The tumour microenvironment after radiotherapy: mechanisms of resistance and recurrence

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Abstract | Radiotherapy plays a central part in curing cancer. For decades, most research on improving treatment outcomes has focused on modulating radiation-induced biological effects on cancer cells. Recently, we have better understood that components within the tumour microenvironment have pivotal roles in determining treatment outcomes. In this Review, we describe vascular, stromal and immunological changes that are induced in the tumour microenvironment by irradiation and discuss how these changes may promote radioresistance and tumour recurrence. We also highlight how this knowledge is guiding the development of new treatment paradigms in which biologically targeted agents will be combined with radiotherapy.

COMMA-D cells
An epithelial cell line derived from the mammary tissue of a mid-pregnant BALB/c mouse, which exhibits many characteristics distinctive of normal mammary epithelial cells.

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Radiotherapy is used to treat up to 50% of cancer patients and to manage 40% of patients who are cured[1]. For decades, research into improving outcomes from radiotherapy focused almost entirely on the cancer cell itself, ignoring complex biological interactions between the tumour and the stroma in which it grows — the so-called tumour microenvironment (TME). As a result, classic radiobiology mostly failed to appreciate that the effects of radiotherapy on the TME, and the responses that are triggered within it, may be crucial in determining the success or failure of therapy. Moreover, preclinical studies in some tumour models have suggested that radiotherapy-induced changes in the TME might, in fact, promote tumour invasion and spread in certain situations — even though decades of clinical experience has failed to show clear proof that radiotherapy promotes invasion and metastasis in patients. Therefore, attempts to combine radiotherapy with new biologically targeted modalities were often predicated on their potential to enhance radiotherapy-induced cancer cell death, rather than on their potential to re-engineer biological processes within the TME[2].

Over the past two decades, this narrow radiobiological view has shifted towards recognizing the central importance of the TME[3–5]. The initial formulation of the hallmarks of cancer described cancers as complex tissues containing multiple cell types that participate in heterotypic interactions with one another[6]. At around the same time as these cancer hallmarks were being defined, evidence that an irradiated stroma might favour tumour growth emerged with the observation that COMMA-D cells, which exhibit several characteristics of normal mammary epithelial cells and are rarely tumorigenic, formed large tumours when implanted into pre-irradiated fat pads of syngeneic hosts[7]. Since then, a considerable body of work has shown that radiation oncologists must take the TME into account, not only its ability to promote radioresistance and recurrence but also as a legitimate therapeutic target in its own right.

Although a detailed explanation of the current state of understanding of the radiobiological model relating to radiotherapy has been reviewed elsewhere[8], in this Review, we focus on the mechanisms of radioresistance that are mediated by the tumour stroma and explore how these can be targeted to improve radiotherapy responses. We briefly discuss early and late radiotherapy-mediated effects on normal tissue, as normal tissue toxicity limits the dose of radiation that can be used in cancer treatment. With respect to tumours, we address the effects of radiotherapy on hypoxia, fibrotic responses and immune activation within the TME to understand how they may confer initial resistance or promote subsequent loco-regional or distant recurrence (FIG. 1). We emphasize throughout the potential for developing novel, mechanism-based, targeted therapies that will exert favourable effects on the TME.

Effects of radiotherapy on the TME

Effects on the vasculature. Possibly the most well-studied components of the TME with respect to radiation are endothelial cells and the tumour vasculature.

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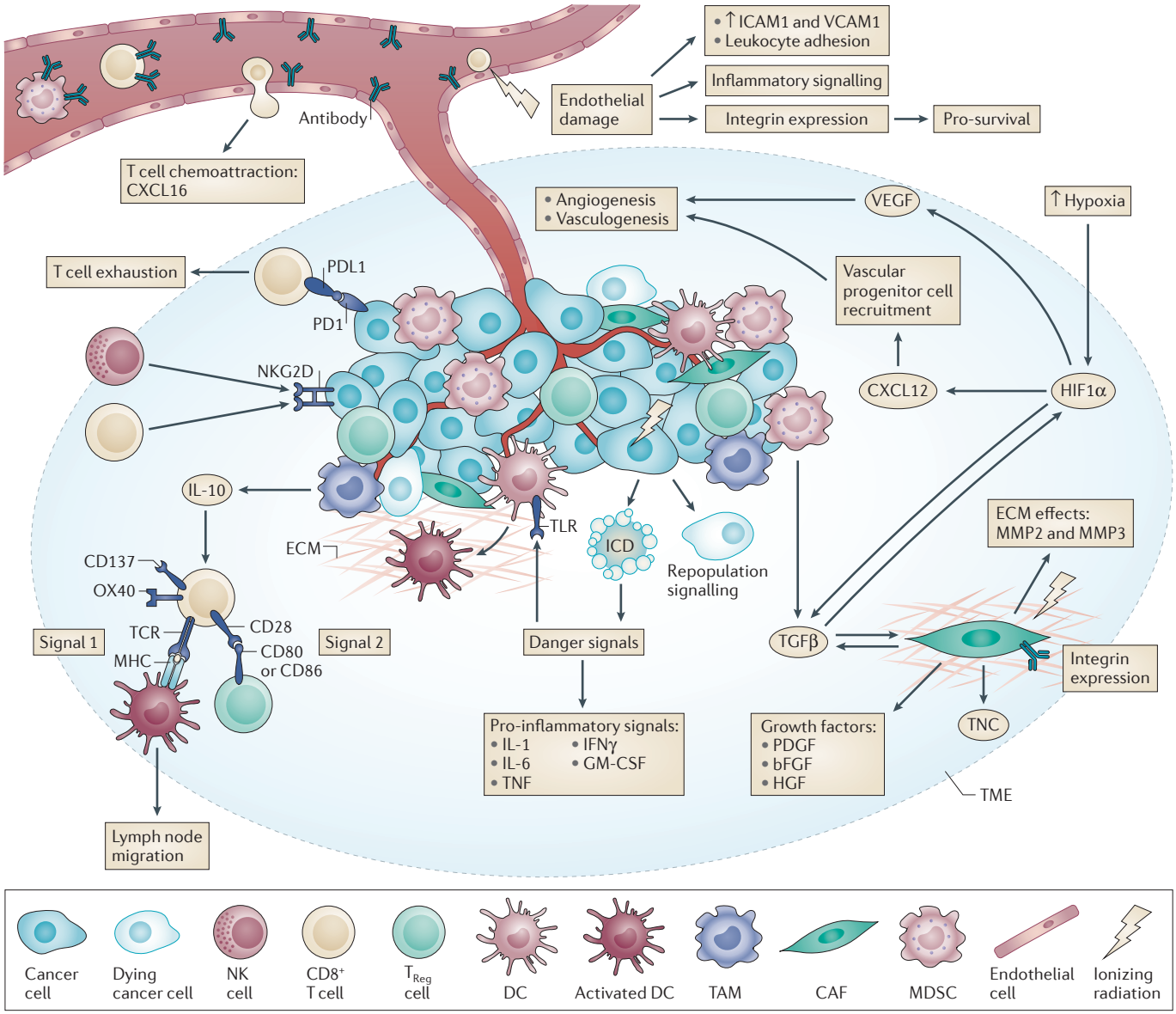


Figure 1 | **Radiation effects on the TME.** Damage from ionizing radiation leads to effects on numerous cell types within the tumour microenvironment (TME). Tumour endothelial cells are sensitive to radiation, and the death of these cells initiates the inflammation cascade. Damage also leads to increased intercellular adhesion molecule 1 (ICAM1) and vascular cell adhesion molecule 1 (VCAM1) expression and leads to the increased attraction of innate immune cells. The upregulation of integrins on endothelial cells leads to increased survival, which acts as a method of radioresistance. Vascular depletion potentiates the effects of hypoxia, leading to hypoxia-inducible factor 1α (HIF1 α) signalling and to pro-angiogenic stimuli through vascular endothelial growth factor (VEGF) and pro-vasculogenic stimuli through chemokine (C-X-C motif) ligand 12 (CXCL12). Cancer-associated fibroblast (CAF) activation following radiation leads to altered growth factor secretion and to the release of numerous modulators of the extracellular matrix (ECM) and cytokines. Transforming growth factor-β (TGFβ) signalling is complex and pleiotropic — it directly affects tumour cells and CAFs, drives HIF1α signalling and reduces the activation of T cells and dendritic cells (DCs). Within the immune compartment, increased tumour cell antigen availability and increased antigen processing by higher mTOR levels combine with a damage-associated molecular pattern (DAMP)-related Toll-like receptor (TLR) response and increased pro-inflammatory cytokine signalling to activate DCs and thus T cells; activated DCs also migrate to proximal lymph nodes. This signalling is often blocked by high expression of cytotoxic T lymphocyte antigen 4 (CTLA4) by regulatory T cell (T_{Reg} cell) which inhibits co-stimulation within the TME. Although radiation also upregulates NKG2D signals on tumour cells, which allows direct cytotoxic effects by natural killer (NK) cells and $CD8^+$ T cells, other tumour escape mechanisms such as programmed cell death protein 1 ligand 1 (PDL1) signalling and myeloid-derived suppressor cell (MDSC)-derived interleukin-10 (IL-10) immunosuppression remain intact. bFGF, basic fibroblast growth factor; GM-CSF, granulocyte–macrophage colony-stimulating factor; HGF, hepatocyte growth factor; ICD, immunogenic cell death; IFNγ, interferon-γ; MHC, major histocompatibility complex; MMP, matrix metalloproteinase; PDGF, platelet-derived growth factor; TAM, tumour-associated macrophage; TCR, T cell receptor; TNC, tenascin C; TNF, tumour necrosis factor.

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Atherosclerosis
Thickening of the artery wall as a result of white blood cell invasion and accumulation.

Medial necrosis
Necrosis of the middle portion of vessel walls (anatomically called the tunica media).

Vessel co-option
A mechanism by which tumours obtain a blood supply by hijacking the existing vasculature.

Radiation induces endothelial cell dysfunction, which is characterized by increased permeability, detachment from the underlying basement membrane and apoptosis[9,10]. High single-fraction doses (8–16 Gy) have been linked to the upregulation of acid sphingomyelinase (ASMase), which induces endothelial cell apoptosis[11]. Endothelial cell dysfunction and apoptosis contribute to post-irradiation inflammation and fibrosis. Within vessels, irradiation generates a pro-thrombotic state, which is characterized by platelet aggregation, microthrombus formation and increased adhesion of inflammatory cells to endothelial cells with subsequent diapedesis into the perivascular space[12].

Structurally, irradiation of the vasculature causes the dose-dependent destruction of blood vessels, which especially affects the microvasculature[13]. This reduced vascular density increases the distances between functioning vessels, meaning that some parenchymal tissues are not perfused. Over time, irradiated blood vessels become thicker — primarily owing to thickening of the intimal layer — and are prone to atherosclerosis[14–17]. Other late morphological changes can also occur, including the development of thrombosis, fibrosis and medial necrosis. Larger single-fraction radiation doses (15–20 Gy) may permanently reduce blood flow, suggesting that irreversible changes to the vascular architecture can occur[18]. Early and late responses of normal tissue to irradiation are dose-limiting factors in radiotherapy, affecting therapeutic efficacy as well as the quality of life of cancer patients (FIG. 2).

Understanding the effect of radiation on the functional status of the tumour microvasculature is important for maximizing the efficacy of radiotherapy. Radiotherapy-induced changes in the tumour vasculature remain dependent on total dose and fraction size, as well as the type, location and stage of the tumour itself[19]. Depending on how they are derived (that is, through angiogenesis, vasculogenesis or vessel co-option), tumour vessels may lack basement membrane and pericyte coverage, rendering them more permeable and leaky and more radiosensitive than vessels in the surrounding normal tissue[20]. Endothelial cells within the TME are characterized by rapid proliferation rates, which contribute to their inherent radiosensitivity. Each endothelial cell supports the growth of around 2,000 cancer cells[21], and their responses to radiotherapy — including mechanisms of recovery and survival — have been the focus of research since they were first postulated as a critical target in tumour control. Similar to high-dose irradiation of normal tissues[11], single, large radiation

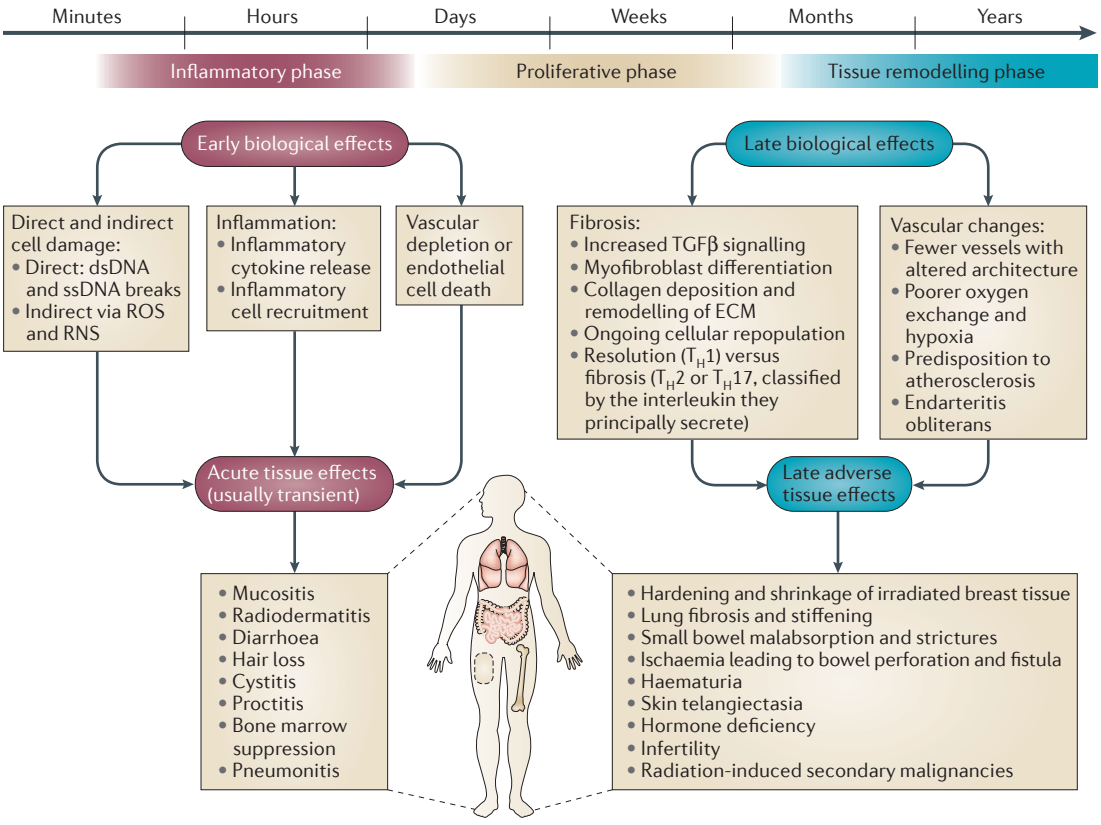


Figure 2 | **Biological effects and normal tissue toxicity after radiotherapy.** Early biological events cause acute tissue effects, which are usually transient and normally resolve within 3 months of completing treatment. These events also result in more protracted biological effects that can manifest in tissues as late biological effects and secondary malignancies. Higher radiation dose per fraction seems to increase the severity of late adverse effects. dsDNA, double-stranded DNA; ECM, extracellular matrix; ROS, reactive oxygen species; RNS, reactive nitrogen species; ssDNA, single-stranded DNA; TGFβ, transforming growth factor-β; T_H, T helper cell.

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Microvascular injury
Injury to the fine network of blood vessels and capillaries that results in changed patterns of blood flow.

doses have been shown to induce ASMase-dependent microvascular damage in fibrosarcoma and melanoma xenograft models in the first 6 hours, with subsequent tumour cell death[22].

Radiation-induced vascular damage potentiates tumour hypoxia and triggers immune responses through the increased production of cytokines and chemokines that induce immune cell recruitment. Subsequent tumour revascularization occurs via hypoxia-inducible factor 1α (HIF1α)-dependent and HIF1α-independent recruitment of bone marrow-derived cells (BMDCs)[23–27].

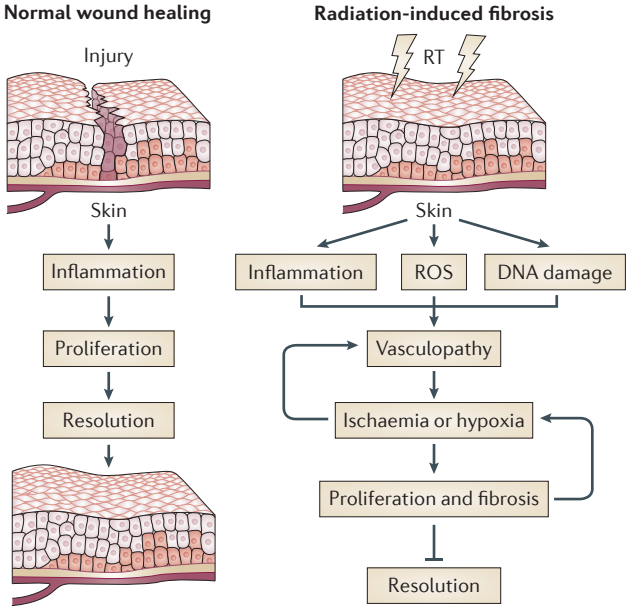
Effects on the stroma. Chronic inflammation is the main driver of fibrosis, in which persistent immune responses occur alongside tissue remodelling and repair processes. Within normal tissues, infiltration of inflammatory cells triggers myofibroblast transformation, resulting in the sustained production of growth factors (such as transforming growth factor-β (TGFβ) and connective tissue growth factor (CTGF)[28,29]), proteolytic enzymes, and angiogenic and/or fibrogenic cytokines that ultimately leads to excess deposition of components of the extracellular matrix (ECM) and scarring of the tissues involved[30]. The inflammatory processes that arise immediately after radiotherapy are not the same as those that occur decades later (FIG. 2). Although the triggers in wound healing, normal tissue fibrosis and radiation fibrosis differ, the pathophysiological processes that ensue show some common features that implicate the immune system, the activation of stromal cells (such as myofibroblasts) and self-propagating signalling networks that result in a progressive phenotype[31] (BOX 1). As with radiotherapy-induced microvascular injury, prevention of fibroproliferative processes is important for maintaining normal organ function and thus improving quality of life following radiotherapy.

Cancer-associated fibroblasts (CAFs) are a heterogeneous cell population that constitute the majority of cells within the stroma in many carcinomas. CAFs often have a spindle-shaped, mesenchymal morphology and are classically identified either as fibroblast activation protein (FAP)- and fibroblast-specific protein (FSP)-positive fibroblasts or as α-smooth muscle actin (sma)-positive myofibroblasts, depending on their origin[32]. They secrete ECM proteins (such as tenascin C (TNC) and collagen I), cytokines (such as hepatocyte growth factor (HGF), platelet-derived growth factor (PDGF) and chemokine (C-X-C motif) ligand 12 (CXCL12; also known as stromal cell-derived factor 1)), and matrix remodelling enzymes (such as matrix metalloproteinases (MMPs))[33,34]. Unlike myofibroblasts that arise in response to inflammation or wound healing, CAFs may be resistant to apoptosis and irreversibly activated[35]. The heterogeneous nature of CAFs varies according to tumour type and stage of disease progression, and may also determine whether they exhibit tumour-promoting or tumour-inhibiting roles[36]. TGFβ is a potent activator of fibroblasts and fibrocytes[37–39], and is also a key CAF-secreted factor, along with CXCL12, which participates in autocrine signalling loops during tumour progression[40]. As well as cytokine-mediated activation, CAFs can obtain an activated phenotype through interaction with other cells in the TME, such as leukocytes and macrophages[41].

Therapeutic irradiation of tumours will affect CAFs, but few detailed studies have been carried out. Moreover, very few studies have used CAFs that have been freshly isolated from human tumours. Hellevik *et al.* demonstrated that CAFs isolated from non-small-cell lung cancer (NSCLC) samples survived ablative radiation but lost their tumour-invasive capability[42]. They proposed that radiation impeded CAF mobility by stabilizing focal contacts through increased integrin (α2, β1 and α5) expression;

Box 1 | **Normal wound healing versus radiation fibrosis**

The normal phases of wound healing progress from the injurious stimulus to inflammation, proliferation and finally resolution. By contrast, radiation generates reactive oxygen species (ROS), DNA damage and inflammation as the early stimuli that mediate the activation of a dysregulated proliferative phase. Unlike wound healing, radiation exerts a field effect on the vascular compartment. Endothelial dysfunction leads to a progressive vasculopathy that is characterized by impaired gaseous exchange and the development of tissue hypoxia, which drive an uncontrolled proliferative stage that replaces the normal proliferative phase of wound healing. This may represent a poorly coordinated haemostatic response that aims to preserve tissue oxygenation. The progressive and perpetuating proliferative phase prevents tissue resolution and results in the development of late adverse effects (see the figure; arrows represent positive feedback loops). RT, radiotherapy.



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formation of integrin-mediated attachments may facilitate CAF survival. Indeed, several preclinical studies have implicated β1 integrin in tumour survival following radiotherapy[43–45]. More recently, it was proposed that β1 integrin signalling in pancreatic cancer cells is required for stromal-mediated radioprotection[46]. Malignancies with a pronounced desmoplastic reaction may therefore be inherently radioresistant owing, in part, to integrin-mediated interactions between cancer cells, stromal cells and the surrounding ECM. In addition, integrin α11β1 has been shown to induce myofibroblast activation[47], and integrin αVβ6 was reported to be upregulated in irradiated lungs immediately before the onset of fibrosis[48], suggesting that integrins may also be involved in the development of radiation fibrosis.

Effects on the immune system. Tumours evolve to evade immune recognition[49], such that immune escape is now considered a hallmark of cancer[50]. This, combined with other intrinsic and extrinsic survival pressures such as resistance to hypoxia and rapid growth, probably results in the observed state of chronic inflammation[51–53] and adaptive immunity suppression. This modulation is maintained by altered cytokine signalling (tumour necrosis factor (TNF), interleukin-1β (IL-1β), IL-6, IL-10 and TGFβ[54,55]) and the recruitment of locally suppressive immune cells (tumour-associated macrophages (TAMs)[56], myeloid-derived suppressor cells (MDSCs) and regulatory T cells (T_{Reg} cells)). After radiotherapy, a relative increase in the number of these locally suppressive cell types, which are less radiosensitive than other lymphocyte subsets[57,58], is counterbalanced by the recruitment of circulating immune cells and increased antigen exposure and presentation. These changes profoundly affect the immune compartment of the TME, potentially re-priming it for an adaptive immune response.

After radiotherapy, inflammatory signals occur through the activation of cell survival pathways and the stimulation of the innate immune system. Both direct and indirect radiation effects trigger inflammatory cytokine signalling (IL-1 and TNF) and immune cell recruitment (for example, by endothelial cell expression of intercellular adhesion molecule 1 (ICAM1), vascular cell adhesion molecule 1 (VCAM1) and E-selectin). These events are principally coordinated by the intracellular actions of reactive oxygen species (ROS) and nuclear factor-κB (NF-κB)[59]. ROS also affect TNF signalling pathways[60] and can activate NF-κB, leading to further TNF production. The resulting cellular stress and death may also stimulate an immune response through the generation of damage-associated molecular patterns (DAMPs) and their corresponding pattern recognition receptors (PRRs)[59].

The PRR family includes Toll-like receptors (TLRs), nucleotide-binding oligomerization domain-like receptors (NLRs), RIG-I-like receptors and C-type lectin receptors[61]. The recognition of pathogen-associated molecular patterns (PAMPs) links stimuli from pathogenic sources to an inflammatory response. There is an analogous pathway that responds to endogenous damage via DAMPs, for example, after radiotherapy[62]. DAMPs can be subdivided into three groups: those exposed on the cell surface (for example, calreticulin); passively released substances (for example, high-mobility-group box 1 (HMGB1) proteins); and those actively secreted (for example, ATP)[63]. After tumour irradiation, a DAMP–PRR-mediated damage response may lead to the immunogenic cell death (ICD) of cancer cells, changing the predominant TME cytokine profile towards an immunostimulatory profile[63], activating dendritic cells (DCs) and maturing them into effective antigen-presenting cells (APCs)[64]. ICD is recognized as being important for triggering an effective antitumour immune response (FIG. 3a). However, the overall effects of radiotherapy-induced ICD responses are complex. Checkpoint inhibition by T_{Reg} cells, which express cytotoxic T lymphocyte antigen 4 (CTLA4)[65] that affects the activation of $CD8^+$ T cells (FIG. 3b), may still prevent the development of effective adaptive immunity to tumour cells. However, signal transducer and activator of transcription 1 (STAT1), downstream of interferon-β (IFNβ) signalling, drives chemokine expression (notably, CXCL9 and CXCL10), which recruits C-X-C chemokine receptor 3 (CXCR3)-positive T cells[66]. Radiotherapy-induced CXCL16 expression in the vasculature of the TME also recruits CXCR6-positive activated $CD8^+$ T cells, which have displayed antitumour effects in a murine breast cancer model[67]. Radiotherapy-induced effects on the TME therefore also lead to enhanced immunostimulatory T cell recruitment[68,69].

T cell activation requires several signals: antigens in an appropriate major histocompatibility complex (MHC) binding to a T cell receptor (TCR); co-stimulatory signals (for example, from an APC); and background levels of cytokine stimulation. Negative feedback from the upregulation of CTLA4 helps to control this process by competitively inhibiting co-stimulatory signals from CD80 (also known as B7-1) and CD86 (also known as B7-2), which are expressed on APCs, to CD28, which is expressed on T cells[65]. This is known as immune tolerization (FIG. 3b).Thus, radiotherapy primes the immune system against cancer cells via ICD, but potentially remains constrained by the enhanced actions of suppressive immune cells. Overcoming these effects to harness the potential of the immune system against cancer cells can generate beneficial local and abscopal effects. Unlocking abscopal effects in particular is the subject of a plethora of active research after case reports suggested that localized radiotherapy combined with immunomodulation may unlock the anti-metastatic potential of the adaptive immune system[70–75].

In summary, complex reactions by the immune system to an irradiated TME are neither wholly immunostimulatory nor immunosuppressive. They comprise effects on cells that are intrinsic to the TME, such as altered production of inflammatory cytokines, antigen exposure and DC priming, as well as relative increases in radioresistant immunosuppressive macrophage and T cell populations. Multifactorial signalling from both tumour ICD and radiotherapy-induced changes to endothelial cells also lead to an influx of circulating immune cells, which are more immunostimulatory than those that are inherent to the TME.

Desmoplastic reaction
A stromal reaction that can be induced by tissue injury, wound repair or cancer growth. Increased extracellular matrix and growth factor production and secretion result in the formation of scar-like fibrotic tissue.

Adaptive immunity
A carefully regulated, adaptable, specific immune response comprising humoral- and cell-mediated components. It is capable of both systemic actions and immunological memory to specific stimuli. It is triggered by known antigens or by appropriate antigen presentation from the innate immune system.

Tumour associated macrophages
(TAMs). Macrophages within the tumour microenvironment; they are generally immunosuppressive and resemble the alternatively activated M2 macrophage.

Regulatory T cells
(T_{Reg} cells). A T cell subset that exerts immunosuppressive and tolerizing effects. T_{Reg} cells have an important role in cancer immune editing, in the maintenance of a permissive cancer microenvironment and in preventing effective adaptive immune recognition of tumour cells.

Innate immune system
The initial immune response that occurs in a generic manner to inflammatory stimuli, comprising complement activation and immune cell recruitment and activation. It coordinates the activation of the adaptive immune system by antigen presentation.

Direct and indirect radiation effects
Radiation damage can be divided into direct effects, where the damage is a result of the ionizing radiation itself, and indirect effects, which refer to the resultant changes in cellular pathways as a result of radiation: for example, as a result of reactive oxygen species.

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Damage-associated molecular patterns (DAMPs). Stimuli released by stressed, dying or injured cells that may trigger an inflammatory response by the activation of a number of pattern recognition receptors.

Therefore, radiotherapy is not a perfect mechanism for triggering ICD of cancer cells with subsequent effective adaptive responses. However, key events, such as the change in immunostimulatory cytokines, DC maturation and activation, and T cell recruitment and stimulation, mean that radiotherapy does have the potential to prime potent responses against tumour cells if important immunosuppressive effects can be overcome.

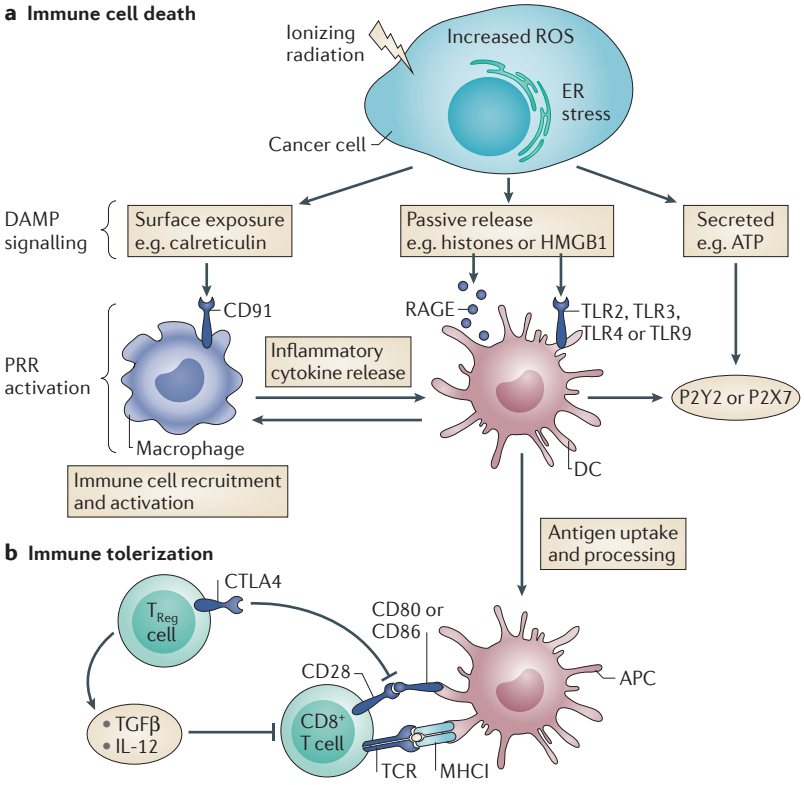


Figure 3 | **ICD of cancer cells and immune tolerization. a** | Radiotherapy damage to cancer cells leads to cell stress, which is mediated by reactive oxygen species (ROS), and endoplasmic reticulum (ER) stress; this in turn results in damage-associated molecular pattern (DAMP) signalling. Three main types of DAMP signalling are now recognized: surface exposure, passive release and active secretion[57]. Cell stress, apoptosis and necrosis probably each result in a different pattern of DAMP signalling. Multiple DAMP signal activation seems to be necessary to achieve effective immunogenic cell death (ICD) in cancer cells to cause an adaptive immune response. DAMPs signal through multiple pattern recognition receptor (PRR) types; for example, HSP90 or calreticulin surface exposure leads to phagocytosis that is mediated by CD91 (expressed on a variety of innate immune cells); high-mobility-group box 1 (HMGB1) release activates Toll-like receptor 2 (TLR2) and TLR4, which in turn lead to MYD88 signalling, dendritic cell (DC) activation and increased production of inflammatory cytokines; ATP secretion activates purinergic receptors P2Y2 and P2X7, which have wide-ranging immunostimulatory actions across DCs, natural killer cells, macrophages and T cells. Effective ICD of cancer cells therefore increases background inflammatory signalling and generates activated DCs to act as antigen-presenting cells (APCs). **b** | APCs present processed antigens on major histocompatibility complex class I (MHCI) receptors to $CD8^+$ T cells via their corresponding T cell receptors (TCRs). Co-stimulation is required to achieve T cell activation. This can be achieved by the interaction of CD80 and CD86 on the APC with CD28 on the $CD8^+$ T cell. Regulatory T cells (T_{Reg} cells) express cytotoxic T lymphocyte antigen 4 (CTLA4), which has a higher affinity for CD80 and CD86 than CD28 and thus effectively inactivates the co-stimulatory signal, leading to ineffective T cell activation. Overcoming this process with appropriate immunomodulation allows for the effective exposure of cancer cells to the immune system and the exciting prospect of novel cancer treatments. IL-12, interleukin-12; RAGE, receptor for advanced glycation end products; TGFβ, transforming growth factor-β.

Targeting TME-mediated radioresistance

Many strategies have been proposed for overcoming radioresistance in tumour cells[2], but too little attention has been given to TME-mediated resistance mechanisms and how they might be circumvented. There are currently drugs that target various aspects of the TME being trialled as monotherapies or in combination with radiotherapy to treat cancer. For example, many drugs have shown efficacy against fibrosis but have so far not been tested for their ability to reduce radiotherapy-mediated late adverse effects in the TME or to prevent tumour recurrence and metastasis. We hypothesize that a number of these will improve the prognosis of patients after radiotherapy and we also propose several immune and vascular targets that may show efficacy in radiosensitizing the TME in future studies (TABLE 1).

Targeting hypoxia. Hypoxia is a key regulatory factor in tumour growth[76] and has long been known to have a crucial role in radiotherapy resistance[77] (BOX 2). Most solid tumours contain zones where oxygen tensions fluctuate between almost anoxic (0% oxygen), hypoxic (1% (7.5 mmHg) oxygen) and normoxic (8% (60 mmHg) oxygen)[78]. High tumour cell proliferation rates and the abnormal structure of the tumour vasculature lead to both chronic, diffusion-limited hypoxia and acute, transient, perfusion-limited hypoxia[79,80]. Furthermore, changes in the TME as a result of radiotherapy can result in regions of cycling hypoxia[81]. Following radiotherapy, lack of oxygen reduces the production of reactive and cytotoxic species, such as ROS, and ultimately prevents irreparable DNA damage from occurring in cancer cells, preventing cancer cell death. Hypoxia also upregulates HIF1α, which independently promotes radioresistance (see below)[82]. Radioresistance manifests in oxygen tensions of <10 mmHg and becomes maximal at ~0.5 mmHg. Brizel *et al.* reported post-radiotherapy disease-free survival rates of 78% in patients with tumours that had median oxygen tensions of >10 mmHg versus rates of 22% in patients with tumours that had median oxygen tensions of <10 mmHg (REF. 83).

Therapeutic attempts to restore tumour oxygenation before or during radiotherapy have included red blood cell transfusion, erythropoietin administration and hyperbaric oxygen (HBO) treatment[84]. Despite HBO treatment having a therapeutic benefit in patients with head and neck squamous cell carcinoma (HNSCC)[77], inconsistent clinical trial data for other cancer types and huge logistical hurdles have impeded the widespread uptake of this approach. Accelerated radiotherapy with carbogen and nicotinamide (ARCON) has since been developed to overcome tumour hypoxia and is currently being evaluated in Phase III clinical trials[85–87]. However, despite promising preclinical studies, clinical trials have so far ended with conflicting and inconclusive results with respect to improving local tumour control. Similarly, clinical trials of early nitroimidazole derivatives that act as oxygen mimetics in the radiochemical process have been inconsistent, and severe dose-limiting toxic effects have restricted their development[88–90]. However, water-soluble compounds with improved toxicity profiles,

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Table 1 | **Current and future TME targets for radiosensitization**

Resistance mechanism	Drugs	Targets	Mode of action
Immune response	Ipilimumab	CTLA4	T cell activation
	Nivolumab and pembrolizumab	PD1	T cell exhaustion
	Imiquimod	TLR7	DC activation
	Oncolytic viruses	Tumour cells	Activate immune response
	Future inhibitors	IL-6 and IL-10	T cell activation
		PDL1, TIM3 and LAG3	Prevent T cell exhaustion
	Future agonists	GM-CSF, CXCL16, OX40, CD40L, CD80 and CD137	T cell recruitment and activation
		CCL3, CCL5, IL-2, IL-4, IL-12 and IRX-2	Activate immune response
Hypoxia	Nitroimidazole derivatives (that is, nimorazole)	Hypoxic cells	Reduce tumour hypoxia
	Bioreactive albumin–MnO_2 nanoparticles	Hypoxic cells	Reduce tumour hypoxia
	Acriflavine and YC-1	HIF1α	Reduce hypoxia response pathway activity
	Aflibercept	All VEGF molecules and PlGF	Vessel normalization
	AMG386	ANG1 and ANG2	Inhibit pBMDC recruitment
	Endostar	VEGF, TGFβ, HIF1α and bFGF	Inhibit angiogenesis
	AMD3100	CXCL12 and CXCR4	Inhibit BMDC recruitment and vasculogenesis
	Integrin inhibitors (cilengitide, vitaxin and volociximab)	Integrins αvβ3, αvβ5 and α5β1	Inhibit angiogenesis
	Future inhibitors	Integrins α6β1 and α6β4	Reduce endothelial cell survival and inhibit angiogenesis
	Future inhibitors	PlGF and ANG2	Vessel normalization and overcome resistance to anti-VEGF therapies
Fibrotic processes	BIBF1000 and BIBF1120	PDGF, VEGF and bFGF receptors	Reduce GF signalling and TME remodelling; fibrosis
	Imatinib, nilotinib and dasatinib	TGFβ and PDGF	GF signalling; collagen synthesis
	Vismodegib, saridegib and sonidegib	SMO	Reduce HH signalling; fibrosis
	Suramin	PDGF, EGF, TGFβ, FGF2 and IGF receptors and heparanase enzymes	Reduce GF signalling and TME remodelling; fibrosis
	ST0001, PG545, M402 and PI-88	Heparanase	Inhibit TME remodelling
	SD-208	TGFβR1	Inhibit TGFβ signalling
	Simtuzumab	LOXL2	Reduce TME remodelling; liver fibrosis
	81C6 and F16SIP	TNC	Reduce CAF-mediated TME remodelling
	Future inhibitors	HGF, CTGF, MMP2, MMP3, and integrins α11β1, αvβ6 and α3β1	TME activation and remodelling; radiation-mediated fibrosis

ANG, angiopoetin; bFGF, basic fibroblast growth factor; BMDC, bone marrow-derived cell; CAF, cancer-associated fibroblast; CTGF, connective tissue growth factor; CTLA4, cytotoxic T lymphocyte antigen 4; CXCL, chemokine (C-X-C motif) ligand; CXCR4, C-X-C chemokine receptor 4; DC, dendritic cell; EGF, epidermal growth factor; FGF2, fibroblast growth factor 2; GF, growth factor; GM-CSF, granulocyte–macrophage colony-stimulating factor; HGF, hepatocyte growth factor; HH, Hedgehog; HIF1α, hypoxia-inducible factor 1α; IGF, insulin-like growth factor; IL, interleukin; IRX2, iroquois homeobox 2; LAG3, lymphocyte activation gene 3; LOXL2, lysyl oxidase-like 2; MMP, matrix metalloproteinase; pBMDC, perivascular bone marrow-derived cell; PD1, programmed cell death protein 1; PDL1, programmed cell death protein ligand 1; PDGF, platelet-derived growth factor; PlGF, placental growth factor; SMO, Smoothened; TGFβ, transforming growth factor-β; TGFβR1, transforming growth factor-β receptor 1; TIM3, T cell immunoglobulin mucin 3; TLR7, Toll-like receptor 7; TME, tumour microenvironment; TNC, tenascin C; VEGF, vascular endothelial growth factor.

Pathogen-associated molecular patterns (PAMPs). Signalling from pathogens by particular stimuli that can be recognized by immune cells, leading to an inflammatory response.

such as doranidazole and nimorazole, have yielded survival benefits for patients with NSCLC when used in combination with chemoradiotherapy in Phase I/II trials[91] and in combination with radiotherapy in supraglottic and pharyngeal tumours in a Phase III study[92]. Nimorazole in combination with radiotherapy is a standard-of-care treatment in Denmark, and a Phase III trial assessing this combination is currently recruiting in the United Kingdom[93]. Finally, a recent development in nanotechnology has allowed the generation of oxygen in hypoxic tumour regions by intratumoural injection of bioreactive albumin–MnO_2 nanoparticles[94]. This therapy has been shown to improve tumour response to radiotherapy in preclinical studies[94].

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Immunogenic cell death
Cell death that triggers an immune reaction by DAMP–PRR signalling. This may occur as a result of different types of tissue damage; for example, damage caused by radiotherapy or chemotherapy.

Immune tolerization
The recognition of 'self' and 'non-self' during antigen presentation is important and carefully regulated to prevent autoimmunity, it is the process of recognizing antigens as 'self'.

Abscopal effects
Irradiation of tumour cells or the adjacent extracellular matrix can induce biologically relevant changes in distant cells, which may or may not have been irradiated themselves. These are distinguished from bystander (which refers to changes affecting nearby unirradiated cells) and cohort effects (which refer to changes affecting off-target irradiated cells).

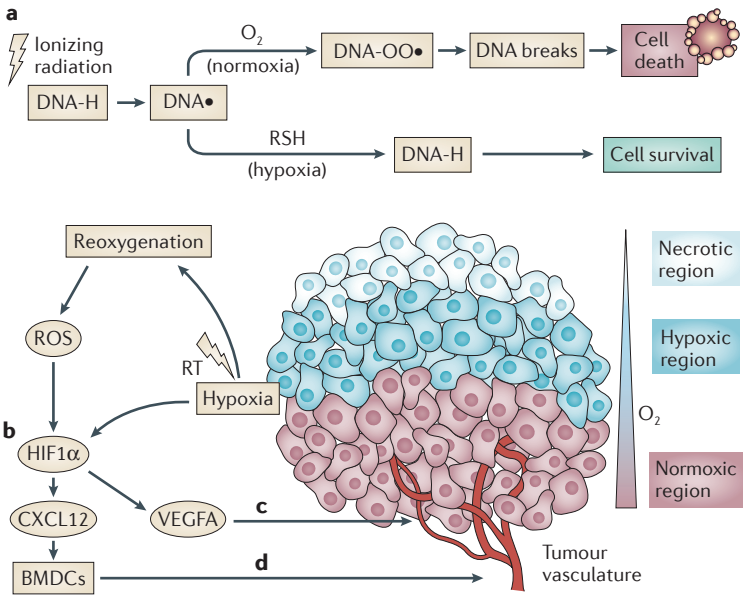
Irradiated tumours have increased expression of HIF1 (REF. 95) (BOX 2), and high tumour HIF1activity is an independent predictor of poor prognosis after radiotherapy[96,97]. Furthermore, markers of both HIF1 and HIF2 hypoxia response pathways are strongly associated with radiotherapy failure in head and neck cancer[98]. Several drugs have been designed to inhibit HIF1 activity and may affect radiosensitivity. Acriflavine directly binds to the HIF1α subunit, thereby inhibiting HIF1 dimerization and transcriptional activity[99]. Acriflavine has been shown in preclinical studies to downregulate mRNA expression of vascular endothelial growth factor (*VEGF*), *CXCL12* and stromal cell factor (*SCF*) and to reduce tumour vasculature[99]. Another HIF1 inhibitor, YC-1, improved treatment outcomes when administered after radiation[100]. Stabilization of the HIF1α subunit can also occur through increased levels of TGFβ in the TME. TGFβ has an important role in maintaining normal vascular homeostasis and has been shown to stimulate angiogenesis in irradiated environments[101]. However, as TGFβ can promote both pro-angiogenic and anti-angiogenic effects[102], the development of strategies that directly target TGFβ is likely to be challenging.

As discussed above, tumours contain abnormal vessels that contribute to tumour hypoxia. Vessel normalization to increase radiotherapy response and to reduce tumour recurrence and metastasis is currently being investigated[103] (FIG. 4). VEGFA is the principal angiogenic molecule implicated in the development of abnormal vasculature in the TME[104]. Pharmacological inhibition of VEGF has been achieved using a VEGF-specific antibody (bevacizumab), a VEGF-trap molecule (aflibercept, which targets all VEGF molecules and placental growth factor (PlGF)), a VEGF receptor (VEGFR)-inhibiting antibody (DC101), and receptor tyrosine kinase inhibitors (TKIs; such as sunitinib, sorafenib and pazopanib)[105]. Although disappointing results have been observed for anti-VEGF monotherapy, the treated tumours were sensitized to chemotherapy and radiotherapy owing to better drug delivery and reduced hypoxia, respectively[106]. TKIs have also been shown to contribute to vessel normalization in many preclinical models and to synergize with radiation in orthotopic models of glioblastoma (GBM), melanoma, and colon, lung, and head and neck cancer[84]. However, anti-VEGF effects are somewhat transient, and targeting the downstream molecules PI3K and mTOR (using NVP-BEZ235 or NVP-BGT226) in combination with radiotherapy could prove to be more efficacious through durable vessel normalization[107]. Vessel normalization occurs during a specific time window following different anti-angiogenic treatments, and this translates into optimal tumour growth delay when radiotherapy is scheduled accordingly[108,109]. Similarly, the dose of anti-angiogenic compounds used in combination therapy needs to be carefully considered. Carmeliet and Jain proposed that higher doses of anti-angiogenic therapy could be harmful as a result of normal tissue toxicity and complete vessel destruction, which may paradoxically increase tumour hypoxia[110].

Two endogenous angiogenesis inhibitors, angiostatin and endostatin, have synergistic antitumour effects in combination with radiotherapy[84]. Likewise, TNP-470, the synthetic analogue of a naturally occurring antibiotic, arrests endothelial cell growth and improves tumour oxygenation and response to radiotherapy[111–113].

Box 2 | **Hypoxia-mediated mechanisms of radioresistance in the tumour microenvironment**

Ionizing radiation produces DNA radicals (DNA•) that are usually fixed by O_2 (in normoxic regions) and causes DNA damage that can lead to cell death if unrepaired. Under hypoxic conditions, DNA damage is reduced (mainly by –SH-containing compounds), which leads to cell survival (see the figure, part **a**). Hypoxia-inducible factor 1α (HIF1α) is stabilized under hypoxic conditions, which leads to the upregulation of genes involved in cell survival. Radiotherapy (RT) causes tumour reoxygenation, an increase in levels of reactive oxygen species (ROS), and contributes to HIF1α stabilization (see the figure, part **b**). Hypoxia increases vascular endothelial growth factor A (VEGFA) production, leading to the formation of abnormal vessels, also contributing to tumour hypoxia (see the figure, part **c**). Vasculogenesis aids tumour recurrence following radiotherapy and can be driven by HIF1α-mediated bone marrow-derived cell (BMDC) recruitment via chemokine (C-X-C motif) ligand 12 (CXCL12) (see the figure, part **d**). RSH, reducing SH-containing compound. Part **a** adapted from REF. 189, Nature Publishing Group.



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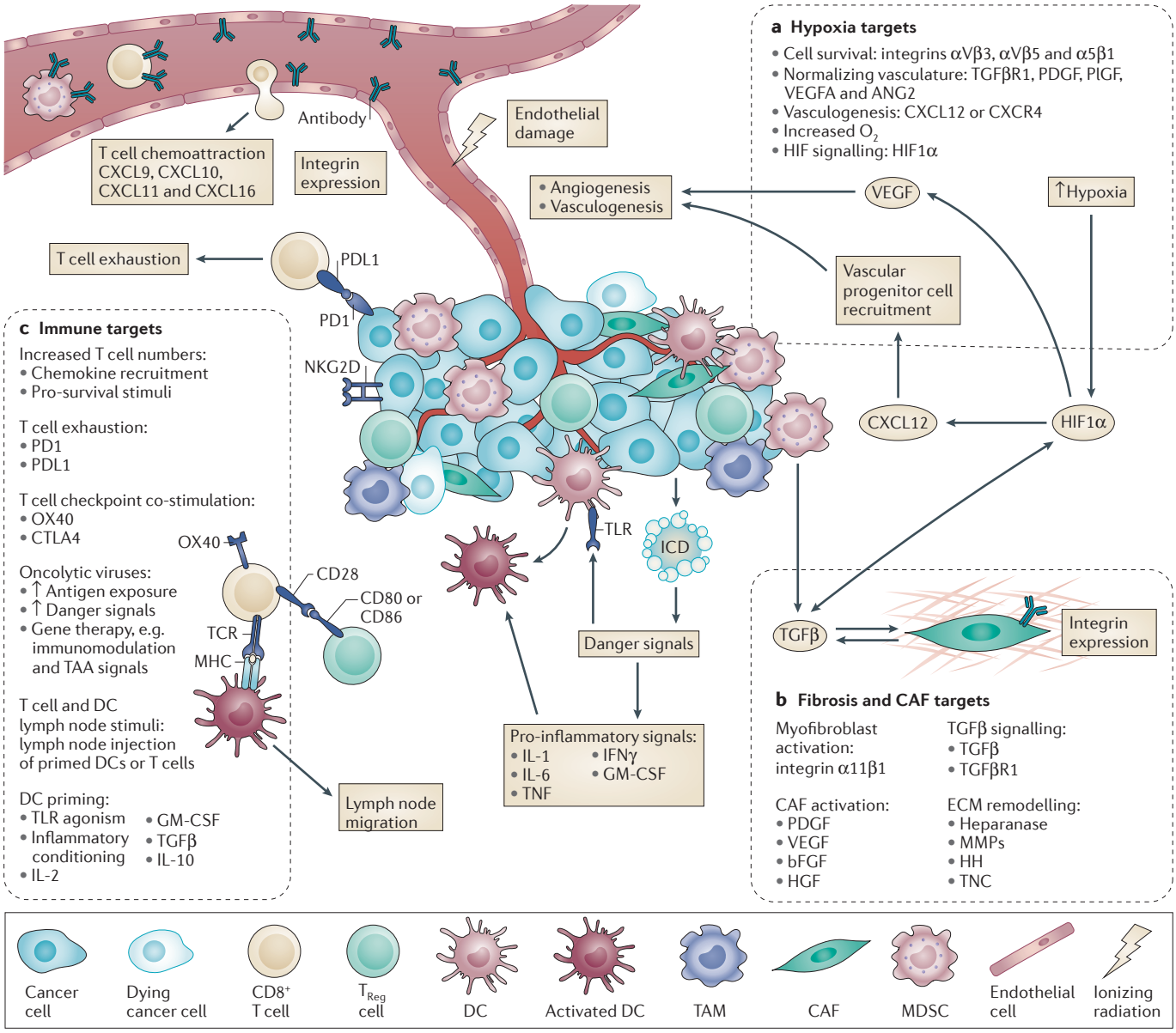


Figure 4 | **Targets for radiosensitization.** After radiotherapy, there are numerous potential targets within the tumour microenvironment (TME) the modulation of which may lead to radiosensitization. These are broadly categorized by area of biological effect — hypoxia, fibrosis and cancer-associated fibroblast (CAF) related, and immune — their actions in relation to the effects of radiotherapy on the TME are summarized in FIG. 1. Ongoing clinical trials addressing these in combination with radiotherapy are addressed in Supplementary information S1 (table). **a** | Hypoxia targets can be further subdivided into those that affect endothelial cell survival (for example, targeting integrins[120]), those that normalize the vasculature (for example, antagonizing vascular endothelial growth factor (VEGF)[106]), those that prevent vasculogenesis (for example, targeting chemokine (C-X-C motif) ligand 12 (CXCL12) recruitment of endothelial progenitor cells[25]), those that alter oxygen delivery (for example, accelerated radiotherapy with carbogen and nicotinamide (ARCON)[85–87]) and those that alter hypoxia-inducible factor 1α (HIF1α) signalling (for example, the HIF1α inhibitor YC-1 (REF. 100)). **b** | Fibrosis targets can be sub-categorized into those that affect stromal activation (for example, by inhibition of growth factors by suramin[148]), those that affect extracellular matrix (ECM) remodelling (for example, targeting tenascin C (TNC) with the antibody 81C6 (REFS 135,136)) and those that affect transforming growth factor β (TGFβ) signalling (for example, by targeting its receptor TGFβR1 with the inhibitor SD-208 (REF. 131)). **c** | Immune targets can be divided into those that prime dendritic cells (DCs) (for example, the Toll-like receptor 7 (TLR7) agonist imiquimod[166]), those that affect T cell checkpoint co-stimulation (for example, the cytotoxic T lymphocyte antigen 4 (CTLA4)-specific antibody ipilimumab[74,75,171]), those that affect T cell exhaustion (for example, the programmed cell death protein 1 (PD1)-specific antibody pembrolizumab[181]) and those that affect T cell recruitment (for example, targeting the chemokine CXCL16). Oncoviruses are also an immunomodulatory stimulus and can be used to affect multiple aspects of the immune pathway in the irradiated TME (for example, granulocyte–macrophage colony-stimulating factor (GM-CSF)-expressing herpes simplex virus[186,188]). ANG2, angiopoietin 2; bFGF, basic fibroblast growth factor; CXCR4, C-X-C chemokine receptor 4; HGF, hepatocyte growth factor; HH, Hedgehog; ICD, immunogenic cell death; IFNγ, interferon-γ; IL, interleukin; MDSC, myeloid-derived suppressor cell; MHC, major histocompatibility complex; MMP, matrix metalloproteinase; PDGF, platelet-derived growth factor; PDL1, PD1 ligand 1; PlGF, placental growth factor; TAA, tumour-associated antigen; TAM, tumour-associated macrophage; TCR, T cell receptor; TNF, tumour necrosis factor.

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Angiostatin and TNP-470 are no longer in clinical trials, owing to poor toxicity profiles, lack of efficacy and the development of more effective anti-angiogenic drugs. Endostar, however, is a more potent and stable version of endostatin[114] and improves radiation responses in preclinical cancer models through the inhibition of VEGF, TGFβ, HIF1α and basic fibroblast growth factor (bFGF)[115,116], and is currently being combined with radiotherapy in Phase II clinical trials (ClinicalTrials.gov identifiers NCT01368419 and NCT01410370).

Blocking vasculogenesis may also improve radiotherapy-mediated local tumour control and prevent recurrence[117] (FIG. 4). In their preclinical study, Kioi *et al.* abrogated BMDC-mediated tumour vasculogenesis through inhibition of HIF1 activity using NSC-34754 (REF. 25). Likewise, administration of AMD3100, which inhibits the interaction between CXCL12 and CXCR4, prevented blood flow returning to irradiated tumours and completely inhibited recurrence for up to 100 days. A similar effect was observed with a CXCR4-neutralizing antibody. Another preclinical study has implicated angiopoietin 2 (ANG2; a molecule proposed to compensate for VEGF inhibition by recruiting perivascular BMDCs to support the weak tumour vasculature) in tumour vasculogenesis. Burrell *et al.* demonstrated that treatment with an inhibitor of both ANG1 and ANG2, AMG386, prevented the influx of perivascular BMDCs to the central vasculature, and it was hypothesized that ANG2 inhibition may overcome resistance to anti-VEGF therapies and prevent tumour recurrence following radiotherapy[118].

Finally, as with CAF-associated integrins, the role of integrins in mediating cell–ECM attachment is important for endothelial cell survival and migration. Early studies revealed that radiotherapy upregulated integrin αvβ3 expression in endothelial cells, and concurrent treatment with an αvβ3 antagonist could enhance radiotherapy-mediated anti-angiogenic and antitumour responses in xenograft models[119]. Integrin inhibitors have been developed (cilengitide against αvβ3 and αvβ5, vitaxin against αvβ3 and volociximab against α5β1) and have shown efficacy alone and in combination with radiotherapy in preclinical and clinical studies. Cilengitide showed encouraging activity in a Phase II trial[120] but has not improved outcomes for patients with GBM in a Phase III trial with chemoradiotherapy[121]. Nevertheless, further trials are ongoing (see Supplementary information S1 (table)). Likewise, volociximab and vitaxin have anti-angiogenic activity, but data on their radiosensitizing capabilities are lacking[122–124].

In conclusion, direct and indirect therapeutics to reduce tumour hypoxia have been developed and show preclinical promise in combination with radiotherapy. However, changes in the TME during therapy and complex issues relating to drug dose and schedule mean that we do not yet know how best to use these agents for clinical benefit.

Targeting the stroma. Inhibition of radiotherapy-induced inflammatory responses may reduce long-term fibrotic processes that could be important if complete eradication of local tumour cells is not achieved. Upregulation of pro-inflammatory NF-κB post-irradiation is often associated with increased IL-1β, IL-6, IL-8, granulocyte–macrophage colony-stimulating factor (GM-CSF) and cyclooxygenase 2 (COX2) in the TME[125]. Therefore, developing inhibitors that interfere with this inflammatory network has been a focus of radiosensitization approaches. Many natural and synthetic anti-inflammatory compounds have proved to be efficacious in tumour radiosensitization[125]. However, their effects have been mediated through anti-angiogenic, antitumour cell survival or decreased BMDC mobilization mechanisms rather than through the inhibition of fibrotic processes. It must also be borne in mind that attempts to reduce inflammation as a means of preventing tumour-promoting fibrotic reactions may have deleterious knock-on consequences for the beneficial effects of inflammation on antitumour immunity (discussed below).

TGFβ signalling is upregulated by radiotherapy[126] and is a key inducer of stromal activation in the TME. Although it has been shown to directly mediate tumour suppression in irradiated environments[127], inhibition of TGFβ in conjunction with radiotherapy has also delayed tumour growth and prevented radiotherapy-mediated increases in circulating levels of TGFβ, thereby reducing cancer metastasis in preclinical models[128,129]. Although these studies mainly focused on TGFβ-mediated effects on the tumour cells, the increased levels of circulating TGFβ that are observed following irradiation may be a consequence of CAF activation and could also influence tumour progression through further modulation of CAF activity. So far, TGFβ inhibition in isolation has not proved to be effective in the clinic[130]. The broad and often opposing activities of TGFβ can pose problems in targeting strategies, and more targeted inhibition of downstream molecules may be more beneficial. Indeed, preclinical studies of a TGFβR1 inhibitor, SD-208, showed reduced tumour growth and decreased fibrosis in the TME[131]. There is, therefore, a rationale for combining TGFβR inhibition with radiotherapy in order to prevent radiation-induced activation of CAFs.

Molecules secreted by CAFs, such as HGF and TNC, could be promising selective targets in the tumour stroma to negate radiotherapy-induced fibrotic phenotypes that may facilitate radioresistance (FIG. 4). Monoclonal antibodies against HGF and MET (also known as hepatocyte growth factor receptor) and MET small-molecule inhibitors have shown efficacy in clinical trials[132]. Preclinical studies have demonstrated that MET inhibition enhances radiotherapy responses and prevents tumour cell invasion mediated by CAF-derived HGF secretion following irradiation[133,134]. However, to this day, HGF and MET inhibitors have not yet been combined with radiotherapy in the clinic. Alternatively, high stromal expression of TNC, as a result of CAF activation, can be targeted with the ^{131}I-labelled TNC-specific antibodies 81C6 and F16SIP. A Phase II clinical trial demonstrated some success for combining 81C6 with radiotherapy[135,136], and a Phase III trial has since been proposed[137].

Several agents with anti-fibrotic actions may affect radiotherapy-mediated inflammation and fibrosis in the TME. Imatinib, nilotinib and dasatinib inhibit TGFβ and

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PDGF signalling and, hence, collagen synthesis[138]. They are currently being evaluated in clinical trials for the treatment of fibrosis. Other inhibitors that affect growth factor signalling include BIBF1000 and BIBF1120, which target PDGFR, VEGFR and bFGFR. These molecules have some efficacy in treating idiopathic pulmonary fibrosis[139,140] and may modulate post-irradiation changes in the TME. Hedgehog (HH) signalling also regulates fibrosis[141] and has been implicated in the desmoplastic reaction in pancreatic ductal adenocarcinoma[142]. Attempts to target this pathway have led to the development of inhibitors (vismodegib, saridegib and sonidegib) of the downstream molecule Smoothened (SMO). Monotherapy preclinical and clinical trials had encouraging outcomes[143–145], and results from early studies combining vismodegib with radiotherapy[146] are also promising. Phase II clinical trials of radiotherapy plus HH inhibition are ongoing (see Supplementary information S1 (table)).

Targeting growth factors that are involved in the activated networks of the TME has led to the development of suramin, which inhibits PDGF, epidermal growth factor (EGF), TGFβ, FGF2 and IGF signalling[147]. Suramin has exhibited anti-proliferative effects against many advanced cancers[148]. Although suramin did not improve survival when combined with cranial radiotherapy in patients with GBM[149], the combination has not yet been tested in cancers in which an activated stroma is common, such as breast and pancreatic cancer. Interestingly, suramin was recently shown to inhibit heparanase enzymes, thereby reducing tissue damage and fibrosis in a rat model of hepatocellular carcinoma[150]. Heparanase is involved in ECM breakdown and regulates the biological availability of growth factors and cytokines in the TME[151]. Most importantly, heparanase has been shown to augment radiation-induced cancer cell invasion[152]. An inhibitor of heparanase, SST0001, has shown potential in preclinical studies in which it reduced the spread of pancreatic cancers in combination with radiotherapy[152]. Other heparanase inhibitors, PG545, M402 and PI-88, have also displayed antitumour activity in murine models and have entered clinical trials[153–155]. The activity of heparanase is also indirectly regulated by MMPs[151]. Many MMP inhibitors, including marimastat, prinomastat, batimastat and tanomastat, have been evaluated in clinical trials for the treatment of various cancers, mostly with disappointing results[156]. The broad-spectrum activity of MMP inhibitors may explain their failure in monotherapy clinical trials. Preclinical studies with more specific MMP inhibition have demonstrated some improvement in the efficacy of radiotherapy (for example, MMP2 inhibition has been shown to abrogate radiotherapy-induced tumour invasion and progression[157–160]), indicating that further research is required.

CAFs are generally genetically more stable than tumour cells, making them good therapeutic targets. However, they display functional duality, meaning that the balance between tumour inhibition and tumour promotion is very delicate. For example, a recent report by Özdemir and colleagues demonstrated that CAF depletion in a mouse model of pancreatic cancer may actually accelerate cancer progression[161]. This suggests that care needs to be taken when specifically targeting CAFs and that blocking downstream mediators of CAF activation may prove to be more efficacious. Whether desmoplasia provides a growth disadvantage for cancer cells or promotes their dissemination is also still under debate. Nevertheless, promising results from trials involving inhibitors of ECM turnover suggest that agents developed for the treatment of fibrotic diseases may enhance radiotherapy-mediated tumour growth delay and reduce subsequent TME changes that could facilitate tumour cell migration and cancer spread.

Immune modulation. Much of the current interest in combining immunomodulation and radiotherapy lies in strategies to overcome the persistent suppression of adaptive immune responses by the tumour and its microenvironment. Of particular interest is the promise of the abscopal effect, whereby localized radiotherapy acts as the catalyst for systemic antitumour immunoreactivity[74,75].

Although radiotherapy does lead to ICD, some tumours exhibit accelerated tumour repopulation by paracrine caspase 3-dependent prostaglandin E_2 (PGE_2)-mediated signalling[162]. Signalling from surviving and recruited MDSCs and TAMs also suppress T cell function through antagonistic cytokine signals, such as IL-6, IL-10 and TGFβ[163]. Their relevance is highlighted in a Phase I/II clinical trial combining radiotherapy and a primed DC vaccine against high-risk soft-tissue sarcoma. A rate of 52.9% for tumour-specific immune responses was reported, but non-responders had significantly higher levels of tumour MDSCs before immunization[164].

Manipulation of the TLR response can increase the activation of DCs, triggering an effective adaptive immune response after radiotherapy[165]. Direct TLR manipulation is currently being trialled using the TLR7 agonist imiquimod, which is approved for the treatment of basal cell skin carcinomas and melanomas. Imiquimod is being used in conjunction with local radiotherapy as a topical treatment for breast cancer metastasis, with promising preclinical data[166]. Using cancer vaccines comprising TLR agonists with tumour antigens to stimulate DCs and T cell responses has been effective in preclinical models and has been translated clinically[167]. GM-CSF has also been used in the clinic, combined with radiotherapy, to treat patients with metastatic disease across multiple cancer types (lung, thyroid, eccrine, cervical and breast cancer)[168]. In that study, patients treated with chemotherapy received radiotherapy (35 Gy in 10 fractions) delivered to a metastatic site followed by daily subcutaneous injections of GM-CSF for 14 days after the first week of radiotherapy. A systemic effect was seen in 27% of the patients, with a corresponding improvement in survival in those who experienced abscopal effects compared with those who did not (median overall survival of 25 months versus 10 months).

Effective T cell activation requires both antigen presentation and co-stimulation from an appropriate APC. Several of the proteins that regulate this process, including

Eccrine
A type of tumour from secretory sweat glands.

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CTLA4, OX40, programmed cell death protein 1 (PD1) and CD137 (also known as 4-1BB), are likely to represent important targets for immunomodulation after radiotherapy-induced immune priming. Ipilimumab (a CTLA4-specific monoclonal antibody) is approved for use in advanced-stage melanoma, as it improved survival in Phase III trials[169,170]. In murine studies, unilateral radiotherapy and systemic CTLA4 blockade delayed the growth of a contralateral, unirradiated tumour[171]. Interestingly, this abscopal response was dependent on radiation dose fractionation, with 24 Gy delivered in three fractions seeming to be optimal[171]. This suggests that there may be preferred fractionation schemes for effective immune priming, although the evidence for this is currently limited (TABLE 2) and may well differ according to therapeutic agent and tumour histology. Scheduling of immunological therapy relative to radiotherapy is also likely to be important, with an initial period of tolerance seen after radiotherapy (TABLE 2). Indeed, there is some early evidence that an interval of around 1 month between radiotherapy and immunomodulation may be optimal for synergy (TABLE 2). Anecdotal clinical reports of radiation-induced abscopal responses have been published, and results from ongoing clinical trials are eagerly awaited (see Supplementary information S1 (table)).

OX40 signalling co-stimulates the activation of several T cell subtypes (for example, $CD4^+$ and $CD8^+$). It mediates positive feedback, potentiating activation and thus polarizing the T cell response. In combination with radiotherapy, in which $CD8^+$ T cell priming or activation already occurs, activation of OX40 signalling might be particularly effective. In a murine model, OX40 agonistic antibodies improved local control after surgery or radiotherapy[172]. It is also thought that OX40 can help to prime low-affinity T cells and reverse T cell tolerance. As such, activating OX40 signalling might work synergistically with CTLA4 blockade to activate T cells post-radiotherapy[173]. T cell co-stimulating proteins, such as CD137 (REF. 174), also represent promising targets.

Induced $CD8^+$ T cell exhaustion is a radioresistance mechanism (FIG. 4). This has been characterized in a murine model of lymphocytic choriomeningitis virus-13 (LCMV-13) infection, where infected T cells were compared with normal uninfected T cells[175]. Similarities between virally induced T cell exhaustion and ineffective T cells in melanoma have been studied[176]. Markedly different gene expression was seen in cancer-infiltrating $CD8^+$ T cells and T cells in the circulation, which showed patterns similar to the exhaustion profiling seen in the LCMV-13 murine model. Modulation of these exhaustion pathways by targeting PD1, T cell immunoglobulin mucin 3 (TIM3; also known as HAVCR2)[177] or lymphocyte activation gene 3 (LAG3)[178] expression represents an exciting area for combination with radiotherapy. Thus far, of these exhaustion pathways, the PD1 receptor ligand

Table 2 | **Fractionation of radiotherapy and timing of immunomodulation**

Study type	Cancer type	Intervention	Outcome	Refs
Fractionation				
Preclinical	Murine glioma	• RT: 10 Gy in one fraction • Immunomodulation: anti-PD1	Longer survival seen (53 days (RT and anti-PD1) versus 25 days (control), 27 days (anti-PD1) or 28 days (RT))	190
Preclinical	Murine breast cancer	• RT: 20 Gy in one fraction, 24 Gy in three fractions or 30 Gy in five fractions • Immunomodulation: anti-CTLA4	Best response seen in 24 Gy in three fractions	171
Preclinical	Murine breast cancer	• RT: 12 Gy in one fraction, 24 Gy in two fractions • Immunomodulation: anti-CTLA4	$CD8^+$ T cell antitumour immunity demonstrated	191
Clinical	Patients with hepatoma	• RT: 8 Gy in one fraction • Immunomodulation: DC vaccine	Two partial responses, four minor ones	192
Clinical	Prostate cancer	• RT: 70 Gy in 30 fractions • Immunomodulation: IL-2 and GM-CSF	Increased levels of PSA-specific T cells	193
Clinical	MF	• RT: 9–18 Gy in nine fractions • Immunomodulation: injected TLR9 agonist	5 out of 15 responses	194
Timing				
Clinical	MM or RCC	• RT: 60 Gy in three fractions • Immunomodulation: IL-2	8 out of 12 responses	195
Preclinical	Murine prostate cancer expressing HA	• RT: 15 Gy in one fraction • Immunomodulation: $CD4^+$ T cells primed against HA (given at points on a time course after RT)	Tolerance seen between days 3 and 16. Normal T cell response by day 33	55
Clinical case report	MM	• RT: 28.5 Gy in three fractions • Immunomodulation: ipilimumab (anti-CTLA4) at 1 month after RT	Complete response	74
Clinical case report	MM	• RT: 54 Gy in three fractions • Immunomodulation: ipilimumab	Complete response	54

CTLA4, cytotoxic T lymphocyte antigen 4; DC, dendritic cell; HA, haemagglutinin antigen; MF, mycosis fungoides; MM, malignant melanoma; PD1, programmed cell death protein 1; PSA, prostate-specific antigen; RCC, renal cell carcinoma; RT, radiotherapy; TLR, Toll-like receptor.

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PDL1–PD1 axis has been the best studied in the context of radiotherapy and immune modulation. Expression of PD1 on T cells in the TME is suggestive of their exhaustion and results in ineffective T cell-mediated immune responses. Upregulation of PDL1 on tumour cells after irradiation[179] also represents a mechanism of radioresistance. Blockade of the PDL1–PD1 axis in combination with radiotherapy is effective at the primary tumour site and in generating abscopal responses in AT-3 mammary tumours[180]. Early clinical results using this strategy against incurable metastatic breast cancer also look promising[181].

Oncolytic viruses preferentially infect and replicate in tumour cells, leading to cell death[182]. They are also recognized as potent immune therapeutics[183]. When combined with radiotherapy, they have demonstrated synergy in preclinical models[184,185]. In early clinical trials of radiotherapy and oncolytic virus combinations, we reported a tolerable toxicity profile that does not overlap with radiotherapy-induced side effects[186]. In the TME, oncolytic virus infection triggers a PAMP- and DAMP-related PRR signal[162,187] and could potentially have a role in ICD, immunomodulation and T cell activation. This viral response may represent another method of overcoming the suppression of adaptive immune responses by the TME.

Viruses can also be used to deliver therapeutic genes that exert immunomodulatory effects. Examples include the overexpression of chemokines (CCL3 and CCL5), immune cytokines (GM-CSF, IL-2, IL-4 and IL-12) and T cell co-stimulators (CD40L, CD80 and 4-1BBL). Talimogene laherparepvec (T-Vec) is a replication-competent GM-CSF-expressing herpes simplex virus that has been tested in a Phase I clinical trial in combination with chemoradiotherapy in head and neck cancer[186,188]. The combination of viral oncolysis, immune stimulation and immunomodulation may represent a powerful approach that is worthy of further evaluation.

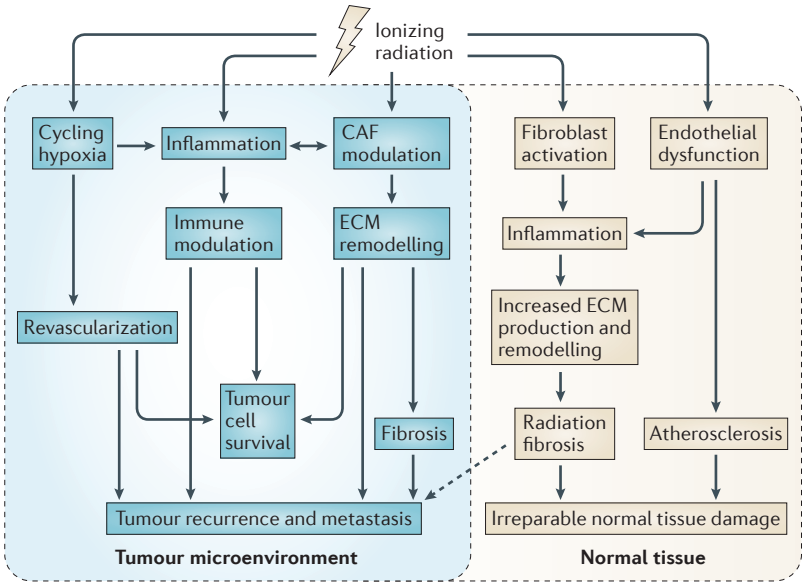


Figure 5 | **The interconnected radiotherapy-mediated changes in the TME.** Hypoxic regions within the tumour microenvironment (TME) may initially affect tumour radiosensitivity. Radiotherapy elicits an inflammatory response in the TME as well as contributing to cycling hypoxia and modulation of cancer-associated fibroblast (CAF) activity. These responses are interconnected with immunomodulation, revascularization, extracellular matrix (ECM) remodelling, and radiation fibrosis that may affect tumour cell survival and/or tumour recurrence and metastasis. Similar processes may occur in the surrounding normal tissue that could result in irreparable damage to critical organs. These events must all be taken into account when combining radiotherapy with therapies to modulate the immune response, as well as reducing hypoxia and ECM remodelling and fibrosis in carefully timed treatment strategies.

Conclusions

Radiotherapy leads to the activation of an interconnected series of processes in the TME: inflammation, cycling hypoxia, immunomodulation, revascularization, CAF-coordinated ECM remodelling and fibrosis. These changes occur in the TME in an interconnected manner (FIG. 5). This is highlighted by the overlapping and widespread effects of several factors, especially growth factors TGFβ, VEGF and PDGF; cytokines TNF, CXCL12, IL-1, IL-2, IL-6 and IL-10; and transcription factors NF-κB and HIF1 (FIG. 1).

Against this background, emerging therapeutic strategies have aimed to tip the balance of the TME radiotherapy response towards more effective cancer therapy while minimizing adverse effects (FIG. 4). Hypoxia directly affects initial radiosensitivity and immune responses, and promotes the development of neovasculature. It also stimulates ongoing CAF activity. Strategies currently under development to increase tumour oxygenation — for example, by modulating oxygen availability or by normalizing the tumour vasculature — have demonstrated great potential. Furthermore, targeting HIF1 could have TME-wide benefits, particularly through its modulation of TGFβ signalling. Related strategies to prevent BMDC-mediated tumour revascularization by using CXCL12 inhibitors also seem to be very promising and are now in early clinical trials (Supplementary information S1 (table)). Integrins, which are central to the survival and repopulation of both endothelial cells and CAFs after radiotherapy, are also important emerging targets (TABLE 1).

Targeting drivers of the initial inflammatory response, such as NF-κB, COX2 and TNF, has been shown to decrease radiotherapy-mediated side effects in surrounding normal tissues without improving tumour responses. Moreover, targeting inflammation may counteract the antitumour benefits of immunomodulation. Therefore, in this regard, emphasis should be placed on reducing late radiation effects on ECM structure rather than the initial inflammatory response. Abrogating excess ECM production by specifically targeting CAFs with FAP-specific antibodies has, so far, not shown efficacy in cancer trials. Indeed, depleting the CAF population may prove to be detrimental in some tumour types. It may be more beneficial to prevent ECM remodelling events in the TME, through prevention of either crosslinking or breakdown of the ECM. Many molecules that impede ECM remodelling, such as inhibitors of growth factors, their receptor kinases, HH signalling and matrix enzymes, are currently being used to treat fibrotic disorders and may show efficacy in preventing the development of a radiotherapy-mediated

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invasive environment (TABLE 1). The most exciting development in the modulation of the tumour ECM could possibly be the generation of heparanase-targeted therapies that aim to prevent tumour cell dissemination by modulating ECM remodelling and by reducing the availability of growth factors and cytokines in the TME.

With respect to immune modulation, the most promising areas of development are in generating an effective adaptive response to treat cancer both locally and abscopally. In summary, there are several strategies involved: increasing ICD and both numbers and activation levels of DCs using a combination of cytokines, growth factors and TLR stimuli; appropriate recruitment and activation of T cells with primed DCs; and the abrogation of inhibitory signalling (CTLA4) and T cell exhaustion (PDL1–PD1). Several of these approaches are now in clinical development both as single agents and in combination with pre-existing chemotherapy and other TME modulators (see Supplementary information S1 (table)). New strategies are also rapidly emerging and warrant investigation (TABLE 1): chemokine recruitment of antitumour T cell subgroups (for example, using CXCL16), targeting T cell exhaustion markers (such as TIM3 and LAG3) and oncolytic immunotherapies (including gene therapy to deliver immunomodulation).

Investigation of the response of the TME to radiotherapy has led to the development of an exciting array of novel treatments. Given the timing and interplay of events in the response of the TME to radiotherapy, it will be a considerable challenge to develop rational radiotherapy–drug combinations in this field. Nonetheless, we believe that carefully designed combinations of radiotherapy with vascular normalization, immunomodulation and antifibrotic therapy will prove to be effective in maximizing therapeutic benefits and minimizing both unwanted off-target effects and the possibility of metastasis and recurrence. Although many questions remain, we believe that the door is beginning to open on the synergistic potential that will be the future of cancer therapy.

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Competing interests statement
The authors declare no competing interests.

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