 hypoxia PET imaging. *Clinical Cancer Research* **21**, 2984–2992.
101. **Yoon C *et al.*** (2015) Hypoxia-activated chemotherapeutic TH-302 enhances the effects of VEGF-A inhibition and radiation on sarcomas. *British Journal of Cancer* **113**, 46–56.
102. **Takakusagi Y *et al.*** (2018) Radiotherapy synergizes with the hypoxia-activated prodrug evofosfamide: *in vitro* and *in vivo* studies. *Antioxidants & Redox Signaling* **28**, 131–140.
103. **Cutsem EV *et al.*** (2016) Evofosfamide (TH-302) in combination with gemcitabine in previously untreated patients with metastatic or locally advanced unresectable pancreatic ductal adenocarcinoma: primary analysis of the randomized, double-blind phase III MAESTRO study. *Journal of Clinical Oncology* **34**, 193.
104. **Larue RT *et al.*** (2016) A phase 1 'window-of-opportunity' trial testing evofosfamide (TH-302), a tumour-selective hypoxia-activated cytotoxic prodrug, with preoperative chemoradiotherapy in oesophageal adenocarcinoma patients. *BMC Cancer* **16**, 644.
105. **Zeman EM *et al.*** (1986) SR-4233: a new bioreductive agent with high selective toxicity for hypoxic mammalian cells. *International Journal of Radiation Oncology Biology Physics* **12**, 1239–1242.

https://doi.org/10.1017/erm.2022.14 Published online by Cambridge University Press

106. **Cowen RL *et al.*** (2004) Hypoxia targeted gene therapy to increase the efficacy of tirapazamine as an adjuvant to radiotherapy: reversing tumor radioresistance and effecting cure. *Cancer Research* **64**, 1396–1402.
107. **Siim BG *et al.*** (1997) Tirapazamine-induced cytotoxicity and DNA damage in transplanted tumors: relationship to tumor hypoxia. *Cancer Research* **57**, 2922–2928.
108. **Rischin D *et al.*** (2005) 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). *Journal of Clinical Oncology* **23**, 79–87.
109. **Rischin D *et al.*** (2010) Tirapazamine, cisplatin, and radiation versus cisplatin and radiation for advanced squamous cell carcinoma of the head and neck (TROG 02.02, HeadSTART): a phase III trial of the Trans-Tasman Radiation Oncology Group. *Journal of Clinical Oncology* **28**, 2989–2995.
110. **McCarthy HO *et al.*** (2003) Bioreductive GDEPT using cytochrome P450 3A4 in combination with AQ4N. *Cancer Gene Therapy* **10**, 40–48.
111. **Mehibel M *et al.*** (2016) Radiation enhances the therapeutic effect of Banoxantrone in hypoxic tumour cells with elevated levels of nitric oxide synthase. *Oncology Reports* **35**, 1925–1932.
112. **Clughsey T *et al.*** (2007) AQ4N in combination with radiotherapy and temozolomide in subjects with newly diagnosed glioblastoma multiforme. Available at https://clinicaltrials.gov/ct2/show/NCT00394628.
113. **Chen FY-S *et al.*** (2019) Mitomycin C modulates tumor microenvironment and enhances radiosensitivity in rectal cancer. *Therapeutic Radiology and Oncology* **3**, 29.
114. **Flam M *et al.*** (1996) Role of mitomycin in combination with fluorouracil and radiotherapy, and of salvage chemoradiation in the definitive nonsurgical treatment of epidermoid carcinoma of the anal canal: results of a phase III randomized intergroup study. *Journal of Clinical Oncology* **14**, 2527–2539.
115. **Huang R and Zhou P-K** (2020) HIF-1 signaling: a key orchestrator of cancer radioresistance. *Radiation Medicine and Protection* **1**, 7–14.
116. **Schönberger T, Fandrey J and Prost-Fingerle K** (2021) Ways into understanding HIF inhibition. *Cancers* **13**, 159.
117. **Okuno T *et al.*** (2018) SN-38 acts as a radiosensitizer for colorectal cancer by inhibiting the radiation-induced up-regulation of HIF-1α. *Anticancer Research* **38**, 3323–3331.
118. **Zhang Y *et al.*** (2017) Targetable T-type calcium channels drive glioblastoma. *Cancer Research* **77**, 3479–3490.
119. **Lester-Coll NH *et al.*** (2018) Mibefradil dihydrochoride with hypofractionated radiation for recurrent glioblastoma: a phase I dose expansion trial. *Journal of Clinical Oncology* **36**, e14046.
120. **Wang X *et al.*** (2020) STAT3 Contributes to radioresistance in cancer. *Frontiers in Oncology* **10**, 1120.
121. **Zhang C *et al.*** (2014) STAT3 inhibitor NSC74859 radiosensitizes esophageal cancer via the downregulation of HIF-1α. *Tumour Biology* **35**, 9793–9799.
122. **Zhang Q *et al.*** (2015) STAT3 inhibitor stattic enhances radiosensitivity in esophageal squamous cell carcinoma. *Tumour Biology* **36**, 2135–2142.
123. **Moeller BJ *et al.*** (2004) Radiation activates HIF-1 to regulate vascular radiosensitivity in tumors: role of reoxygenation, free radicals, and stress granules. *Cancer Cell* **5**, 429–441.
124. **Oike T *et al.*** (2012) Suppression of HIF-1α expression and radiation resistance in acute hypoxic conditions. *Experimental and Therapeutic Medicine* **3**, 141–145.
125. **Lee DE *et al.*** (2018) A radiosensitizing inhibitor of HIF-1 alters the optical redox state of human lung cancer cells in vitro. *Scientific Reports* **8**, 8815.
126. **Palayoor ST *et al.*** (2008) PX-478, an inhibitor of hypoxia-inducible factor-1alpha, enhances radiosensitivity of prostate carcinoma cells. *International Journal of Cancer* **123**, 2430–2437.
127. **Courtney KD *et al.*** (2020) HIF-2 complex dissociation, target inhibition, and acquired resistance with PT2385, a first-in-class HIF-2 inhibitor, in patients with clear cell renal cell carcinoma. *Clinical Cancer Research* **26**, 793–803.
128. **Choueiri TK *et al.*** (2021) Inhibition of hypoxia-inducible factor-2α in renal cell carcinoma with belzutifan: a phase 1 trial and biomarker analysis. *Nature Medicine* **27**, 802–805.
129. **Chen W *et al.*** (2016) Targeting renal cell carcinoma with a HIF-2 antagonist. *Nature* **539**, 112–117.
130. **Kaplan AR and Glazer PM** (2020) Impact of hypoxia on DNA repair and genome integrity. *Mutagenesis* **35**, 61–68.
131. **Brown JS *et al.*** (2017) Targeting DNA repair in cancer: beyond PARP inhibitors. *Cancer Discovery* **7**, 20–37.
132. **O'Neil NJ, Bailey ML and Hieter P** (2017) Synthetic lethality and cancer. *Nature Reviews Genetics* **18**, 613–623.
133. **Mateo J *et al.*** (2019) A decade of clinical development of PARP inhibitors in perspective. *Annals of Oncology* **30**, 1437–1447.
134. **Lord CJ and Ashworth A** (2017) PARP inhibitors: synthetic lethality in the clinic. *Science* **355**, 1152–1158.
135. **Chan N *et al.*** (2010) Contextual synthetic lethality of cancer cell kill based on the tumor microenvironment. *Cancer Research* **70**, 8045–8054.
136. **Begg K and Tavassoli M** (2020) Inside the hypoxic tumour: reprogramming of the DDR and radioresistance. *Cell Death Discovery* **6**, 77.
137. **Michmerhuizen AR *et al.*** (2019) PARP1 Inhibition radiosensitizes models of inflammatory breast cancer to ionizing radiation. *Molecular Cancer Therapeutics* **18**, 2063–2073.
138. **Kirova YM *et al.*** (2020) Radioparp: a phase I of olaparib with radiation therapy (RT) in patients with inflammatory, locoregionally advanced or metastatic triple-negative breast cancer (TNBC) or patient with operated TNBC with residual disease – preliminary results. *Journal of Clinical Oncology* **38**, 571.
139. **Lesueur P *et al.*** (2019) Phase I/IIa study of concomitant radiotherapy with olaparib and temozolomide in unresectable or partially resectable glioblastoma: OLA-TMZ-RTE-01 trial protocol. *BMC Cancer* **19**, 198.
140. **de Haan R *et al.*** (2019) Study protocols of three parallel phase 1 trials combining radical radiotherapy with the PARP inhibitor olaparib. *BMC Cancer* **19**, 901.
141. **Merck Sharp & Dohme Corp** (2020) Study of pembrolizumab with concurrent chemoradiation therapy followed by pembrolizumab with or without olaparib in stage III non-small cell lung cancer (NSCLC) (MK-7339-012/KEYLYNK-012). Available at https://clinicaltrials.gov/ct2/show/NCT04380636.
142. **Forster MD *et al.*** (2016) ORCA-2: a phase I study of olaparib in addition to cisplatin-based concurrent chemoradiotherapy for patients with high risk locally advanced squamous cell carcinoma of the head and neck. *Journal of Clinical Oncology* **34**, TPS6108.
143. **McKay RR** (2017) Testing the safety of different doses of olaparib given radium-223 for men with advanced prostate cancer with bone metastasis. Available at https://clinicaltrials.gov/ct2/show/NCT03317392.
144. **Negrao MV** (2021) Olaparib and durvalumab with carboplatin, etoposide, and/or radiation therapy for the treatment of extensive-stage small cell lung cancer, PRIO Trial. Available at https://clinicaltrials.gov/ct2/show/NCT04728230.
145. **Reiss KA *et al.*** (2017) A final report of a phase I study of veliparib (ABT-888) in combination with low-dose fractionated whole abdominal radiation therapy (LDFWAR) in patients with advanced solid malignancies and peritoneal carcinomatosis with a dose escalation in ovarian and fallopian tube cancers. *Gynecologic Oncology* **144**, 486–490.
146. **Chabot P *et al.*** (2017) Veliparib in combination with whole-brain radiation therapy for patients with brain metastases from non-small cell lung cancer: results of a randomized, global, placebo-controlled study. *Journal of Neuro-Oncology* **131**, 105–115.
147. **Kozono DE *et al.*** (2019) Veliparib (Vel) in combination with chemoradiotherapy (CRT) of carboplatin/paclitaxel (C/P) plus radiation in patients (pts) with stage III non-small cell lung cancer (NSCLC) (M14-360/AFT-07). *Journal of Clinical Oncology* **37**, 8510.
148. **Czito BG *et al.*** (2017) Safety and tolerability of veliparib combined with capecitabine plus radiotherapy in patients with locally advanced rectal cancer: a phase 1b study. *The Lancet Gastroenterology and Hepatology* **2**, 418–426.
149. **Jelinek MJ *et al.*** (2018) A phase I/II trial adding poly(ADP-ribose) polymerase (PARP) inhibitor veliparib to induction carboplatin-paclitaxel (Carbo-Tax) in patients with head and neck squamous cell carcinoma (HNSCC) Alliance A091101. *Journal of Clinical Oncology* **36**, 6031.
150. **Mehta MP *et al.*** (2015) Veliparib in combination with whole brain radiation therapy in patients with brain metastases: results of a phase 1 study. *Journal of Neuro-Oncology* **122**, 409–417.
151. **Tuli R *et al.*** (2019) A phase 1 study of veliparib, a PARP-1/2 inhibitor, with gemcitabine and radiotherapy in locally advanced pancreatic cancer. *EBioMedicine* **40**, 375–381.
152. **Jagsi R *et al.*** (2018) Concurrent veliparib with chest wall and nodal radiotherapy in patients with inflammatory or locoregionally recurrent

https://doi.org/10.1017/erm.2022.14 Published online by Cambridge University Press

breast cancer: the TBCRC 024 phase I multicenter study. *Journal of Clinical Oncology* **36**, 1317–1322.
153. **Baxter PA *et al.*** (2020) A phase I/II study of veliparib (ABT-888) with radiation and temozolomide in newly diagnosed diffuse pontine glioma: a Pediatric Brain Tumor Consortium study. *Neuro-Oncology* **22**, 875–885.
154. **Argiris A *et al.*** (2019) S1206: a dose-finding study followed by a phase II randomized placebo-controlled trial of chemoradiotherapy (CRT) with or without veliparib in stage III non-small cell lung cancer (NSCLC). *Journal of Clinical Oncology* **37**, 8523.
155. **Spratt D and Jackson W** (2019) A multi-center trial of androgen suppression with abiraterone acetate, leuprolide, PARP inhibition and stereotactic body radiotherapy in prostate cancer (ASCLEPIuS). Available at https://clinicaltrials.gov/ct2/show/NCT04194554.
156. **Ludwig MS** (2018) Study of niraparib with radiotherapy for treatment of metastatic invasive carcinoma of the cervix (NIVIX). Available at https://clinicaltrials.gov/ct2/show/NCT03644342.
157. **Ho A** (2021) Radiation, immunotherapy and PARP inhibitor in triple negative breast cancer (NADiR). Available at https://clinicaltrials.gov/ct2/show/NCT04837209.
158. **Triest BV *et al.*** (2018) A phase Ia/Ib trial of the DNA-PK inhibitor M3814 in combination with radiotherapy (RT) in patients (pts) with advanced solid tumors: dose-escalation results. *Journal of Clinical Oncology* **36**, 2518.
159. **Romesser P *et al.*** (2020) A multicenter phase Ib/II study of DNA-PK inhibitor peposertib (M3814) in combination with capecitabine and radiotherapy in patients with locally advanced rectal cancer. *Journal of Clinical Oncology* **38**, TPS4117.
160. **Bendell JC *et al.*** (2019) Phase 1, open-label, dose-escalation study of M3814 + avelumab ± radiotherapy (RT) in patients (pts) with advanced solid tumors. *Journal of Clinical Oncology* **37**, TPS3169.
161. **Majd N** (2020) Nedisertib and radiation therapy, followed by temozolomide for the treatment of patients with newly diagnosed MGMT unmethylated glioblastoma or gliosarcoma. Available at https://clinicaltrials.gov/ct2/show/NCT04555577.
162. **Gillison ML** (2020) Testing the addition of M3814 (peposertib) to radiation therapy for patients with advanced head and neck cancer who cannot take cisplatin. Available at https://clinicaltrials.gov/ct2/show/NCT04533750.
163. **Reddy VP *et al.*** (2019) Abstract 4868: a preclinical PK/PD model based on a mouse glioblastoma survival model for AZD1390, a novel, brain-penetrant ATM kinase inhibitor, to predict the inhibition of DNA damage response induced by radiation and the human efficacious dose. *Cancer Research* **79**, 4868.
164. **Dillon MT *et al.*** (2018) PATRIOT: a phase I study to assess the tolerability, safety and biological effects of a specific ataxia telangiectasia and Rad3-related (ATR) inhibitor (AZD6738) as a single agent and in combination with palliative radiation therapy in patients with solid tumours. *Clinical and Translational Radiation Oncology* **12**, 16–20.
165. **Owonikoko TK** (2015) Testing the addition of M6620 (VX-970, berzosertib) to usual chemotherapy and radiation for head and neck cancer. Available at https://clinicaltrials.gov/ct2/show/NCT02567422.
166. **Mohindra P** (2015) Testing the safety of M6620 (VX-970) when given with standard whole brain radiation therapy for the treatment of brain metastases from non-small cell lung cancer, small cell lung cancer, or neuroendocrine tumors. Available at https://clinicaltrials.gov/ct2/show/NCT02589522.
167. **van Werkhoven E *et al.*** (2020) Practicalities in running early-phase trials using the time-to-event continual reassessment method (TiTE-CRM) for interventions with long toxicity periods using two radiotherapy oncology trials as examples. *BMC Medical Research Methodology* **20**, 162.
168. **Mowery YM** (2020) Testing the addition of an anti-cancer drug, BAY1895344, with radiation therapy to the usual pembrolizumab treatment for recurrent head and neck cancer. Available at https://clinicaltrials.gov/ct2/show/NCT04576091.
169. **Kong A *et al.*** (2020) Phase I trial of WEE1 inhibition with chemotherapy and radiotherapy as adjuvant treatment, and a window of opportunity trial with cisplatin in patients with head and neck cancer: the WISTERIA trial protocol. *BMJ Open* **10**, e033009.
170. **Cuneo KC *et al.*** (2019) Dose escalation trial of the wee1 inhibitor adavosertib (AZD1775) in combination with gemcitabine and radiation for patients with locally advanced pancreatic cancer. *Journal of Clinical Oncology* **37**, 2643–2650.
171. **Lheureux S** (2017) Testing AZD1775 in combination with radiotherapy and chemotherapy in cervical, upper vaginal and uterine cancers. Available at https://clinicaltrials.gov/ct2/show/NCT03345784.
172. **Siddharth Sheth D** (2015) Dose-escalating AZD1775 + concurrent radiation + cisplatin for intermediate/high risk HNSCC. Available at https://clinicaltrials.gov/ct2/show/NCT02585973.
173. **Alexander BM *et al.*** (2017) Phase I study of AZD1775 with radiation therapy (RT) and temozolomide (TMZ) in patients with newly diagnosed glioblastoma (GBM) and evaluation of intratumoral drug distribution (IDD) in patients with recurrent GBM. *Journal of Clinical Oncology* **35**, 2005.
174. **Gogola E *et al.*** (2018) Selective loss of PARG restores PARylation and counteracts PARP inhibitor-mediated synthetic lethality. *Cancer Cell* **33**, 1078–1093. e1012.
175. **Petrucelli N, Daly MB and Pal T** (1998) BRCA1- and BRCA2-associated hereditary breast and ovarian cancer. *GeneReviews® [Internet]. Seattle (WA): University of Washington, Seattle; 1993–2021.*
176. **Lord CJ and Ashworth A** (2016) BRCAness revisited. *Nature Reviews Cancer* **16**, 110–120.
177. **Lesueur P *et al.*** (2017) Poly-(ADP-ribose)-polymerase inhibitors as radiosensitizers: a systematic review of pre-clinical and clinical human studies. *Oncotarget* **8**, 69105–69124.
178. **Jiang Y *et al.*** (2016) Hypoxia potentiates the radiation-sensitizing effect of olaparib in human non-small cell lung cancer xenografts by contextual synthetic lethality. *International Journal of Radiation Oncology Biology Physics* **95**, 772–781.
179. **Ramakrishnan Geethakumari P *et al.*** (2017) PARP inhibitors in prostate cancer. *Current Treatment Options in Oncology* **18**, 37.
180. **Mahaney BL, Meek K and Lees-Miller SP** (2009) Repair of ionizing radiation-induced DNA double-strand breaks by non-homologous end-joining. *Biochemical Journal* **417**, 639–650.
181. **Goodwin JF and Knudsen KE** (2014) Beyond DNA repair: DNA-PK function in cancer. *Cancer Discovery* **4**, 1126–1139.
182. **Hsu FM, Zhang S and Chen BP** (2012) Role of DNA-dependent protein kinase catalytic subunit in cancer development and treatment. *Translational Cancer Research* **1**, 22–34.
183. **Geng W *et al.*** (2019) DNA-PKcs inhibitor increases the sensitivity of gastric cancer cells to radiotherapy. *Oncology Reports* **42**, 561–570.
184. **Jiang Y *et al.*** (2021) DNAPK inhibition preferentially compromises the repair of radiation-induced DNA double-strand breaks in chronically hypoxic tumor cells in xenograft models. *Molecular Cancer Therapeutics* **20**, 1663–1671.
185. **Klein C *et al.*** (2017) Overcoming hypoxia-induced tumor radioresistance in non-small cell lung cancer by targeting DNA-dependent protein kinase in combination with carbon ion irradiation. *Radiation Oncology* **12**, 208.
186. **Meschini R *et al.*** (2015) Role of chromatin structure modulation by the histone deacetylase inhibitor trichostatin A on the radio-sensitivity of ataxia telangiectasia. *Mutation Research* **777**, 52–59.
187. **Szurman-Zubrzycka M *et al.*** (2019) ATR, a DNA damage signaling kinase, is involved in aluminum response in barley. *Frontiers in Plant Science* **10**, 1299.
188. **Pires IM *et al.*** (2012) Targeting radiation-resistant hypoxic tumour cells through ATR inhibition. *British Journal of Cancer* **107**, 291–299.
189. **Olcina MM, Grand RJ and Hammond EM** (2014) ATM activation in hypoxia – causes and consequences. *Molecular & Cellular Oncology* **1**, e29903.
190. **Bencokova Z *et al.*** (2009) ATM activation and signaling under hypoxic conditions. *Molecular and Cellular Biology* **29**, 526–537.
191. **Prevo R *et al.*** (2012) The novel ATR inhibitor VE-821 increases sensitivity of pancreatic cancer cells to radiation and chemotherapy. *Cancer Biology & Therapy* **13**, 1072–1081.
192. **Leszczynska KB *et al.*** (2016) Preclinical testing of an Atr inhibitor demonstrates improved response to standard therapies for esophageal cancer. *Radiotherapy & Oncology* **121**, 232–238.
193. **Zeng L *et al.*** (2020) CHK1/2 inhibitor prexasertib suppresses NOTCH signaling and enhances cytotoxicity of cisplatin and radiation in head and neck squamous cell carcinoma. *Molecular Cancer Therapeutics* **19**, 1279–1288.

https://doi.org/10.1017/erm.2022.14 Published online by Cambridge University Press

194. **Zeng L *et al.*** (2017) Combining Chk1/2 inhibition with cetuximab and radiation enhances in vitro and in vivo cytotoxicity in head and neck squamous cell carcinoma. *Molecular Cancer Therapeutics* **16**, 591–600.
195. **Li Z *et al.*** (2020) Development and characterization of a wee1 kinase degrader. *Cell Chemical Biology* **27**, 57–65. e59.
196. **Lee Y-Y *et al.*** (2019) Anti-tumor effects of wee1 kinase inhibitor with radiotherapy in human cervical cancer. *Scientific Reports* **9**, 15394.
197. **Cuneo KC *et al.*** (2016) Wee1 kinase inhibitor AZD1775 radiosensitizes hepatocellular carcinoma regardless of TP53 mutational status through induction of replication stress. *International Journal of Radiation Oncology, Biology, Physics* **95**, 782–790.
198. **O'Brien EM *et al.*** (2013) Impact of Wee1 inhibition on the hypoxia-induced DNA damage response.
199. **McCann E, O'Sullivan J and Marcone S** (2021) Targeting cancer-cell mitochondria and metabolism to improve radiotherapy response. *Translational Oncology* **14**, 100905.
200. **Zhang Z *et al.*** (2018) PI3K/Akt and HIF-1 signaling pathway in hypoxia-ischemia (Review). *Molecular Medicine Reports* **18**, 3547–3554.
201. **Lee CM *et al.*** (2006) Phosphatidylinositol 3-kinase inhibition by LY294002 radiosensitizes human cervical cancer cell lines. *Clinical Cancer Research* **12**, 250–256.
202. **Chang L *et al.*** (2014) PI3K/Akt/mTOR pathway inhibitors enhance radiosensitivity in radioresistant prostate cancer cells through inducing apoptosis, reducing autophagy, suppressing NHEJ and HR repair pathways. *Cell Death & Disease* **5**, e1437.
203. **Fokas E *et al.*** (2012) Dual inhibition of the PI3K/mTOR pathway increases tumor radiosensitivity by normalizing tumor vasculature. *Cancer Research* **72**, 239–248.
204. **Kelly CJ *et al.*** (2014) Regulation of O2 consumption by the PI3K and mTOR pathways contributes to tumor hypoxia. *Radiotherapy & Oncology* **111**, 72–80.
205. **Sastri SJ and Goda J** (2020) Radiosensitizing effect of nelfinavir in locally advanced carcinoma of cervix (NELCER). Available at https://clinicaltrials.gov/ct2/show/NCT03256916.
206. **Rengan R *et al.*** (2012) 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. *Journal of Thoracic Oncology* **7**, 709–715.
207. **Yang TJ** (2019) GDC-0084 with radiation therapy for people with PIK3CA-mutated solid tumor brain metastases or leptomeningeal metastases. Available at https://clinicaltrials.gov/ct2/show/NCT04192981.
208. **Tinkle C and Gajjar A** (2018) Study of GDC-0084 in pediatric patients with newly diagnosed diffuse intrinsic pontine glioma or diffuse midline gliomas. Available at https://clinicaltrials.gov/ct2/show/NCT03696355.
209. **Mueller S** (2021) Combination therapy for the treatment of diffuse midline gliomas. Available at https://clinicaltrials.gov/ct2/show/NCT05009992.
210. **Glorieux M, Dok R and Nuyts S** (2020) The influence of PI3K inhibition on the radiotherapy response of head and neck cancer cells. *Scientific Reports* **10**, 16208.
211. **Brunner TB *et al.*** (2008) Phase I trial of the human immunodeficiency virus protease inhibitor nelfinavir and chemoradiation for locally advanced pancreatic cancer. *Journal of Clinical Oncology* **26**, 2699–2706.
212. **Buijsen J *et al.*** (2013) Phase I trial of the combination of the Akt inhibitor nelfinavir and chemoradiation for locally advanced rectal cancer. *Radiotherapy and Oncology* **107**, 184–188.
213. **Garcia-Soto AE *et al.*** (2015) Phase I trial of nelfinavir added to cisplatin chemotherapy with concurrent pelvic radiation for locally advanced cervical cancer. *Journal of Clinical Oncology* **33**, TPS5619.
214. **Baumert B** (2015) Study with nelfinavir and combined radiochemotherapy for glioblastoma. Available at https://clinicaltrials.gov/ct2/show/NCT00694837.
215. **Tran P** (2021) Stereotactic body radiation with nelfinavir for oligometastases. Available at https://clinicaltrials.gov/ct2/show/NCT01728779.
216. **Lin C *et al.*** (2019) Phase I trial of concurrent stereotactic body radiotherapy and nelfinavir for locally advanced borderline or unresectable pancreatic adenocarcinoma. *Radiotherapy & Oncology* **132**, 55–62.
217. **Hansen A** (2015) Phase I study of BYL719 in combination with cisplatin and radiotherapy in patients with squamous cell head and neck cancer. Available at https://clinicaltrials.gov/ct2/show/NCT02537223.
218. **Operations CS** (2013) A study of XL765 (SAR245409) in combination with temozolomide with and without radiation in adults with malignant gliomas. Available at https://clinicaltrials.gov/ct2/show/NCT00704080.
219. **Graillon T and Arnaud J-O** (2018) Combination of alpelisib and trametinib in progressive refractory meningiomas (ALTREM). Available at https://clinicaltrials.gov/ct2/show/NCT03631953.
220. **Pharmaceuticals N** (2010) A study of mTOR inhibitor everolimus (RAD001) in association with cisplatin and radiotherapy for locally advanced cervix cancer (PHOENIX I). Available at https://clinicaltrials.gov/ct2/show/NCT01217177.
221. **Narayan V *et al.*** (2016) Phase I trial of everolimus plus radiation therapy for salvage treatment of biochemical recurrence in prostate cancer patients following prostatectomy. *Journal of Clinical Oncology* **34**, e16617.
222. **Buijsen J** (2019) Safety study of rapamycin administered before and during radiotherapy to treat rectum cancer. Available at https://clinicaltrials.gov/ct2/show/NCT00409994.
223. **Williams KJ *et al.*** (2005) Enhanced response to radiotherapy in tumours deficient in the function of hypoxia-inducible factor-1. *Radiotherapy & Oncology* **75**, 89–98.
224. **Hudes G *et al.*** (2007) Temsirolimus, interferon alfa, or both for advanced renal-cell carcinoma. *New England Journal of Medicine* **356**, 2271–2281.
225. **Kido T *et al.*** (2020) Glucose transporter 1 is important for the glycolytic metabolism of human endometrial stromal cells in hypoxic environment. *Heliyon* **6**, e03985.
226. **Boström PJ *et al.*** (2016) Hypoxia marker GLUT-1 (glucose transporter 1) is an independent prognostic factor for survival in bladder cancer patients treated with radical cystectomy. *Bladder Cancer* **2**, 101–109.
227. **Tang L *et al.*** (2018) Role of metabolism in cancer cell radioresistance and radiosensitization methods. *Journal of Experimental & Clinical Cancer Research* **37**, 87.
228. **Kunkel M *et al.*** (2007) Overexpression of GLUT-1 is associated with resistance to radiotherapy and adverse prognosis in squamous cell carcinoma of the oral cavity. *Oral Oncology* **43**, 796–803.
229. **Zhao F *et al.*** (2016) Inhibition of Glut1 by WZB117 sensitizes radioresistant breast cancer cells to irradiation. *Cancer Chemotherapy and Pharmacology* **77**, 963–972.
230. **Shen H *et al.*** (2015) Sensitization of glioblastoma cells to irradiation by modulating the glucose metabolism. *Molecular Cancer Therapeutics* **14**, 1794–1804.
231. **Storozynsky Q and Hitt MM** (2020) The impact of radiation-induced DNA damage on cGAS-STING-mediated immune responses to cancer. *International Journal of Molecular Sciences* **21**, 8877.
232. **Deng J *et al.*** (2019) Hypoxia-Induced VISTA promotes the suppressive function of myeloid-derived suppressor cells in the tumor microenvironment. *Cancer Immunology Research* **7**, 1079–1090.
233. **Boutilier AJ and Elsawa SF** (2021) Macrophage polarization states in the tumor microenvironment. *International Journal of Molecular Sciences* **22**, 6995.
234. **Winning S and Fandrey J** (2016) Dendritic cells under hypoxia: how oxygen shortage affects the linkage between innate and adaptive immunity. *Journal of Immunology Research* **2016**, 5134329.
235. **Kumar V and Gabrilovich DI** (2014) Hypoxia-inducible factors in regulation of immune responses in tumour microenvironment. *Immunology* **143**, 512–519.
236. **Vanpouille-Box C, Formenti SC and Demaria S** (2018) Toward precision radiotherapy for use with immune checkpoint blockers. *Clinical Cancer Research* **24**, 259–265.
237. **Pilones KA, Vanpouille-Box C and Demaria S** (2015) Combination of radiotherapy and immune checkpoint inhibitors. *Seminars in Radiation Oncology* **25**, 28–33.
238. **Jing Z *et al.*** (2018) Combination of radiation therapy and anti-PD-1 antibody SHR-1210 in treating patients with esophageal squamous cell cancer. *International Journal of Radiation Oncology, Biology, Physics* **102**, e31.
239. **Pang Q *et al.*** (2018) Safety and effect of radiation therapy combined with anti-PD-1 antibody SHR-1210 as first-line treatment on patients with intolerable concurrent chemoradiotherapy esophageal cancer: a phase 1B clinical trial. *International Journal of Radiation Oncology, Biology, Physics* **102**, e39.
240. **Shaverdian N *et al.*** (2017) Previous radiotherapy and the clinical activity and toxicity of pembrolizumab in the treatment of non-small-cell lung cancer: a secondary analysis of the KEYNOTE-001 phase 1 trial. *The Lancet. Oncology* **18**, 895–903.

https://doi.org/10.1017/erm.2022.14 Published online by Cambridge University Press

241. **Kim C *et al.*** (2020) Phase I study of the (177)Lu-DOTA(0)-Tyr (3)-Octreotate (lutathera) in combination with nivolumab in patients with neuroendocrine tumors of the lung. *Journal for Immunotherapy of Cancer* **8**, e000980.
242. **El-Khoueiry AB *et al.*** (2017) Nivolumab in patients with advanced hepatocellular carcinoma (CheckMate 040): an open-label, non-comparative, phase 1/2 dose escalation and expansion trial. *Lancet* **389**, 2492–2502.
243. **Bozorgmehr F *et al.*** (2019) Fostering efficacy of anti-PD-1-treatment: Nivolumab plus radiotherapy in advanced non-small cell lung cancer – study protocol of the FORCE trial. *BMC Cancer* **19**, 1074.
244. **Pryor D *et al.*** (2021) A phase I/II study of stereotactic radiotherapy and pembrolizumab for oligometastatic renal tumours (RAPPORT): clinical trial protocol. *Contemporary Clinical Trials Communications* **21**, 100703.
245. **Takiar V and Corp MSD** (2021) A study of chemoradiation plus pembrolizumab for locally advanced laryngeal squamous cell carcinoma. Available at https://clinicaltrials.gov/ct2/show/NCT02759575.
246. **Kolstad A** (2021) Sequential intranodal immunotherapy (SIIT) combined with anti-PD1 (pembrolizumab) in follicular lymphoma (Lymvac-2). Available at https://clinicaltrials.gov/ct2/show/NCT02677155.
247. **Maity A *et al.*** (2018) A phase I trial of pembrolizumab with hypofractionated radiotherapy in patients with metastatic solid tumours. *British Journal of Cancer* **119**, 1200–1207.
248. **Loi S and David S** (2017) Pilot study of stereotactic ablation for oligometastatic breast neoplasia in combination with the anti-PD-1 antibody MK-3475 (BOSTON II). Available at https://clinicaltrials.gov/ct2/show/NCT02303366.
249. **Park H *et al.*** (2019) Abstract CT212: combining pembrolizumab with locally delivered radiation therapy for the treatment of metastatic esophageal cancer. *Cancer Research* **79**, CT212.
250. **Bjarnason G** (2021) A proof of principle study of pembrolizumab with SBRT in TKI mRCC patients (OZM-065). Available at https://clinicaltrials.gov/ct2/show/NCT02599779.
251. **Monjazeb A** (2020) UCDCC#269: a pilot study of interlesional IL-2 and RT in patients with NSCLC. Available at https://clinicaltrials.gov/ct2/show/NCT03224871.
252. **Floudas CS *et al.*** (2019) A pilot study of the PD-1 targeting agent AMP-224 used with low-dose cyclophosphamide and stereotactic body radiation therapy in patients with metastatic colorectal cancer. *Clinical Colorectal Cancer* **18**, e349–e360.
253. **Daly M** (2021) Avelumab and stereotactic ablative radiotherapy in non-responding and progressing NSCLC patients. Available at https://clinicaltrials.gov/ct2/show/NCT03158883.
254. **Kurz S** (2021) Avelumab with hypofractionated radiation therapy in adults with isocitrate dehydrogenase (IDH) mutant glioblastoma. Available at https://clinicaltrials.gov/ct2/show/NCT02968940.
255. **Babiker H *et al.*** (2021) Phase I trial of cemiplimab, radiotherapy, cyclophosphamide, and granulocyte macrophage colony-stimulating factor in patients with recurrent or metastatic head and neck squamous cell carcinoma. *The Oncologist* **26**, e1508–e1513.
256. **Rischin D *et al.*** (2020) Phase 2 study of cemiplimab in patients with metastatic cutaneous squamous cell carcinoma: primary analysis of fixed-dosing, long-term outcome of weight-based dosing. *Journal for Immunotherapy of Cancer* **8**, e000775.
257. **Chachoua A** (2020) Study of combined ionizing radiation and ipilimumab in metastatic non-small cell lung cancer (NSCLC). Available at https://clinicaltrials.gov/ct2/show/NCT02221739.
258. **Cheson BD *et al.*** (1999) Report of an international workshop to standardize response criteria for non-Hodgkin's lymphomas. NCI Sponsored International Working Group. *Journal of Clinical Oncology* **17**, 1244.
259. **Hiniker SM *et al.*** (2016) A prospective clinical trial combining radiation therapy with systemic immunotherapy in metastatic melanoma. *International Journal of Radiation Oncology Biology Physics* **96**, 578–588.
260. **Ost P** (2017) Trial of SBRT with concurrent ipilimumab in metastatic melanoma. Available at https://clinicaltrials.gov/ct2/show/NCT02406183.
261. **López-Martín JA** (2021) GEM STUDY: radiation and Yervoy in patients with melanoma and brain metastases (GRAY-B). Available at https://clinicaltrials.gov/ct2/show/NCT02115139.
262. **Da Silva DM *et al.*** (2020) Immune activation in patients with locally advanced cervical cancer treated with ipilimumab following definitive chemoradiation (GOG-9929). *Clinical Cancer Research* **26**, 5621–5630.
263. **Reyes-Gibby CC *et al.*** (2007) Patterns of self-reported symptoms in pancreatic cancer patients receiving chemoradiation. *Journal of Pain and Symptom Management* **34**, 244–252.
264. **Pakkala S *et al.*** (2020) Durvalumab and tremelimumab with or without stereotactic body radiation therapy in relapsed small cell lung cancer: a randomized phase II study. *Journal for Immunotherapy of Cancer* **8**, e001302.
265. **Jones KI *et al.*** (2018) Radiation combined with macrophage depletion promotes adaptive immunity and potentiates checkpoint blockade. *EMBO Molecular Medicine* **10**, e9342.
266. **Eckert F *et al.*** (2019) Rationale for combining radiotherapy and immune checkpoint inhibition for patients with hypoxic tumors. *Frontiers in Immunology* **10**, 407.
267. **Cho B** (2018) Intensity-modulated radiation therapy: a review with a physics perspective. *Radiation Oncology Journal* **36**, 1–10.
268. **Marta GN *et al.*** (2014) Intensity-modulated radiation therapy for head and neck cancer: systematic review and meta-analysis. *Radiotherapy & Oncology* **110**, 9–15.
269. **Wang H *et al.*** (2018) Cancer radiosensitizers. *Trends in Pharmacological Sciences* **39**, 24–48.
270. **Kim MS *et al.*** (2016) Gold nanoparticles enhance anti-tumor effect of radiotherapy to hypoxic tumor. *Radiation Oncology Journal* **34**, 230–238.
271. **Huefner ND *et al.*** (2014) Genomic stability in response to high versus low linear energy transfer radiation in *Arabidopsis thaliana*. *Frontiers in Plant Science* **5**, 206.
272. **Ilicic K, Combs SE and Schmid TE** (2018) New insights in the relative radiobiological effectiveness of proton irradiation. *Radiation Oncology* **13**, 6.
273. **Costes SV *et al.*** (2007) Image-based modeling reveals dynamic redistribution of DNA damage into nuclear sub-domains. *PLoS Computational Biology* **3**, e155.
274. **Wenzl T and Wilkens JJ** (2011) Modelling of the oxygen enhancement ratio for ion beam radiation therapy. *Physics in Medicine and Biology* **56**, 3251–3268.
275. **Nikjoo H *et al.*** (2001) Computational approach for determining the spectrum of DNA damage induced by ionizing radiation. *Radiation Research* **156**, 577–583. 577.
276. **Roobol SJ *et al.*** (2020) Comparison of high- and low-LET radiation-induced DNA double-strand break processing in living cells. *International Journal of Molecular Sciences* **21**, 6602.
277. **Cao W *et al.*** (2017) Linear energy transfer incorporated intensity modulated proton therapy optimization. *Physics in Medicine and Biology* **63**, 015013.
278. **Le Caër S** (2011) Water radiolysis: influence of oxide surfaces on H2 production under ionizing radiation. *Water* **3**, 2073–4441.
279. **Desouky O, Ding N and Zhou G** (2015) Targeted and non-targeted effects of ionizing radiation. *Journal of Radiation Research and Applied Sciences* **8**, 247–254.
280. **Wilson JD *et al.*** (2019) Ultra-high dose rate (FLASH) radiotherapy: silver bullet or fool's gold? *Frontiers in Oncology* **9**, 1563.
281. **Diffenderfer ES *et al.*** (2020) Design, implementation, and *in vivo* validation of a novel proton FLASH radiation therapy system. *International Journal of Radiation Oncology Biology Physics* **106**, 440–448.
282. **Moon EJ, Petersson K and Olcina MM** (2022) The importance of hypoxia in radiotherapy for the immune response, metastatic potential and FLASH-RT. *International Journal of Radiation Biology* **98**, 439–451.
283. **Fouillade C *et al.*** (2020) FLASH irradiation spares lung progenitor cells and limits the incidence of radio-induced senescence. *Clinical Cancer Research* **26**, 1497–1506.
284. **Hughes JR and Parsons JL** (2020) FLASH radiotherapy: current knowledge and future insights using proton-beam therapy. *International Journal of Molecular Sciences* **21**, 6492.
285. **Adrian G *et al.*** (2020) The FLASH effect depends on oxygen concentration. *The British Journal of Radiology* **93**, 20190702.
286. **Busk M, Horsman MR and Overgaard J** (2008) Resolution in PET hypoxia imaging: voxel size matters. *Acta Oncologica* **47**, 1201–1210.
287. **van der Heide UA *et al.*** (2012) Functional MRI for radiotherapy dose painting. *Magnetic Resonance Imaging* **30**, 1216–1223.
288. **Donche S *et al.*** (2019) The path toward PET-guided radiation therapy for glioblastoma in laboratory animals: a mini review. *Frontiers in Medicine* **6**, 5.

https://doi.org/10.1017/erm.2022.14 Published online by Cambridge University Press