Cancer Letters 502 (2021) 84–96

Contents lists available at ScienceDirect

Cancer Letters

journal homepage: www.elsevier.com/locate/canlet

Radiotherapy, immunotherapy, and the tumour microenvironment: Turning an immunosuppressive milieu into a therapeutic opportunity

N.E. Donlon [a,b], R. Power [a,b], C. Hayes [a,b], J.V. Reynolds [a,b], J. Lysaght [a,b,*]

[a] Department of Surgery, School of Medicine, Trinity College Dublin, Dublin, Ireland
[b] Trinity St James' Cancer Institute, St James's Hospital Dublin, Ireland

ARTICLE INFO

Keywords:
Radiotherapy
Immunotherapy
Abscopal effect
Immunogenic cell death
DAMPS
Hypofractionation

ABSTRACT

Immune checkpoint blockade (ICB) has revolutionised the treatment of solid tumours, yet most patients do not derive a clinical benefit. Resistance to ICB is often contingent on the tumour microenvironment (TME) and modulating aspects of this immunosuppressive milieu is a goal of combination treatment approaches. Radiation has been used for over a century in the management of cancer with more than half of all cancer patients receiving radiotherapy. Here, we outline the rationale behind combining radiotherapy with ICB, a potential synergy through mutually beneficial remodelling of the TME. We discuss the pleiotropic effects radiation has on the TME including immunogenic cell death, activation of cytosolic DNA sensors, remodelling the stroma and vasculature, and paradoxical infiltration of both anti-tumour and suppressive immune cell populations. These events depend on the radiation dose and fractionation and optimising these parameters will be key to develop safe and effective combination regimens. Finally, we highlight ongoing efforts that combine radiation, immunotherapy and inhibitors of DNA damage response, which can help achieve a favourable equilibrium between the immunogenic and tolerogenic effects of radiation on the immune microenvironment.

1. Introduction

Radiotherapy has been one of the pillars for the management of neoplastic burden in cancer patients for over a century. It is used as a treatment modality in approximately 50% of cancer patients in the neoadjuvant, adjuvant, curative and palliative settings. Radiotherapy was employed for its ability to induce double strand DNA breaks, resulting in cell death mainly through mitotic catastrophe and less commonly by apoptosis and cellular senescence [1,2]. Since then, radiation has been successfully integrated with surgery and chemotherapy, and ongoing efforts seek to combine radiotherapy with novel systemic agents. Antibodies against cytotoxic T lymphocyte antigen 4 (CTLA-4), programmed cell death 1 (PD-1) or programmed cell death ligand 1 (PD-L1) [3,4] aim to block negative regulators of immune homeostasis known as immune checkpoints and reinvigorate exhausted $CD8^+$ T cells [5]. This immune checkpoint blockade (ICB) has reshaped the treatment landscape of certain solid tumours, including non-small cell lung cancer, melanoma, urothelial cancer and head and neck squamous cell carcinoma [6–8]. Although some patients display dramatic and durable responses to ICB, the majority derive no benefit. Therefore, there is considerable interest in combination regimens of ICB and potential sensitising agents, including radiation [9].

Traditionally, radiotherapy research has focused on radiation induced biological effects on cancer cells, with little focus on the surrounding stroma. However, cancer cells can reprogram the local environment to induce a tumourigenic milieu. This tumour microenvironment (TME) is composed of blood vessels, extracellular matrix, cancer-associated fibroblasts (CAFs) and a range of immune cells including T and B lymphocytes, tumour-associated macrophages (TAMs), natural killer (NK) cells and myeloid derived suppressor cells (MDSCs) [10]. A growing body of evidence suggests the TME is not only important in cancer development and progression but is altered dynamically in response to radiotherapy. As these changes may be critical in determining treatment success or failure, a deeper knowledge of the TME following radiotherapy is needed to understand radioresistance and develop effective combination strategies. In this review, we discuss the effect of radiation on the stroma, the vasculature and immune cell composition of the TME. We outline a 'double edged sword' effect, where radiation can induce both immunogenic and immunosuppressive changes in the immune microenvironment. Finally, we

* Corresponding author. Cancer Immunology and Immunotherapy Group, Department of Surgery, Trinity Translational Medicine Institute, St. James's Hospital, Dublin 8, Ireland.
E-mail address: jlysaght@tcd.ie (J. Lysaght).

https://doi.org/10.1016/j.canlet.2020.12.045
Received 9 October 2020; Received in revised form 7 December 2020; Accepted 28 December 2020
Available online 12 January 2021

propose how this milieu can be therapeutically targeted using a combination of radiation and immune-based approaches to optimise radiation induced microenvironment remodelling.

2. Immunogenic effects of radiation

2.1. The abscopal effect

The 'abscopal effect', first described in 1953 by Mole [11], refers to regression of metastases outside the radiation field after irradiation of one tumour site. This rare phenomenon could not be explained for decades, but it has recently been linked to radiation-induced systemic antitumour immunity [12]. Although the mechanism is not fully understood, it is thought that the initial step is radiation liberating neoantigens from tumour cells [13]. These antigens can be taken up by antigen-presenting cells (APCs), which migrate to lymph nodes and participate in the cross priming of naïve $CD8^+$ T cell. Activated tumour-specific $CD8^+$ cytotoxic T cells infiltrate both the primary tumour and the non-irradiated metastatic lesion, where they contribute to tumour cell elimination [13]. This effect is rare in tumours treated with radiotherapy alone but is more common when radiation is combined with ICB, both in preclinical and clinical models of PD-1 and CTLA-4 blockade [12,14–18].

2.2. Immunogenic cell death

The precise delivery of radiotherapy can convert tumours into an *in-situ* vaccine and augment the adjuvanticity of the TME. This is through induction of immunogenic cell death, whereby activation of cell death pathways in tumours promotes processing of tumour neoantigens. This represents the first step in the cancer immunity cycle [19], mediated by damage associated molecular patterns (DAMPs) including ATP, high mobility group box 1 (HMGB1), calreticulin and heat shock proteins (HSPs). HMGB1 activates toll like receptor-4 and activates cross-presentation of neoantigens by dendritic cells (DCs) as a result of a reduction in phagosome degradation [20]. Calreticulin acts a pro-phagocytic signal by binding to the CD91 receptor on macrophages and DCs, promoting tumour antigen presentation [21]. Immunogenic cell death releases intracellular ATP which activates the P2X7 purinergic receptor on DCs [22]. This is an activation signal for the NLRP3 inflammasome, enhancing maturation and release of IL-1β which plays a role $CD8^+$ T cell priming. The net effect of DAMP release is increased cross presentation of tumour antigens which has been demonstrated preclinically. Therefore, radiation can profoundly modify the immunogenicity of the TME by promoting the release and presentation of tumour neoantigens.

2.3. The cGAS-STING pathway

The delivery of ionising radiation to cancer cells results in double-strand DNA breaks. The main mechanism of DNA-damage induced cell death is through mitotic catastrophe, where a dysregulated G2/M cell cycle checkpoint allows aberrant mitotic entry with nuclear fragmentation and micronuclei production, ultimately resulting in delayed cell death [1]. DNA released following radiation-induced cell death has been found to activate the cyclic GMP-AMP synthase (cGAS)-stimulator of interferon genes (STING) pathway, a pattern recognition receptor that senses cytosolic DNA [23]. DNA binds to cGAS, which converts ATP and GTP to 2,3 -cGAMP, a second messenger that activates the STING protein attached to the endoplasmic reticulum. STING activates interferon regulatory factor 3 (IRF3) that results in production of type I interferons [24]. The substrate of this DNA-induced interferon production is thought to be $CD11c^+CD8\alpha^+$ BATF-lineage of DCs, a subtype specialised in antigen cross presentation [25]. The classical model of the cGAS-STING pathway involves type I interferon production in response to cytosolic DNA, but newer data indicate that DNA recognition occurs within cGAS containing micronuclei [26,27]. These micronuclei are produced by aberrant progression to mitosis in DNA damaged cells, elegantly explaining the link between radiotherapy and delayed onset DNA damage and immune signalling. There is debate regarding the cellular compartment of STING signalling in the TME. Some preclinical models indicate tumour cell intrinsic STING activation is necessary [28,29], while others suggest tumour cell derived DNA in exosomes can activate STING signalling *in trans,* which is responsible for radiation induced anti-tumour immunity [30,31]. Type I interferons stimulate cross presentation of tumour antigens by DCs and subsequently lead to cross priming of $CD8^+$ effector T cells.

2.4. Infiltration and activation of T cells

The cytokine milieu is critically important for the immune cell composition of the TME and is altered following radiotherapy. Effector $CD8^+$ T cells, interferon (IFN)-γ expression, T helper 1 cells (T_H1) cells and NK cells have potent anti-tumour effects. These cells express the CXC-chemokine receptor 3 (CXCR3) which binds to Th1 chemokines CXC-chemokine ligand 9 (CXCL9) and CXCL10, which allow migration into tumours [32]. In mice, radiation promotes transcription of CXCL10, which can bind to CXCR3 and promote migration of $CD8^+$ effector T cells [33]. $CD11c^+CD8\alpha^+$ BATF-lineage DCs were found to be important in this CXCL10 and CXCL9 production [25]. Another preclinical study found the addition of cisplatin to radiation and *anti*-PD-1 therapy can promote abscopal responses, and this was contingent on CXCL10/CXCR3 mediated $CD8^+$ T cell recruitment [34]. Radiation can also induce the release of CXCL16 by tumour cells, which binds CXCR6 on Th1 cells and $CD8^+$ T cells to encourage tumour infiltration [35]. In addition to chemokines, radiation affects leukocyte infiltration through changes in cell adhesion molecule expression. IL-1β, TNF-α and type I and II interferons are upregulated in response to radiation [36]. These cytokines induce the upregulation of ICAM-1 and VCAM-1 on tumour endothelium [37,38], promoting migration of lymphocytes into the tumour parenchyma.

Radiotherapy can upregulate signals in the TME that promote effector $CD8^+$ T cell mediated killing of tumour cells as $CD8^+$ T cells recognise tumour cells by presentation of neoantigens on major histocompatibility complex (MHC)-I complex and radiation increases its expression on tumour cells [39]. Radiation alters the intracellular peptide pool, which can alter the repertoire of cellular MHC-I associated peptide profiles [39,40]. DNA damage caused by radiotherapy induces cellular stress, which can elevate expression of poorly expressed neoantigens [41]. An example is *KPNA2* in non-small-cell lung cancer, where expression was upregulated by radiation, and peptide fragments trigger IFN production in patient-derived effector T cells [42]. Other molecules including ICAM-1 and MIC A/B (an NKG2D ligand) are upregulated by radiation *in vivo* and are important in $CD8^+$ T cell mediated killing [42]. In mice, MHC-I, ICAM-1, RAE-1γ and NKG2D are central in T cell functional arrest, immune synapse formation and tumour regression in response to a combination of radiation and anti CTLA-4 therapy [43]. Therefore, inflammatory remodelling in the microenvironment following radiation allows enhanced recruitment and activation of effector T cells and enhanced immunogenicity in tumours (Fig. 1).

2.5. Paradoxical effects of immunogenic signalling

The same immunogenic pathways highlighted above can also have immunosuppressive effects and may even promote radio-resistance. STING activation following high dose radiation (20Gy) upregulates CC chemokine ligand 2 (CCL2), CCL7 and CCL12 leading to accumulation of the monocytic MDSCs in the TME through the action of the CCR2 receptor [44]. Similar (>18 Gy) doses of radiation activate TREX1, an endonuclease that cleaves cytosolic DNA and attenuates STING mediated radiation induced anti-tumour immunity [45]. Moreover, STING

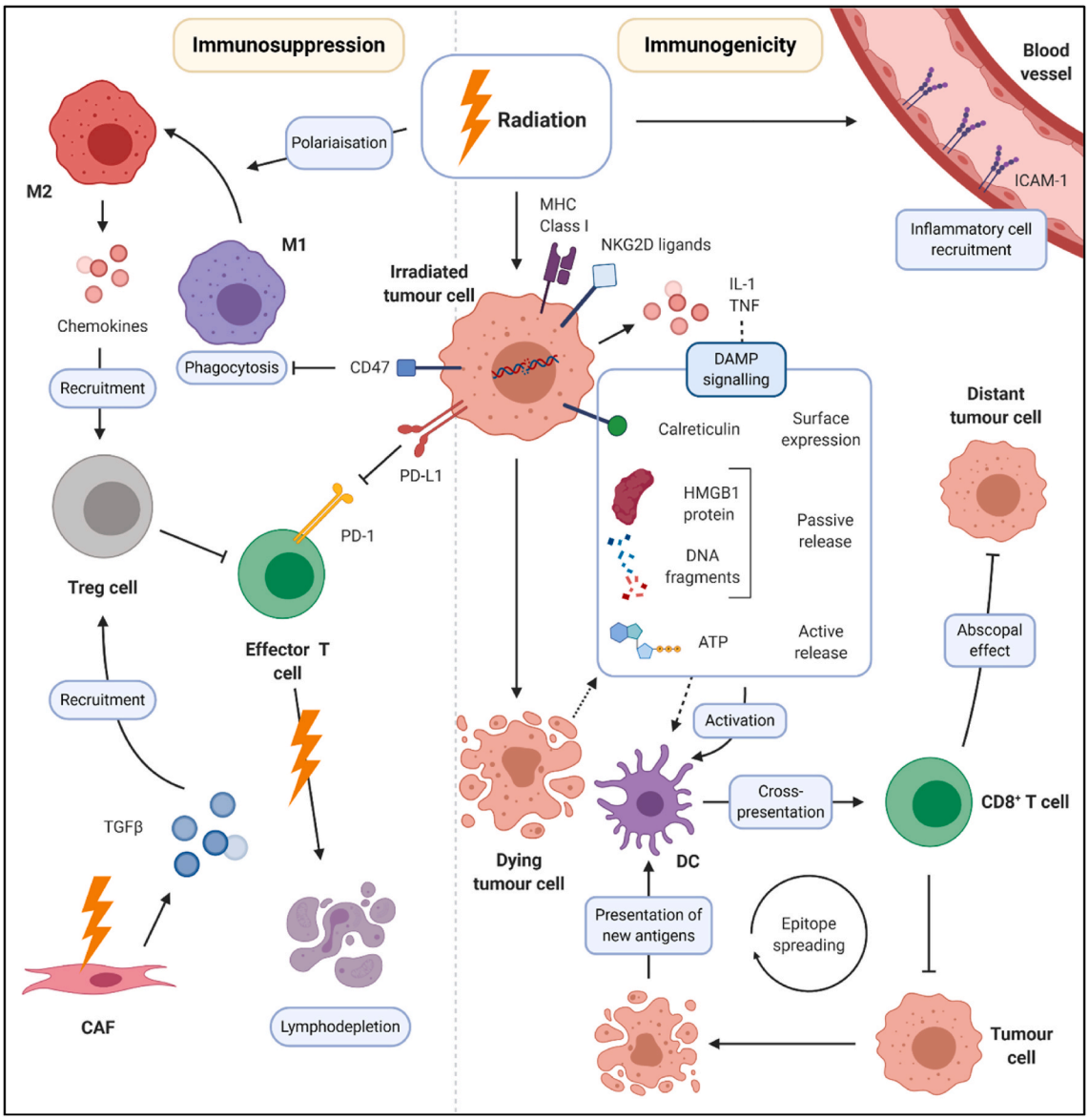


Fig. 1. Double-edged sword of radiotherapy: immunosuppression and immunogenicity. DNA damage induced by radiation therapy (RT) leads to tumour cell stress and death if the former surpasses a cell's reparative capacity. Radiation can promote cell death by up-regulating major histocompatibility (MHC) class I and NKG2D ligands which T cells and natural killer (NK) cells respectively recognise. Furthermore, damaged cells express and release damage-associated molecular patterns (DAMPs): calreticulin expressed on the cell surface, and release of intracellular molecules including high-mobility group box 1 (HMGB1), DNA and ATP by passive or active mechanisms activating antigen presenting cells such as dendritic cells (DCs) a key step in adaptive immunity. Cytokine release, including interleukin (IL)-1 and tumour necrosis factor (TNF), by irradiated tumour cells also contributes to DC activation. DCs can cross-present tumour antigens to $CD8^+$ T cells. $CD8^+$ T cells mature into cytotoxic lymphocytes and drive destruction of tumour cells; this process can lead to the phenomenon of epitope spreading as new antigens are released and presented following destruction of heterogeneous cancer cells. Tumour-specific activated T lymphocytes can mediate regression of distant tumours which may not have been irradiated themselves: known as the abscopal effect. Irradiation of tumour blood vessels leads to increased expression of leukocyte adhesion molecules such as intercellular adhesion molecule 1 (ICAM-1) facilitating recruitment of anti-tumour immune cells. RT can also favour polarisation of M1 macrophages towards a pro-tumour M2 phenotype in addition to activating cancer-associated fibroblasts (CAFs). Chemokines released by M2 macrophages and transforming growth factor-β (TGF-β) secreted by CAFs work in tandem to recruit regulatory T (Treg) cells which suppress anti-tumour immunity. RT also causes a relative increase in these immunosuppressive cell types by driving apoptosis of effector lymphocytes known as lymphodepletion. Furthermore, induction of co-inhibitory molecules on tumour cells such as programmed death-ligand 1 (PD-L1) and CD47 suppress effector lymphocyte activity and phagocytosis by macrophages and DCs, respectively. PD-L1 mediates this effect via interactions with programmed cell death protein 1 (PD-1) on lymphocytes.

can activate the noncanonical NF-κB pathway in response to radiation, which can reduce production of IFN-β in DCs [46]. Inhibiting this pathway can enhance DC priming and treatment efficacy. Therefore, like much of radiation's effects on the TME, STING has a dual role in anti-tumour immunity.

Type I interferons have opposing effects on tumour and immune cells, augmenting the anti-tumour immune response while promoting tumour cell survival. Basal high expression of interferon stimulated genes (ISGs) in tumours is linked to radioresistance and recent evidence indicates this is mediated by autocrine and/or paracrine IFN signalling [47]. Type I interferon signalling in tumour cells can upregulate a myriad of T cell inhibitory ligands, including PD-L1, galectin 9 and HVEM [48]. Furthermore, a pan-cancer analysis of tumours with dysfunctional infiltrating lymphocytes showed upregulation of interferon regulated pathways [49]. Once example is *SERPINB9*, a serine protease inhibitor that inactivates granzyme B, thus preventing T cell

mediated cytotoxicity [50]. *IFNAR1* knockout or JAK inhibition in mouse models enhanced the tumour response to radiation due to increased effector $CD8^+$ T cell mediated killing linked to a reduction in SERPINB9 [51]. Overexpression of SERPINB9 reversed this effect, suggesting that radiation-induced interferon signalling restrains T cell mediated cytotoxicity.

2.6. Immunosuppressive myeloid populations

Radiation can also induce changes in the cytokine profile of the TME to promote an immunosuppressive milieu. Myeloid derived suppressor cells (MDSCs) are increased in the TME following radiotherapy in mouse models [52]. MDSCs inhibit T cell function and anti-tumour immunity through arginase mediated arginine depletion, nitric oxide production and reactive oxygen species release [52]. Accumulation in the TME following radiotherapy is through CCL2 - CCR2 signalling, and CCL2 may be derived from tumour cells [53]. Therapies targeting CCL2 or CCR2 can enhance radiotherapy efficacy in preclinical models, and has been demonstrated in pancreatic cell line models [54]. Another chemokine axis involved in immune infiltration is CCR5, a receptor for CCL5. Radiation upregulates CCL2 and CCL5 production, which recruits $CCR2^+CCR5^+$ suppressive monocytes to the TME [54]. Similarly, administration of a dual CCR2 and CCR5 antagonist reversed this infiltration and increased radiotherapy efficacy. Radiation also upregulates complement component C5a, which may activate DCs [55] but is also a potent recruiter of MDSCs [56].

Another myeloid cell population, abundant in the irradiated TME are tumour associated macrophages (TAMs), which generally exhibit pro-tumour (M2-phenotype) properties. TAMs induce angiogenesis and secrete immunosuppressive mediators like IL-10 and TGF-β, thus inhibiting anti-tumour immunity and promote a radioresistant phenotype [57,58]. In autochthonous mouse models of glioblastoma, radiation upregulates stromal derived factor 1 (SDF-1) which recruits TAMs through the CXCR4 receptor, and inhibiting SDF-1 can prevent tumour recurrence following radiotherapy [59]. Colony stimulating factor-1 (CSF-1) is upregulated in the irradiated TME, and is important in recruitment of TAMs and MDSCs [60]. In preclinical models of glioblastoma and prostate cancer, CSF-1 receptor signalling blockade reduces accumulation of TAMs and MDSCs in the TME and suppresses tumour growth more than radiation alone [60,61].

2.7. Regulatory T cells

Regulatory T cells (T_{regs}) are also increased in the TME following radiotherapy. T_{regs} induce immunosuppression by CTLA-4 expression, IL-10 release and the production of adenosine by the CD39 and CD73 ectonucleotidases [62]. Cells expressing CD39 and/or CD73 include tumour cells, cancer-associated fibroblasts (CAF), endothelial cells, $Foxp3^+$ T_{regs}, Th17 cells, γδ T cells, NK cells, effector and memory T cells, B regulatory cells (B_{regs}), myeloid-derived suppressor cells (MDSC), macrophages and neutrophils, inter alia. Their presence is a poor prognostic indicator across solid tumours [63]. Release of adenosine by tumour cells, as well as upregulation of TGF-β, both mediators of homeostatic tissue repair may contribute to T_{reg} accumulation [64]. Another mechanism is tumour cell derived CCL2 release and release of TNF-α from MDSCs which can activate $CCR2^+$ T_{reg} cells to promote immunosuppression [65]. T_{reg} cells are more radioresistant than other T and B cells, due to increased expression of the PI3K/Akt pathway which renders them less susceptible to radiation induced apoptosis [66,67]. The effect of selective T_{reg} cell ablation on tumour regression was not increased by the addition of PD-1 or CTLA-4 blockade, but combined with radiation reduced tumour burden and enhanced survival, highlighting a potential future therapeutic strategy [68].

3. The tumour stroma and vasculature

3.1. Endothelial cells

The vascular component of the TME is composed of endothelial cells, pericytes and supportive stroma. The tumour vasculature and endothelial cells are perhaps the most well studied component of TME following radiation, but changes depend on dose, fractionation, as well as the type and location of the tumour [10]. Radiation induces endothelial cell dysfunction which is characterised by increased permeability, detachment and apoptosis [69,70] and is associated with the expression of acid sphingomyelinase, which in turn induces endothelial cell apoptosis [71]. This promotes a prothrombotic state, encouraging platelet aggregation, microthrombus formation and adhesion of inflammatory cells with transmigration into the interstitial space [72]. Radiation can induce a senescent phenotype in endothelial cells [73], where suppression of angiogenesis, oxidative stress and inflammation contribute to long term vascular dysfunction [74]. Radiation induced endothelial cell death can also promote anti-tumour immune signals, including CXCL16, which activates macrophages and T cells [35]. Pro-survival processes in cancer cells can be upregulated following endothelial cell irradiation. For example, tumour cells may secrete vascular endothelial growth factor (VEGF) and fibroblast growth factor (FGF), promoting survival of endothelial cells, maintaining vascular function post radiation which in turn can promote cancer progression [75]. Endothelial cells also induce other pro-survival processes, including the phosphatidylinositol 3-kinase (PI3K)/Akt pathway [76] and overexpression of the $\alpha_v\beta_3$ integrin, which promotes radioresistance with epithelial mesenchymal transition (EMT) [77]. Finally, adhesion molecule expression, such as focal adhesion kinase (FAK), which is important in the regulation of integrin signaling, cell adhesion, migration and proliferation of cells and its downstream effector paxillin are increased following irradiation, which may help adherence of tumour cells and the initiation of the metastatic cascade [78]. In summary, activation, apoptosis and senescence of the endothelium can have pleotropic pro- and anti-tumour effects.

3.2. The tumour vasculature

Understanding the functional impact of radiation on the tumour vasculature is key to maximise therapeutic efficacy. On a macroscopic level, irradiation of the vasculature results in dose dependant destruction of blood vessels, especially potent at the level of the microvessel network [79]. This, in turn, reduces vascular density and the distance between functioning vessels to promote an area of vessels with hypoperfusion. Vessels become thicker, with intimal proliferation and an increased risk of atherosclerotic changes [80]. Later changes include fibrosis and medial necrosis and high doses of radiation may induce a permanent reduction in blood flow, implying that the post radiation effects are irreversible [81]. Vessels in the TME may arise through angiogenesis, vasculogenesis or vessel co-option and may lack basement membrane or supporting pericytes, increasing radiosensitivity of the local TME vasculature [82]. Moreover, endothelial cells in the TME have high proliferation rates, which increases inherent radiosensitivity [83].

Similar to normal tissue, high dose radiation promotes acid sphingomyelinase dependant endothelial apoptosis, microvascular damage and tumour cell death in melanoma and fibrosarcoma xenografts [84]. Subsequent tumour revascularisation is by vasculogenesis, a less efficient method of vessel development compared to angiogenesis, and occurs through hypoxia inducible factor 1α (HIF-1α) dependant and HIF-1α independent mechanisms [85]. HIF-dependant induction of stromal cell derived factor (SDF-1) production in the TME is required for recruitment of matrix metalloprotease 9 bone marrow derived cells through the CXCR4 chemokine receptor [86]. Radiation can also activate a novel pathway of HIF-1α independent SDF-1 induction to promote endothelial cell migration [87]. Inhibiting CXCR4 reduces

vasculogenesis and prevents tumour recurrence post radiotherapy [85], suggesting another pathway of vessel remodelling which could be targeted by radiosensitisers.

3.3. Hypoxia

The dysfunctional microvasculature, inherent to the TME, contributes to radioresistance by promoting hypoxia and radiation-induced microvascular damage further potentiates this as shown in Fig. 2 [88]. Hypoxia correlates with tumour recurrence and poor prognosis in response to radiotherapy [89], and hypoxia directly and indirectly leads to radioresistance through a number of mechanisms. Firstly, hypoxia supports development of a cancer stem cell like phenotype through epigenetic reprogramming [90], and has been shown to encourage radioresistance in pancreatic cancer models [91]. Secondly, low intracellular levels of oxygen prevent radiation induced reactive oxygen species production (ROS), which is responsible for the indirect method of radiation induced DNA damage [92].

Additionally, hypoxia leads to accumulation and stabilisation of HIF-1α [93], which has been demonstrated in irradiated glioma cells, promoting angiogenesis and tumour progression [94]. This, amongst other pathways, activates anaerobic glycolysis by expression of pyruvate kinase isoform M2, aldolase A, enolase and lactate dehydrogenase [95,96]. Anaerobic glycolysis involves conversion of pyruvate to lactate which is then secreted into the TME to prevent feedback inhibition. Lactate levels in the TME diminish T and NK cell activation, leading to immune escape and resistance to radiotherapy [97,98]. Lactate also upregulates the HIF-1α pathway, creating a futile cycle of radio- and immune resistance [99]. HIF-1α also upregulates the pentose phosphate and the serine synthesis pathway, which increase the production of NADPH [100]. NADPH is vital in the glutathione dependent antioxidant pathway and upregulation further impedes ROS induced cell death in the irradiated TME [100].

The HIF pathway can also modify the immune microenvironment. HIF-1α dependent production of the chemokine CCL28 in hepatocellular carcinoma cells recruits T_{reg} cells to the TME [101]. This dampens effector T cell function and promotes angiogenesis. Hypoxia also leads to recruitment of TAMs with an M2 phenotype through p38 mitogen activated protein kinase (MAPK) signalling [102]. In response to hypoxia, TAMs can inhibit T cell proliferation in a HIF-1α dependent manner and targeted deletion of HIF in myeloid cells reduced tumour growth in mouse models of breast cancer [103]. HIF-1α can also regulates MDSC differentiation and function [104], signals secreted from hypoxic tumours can promote the establishment of a premetastatic niche

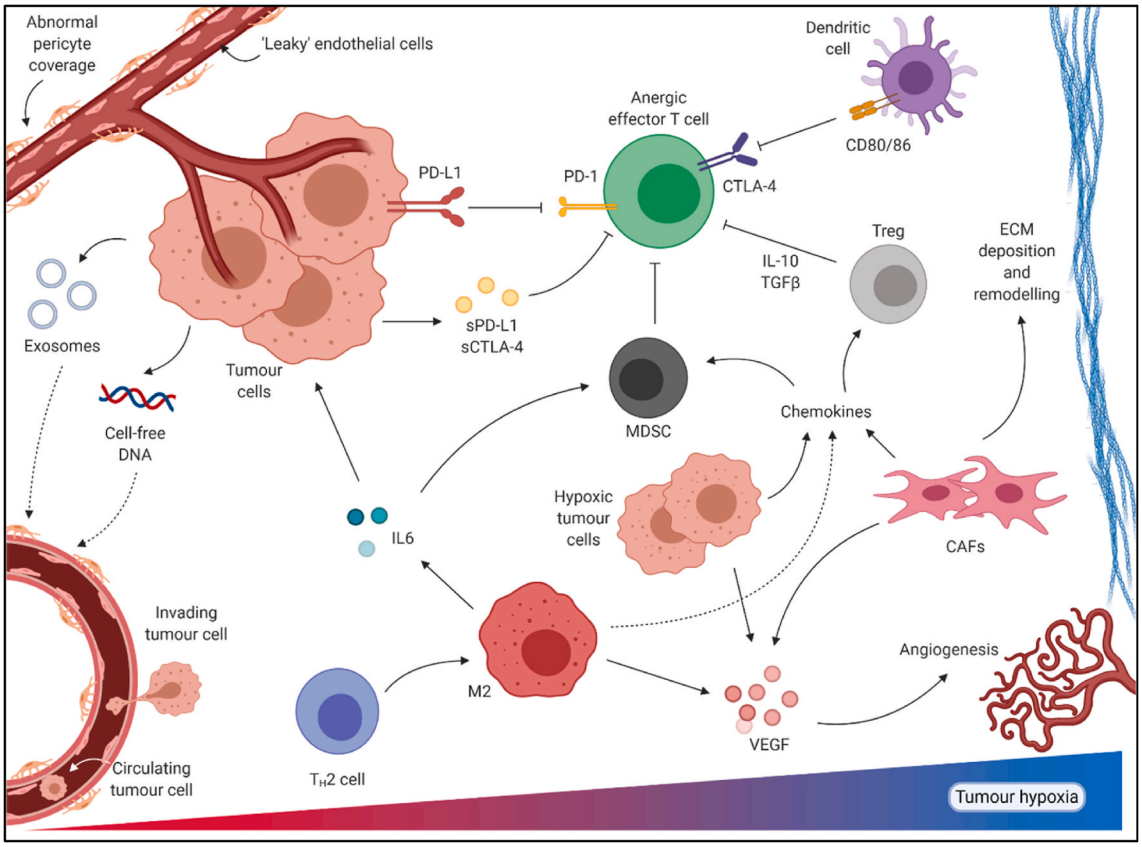


Fig. 2. The tumour microenvironment: a team effort. A key concept in tumourigenesis and progression is the corruption of surrounding stromal and immune cells and soluble mediators to form a network, dubbed the tumour microenvironment, that aids, nourishes and protects the tumour. A major role of the tumour microenvironment is to subvert anti-tumour immunity. This can be achieved by the expression of immune checkpoints such as programmed death 1 (PD-1) or cytotoxic T-lymphocyte antigen 4 (CTLA-4) which induce anergy following ligation with their ligands programmed cell death protein 1 (PD-1) and CD80/86, respectively. Tumours can also release soluble immune checkpoints, including sPD-L1 and sCTLA-4, to dampen anti-tumour responses. Recruitment of immunosuppressive cell types such as regulatory T (Treg) cells and myeloid-derived suppressor cells (MDSCs) is encouraged by expression of chemokines and cytokines from transformed cells, M2 macrophages and cancer-associated fibroblasts (CAFs) and act to limit effector lymphocyte responses. CAFs are also critical in the remodelling of the tumour microenvironment by depositing extracellular matrix (ECM) and promoting angiogenesis through expression of vascular endothelial growth factor (VEGF) under hypoxic conditions along with tumour cells and macrophages. Stromal remodelling and angiogenesis are key facets of tumour progression: remodelling of the ECM promotes invasion and metastasis while the nascent vasculature facilitates nutrient and oxygen delivery. Additionally, tumour blood vessels are characterised by dysfunctional endothelium and abnormal pericyte coverage leading to increased vessel permeability; these factors assist the intravasation of potential metastatic seedlings as well as exosomes and cell-free DNA which can circulate in the blood and prime pre-metastatic niches.

by MDSC recruitment and suppression of NK cell cytotoxicity [105]. Hypoxia induced accumulation of these suppressive cell populations in the TME is a potent means of radioresistance.

3.4. Cancer associated fibroblasts

Cancer associated fibroblasts (CAFs) are a heterogeneous population that make up the majority of stromal cells in many carcinomas. CAFs secrete extracellular matrix proteins and cytokines (such as SDF-1 and TGF-β) that have diverse immunomodulatory roles but generally promote tumour progression [106,107]. Radiation can recruit CAFs and the irradiated TME myofibroblasts undergo phenotypic transformation to CAFs [108]. The role of CAFs in the immune response to radiotherapy is poorly understood, largely due to heterogeneity in CAF function. CAFs are predominantly immunosuppressive and can contribute to radioresistance by secretion of TGF-β to induce a radioresistance cancer stem cell phenotype [91]. Cancer stem cells (CSC) represent an individual subpopulation within a tumour exhibiting the capacity to self-renew and differentiate, rendering CSC resistant to various therapies including radiation therapy [109]. CAFs can also secrete RNA containing exosomes that interact with tumour cell RIG-I, a pattern recognition receptor, and activates STAT1 dependent signalling, which facilitates transcriptional responses to NOTCH3 and expands therapy resistant tumour-initiating cells to potentiate radioresistance in breast cancer [110]. Radiation induced DNA damage can potentiate immune resistance, through conferring a senescence CAF phenotype, via the release of different factors by CAFs mediating fibrosis, EMT/invasion and treatment resistance promoting tumour cell survival by promoting immunomodulation, metabolism, ECM remodelling, autophagy and treatment resistance to radiation therapy through a β_1 integrin mediated mechanism [111,112]. However, further work is needed to characterise the specific immunosuppressive role of irradiated CAFs in this context.

4. The effect of radiation dosing and fractionation

Conventional fractionated radiation is delivered in small 1.8–2 Gray (Gy) daily fractions, but may provide a total radiation dose of up to 66 Gy [113]. Recent advances in radiation technique and delivery, including intensity modulated radiotherapy (IMRT), volumetric-modulated arc therapy (VMAT) and proton beam therapy allow delivery of higher radiation doses while minimising acute and long-term toxicity [114]. This has allowed a shift to 'hypo-fractionated' approaches, ranging from 5 to 10 Gy over three to five fractions to single doses of up to 24 Gy using stereotactic ablative radiotherapy (SABR) or stereotactic body radiotherapy (SBRT) [115]. Different cellular responses in the TME depend on the dose of radiation delivered, mediated in part, by the intrinsic radioresistance of different immune cells [116]. The sensitivity of T cells to radiation depends on their activation state; resting lymphocytes are more radiosensitive than activated forms. T_{reg} cells are more radioresistant than other T cells, allowing persistence following radiation, while B lymphocytes are highly radiosensitive [116]. Macrophages are generally more resistant to radiation than monocytes under higher radiation doses [117,118], however, single high doses (>30 Gy) reduce co-stimulatory receptor expression in immature DCs, down-regulating the expression of CD86 and CD80 compromising their ability to capture and present antigens [119].

4.1. Dosing, fractionation, and immunity

Conventional fractionated radiotherapy is designed to exploit vulnerabilities in DNA repair and cell cycle arrest as described in Fig. 3, but the fractionation required for the most effective anti-tumour immune response is yet to be determined. Conventional fractionation is associated with increased MDSCs and T_{reg} cells in the TME [15,120], but this effect on MDSCs is also seen in single high (20 Gy) dose radiation [44]. Low dose radiotherapy also promotes TAM accumulation, but may also promote the polarisation of TAMs to an immunogenic M1 phenotype [121] and a Th1 cytokine milieu [122]. Therefore, despite these putative immunosuppressive effects, conventional radiation doses induce some level of anti-tumour immunity, as tumour antigen specific T cells have been isolated in prostate [123] and colorectal [124] cancer patients receiving radiation treatment.

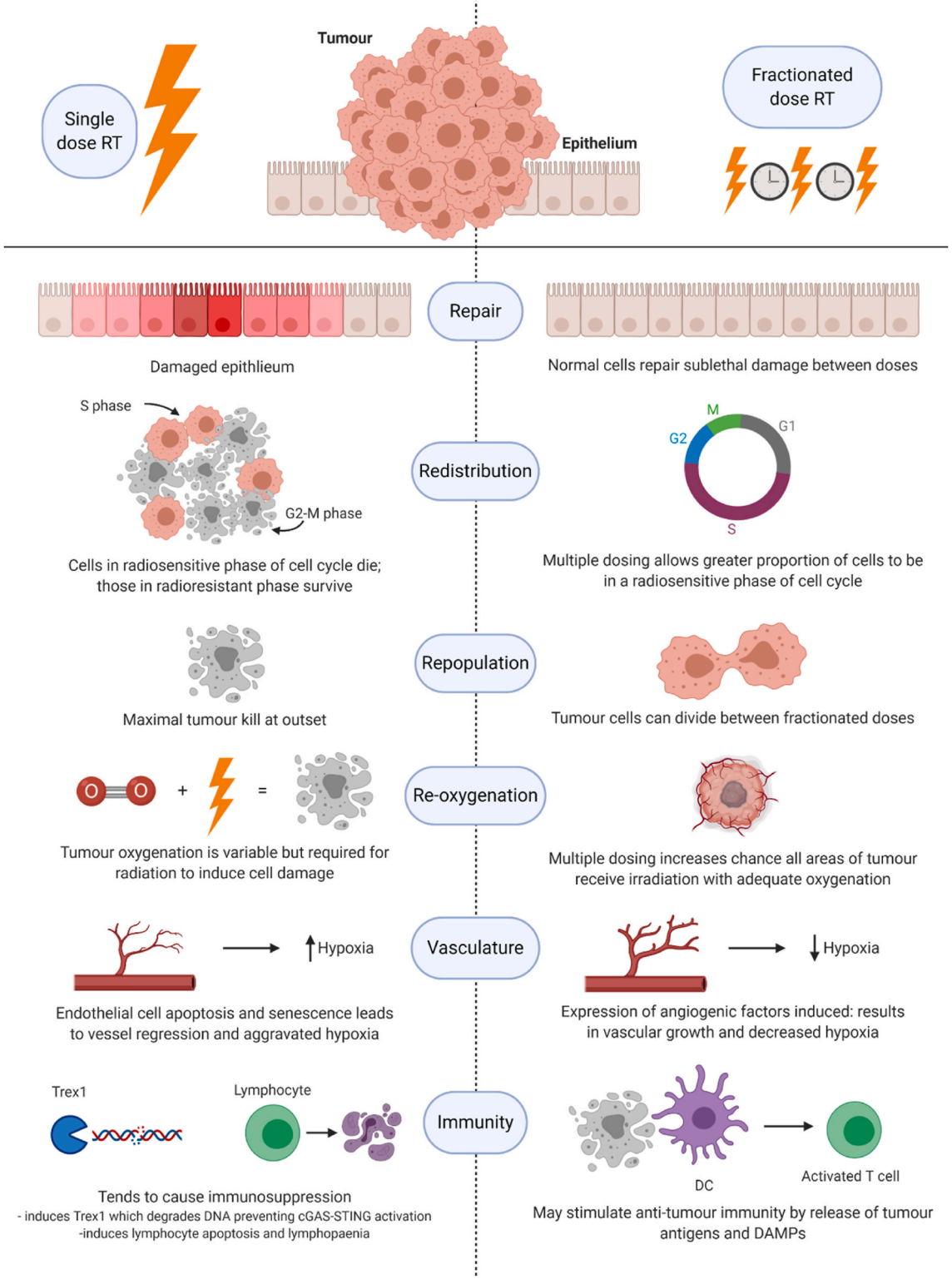
There is some evidence that hypofractionated radiotherapy (eg. 3 × 8 Gy) promotes more immunogenic changes in the TME. Upregulation of MHC-I on tumour cells and expanding the peptide repertoire is dose dependant, having an effect at single doses of >4 Gy in melanoma and >8–20 Gy in colon cancer cells [39]. However, these preclinical studies included single doses of radiation, and repeated high doses (as in hypofractionation) was not directly compared to conventional fractionation. Upregulation of ICAM-1 on tumour cells, as well as the CD95 death receptor are also contingent on total radiation dose [125]. Hypofractionated radiotherapy can promote cell death by necrosis and senescence, which has traditionally been regarded as a more immunogenic form of cell death compared to apoptosis [20], but the increased exposure of calreticulin in apoptotic cells is also involved in the immune response to radiation [126]. Indeed, a single dose of 15 Gy radiation has been found to enhance DC maturation compared to conventional fractionation (5 × 3 Gy) in mice [127]. These immunogenic effects might have implications for combining with ICB. In mouse models of oral cancer, a hypofractionated regimen (2 × 8 Gy) induced greater $CD8^+$ T cell infiltration and reduced MDSC accumulation compared to low dose, conventionally fractionated radiotherapy (10 × 2 Gy) [16]. Furthermore, *anti*-PD-1 therapy reversed adaptive immune resistance and promoted $CD8^+$ dependant local and distant tumour regression.

High ablative single doses (>20 Gy), such as those delivered by SABR/SBRT have been shown to dramatically increase T cell priming, $CD8^+$ T cell infiltration and the induction of tumour regression in breast, lung and melanoma mouse models [128,129]. Another preclinical study in an inherent poorly immunogenic colon cancer mouse model found a similar increase in $CD8^+$ infiltration with a single high (30 Gy) dose compared to extended fractionation regimen (10 × 3 Gy), alongside MDSC depletion [130]. These immunogenic effects of ablative radiotherapy are not universal. In combination with *anti*-CTLA-4 therapy, hypofractionated (3 × 8 Gy) radiotherapy was superior to an ablative (20Gy) dose in promoting T cell infiltration and local and distant tumour regression [14]. Consistent with this, single higher ablative doses (>20 Gy) of radiation induce TREX1, as mentioned previously, an exonuclease that degrades cytoplasmic DNA and abrogates STING mediated IFN production in mice [45]. This induction of TREX1 was not seen in a hypofractionated (3 × 8 Gy) regimen, which instead amplified IFN-β and resulted in accumulation of intratumoral $Batf3^+$ antigen presentation cells. Finally, a single ablative (20 Gy) dose can also lead to MDSC recruitment [54], further highlighting a ceiling on radiation's dose dependant immunogenicity.

4.2. Dosing, fractionation and the tumour vasculature

Radiation dosing and fractionation also have divergent effects on the tumour vasculature. Low single doses (<5 Gy) of radiation not only normalise vessels but even stimulate angiogenesis and vasculogenesis through VEGF and SDF-1/CXCR12 signalling [87]. Even smaller single doses (0.5–2 Gy) radiation can normalise dysfunctional vessels of the TME, promoting inducible nitric oxide synthase (iNOS) and an M1-phenotype in TAMs, allowing NO dependant vascular normalisation [121]. The subsequent Th1 pattern of cytokines allows recruitment of $CD8^+$ T cells and tumour rejection in mouse models. Therefore, conventional radiation fractionation that uses repeated doses in this range may be useful in immune-excluded tumours, where a dense stroma prevents effector T cells from accessing the tumour parenchyma [9].

Ablative doses of radiation leads to the destruction of the tumour vasculature. High single doses (>8–10 Gy) of radiation delivered through SBRT/SABR promote endothelial cell apoptosis, through direct



(caption on next page)

Fig. 3. Fractionation: more than the sum of its parts. Radiation therapy (RT) can be administered to patients in a variety of regimens: single or fractionated dose. In contrast to a high dose of radiation administered at a single time-point, fractionated RT entails division of the total radiation dose into multiple, smaller doses which are administered to a patient over a period of several days. Rationing the dose allows normal cells to recover or repair sublethal DNA damage whereas administering a high single dose often overwhelms a cell's ability to repair itself leading to toxicity. Cancer cells are often characterised by defective or repressed DNA repair, and therefore, single and fractionated radiation exposure leads to accumulation of genomic aberrations driving cell death. The radiosensitivity of tumour cells varies depending on progress through the cell cycle, with cells in S phase most radioresistant compared to those in G2-M phase. Administering multiple doses of radiation subjects a higher proportion of cells to radiation in the appropriate cell cycle phase in a concept known as redistribution. However, the gaps between fractionated treatments provide an opportunity for tumours to repopulate – this effect is mitigated somewhat by administering a single dose of radiation. RT is highly dependent on the oxygenation status of a tumour as oxygen free radicals are a major mediator of cell damage; however, malignant tumours are renowned for their hostile hypoxic microenvironments which can limit the success of RT. Additionally, the location of the hypoxic areas within tumours are highly variable, therefore, fractionating the dose increases the number of cells with adequate oxygenation (re-oxygenation) and the yield of cells killed. High single dose radiation causes regression of tumour blood vessels; however, the ensuing lack of perfusion may aggravate tumour hypoxia leading to angiogenesis and tumour progression. Conversely, fractionated RT may induce vascular growth and normalisation via pro-angiogenic factor expression with resultant normoxia. While the differing effects of RT dosing regimens on the immune system have yet to be fully elucidated it seems that a high single dose of radiation contributes to immunosuppression within the tumour microenvironment while the fractionated RT appears to have immunostimulatory effects. High dose radiation can induce expression of Trex1 which degrades DNA fragments that otherwise would have triggered cytokine production via the cGAS-STING pathway. Moreover, ablative doses of radiation are inherently lymphotoxic and lymphopaenia often follows such treatments. In contrast, fractionated RT may stimulate adaptive immune responses via release of tumour antigens and damage-associated molecular patterns (DAMPs) which prime DCs to activate anti-tumour T cells.

DNA damage and induced acid sphingomyelinase [84]. Although this disruption of the tumour vasculature can lead to cancer cell death, they may generate regions of hypoxia and promote radioresistance [89,92,131]. A computational model of tumour growth probability found that single ablative doses result in impaired local control of hypoxic tumours compared to hypofractionated regimens [131]. Therefore, the choice of radiation may be tailored based on the specific goals of microenvironment modification, whether destruction or normalisation of vessels is favoured.

5. The microenvironment as a therapeutic target

Radiation has profound effects on the composition and organisation of the TME, depending on the dose, location and tumour type. The immunogenic effects of radiotherapy promote tumour regression, whereas recruitment of immunosuppressive cell populations and exacerbation of tumour hypoxia can engender a radioresistant phenotype. Boosting these positive effects while mitigating these undesirable effects can be exploited to improve clinical responses.

5.1. Immune checkpoint blockade

The induction of immunogenic cell death, enhanced neoantigen expression and presentation, cGAS-STING activation and $CD8^+$ T cell activation and infiltration provide preclinical rationale for combinatorial approaches of radiation and ICB. Radiation can also increase tumour and immune cell expression of PD-L1, thought to be related somewhat to $CD8^+$ T cell derived IFN-γ secretion [132]. This is a method for adaptive immune resistance to radiotherapy which can be overcome by PD-1/PD-L1 blockade [15]. The expression of PD-L1 was found to be essential for ICB efficacy in some preclinical models [133] and a robust predictor of benefit of ICB in clinical trials [134], suggesting this means of radioresistance may also sensitise tumours to checkpoint inhibition. These immunogenic effects of radiation are more pronounced in hypofractionated radiotherapy or SBRT, but these doses promote vessel destruction, potentially exacerbating tumour hypoxia. Interestingly, anti PD-1 and *anti*-CTLA-4 therapy can result not only in tumour regression, but can also reduce tumour hypoxia [135]. This vessel normalisation was mediated by IFN-γ producing $CD8^+$ T cells and was correlated with clinical efficacy. Another *in silico* analysis found that gene expression related to vessel normalisation correlates to immunostimulatory pathways with Th1 derived IFN-γ normalising vessels [136]. This reduces hypoxia *in vivo* and provides evidence of the symbiotic relationship between ICB and RT, through mutually favourable remodelling of the microenvironment.

Prospective clinical data combining immunotherapy and radiation are in a nascent phase; phase III trials have not been published outside of prostate and non-small cell lung cancer (NSCLC). The phase III PACIFIC trial investigated durvalumab (*anti*-PD-L1) following chemoradiotherapy in locally advanced NSCLC [113]. Compared to placebo, durvalumab improved overall survival (28.3 vs 16.2 months, hazard ratio 0.68, $p = 0.0025$) and both arms reported similar rates of treatment related adverse events (NCT02125461). A phase III trial in metastatic castration resistant prostate cancer found no survival benefit of ipilimumab (*anti*-CTLA-4) following a single 8Gy dose of radiation, but a benefit in the combination arm was reported among those with favourable prognostic features [137]. These trials have employed conventional fractionation or a single dose, whereas some conflicting preclinical data indicate that hypofractionated radiotherapy could potentially be more immunogenic. One phase I trial of pembrolizumab (*anti*-PD-1) alongside hypofractionated radiotherapy (3×8 Gy) in metastatic solid tumours has reported early results [138]. No dose limiting toxicities were observed and three patients displayed durable or complete responses to therapy. However, another trial of a similar regimen in bladder cancer was terminated early due to adverse events, highlighting the potential toxicity issues of this combination [139]. Efficacy data is eagerly awaited from ongoing trials of hypofractionated radiation and immunotherapy in NSCLC (NCT04351256), melanoma (NCT03646617), renal cell carcinoma (NCT04090710) and glioblastoma (NCT03743662).

Although pivotal trials to date have focused on the PD-1/PD-L1 and CTLA-4 axes, targeting other immune surface receptors represent promising means of modifying the immunosuppressive TME. One example is CD47, a macrophage immune checkpoint that opposes the effect of calreticulin in immunogenic cell death following radiotherapy [126]. CD47-mediated antiphagocytosis mediates radioresistance in mouse models of HER2 breast cancer, and dual blockade of HER2 and CD47 synergise with radiation to promote tumour elimination [140]. Another preclinical study suggests anti-CD47 therapy can enhance the efficacy of radiation in glioblastoma [141]. This indicates a potential for combining anti-CD47 with radiation, and one phase I trial is currently investigating an intratumoral injection of CD47 inhibitor alongside radiation in advanced solid tumours (NCT02890368). Interestingly, a triple regimen of *anti*-CTLA-4, anti-CD47 and radiation provided a survival benefit in mouse models of melanoma [142]. This synergy was mediated by enhanced adaptive T and NK cell immunity, providing rationale for investigating the use of anti-CD47 therapy to complement ICB and radiotherapy in future trials.

5.2. Soluble factor inhibition

Targeting chemokines has the potential to mitigate the immunosuppressive effects of MDSCs, T_{regs} and TAMs on the irradiated immune microenvironment. MDSCs are recruited following radiation due to

interactions of CCL2/CCR2 and CCL2/CCR5, and inhibition of these chemokine axes can increase response to radiotherapy in preclinical models [143]. This approach is being evaluated in a phase I/II trial, combining a CCR2/5 dual antagonist (BMS-813160) with anti PD-1 therapy and hypofractionated radiation (5 × 6.6Gy) in pancreatic cancer (NCT03767582). T_{reg} cells are recruited by CCL2 and TGF-β in an immunosuppressive TME. Targeting TGF-β alongside hypofractionated radiotherapy (3 × 7.5 Gy) has been examined in a phase I trial of metastatic breast cancer [144]. This combination was associated with grade 3/4 adverse events in 5/11 patients in the 1 mg/kg arm and in 2/12 patients in the 10 mg/kg arm, respectively. In addition to this, the higher dose cohort had favourable immune markers and longer median survival (16 vs 7.6 months) than those in the lower dose cohort, but this trial was not adequately powered to detect a survival difference. Production of adenosine by CD73 is a potent means of T_{reg} induced immunosuppression and a recent preclinical study reported that combining CD73 inhibition with radiation promotes DC infiltration and tumour rejection in combination with *anti*-PD-L1 and *anti*-CTLA-4 therapy [145]. Anti-CD73 therapy alongside PD-L1 blockade and SBRT is being investigated in an ongoing breast cancer trial by Institut Curie in collaboration with AstraZeneca (NCT03875573). Another approach is inhibiting JAK1/2, tyrosine kinases that play important roles in type I interferon signalling, a mediator of response and resistance to radiotherapy [146]. A phase I trial of ruxolitinib, a small molecule JAK1 and JAK2 inhibitor, is ongoing in combination with temozolomide and radiation (30 × 2 Gy) for glioblastoma patients (NCT03514069). There is also a strong preclinical mechanistic rationale for combining JAK inhibition with CCR2 and CCR5 antagonists [44,48,53,54,65], and replication in clinical trials would provide important insights.

5.3. Anti-angiogenic therapy

The combination of anti-angiogenic therapy and immunotherapy has recently emerged as a novel therapeutic strategy. Anti-angiogenic therapy can promote vessel normalisation, increasing immune effector cell trafficking to the tumour site, and reduce hypoxia which is linked to an immunosuppressive TME [147]. Indeed, this approach has led to a robust overall survival benefit compared to sorafenib in the first line treatment of advanced hepatocellular carcinoma [148], and promising data is emerging in other tumour types [149,150]. As previously mentioned, both ICB and radiation can promote vessel normalisation through IFN-γ producing $CD8^+$ T cells and $iNOS^+$ macrophages respectively [121,136]. This mutually favourable feedback loop could be further amplified by the addition of anti-angiogenic therapy, and could reduce hypoxia that is linked to resistance to both ICB and radiation [147]. Furthermore, in melanoma models the improved anti-tumour immune response following STING activation depends on endothelial production of type I IFN [151], indicating that targeting endothelial cells could be relevant to the synergy between ICB and radiation. However, this may lead to additional challenges regarding dosage, timing, but importantly the toxicities associated with combining more than two treatments. For example, 50% (93/147) patients had grade ≥3 adverse events in one phase I trial of pembrolizumab and lenvatinib, an anti-angiogenic kinase inhibitor [152]. Nevertheless, ongoing phase II trials are evaluating early efficacy and tolerability of the addition of anti-angiogenic agents to ICB and radiotherapy in non-small cell lung cancer (NCT04517526) and glioblastoma (NCT03661723).

5.4. The DNA damage response

Cancer associated DNA damage response (DDR) defects can be targeted to amplify radiation induced DNA damage [153]. Checkpoint kinase 1 (CHK1), WEE1 and ataxia telangiectasia and Rad3-related protein (ATR) are involved in the S phase and G2/M arrest following DNA damage [154]. Inhibiting this pathway leads to premature M phase entry and results in increased micronuclei formation when combined with radiation, which can enhance the STING induced type I interferon release [155]. However, given the paradoxical effects of type I interferon on the tumour and immune cells, further work is needed to elucidate the full downstream therapeutic effects of this combination. Studies in mice indicate that AZD6738, an ATR inhibitor, potentiates radiation induced type I interferon production and modifies the TME; with an increase in $CD8^+$ T cells, NK cell and DC infiltration noted alongside MDSC depletion compared to controls [40,156]. A phase I trial is evaluating the addition of AZD6738 as a radiosensitiser to palliative radiotherapy [157]. Another approach, the combination of a WEE1 inhibitor alongside radiation and PD-1 blockade enhanced the efficacy of $CD8^+$ T cell mediated cytotoxic activity in mice [158]. DNA protein kinase (DNA-PK) is involved in non-homologous end joining repair of double strand DNA breaks [153], and although it has not been studied preclinically alongside radiation and ICB, clinical trials evaluating this triplet combination are ongoing (NCT04068194, NCT03724890). As these strategies of DDR inhibition represents a novel approach to amplify the immunogenic effects of radiation, it may prove useful as a radiosensitiser alone or to further optimise the combination of radiotherapy and ICB.

5.5. Future prospects

Despite immense progress leveraging knowledge of the TME to increase response to radiotherapy, certain challenges remain. First, there is a paucity of studies that directly compares the immunogenicity of different dosing and fractionation regimens, both in preclinical studies and clinical trials. Many studies vary the overall radiation dose but do not directly ascertain whether this dose would be better delivered in a single ablative dose, a hypofractionated regimen or a conventional 1.8–2 Gy fractionation. A well-controlled comparison in preclinical studies would provide useful mechanistic data, and a platform clinical trial adequately powered for overall survival would determine the optimal dosing regimen. Second, clinical trials of immunotherapy and radiotherapy should incorporate basic and translational study endpoints. These will be useful to dissect mechanisms of action and resistance to ICB and radiotherapy, complementing the existing data available for single agent ICB [134,159]. Third, there is a need to account for TME heterogeneity, both within and between patients, in deciding whether to add DDR inhibitors, anti-angiogenic therapies or other immunomodulators to radiation and ICB [160]. Just like genomic heterogeneity is a significant barrier to effective targeted and cytotoxic therapies, heterogeneity in the cellular composition of TME could limit combining immunotherapy with radiation [161,162]. A precision oncology approach, incorporating interpatient microenvironment diversity, would aid development of effective combination strategies.

6. Conclusion

Signalling events following radiotherapy have profound effects in altering the immune landscape of the TME. Advances have been made in recent years to untangle the biology of radiation induced anti-tumour immunity and how this knowledge can be used to design rational therapeutic approaches. However, issues remain regarding the paradoxical effects of radiation in recruiting suppressive cell populations, the complex effects that radiation invokes on the tumour vasculature and the radiation dosage and fractionation that influences these effects. Although hypofractionated regimens display the promising immunomodulation in some preclinical studies, there is a lack of prospective clinical data that directly compare different fractionation and dosing approaches. Questions remain around the best way to promote immunostimulatory effects of radiotherapy while minimising immunosuppression. We propose that combining radiation with immunotherapy, DDR inhibition or *anti*-antiangiogenic therapy is a promising approach to shift this balance and exploit the microenvironment's untapped

therapeutic potential.

Author contributions

Conceptualization: NED, RP, CH, JVR, JL; writing - original draft: NED; writing - review & editing: NED, RP, CH; JVR, JL; supervision: JVR and JL.

Funding

This work was supported by the CROSS Cancer Charity (RCN: 15364).

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

[1] D. Eriksson, P.O. Lofroth, L. Johansson, K.A. Riklund, T. Stigbrand, Cell cycle disturbances and mitotic catastrophes in HeLa Hep2 cells following 2.5 to 10 Gy of ionizing radiation, Clin. Canc. Res. 13 (18 Pt 2) (2007) 8s–5501s.
[2] D. Eriksson, T. Stigbrand, Radiation-induced cell death mechanisms, Tumour Biol 31 (4) (2010) 363–372.
[3] D.R. Leach, M.F. Krummel, J.P. Allison, Enhancement of antitumor immunity by CTLA-4 blockade, Science 271 (5256) (1996) 1734–1736.
[4] Y. Iwai, M. Ishida, Y. Tanaka, T. Okazaki, T. Honjo, N. Minato, Involvement of PD-L1 on tumor cells in the escape from host immune system and tumor immunotherapy by PD-L1 blockade, Proc. Natl. Acad. Sci. U. S. A. 99 (19) (2002) 12293–12297.
[5] D.S. Chen, I. Mellman, Elements of cancer immunity and the cancer-immune set point, Nature 541 (7637) (2017) 321–330.
[6] R.L. Ferris, G. Blumenschein, J. Fayette, J. Guigay, A.D. Colevas, L. Licitra, et al., Nivolumab for recurrent squamous-cell carcinoma of the head and neck, N. Engl. J. Med. 375 (19) (2016) 1856–1867.
[7] C. Robert, J. Schachter, G.V. Long, A. Arance, J.J. Grob, L. Mortier, et al., Pembrolizumab versus ipilimumab in advanced melanoma, N. Engl. J. Med. 372 (26) (2015) 2521–2532.
[8] M. Reck, D. Rodriguez-Abreu, A.G. Robinson, R. Hui, T. Csoszi, A. Fulop, et al., Pembrolizumab versus chemotherapy for PD-L1-positive non-small-cell lung cancer, N. Engl. J. Med. 375 (19) (2016) 1823–1833.
[9] J. Galon, D. Bruni, Approaches to treat immune hot, altered and cold tumours with combination immunotherapies, Nat. Rev. Drug Discov. 18 (3) (2019) 197–218.
[10] H.E. Barker, J.T.E. Paget, A.A. Khan, K.J. Harrington, Erratum: the tumour microenvironment after radiotherapy: mechanisms of resistance and recurrence, Nat. Rev. Canc. 15 (8) (2015) 509.
[11] R.H. Mole, Whole body irradiation; radiobiology or medicine? Br. J. Radiol. 26 (305) (1953) 234–241.
[12] S. Demaria, B. Ng, M.L. Devitt, J.S. Babb, N. Kawashima, L. Liebes, et al., Ionizing radiation inhibition of distant untreated tumors (abscopal effect) is immune mediated, Int. J. Radiat. Oncol. Biol. Phys. 58 (3) (2004) 862–870.
[13] W. Ngwa, O.C. Irabor, J.D. Schoenfeld, J. Hesser, S. Demaria, S.C. Formenti, Using immunotherapy to boost the abscopal effect, Nat. Rev. Canc. 18 (5) (2018) 313–322.
[14] M.Z. Dewan, A.E. Galloway, N. Kawashima, J.K. Dewyngaert, J.S. Babb, S. C. Formenti, et al., Fractionated but not single-dose radiotherapy induces an immune-mediated abscopal effect when combined with anti-CTLA-4 antibody, Clin. Canc. Res. 15 (17) (2009) 5379–5388.
[15] S.J. Dovedi, E.J. Cheadle, A.L. Popple, E. Poon, M. Morrow, R. Stewart, et al., Fractionated radiation therapy stimulates antitumor immunity mediated by both resident and infiltrating polyclonal T-cell populations when combined with PD-1 blockade, Clin. Canc. Res. 23 (18) (2017) 5514–5526.
[16] M. Morisada, P.E. Clavijo, E. Moore, L. Sun, M. Chamberlin, C. Van Waes, et al., PD-1 blockade reverses adaptive immune resistance induced by high-dose hypofractionated but not low-dose daily fractionated radiation, OncoImmunology 7 (3) (2018), e1395996.
[17] S.S. Park, H. Dong, X. Liu, S.M. Harrington, C.J. Krco, M.P. Grams, et al., PD-1 restrains radiotherapy-induced abscopal effect, Canc. Immunol. Res. 3 (6) (2015) 610–619.
[18] M.A. Postow, M.K. Callahan, C.A. Barker, Y. Yamada, J. Yuan, S. Kitano, et al., Immunologic correlates of the abscopal effect in a patient with melanoma, N. Engl. J. Med. 366 (10) (2012) 925–931.
[19] D.S. Chen, I. Mellman, Oncology meets immunology: the cancer-immunity cycle, Immunity 39 (1) (2013) 1–10.
[20] L. Apetoh, F. Ghiringhelli, A. Tesniere, M. Obeid, C. Ortiz, A. Criollo, et al., Toll-like receptor 4–dependent contribution of the immune system to anticancer chemotherapy and radiotherapy, Nat. Med. 13 (9) (2007) 1050–1059.
[21] M. Obeid, A. Tesniere, F. Ghiringhelli, G.M. Fimia, L. Apetoh, J.L. Perfettini, et al., Calreticulin exposure dictates the immunogenicity of cancer cell death, Nat. Med. 13 (1) (2007) 54–61.
[22] F. Ghiringhelli, L. Apetoh, A. Tesniere, L. Aymeric, Y. Ma, C. Ortiz, et al., Activation of the NLRP3 inflammasome in dendritic cells induces IL-1beta-dependent adaptive immunity against tumors, Nat. Med. 15 (10) (2009) 1170–1178.
[23] L. Deng, H. Liang, M. Xu, X. Yang, B. Burnette, A. Arina, et al., STING-dependent cytosolic DNA sensing promotes radiation-induced type I interferon-dependent antitumor immunity in immunogenic tumors, Immunity 41 (5) (2014) 843–852.
[24] L. Sun, J. Wu, F. Du, X. Chen, Z.J. Chen, Cyclic GMP-AMP synthase is a cytosolic DNA sensor that activates the type I interferon pathway, Science (New York, NY) 339 (6121) (2013) 786–791.
[25] S. Spranger, D. Dai, B. Horton, T.F. Gajewski, Tumor-residing Batf3 dendritic cells are required for effector T cell trafficking and adoptive T cell therapy, Canc. Cell 31 (5) (2017) 711–723, e4.
[26] S.M. Harding, J.L. Benci, J. Irianto, D.E. Discher, A.J. Minn, R.A. Greenberg, Mitotic progression following DNA damage enables pattern recognition within micronuclei, Nature 548 (7668) (2017) 466–470.
[27] K.J. Mackenzie, P. Carroll, C.A. Martin, O. Murina, A. Fluteau, D.J. Simpson, et al., cGAS surveillance of micronuclei links genome instability to innate immunity, Nature 548 (7668) (2017) 461–465.
[28] T. Sen, B.L. Rodriguez, L. Chen, C.M.D. Corte, N. Morikawa, J. Fujimoto, et al., Targeting DNA damage response promotes antitumor immunity through STING-mediated T-cell activation in small cell lung cancer, Canc. Discov. 9 (5) (2019) 646–661.
[29] Z. Wang, J. Chen, J. Hu, H. Zhang, F. Xu, W. He, et al., cGAS/STING axis mediates a topoisomerase II inhibitor-induced tumor immunogenicity, J. Clin. Invest. 130 (2019) 4850–4862.
[30] J.M. Diamond, C. Vanpouille-Box, S. Spada, N.P. Rudqvist, J.R. Chapman, B. M. Ueberheide, et al., Exosomes shuttle TREX1-sensitive IFN-stimulatory dsDNA from irradiated cancer cells to DCs, Canc. Immunol. Res. 6 (8) (2018) 910–920.
[31] A. Marcus, A.J. Mao, M. Lensink-Vasan, L. Wang, R.E. Vance, D.H. Raulet, Tumor-derived cGAMP triggers a STING-mediated interferon response in non-tumor cells to activate the NK cell response, Immunity 49 (4) (2018) 754–763, e4.
[32] N. Nagarsheth, M.S. Wicha, W. Zou, Chemokines in the cancer microenvironment and their relevance in cancer immunotherapy, Nat. Rev. Immunol. 17 (9) (2017) 559–572.
[33] E.E. Parkes, S.M. Walker, L.E. Taggart, N. McCabe, L.A. Knight, R. Wilkinson, et al., Activation of STING-dependent innate immune signaling by S-Phase-Specific DNA damage in breast cancer, J. Nat. Canc. Inst. 109 (1) (2016) djw199.
[34] R. Luo, E. Firat, S. Gaedicke, E. Guffart, T. Watanabe, G. Niedermann, Cisplatin facilitates radiation-induced abscopal effects in conjunction with PD-1 checkpoint blockade through CXCR3/CXCL10-mediated T-cell recruitment, Clin. Canc. Res. 25 (23) (2019) 7243.
[35] S. Matsumura, B. Wang, N. Kawashima, S. Braunstein, M. Badura, T.O. Cameron, et al., Radiation-induced CXCL16 release by breast cancer cells attracts effector T cells, J. Immunol. 181 (5) (2008) 3099–3107.
[36] F.M. Di Maggio, L. Minafra, G.I. Forte, F.P. Cammarata, D. Lio, C. Messa, et al., Portrait of inflammatory response to ionizing radiation treatment, J. Inflamm. 12 (2015) 14.
[37] D. Hallahan, J. Kuchibhotla, C. Wyble, Cell adhesion molecules mediate radiation-induced leukocyte adhesion to the vascular endothelium, Canc. Res. 56 (22) (1996) 5150–5155.
[38] S.C. Formenti, S. Demaria, Combining radiotherapy and cancer immunotherapy: a paradigm shift, JNCI (J. Natl. Cancer Inst.): J. Nat. Canc. Inst. 105 (4) (2013) 256–265.
[39] E.A. Reits, J.W. Hodge, C.A. Herberts, T.A. Groothuis, M. Chakraborty, E. K. Wansley, et al., Radiation modulates the peptide repertoire, enhances MHC class I expression, and induces successful antitumor immunotherapy, J. Exp. Med. 203 (5) (2006) 1259–1271.
[40] M.T. Dillon, K.F. Bergerhoff, M. Pedersen, H. Whittock, E. Crespo-Rodriguez, E. C. Patin, et al., ATR inhibition potentiates the radiation-induced inflammatory tumor microenvironment, Clin. Canc. Res. 25 (11) (2019) 3392–3403.
[41] A. Sharma, B. Bode, R.H. Wenger, K. Lehmann, A.A. Sartori, H. Moch, et al., gamma-Radiation promotes immunological recognition of cancer cells through increased expression of cancer-testis antigens in vitro and in vivo, PLoS One 6 (11) (2011), e28217.
[42] S.C. Formenti, N.P. Rudqvist, E. Golden, B. Cooper, E. Wennerberg, C. Lhuillier, et al., Radiotherapy induces responses of lung cancer to CTLA-4 blockade, Nat. Med. 24 (12) (2018) 1845–1851.
[43] M.G. Ruocco, K.A. Pilones, N. Kawashima, M. Cammer, J. Huang, J.S. Babb, et al., Suppressing T cell motility induced by anti-CTLA-4 monotherapy improves antitumor effects, J. Clin. Invest. 122 (10) (2012) 3718–3730.
[44] H. Liang, L. Deng, Y. Hou, X. Meng, X. Huang, E. Rao, et al., Host STING-dependent MDSC mobilization drives extrinsic radiation resistance, Nat. Commun. 8 (1) (2017) 1736.
[45] C. Vanpouille-Box, A. Alard, M.J. Aryankalayil, Y. Sarfraz, J.M. Diamond, R. J. Schneider, et al., DNA exonuclease Trex1 regulates radiotherapy-induced tumour immunogenicity, Nat. Commun. 8 (2017) 15618.
[46] Y. Hou, H. Liang, E. Rao, W. Zheng, X. Huang, L. Deng, et al., Non-canonical NF-κB antagonizes STING sensor-mediated DNA sensing in radiotherapy, Immunity 49 (3) (2018) 490–503, e4.
[47] J.L. Benci, L.R. Johnson, R. Choa, Y. Xu, J. Qiu, Z. Zhou, et al., Opposing functions of interferon coordinate adaptive and innate immune responses to cancer immune checkpoint blockade, Cell 178 (4) (2019) 933–948, e14.

[48] J.L. Benci, B. Xu, Y. Qiu, T.J. Wu, H. Dada, C. Twyman-Saint Victor, et al., Tumor interferon signaling regulates a multigenic resistance program to immune checkpoint blockade, Cell 167 (6) (2016) 1540–1554, e12.

[49] P. Jiang, S. Gu, D. Pan, J. Fu, A. Sahu, X. Hu, et al., Signatures of T cell dysfunction and exclusion predict cancer immunotherapy response, Nat. Med. 24 (10) (2018) 1550–1558.

[50] H.W. Stout-Delgado, Y. Getachew, B.C. Miller, D.L. Thiele, Intrahepatic lymphocyte expression of dipeptidyl peptidase I-processed granzyme B and perforin induces hepatocyte expression of serine proteinase inhibitor 6 (Serpinb9/SPI-6), J. Immunol. 179 (10) (2007) 6561–6567.

[51] J. Chen, Y. Cao, B. Markelc, J. Kaeppler, J.A. Vermeer, R.J. Muschel, Type I IFN protects cancer cells from CD8+ T cell-mediated cytotoxicity after radiation, J. Clin. Invest. 129 (10) (2019) 4224–4238.

[52] D.I. Gabrilovich, S. Nagaraj, Myeloid-derived suppressor cells as regulators of the immune system, Nat. Rev. Immunol. 9 (3) (2009) 162–174.

[53] A. Kalbasi, C. Komar, G.M. Tooker, M. Liu, J.W. Lee, W.L. Gladney, et al., Tumor-derived CCL2 mediates resistance to radiotherapy in pancreatic ductal adenocarcinoma, Clin. Canc. Res. 23 (1) (2017) 137–148.

[54] K.A. Connolly, B.A. Belt, N.M. Figueroa, A. Murthy, A. Patel, M. Kim, et al., Increasing the efficacy of radiotherapy by modulating the CCR2/CCR5 chemokine axes, Oncotarget 7 (52) (2016) 86522–86535.

[55] L. Surace, V. Lysenko, O. Fontana Andrea, V. Cecconi, H. Janssen, A. Bicvic, et al., Complement is a central mediator of radiotherapy-induced tumor-specific immunity and clinical response, Immunity 42 (4) (2015) 767–777.

[56] M. Yoneda, R. Imamura, H. Nitta, K. Taniguchi, F. Saito, K. Kikuchi, et al., Enhancement of cancer invasion and growth via the C5a-C5a receptor system: implications for cancer promotion by autoimmune diseases and association with cervical cancer invasion, Oncol. Lett. 17 (1) (2019) 913–920.

[57] E. Wennerberg, C. Lhuillier, C. Vanpouille-Box, K.A. Pilones, E. García-Martínez, N.-P. Rudqvist, et al., Barriers to radiation-induced in situ tumor vaccination, Front. Immunol. 8 (2017) 229.

[58] E.Y. Lin, J.-F. Li, L. Gnatovskiy, Y. Deng, L. Zhu, D.A. Grzesik, et al., Macrophages regulate the angiogenic switch in a mouse model of breast cancer, Canc. Res. 66 (23) (2006) 11238.

[59] S.-C. Liu, R. Alomran, S.B. Chernikova, F. Lartey, J. Stafford, T. Jang, et al., Blockade of SDF-1 after irradiation inhibits tumor recurrences of autochthonous brain tumors in rats, Neuro Oncol. 16 (1) (2013) 21–28.

[60] J.H. Stafford, T. Hirai, L. Deng, S.B. Chernikova, K. Urata, B.L. West, et al., Colony stimulating factor 1 receptor inhibition delays recurrence of glioblastoma after radiation by altering myeloid cell recruitment and polarization, Neuro Oncol. 18 (6) (2016) 797–806.

[61] J. Xu, J. Escamilla, S. Mok, J. David, S. Priceman, B. West, et al., CSF1R signaling blockade stanches tumor-infiltrating myeloid cells and improves the efficacy of radiotherapy in prostate cancer, Canc. Res. 73 (9) (2013) 2782–2794.

[62] B. Allard, M.S. Longhi, S.C. Robson, J. Stagg, The ectonucleotidases CD39 and CD73: novel checkpoint inhibitor targets, Immunol. Rev. 276 (1) (2017) 121–144.

[63] A. Schmidt, N. Oberle, P.H. Krammer, Molecular mechanisms of treg-mediated T cell suppression, Front. Immunol. 3 (2012) 51.

[64] B. Hosek, J. Bohacek, J. Sikulova, M. Pospisil, A. Vacek, Protection of early cellular damage in 1 Gy-irradiated mice by the elevation of extracellular adenosine, Radiat. Environ. Biophys. 31 (4) (1992) 289–297.

[65] M. Mondini, P.L. Loyher, P. Hamon, M. Gerbe de Thore, M. Laviron, K. Berthelot, et al., CCR2-Dependent recruitment of Tregs and monocytes following radiotherapy is associated with TNFalpha-mediated resistance, Canc. Immunol. Res. 7 (3) (2019) 376–387.

[66] M. Wang, X. Gou, L. Wang, Protein kinase B promotes radiation-induced regulatory T cell survival in bladder carcinoma, Scand. J. Immunol. 76 (1) (2012) 70–74.

[67] C.G. Li, M.R. He, F.L. Wu, Y.J. Li, A.M. Sun, Akt promotes irradiation-induced regulatory T-cell survival in hepatocellular carcinoma, Am. J. Med. Sci. 346 (2) (2013) 123–127.

[68] P.D. Bos, G. Plitas, D. Rudra, S.Y. Lee, A.Y. Rudensky, Transient regulatory T cell ablation deters oncogene-driven breast cancer and enhances radiotherapy, J. Exp. Med. 210 (11) (2013) 2435–2466.

[69] R.E. Langley, E.A. Bump, S.G. Quartuccio, D. Medeiros, S.J. Braunhut, Radiation-induced apoptosis in microvascular endothelial cells, Br. J. Canc. 75 (5) (1997) 666–672.

[70] M. Heckmann, K. Douwes, R. Peter, K. Degitz, Vascular activation of adhesion molecule mRNA and cell surface expression by ionizing radiation, Exp. Cell Res. 238 (1) (1998) 148–154.

[71] F. Paris, Z. Fuks, A. Kang, P. Capodieci, G. Juan, D. Ehleiter, et al., Endothelial apoptosis as the primary lesion initiating intestinal radiation damage in mice, Science 293 (5528) (2001) 293–297.

[72] J. Wang, M. Boerma, Q. Fu, M. Hauer-Jensen, Significance of endothelial dysfunction in the pathogenesis of early and delayed radiation enteropathy, World J. Gastroenterol. 13 (22) (2007) 3047–3055.

[73] C.W. Oh, E.A. Bump, J.S. Kim, D. Janigro, M.R. Mayberg, Induction of a senescence-like phenotype in bovine aortic endothelial cells by ionizing radiation, Radiat. Res. 156 (3) (2001) 232–240.

[74] Y. Wang, M. Boerma, D. Zhou, Ionizing radiation-induced endothelial cell senescence and cardiovascular diseases, Radiat. Res. 186 (2) (2016) 153–161.

[75] B.J. Moeller, Y. Cao, C.Y. Li, M.W. Dewhirst, Radiation activates HIF-1 to regulate vascular radiosensitivity in tumors: role of reoxygenation, free radicals, and stress granules, Canc. Cell 5 (5) (2004) 429–441.

[76] E. Edwards, L. Geng, J. Tan, H. Onishko, E. Donnelly, D.E. Hallahan, Phosphatidylinositol 3-kinase/Akt signaling in the response of vascular endothelium to ionizing radiation, Canc. Res. 62 (16) (2002) 4671–4677.

[77] A. Abdollahi, D.W. Griggs, H. Zieher, A. Roth, K.E. Lipson, R. Saffrich, et al., Inhibition of alpha(v)beta3 integrin survival signaling enhances antiangiogenic and antitumor effects of radiotherapy, Clin. Canc. Res. 11 (17) (2005) 6270–6279.

[78] P. Nguemgo Kouam, H. Bühler, T. Hero, I.A. Adamietz, The increased adhesion of tumor cells to endothelial cells after irradiation can be reduced by FAK-inhibition, Radiat. Oncol. 14 (1) (2019) 25.

[79] D.G. Baker, R.J. Krochak, The response of the microvascular system to radiation: a review, Canc. Invest. 7 (3) (1989) 287–294.

[80] S. Hoving, S. Heeneman, M.J. Gijbels, J.A. te Poele, N.S. Russell, M.J. Daemen, et al., Single-dose and fractionated irradiation promote initiation and progression of atherosclerosis and induce an inflammatory plaque phenotype in ApoE(-/-) mice, Int. J. Radiat. Oncol. Biol. Phys. 71 (3) (2008) 848–857.

[81] M.S. Chin, B.B. Freniere, C.F. Bonney, L. Lancerotto, J.H. Saleeby, Y.C. Lo, et al., Skin perfusion and oxygenation changes in radiation fibrosis, Plast. Reconstr. Surg. 131 (4) (2013) 707–716.

[82] R.K. Jain, Molecular regulation of vessel maturation, Nat. Med. 9 (6) (2003) 685–693.

[83] J. Denekamp, Vascular endothelium as the vulnerable element in tumours, Acta Radiol. Oncol. 23 (4) (1984) 217–225.

[84] M. Garcia-Barros, F. Paris, C. Cordon-Cardo, D. Lyden, S. Rafii, A. Haimovitz-Friedman, et al., Tumor response to radiotherapy regulated by endothelial cell apoptosis, Science 300 (5622) (2003) 1155–1159.

[85] M. Kioi, H. Vogel, G. Schultz, R.M. Hoffman, G.R. Harsh, J.M. Brown, Inhibition of vasculogenesis, but not angiogenesis, prevents the recurrence of glioblastoma after irradiation in mice, J. Clin. Invest. 120 (3) (2010) 694–705.

[86] S.V. Kozin, W.S. Kamoun, Y. Huang, M.R. Dawson, R.K. Jain, D.G. Duda, Recruitment of myeloid but not endothelial precursor cells facilitates tumor regrowth after local irradiation, Canc. Res. 70 (14) (2010) 5679–5685.

[87] O.Z. Lerman, M.R. Greives, S.P. Singh, V.D. Thanik, C.C. Chang, N. Seiser, et al., Low-dose radiation augments vasculogenesis signaling through HIF-1-dependent and -independent SDF-1 induction, Blood 116 (18) (2010) 3669–3676.

[88] M.W. Dewhirst, H. Kimura, S.W. Rehmus, R.D. Braun, D. Papahadjopoulos, K. Hong, et al., Microvascular studies on the origins of perfusion-limited hypoxia, Br. J. Canc. Suppl. 27 (1996) S247–S251.

[89] D.M. Brizel, G.S. Sibley, L.R. Prosnitz, R.L. Scher, M.W. Dewhirst, Tumor hypoxia adversely affects the prognosis of carcinoma of the head and neck, Int. J. Radiat. Oncol. Biol. Phys. 38 (2) (1997) 285–289.

[90] T. van den Beucken, E. Koch, K. Chu, R. Rupaimoole, P. Prickaerts, M. Adriaens, et al., Hypoxia promotes stem cell phenotypes and poor prognosis through epigenetic regulation of DICER, Nat. Commun. 5 (2014) 5203.

[91] O. Al-Assar, F. Demiciorglu, S. Lunardi, M.M. Gaspar-Carvalho, W.G. McKenna, R. M. Muschel, et al., Contextual regulation of pancreatic cancer stem cell phenotype and radioresistance by pancreatic stellate cells, Radiother. Oncol. 111 (2) (2014) 243–251.

[92] S. Rockwell, I.T. Dobrucki, E.Y. Kim, S.T. Marrison, V.T. Vu, Hypoxia and radiation therapy: past history, ongoing research, and future promise, Curr. Mol. Med. 9 (4) (2009) 442–458.

[93] G.L. Semenza, G.L. Wang, A nuclear factor induced by hypoxia via de novo protein synthesis binds to the human erythropoietin gene enhancer at a site required for transcriptional activation, Mol. Cell Biol. 12 (12) (1992) 5447–5454.

[94] Y.-H. Kim, K.-C. Yoo, Y.-H. Cui, N. Uddin, E.-J. Lim, M.-J. Kim, et al., Radiation promotes malignant progression of glioma cells through HIF-1alpha stabilization, Canc. Lett. 354 (1) (2014) 132–141.

[95] W. Luo, H. Hu, R. Chang, J. Zhong, M. Knabel, R. O'Meally, et al., Pyruvate kinase M2 is a PHD3-stimulated coactivator for hypoxia-inducible factor 1, Cell 145 (5) (2011) 732–744.

[96] G.L. Semenza, B.H. Jiang, S.W. Leung, R. Passantino, J.P. Concordet, P. Maire, et al., Hypoxia response elements in the aldolase A, enolase 1, and lactate dehydrogenase A gene promoters contain essential binding sites for hypoxia-inducible factor 1, J. Biol. Chem. 271 (51) (1996) 32529–32537.

[97] M.I. Koukourakis, A. Giatromanolaki, M. Panteliadou, S.E. Pouliliou, P. S. Chondrou, S. Mavropoulou, et al., Lactate dehydrogenase 5 isoenzyme overexpression defines resistance of prostate cancer to radiotherapy, Br. J. Canc. 110 (9) (2014) 2217–2223.

[98] A. Brand, K. Singer, E. Koehl Gudrun, M. Kolitzus, G. Schoenhammer, A. Thiel, et al., LDHA-associated lactic acid production blunts tumor immunosurveillance by T and NK cells, Cell Metabol. 24 (5) (2016) 657–671.

[99] D.C. Lee, H.A. Sohn, Z.Y. Park, S. Oh, Y.K. Kang, K.M. Lee, et al., A lactate-induced response to hypoxia, Cell 161 (3) (2015) 595–609.

[100] D. Singh, R. Arora, P. Kaur, B. Singh, R. Mannan, S. Arora, Overexpression of hypoxia-inducible factor and metabolic pathways: possible targets of cancer, Cell Biosci. 7 (2017) 62.

[101] L. Ren, Y. Yu, L. Wang, Z. Zhu, R. Lu, Z. Yao, Hypoxia-induced CCL28 promotes recruitment of regulatory T cells and tumor growth in liver cancer, Oncotarget 7 (46) (2016) 75763–75773.

[102] X. Ke, C. Chen, Y. Song, Q. Cai, J. Li, Y. Tang, et al., Hypoxia modifies the polarization of macrophages and their inflammatory microenvironment, and inhibits malignant behavior in cancer cells, Oncol. Lett. 18 (6) (2019) 5871–5878.

[103] A.L. Doedens, C. Stockmann, M.P. Rubinstein, D. Liao, N. Zhang, D.G. DeNardo, et al., Macrophage expression of hypoxia-inducible factor-1 alpha suppresses T-cell function and promotes tumor progression, Canc. Res. 70 (19) (2010) 7465–7475.

[104] C.A. Corzo, T. Condamine, L. Lu, M.J. Cotter, J.I. Youn, P. Cheng, et al., HIF-1alpha regulates function and differentiation of myeloid-derived suppressor cells in the tumor microenvironment, J. Exp. Med. 207 (11) (2010) 2439–2453.
[105] J. Sceneay, M.T. Chow, A. Chen, H.M. Halse, C.S. Wong, D.M. Andrews, et al., Primary tumor hypoxia recruits CD11b+/Ly6Cmed/Ly6G+ immune suppressor cells and compromises NK cell cytotoxicity in the premetastatic niche, Canc. Res. 72 (16) (2012) 3906–3911.
[106] Y. Kojima, A. Acar, E.N. Eaton, K.T. Mellody, C. Scheel, I. Ben-Porath, et al., Autocrine TGF-beta and stromal cell-derived factor-1 (SDF-1) signaling drives the evolution of tumor-promoting mammary stromal myofibroblasts, Proc. Natl. Acad. Sci. U. S. A. 107 (46) (2010) 20009–20014.
[107] Y. Liu, C. Meyer, A. Muller, F. Herweck, Q. Li, R. Mullenbach, et al., IL-13 induces connective tissue growth factor in rat hepatic stellate cells via TGF-beta-independent Smad signaling, J. Immunol. 187 (5) (2011) 2814–2823.
[108] Z. Wang, Y. Tang, Y. Tan, Q. Wei, W. Yu, Cancer-associated fibroblasts in radiotherapy: challenges and new opportunities, Cell Commun. Signal. : CCS 17 (1) (2019) 47.
[109] C.R. Arnold, J. Mangesius, Skvortsova II, U. Ganswindt, The role of cancer stem cells in radiation resistance, Front. Oncol. 10 (2020) 164.
[110] M.C. Boelens, T.J. Wu, B.Y. Nabet, B. Xu, Y. Qiu, T. Yoon, et al., Exosome transfer from stromal to breast cancer cells regulates therapy resistance pathways, Cell 159 (3) (2014) 499–513.
[111] C.C. Park, H.J. Zhang, E.S. Yao, C.J. Park, M.J. Bissell, Beta1 integrin inhibition dramatically enhances radiotherapy efficacy in human breast cancer xenografts, Canc. Res. 68 (11) (2008) 4398–4405.
[112] Y. Sun, J. Campisi, C. Higano, T.M. Beer, P. Porter, I. Coleman, et al., Treatment-induced damage to the tumor microenvironment promotes prostate cancer therapy resistance through WNT16B, Nat. Med. 18 (9) (2012) 1359–1368.
[113] S.J. Antonia, A. Villegas, D. Daniel, D. Vicente, S. Murakami, R. Hui, et al., Overall survival with durvalumab after chemoradiotherapy in stage III NSCLC, N. Engl. J. Med. 379 (24) (2018) 2342–2350.
[114] R.R. Weichselbaum, H. Liang, L. Deng, Y.X. Fu, Radiotherapy and immunotherapy: a beneficial liaison? Nat. Rev. Clin. Oncol. 14 (6) (2017) 365–379.
[115] C. Wujanto, B. Vellayappan, S. Siva, A.V. Louie, M. Guckenberger, B.J. Slotman, et al., Stereotactic body radiotherapy for oligometastatic disease in non-small cell lung cancer, Front. Oncol. 9 (2019) 1219.
[116] K. Manda, A. Glasow, D. Paape, G. Hildebrandt, Effects of ionizing radiation on the immune system with special emphasis on the interaction of dendritic and T cells, Front. Oncol. 2 (2012) 102.
[117] M. Bauer, M. Goldstein, M. Christmann, H. Becker, D. Heylmann, B. Kaina, Human monocytes are severely impaired in base and DNA double-strand break repair that renders them vulnerable to oxidative stress, Proc. Natl. Acad. Sci. Unit. States Am. 108 (52) (2011) 21105.
[118] M.P. Hildebrandt, C.N. Seed, C.A.S. Freemantle, P.R. Alam, K.R. Colville-Nash, G. Trott, Mechanisms of the anti-inflammatory activity of low-dose radiation therapy, Int. J. Radiat. Biol. 74 (3) (1998) 367–378.
[119] M.D. Cao, Z.D. Chen, Y. Xing, Gamma irradiation of human dendritic cells influences proliferation and cytokine profile of T cells in autologous mixed lymphocyte reaction, Cell Biol. Int. 28 (3) (2004) 223–228.
[120] E.L. Kachikwu, K.S. Iwamoto, Y.P. Liao, J.J. DeMarco, N. Agazaryan, J. S. Economou, et al., Radiation enhances regulatory T cell representation, Int. J. Radiat. Oncol. Biol. Phys. 81 (4) (2011) 1128–1135.
[121] F. Klug, H. Prakash, P.E. Huber, T. Seibel, N. Bender, N. Halama, et al., Low-dose irradiation programs macrophage differentiation to an iNOS(+)/M1 phenotype that orchestrates effective T cell immunotherapy, Canc. Cell 24 (5) (2013) 589–602.
[122] H. Gao, Z. Dong, X. Gong, J. Dong, Y. Zhang, W. Wei, et al., Effects of various radiation doses on induced T-helper cell differentiation and related cytokine secretion, J. Radiat. Res. 59 (4) (2018) 395–403.
[123] Z. Tabi, L.K. Spary, S. Coleman, A. Clayton, M.D. Mason, J. Staffurth, Resistance of CD45RA- T cells to apoptosis and functional impairment, and activation of tumor-antigen specific T cells during radiation therapy of prostate cancer, J. Immunol. 185 (2) (2010) 1330–1339.
[124] D. Schaue, B. Comin-Anduix, A. Ribas, L. Zhang, L. Goodglick, J.W. Sayre, et al., T-cell responses to survivin in cancer patients undergoing radiation therapy, Clin. Canc. Res. 14 (15) (2008) 4883–4890.
[125] M. Hareyama, K. Imai, A. Oouchi, H. Takahashi, Y. Hinoda, M. Tsujisaki, et al., The effect of radiation on the expression of intercellular adhesion molecule-1 of human adenocarcinoma cells, Int. J. Radiat. Oncol. Biol. Phys. 40 (3) (1998) 691–696.
[126] L. Galluzzi, A. Buqué, O. Kepp, L. Zitvogel, G. Kroemer, Immunogenic cell death in cancer and infectious disease, Nat. Rev. Immunol. 17 (2) (2017) 97–111.
[127] A.A. Lugade, J.P. Moran, S.A. Gerber, R.C. Rose, J.G. Frelinger, E.M. Lord, Local radiation therapy of B16 melanoma tumors increases the generation of tumor antigen-specific effector cells that traffic to the tumor, J. Immunol. 174 (12) (2005) 7516–7523.
[128] Y. Lee, S.L. Auh, Y. Wang, B. Burnette, Y. Meng, M. Beckett, et al., Therapeutic effects of ablative radiation on local tumor require CD8+ T cells: changing strategies for cancer treatment, Blood 114 (3) (2009) 589–595.
[129] I. Verbrugge, J. Hagekyriakou, L.L. Sharp, M. Galli, A. West, N.M. McLaughlin, et al., Radiotherapy increases the permissiveness of established mammary tumors to rejection by immunomodulatory antibodies, Canc. Res. 72 (13) (2012) 3163–3174.
[130] A. Filatenkov, J. Baker, A.M. Mueller, J. Kenkel, G.O. Ahn, S. Dutt, et al., Ablative tumor radiation can change the tumor immune cell microenvironment to induce durable complete remissions, Clin. Canc. Res. 21 (16) (2015) 3727–3739.
[131] D.J. Carlson, K.M. Yenice, C.G. Orton, Tumor hypoxia is an important mechanism of radioresistance in hypofractionated radiotherapy and must be considered in the treatment planning process, Med. Phys. 38 (12) (2011) 6347–6350.
[132] S.J. Dovedi, A.L. Adlard, G. Lipowska-Bhalla, C. McKenna, S. Jones, E.J. Cheadle, et al., Acquired resistance to fractionated radiotherapy can be overcome by concurrent PD-L1 blockade, Canc. Res. 74 (19) (2014) 5458–5468.
[133] H. Tang, Y. Liang, R.A. Anders, J.M. Taube, X. Qiu, A. Mulgaonkar, et al., PD-L1 on host cells is essential for PD-L1 blockade-mediated tumor regression, J. Clin. Invest. 128 (2) (2018) 580–588.
[134] R.S. Herbst, J.C. Soria, M. Kowanetz, G.D. Fine, O. Hamid, M.S. Gordon, et al., Predictive correlates of response to the anti-PD-L1 antibody MPDL3280A in cancer patients, Nature 515 (7528) (2014) 563–567.
[135] X. Zheng, Z. Fang, X. Liu, S. Deng, P. Zhou, X. Wang, et al., Increased vessel perfusion predicts the efficacy of immune checkpoint blockade, J. Clin. Invest. 128 (5) (2018) 2104–2115.
[136] L. Tian, A. Goldstein, H. Wang, H. Ching Lo, I. Sun Kim, T. Welte, et al., Mutual regulation of tumour vessel normalization and immunostimulatory reprogramming, Nature 544 (7649) (2017) 250–254.
[137] E.D. Kwon, C.G. Drake, H.I. Scher, K. Fizazi, A. Bossi, A.J. van den Eertwegh, et al., Ipilimumab versus placebo after radiotherapy in patients with metastatic castration-resistant prostate cancer that had progressed after docetaxel chemotherapy (CA184-043): a multicentre, randomised, double-blind, phase 3 trial, Lancet Oncol. 15 (7) (2014) 700–712.
[138] A. Maity, R. Mick, A.C. Huang, S.M. George, M.D. Farwell, J.N. Lukens, et al., A phase I trial of pembrolizumab with hypofractionated radiotherapy in patients with metastatic solid tumours, Br. J. Canc. 119 (10) (2018) 1200–1207.
[139] A.C. Tree, K. Jones, S. Hafeez, M.T.A. Sharabiani, K.J. Harrington, S. Lalondrelle, et al., Dose-limiting urinary toxicity with pembrolizumab combined with weekly hypofractionated radiation therapy in bladder cancer, Int. J. Radiat. Oncol. Biol. Phys. 101 (5) (2018) 1168–1171.
[140] D. Candas-Green, B. Xie, J. Huang, M. Fan, A. Wang, C. Menaa, et al., Dual blockade of CD47 and HER2 eliminates radioresistant breast cancer cells, Nat. Commun. 11 (1) (2020) 4591.
[141] S. Gholamin, O.A. Youssef, M. Rafat, R. Esparza, S. Kahn, M. Shahin, et al., Irradiation or temozolomide chemotherapy enhances anti-CD47 treatment of glioblastoma, Innate Immun. 26 (2) (2020) 130–137.
[142] A.L. Schwartz, P.R. Nath, M. Allgauer, E.C. Lessey-Morillon, J.M. Sipes, L. A. Ridnour, et al., Antisense targeting of CD47 enhances human cytotoxic T-cell activity and increases survival of mice bearing B16 melanoma when combined with anti-CTLA4 and tumor irradiation, Canc. Immunol. Immunother.: CII 68 (11) (2019) 1805–1817.
[143] D. Argyle, T. Kitamura, Targeting macrophage-recruiting chemokines as a novel therapeutic strategy to prevent the progression of solid tumors, Front. Immunol. 9 (2018) 2629.
[144] S.C. Formenti, P. Lee, S. Adams, J.D. Goldberg, X. Li, M.W. Xie, et al., Focal irradiation and systemic TGFbeta blockade in metastatic breast cancer, Clin. Canc. Res. 24 (11) (2018) 2493–2504.
[145] E. Wennerberg, S. Spada, N.-P. Rudqvist, C. Lhuillier, S. Gruber, Q. Chen, et al., CD73 blockade promotes dendritic cell infiltration of irradiated tumors and tumor rejection, Canc. Immunol. Res. 8 (4) (Apr 2020) 465–478, canimm.0449.2019.
[146] Y. Sun, L. Moretti, N.J. Giacalone, S. Schleicher, C.K. Speirs, D.P. Carbone, et al., Inhibition of JAK2 signaling by TG101209 enhances radiotherapy in lung cancer models, J. Thorac. Oncol. 6 (4) (2011) 699–706.
[147] R.S.A. Goedegebuure, L.K. de Klerk, A.J. Bass, S. Derks, V.L.J.L. Thijssen, Combining radiotherapy with anti-angiogenic therapy and immunotherapy; A therapeutic triad for cancer? Front. Immunol. 9 (2019) 3107.
[148] R.S. Finn, S. Qin, M. Ikeda, P.R. Galle, M. Ducreux, T.-Y. Kim, et al., Atezolizumab plus bevacizumab in unresectable hepatocellular carcinoma, N. Engl. J. Med. 382 (20) (2020) 1894–1905.
[149] A. Kawazoe, S. Fukuoka, Y. Nakamura, Y. Kuboki, M. Wakabayashi, S. Nomura, et al., Lenvatinib plus pembrolizumab in patients with advanced gastric cancer in the first-line or second-line setting (EPOC1706): an open-label, single-arm, phase 2 trial, Lancet Oncol. 21 (8) (2020) 1057–1065.
[150] V. Makker, D. Rasco, N.J. Vogelzang, M.S. Brose, A.L. Cohn, J. Mier, et al., Lenvatinib plus pembrolizumab in patients with advanced endometrial cancer: an interim analysis of a multicentre, open-label, single-arm, phase 2 trial, Lancet Oncol. 20 (5) (2019) 711–718.
[151] O. Demaria, A. De Gassart, S. Coso, N. Gestermann, J. Di Domizio, L. Flatz, et al., STING activation of tumor endothelial cells initiates spontaneous and therapeutic antitumor immunity, Proc. Natl. Acad. Sci. Unit. States Am. 112 (50) (2015) 15408.
[152] Z. Lwin, C. Gomez-Roca, E. Saada-Bouzid, E. Yanez, F.L. Muñoz, S.A. Im, et al., LBA41 LEAP-005: phase II study of lenvatinib (len) plus pembrolizumab (pembro) in patients (pts) with previously treated advanced solid tumours, Ann. Oncol. 31 (2020) S1170.
[153] M. McLaughlin, E.C. Patin, M. Pedersen, A. Wilkins, M.T. Dillon, A.A. Melcher, et al., Inflammatory microenvironment remodelling by tumour cells after radiotherapy, Nat. Rev. Canc. (2020).
[154] P.G. Pilié, C. Tang, G.B. Mills, T.A. Yap, State-of-the-art strategies for targeting the DNA damage response in cancer, Nat. Rev. Clin. Oncol. 16 (2) (2019) 81–104.
[155] M.T. Dillon, H.E. Barker, M. Pedersen, H. Hafsi, S.A. Bhide, K.L. Newbold, et al., Radiosensitization by the ATR inhibitor AZD6738 through generation of acentric micronuclei, Mol. Canc. Therapeut. 16 (1) (2017) 25–34.

[156] F.P. Vendetti, P. Karukonda, D.A. Clump, T. Teo, R. Lalonde, K. Nugent, et al., ATR kinase inhibitor AZD6738 potentiates CD8+ T cell-dependent antitumor activity following radiation, J. Clin. Invest. 128 (9) (2018) 3926–3940.
[157] M.T. Dillon, Z. Boylan, D. Smith, J. Guevara, K. Mohammed, C. Peckitt, et al., 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, Clin. Transl. Radiat. Oncol. 12 (2018) 16–20.
[158] P. Patel, L. Sun, Y. Robbins, P.E. Clavijo, J. Friedman, C. Silvin, et al., Enhancing direct cytotoxicity and response to immune checkpoint blockade following ionizing radiation with Wee1 kinase inhibition, OncoImmunology 8 (11) (2019), e1638207.
[159] J.M. Zaretsky, A. Garcia-Diaz, D.S. Shin, H. Escuin-Ordinas, W. Hugo, S. Hu-Lieskovan, et al., Mutations associated with acquired resistance to PD-1 blockade in melanoma, N. Engl. J. Med. 375 (9) (2016) 819–829.
[160] L.J.M. Perus, L.A. Walsh, Microenvironmental heterogeneity in brain malignancies, Front. Immunol. 10 (2019) 2294.
[161] Y. Yan, A.A. Leontovich, M.J. Gerdes, K. Desai, J. Dong, A. Sood, et al., Understanding heterogeneous tumor microenvironment in metastatic melanoma, PloS One 14 (6) (2019), e0216485.
[162] F. Mpekris, C. Voutouri, J.W. Baish, D.G. Duda, L.L. Munn, T. Stylianopoulos, et al., Combining microenvironment normalization strategies to improve cancer immunotherapy, Proc. Natl. Acad. Sci. Unit. States Am. 117 (7) (2020) 3728.