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The Role of Macrophage Phenotype in Regulating the Response to Radiation Therapy

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Abstract

Increasing experimental and clinical evidence has revealed a critical role for myeloid cells in the development and progression of cancer. The ability of monocytes and macrophages to regulate inflammation allows them to manipulate the tumor microenvironment to support the growth and development of malignant cells. Recent studies have shown that macrophages can exist in several functional states depending on the microenvironment they encounter in the tissue. These functional phenotypes not only influence the genesis and propagation of tumors, but also the efficacy of cancer therapies particularly radiation. Early classification of the macrophage phenotypes, or “polarization states”, identified two major states, M1 and M2, that have cytotoxic and wound repair capacity respectively. In the context of tumors, classically activated or M1 macrophages driven by IFN-gamma support anti-tumor immunity while alternatively activated or M2 macrophages generated in part from interleukin-4 exposure hinder anti-tumor immunity by suppressing cytotoxic responses against a tumor. In this review, we discuss the role that the functional phenotype of a macrophage population plays in tumor development. We will then focus more specifically on how macrophages and myeloid cells regulate the tumor response to radiation therapy.

Keywords

macrophage; macrophage polarization; radiation therapy; tumorassociated macrophages

Introduction

Macrophages arise from the myeloid cell lineage in the bone marrow. They begin life by entering the blood stream as monocytes and in response to a variety of inflammatory stimuli migrate into the tissue and become mature macrophages. Once in the tissue they can differentiate into several different types of mature macrophages. These macrophages play pivotal roles in the initiation, propagation and resolution of inflammation.[1] In the tissue, macrophages are highly responsive to environmental cues including cytokines and other

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inflammatory stimuli. Macrophages undergo phenotypic changes upon encountering these triggers to acquire functions that can support or inhibit an inflammatory response.[2] Tumors actively recruit myeloid cells and express various cytokines and cell surface molecules that push recruited myeloid cells to differentiate into macrophages that can support tumor growth and inhibit the tumor response to therapies such chemotherapy and radiation.[3] Tumor-associated macrophages inhibit the response to therapies through multiple mechanisms including inhibition of the anti-tumor immune response stimulated by therapy-induced cell death and production of vascular and epithelial growth factors. In this review, we will discuss the current understanding of macrophage phenotypes, the role of macrophages in cancer and finally how macrophages and their functional phenotypes regulate the response to radiation therapy.

Macrophage polarization: functional diversity of macrophages

Recent studies have revealed tremendous functional diversity among macrophages ranging from their cytotoxic killing abilities to their central role in tissue repair. This diversity likely arises from the multitude of situations requiring phagocytic cells throughout the body. Found in virtually every tissue of the body, tissue and bone marrow derived macrophages encounter a tremendous number of agents they have to deal with in order to maintain tissue homeostasis. Thus, their diversity arises from the need to prepare macrophages for the things they will encounter anywhere from infected cells to damaged tissue. Early classification schemas attempted to categorize macrophage functional states into either the classically activated macrophages (M1) phenotype or the alternatively activated macrophages (M2) phenotype, mirroring the Th1/Th2 polarization states found in T cells.[4] With respect to tumors, M1 macrophages due to their cytotoxic capacity is often considered the “anti-tumor” phenotype whereas the M2 macrophages due to their immunosuppressive and angiogenic capacioty are thought to be the “pro-tumor” phenotype. More recent studies examining macrophage populations in vivo have shown that the M1/M2 classification grossly oversimplifies the wide spectrum of functional macrophage phenotypes found in the body.[5,6] Nevertheless, for the purposes of this discussion it remains helpful to broadly classify macrophages into their earlier M1/M2 nomenclature recognizing that this subdivision likely does not capture a complete understanding of the macrophage phenotypes involved.

M1 macrophages: the “anti-tumor” phenotype

Macrophages have long been recognized as the first line of defense against foreign pathogens in innate immunity. [7] Th1-related cytokines like interferon-γ (IFN-γ) and microbicidal stimuli such as lipopolysaccharide (LPS) prime macrophages to produce a cytotoxic activation state that characterizes the M1 phenotype.[8] In these M1 macrophages, downstream signaling of IFNs and toll-like receptors (TLRs), through activation of signal transducer and activator of transcription 1 (STAT1) and nuclear factor-kB (NF-kB) drives the expression of a transcriptional program including the chemokines C-C motif ligand 15 (CCL15), C-X-C motif ligand 10 (CXCL10), chemokine receptors such as C-C chemokine receptor type 7 (CCR7) and reactive oxygen species (particularly inducible nitric oxide synthase, iNOS).[9] M1 macrophages have been identified by both surface markers and expression of several key genes such as interleukin (IL)-12, IL-1 and tumor necrosis factor

(TNF).[10–12] Studies examining the interaction between macrophages and cancer cells suggest that M1 macrophages both directly kill cancer cells and support the cytotoxic activity of other immune cells including T cell and NK cells thus the M1 phenotype is often considered the “anti-tumor” phenotype.[13]

M2 macrophages: “pro-tumor” phenotype

Originally identified in response to metazoan parasite infections and allergens, M2 macrophages form when macrophages encounter Th2-associated cytokines including IL-4 and IL-13 which activate STAT6 leading to expression of targets that were found to be key not only in mediating anti-helminthic immunity but also tissue repair.[14] M2-polarized macrophages possess higher levels of arginase (Arg-1) activity, allowing them to convert arginine to ornithine, a precursor of polyamines and collagen, contributing to the production of extracellular matrix.[15] M2 macrophages are also known to secrete other factors associated with wound healing such as vascular endothelial growth factor (VEGF), colony stimulating factor 1 (CSF1) and IL-8 which promote angiogenesis, lymphangiogenesis and fibrosis.[2,16] They are characterized by expression of immunosuppressive cytokines, chemokines and surface markers such as IL-10, CCL17 and CD206.[9] Tumor-associated macrophages (TAMs) share many of the same expression patterns as M2 macrophages. TAMs play a crucial role in the initiation, promotion and metastasis of cancer cells by encouraging angiogenesis and remodeling of the stromal matrix to help establish the premalignant niche. Thus, M2-polarized macrophages are often considered a “pro-tumor” phenotype.[17,18]

Myeloid-macrophage cells in cancer

Myeloid cells and macrophages have been associated with both the development and progression of cancer.[16] Several larger retrospective clinical studies found that increasing numbers of TAMs correlate with higher grade tumors in multiple tumor types including breast, lung and prostate.[19] Tumors actively recruit macrophages as they grow in order to establish a favorable microenvironment through macrophage-derived growth signals, tissue remodeling and immunosuppression.[20]

Recruitment of TAMs to tumors

Multiple cytokines and chemokines coordinate the recruitment and differentiation of macrophages in sites of tumor formation. Tumors produce macrophage colony-stimulating factor (M-CSF) and CCL-2 both of which regulate the influx and survival of TAMs.[21] CSF1 regulates the production of myeloid cells in the bone marrow and is also responsible for attracting macrophages to the tumor from the circulation. CCL2 and ligation of its receptor CCR2 on macrophages induce their chemotaxis and retention in tumors. Other inflammatory mediators released by tumors such as TNF-α, IL-6 and VEGF also play a role in macrophage recruitment to the tumors.[22]

TAMs promote tumor growth

Once in the tumors, TAMs secrete a series of cytokines, chemokines and growth factors to promote tumor growth by setting up an immunosuppressive microenvironment, supporting de novo angiogenesis and enhancing the metastatic potential of malignant cells (Table 1).

In accordance with their ability to set up a favorable immune environment for tumor growth, TAMs possess the ability to strongly suppress anti-tumor immunity. One mechanism employed by macrophages to suppress anti-tumor immunity is expression of the inhibitory programmed death-1 (PD-1) and its ligands, PD-L1 and PD-L2.[23,24] Engagement of PD-1 on T cells and macrophages themselves leads to suppression of CTLs and inhibition of phagocytosis respectively.[23,25,26] In addition to the expression of inhibitory signals such as PD-L1 and 2, TAMs also produce Arg-1 which depletes arginine from the tumor microenvironment leading to further inhibition of effector T cell responses as T cells require arginine for activation and because the catabolic byproducts of arginine themselves are immunosuppressive.[27–29]

In addition to establishing an immunosuppressive microenvironment in tumors, TAMs also enhance angiogenesis in tumors. Often found in association with tumor vasculature, macrophages are drawn to hypoxic areas within tissue.[30] Once there, lactic acid-mediated hypoxia-inducible factor-1α (HIF-1α) activation in macrophages leads to transcription of VEGF which induces angiogenesis in tumors.[31,32] Macrophages also alter the tumor matrix making it more favorable for tumor growth through the release of tissue remodeling factors such as matrix metalloproteinase (MMP-9).[33] MMP-mediated remodeling of the extracellular matrix liberates “damage” signals which promotes ongoing chronic inflammation as well as altering the architecture of the tissue both of which further stimulate tumor cell invasion and subsequent metastases.[34,35]

As a result of the crucial role that macrophages play in the development and progression of cancer, many preclinical and clinical studies have directed their efforts at targeting macrophages and their activity.

Targeting macrophages in cancer

The presence of TAMs has been associated with chemotherapy resistance and poor clinical prognosis in multiple cancer types including pancreatic, prostate and breast.[36–38] Hence, multiple agents that deplete and/or prevent the infiltration of TAMs are currently under investigation in several disease sites (Table 2).

Experimental studies

Myeloid recruitment and expansion from progenitors into tumor-associated macrophages and other myeloid subpopulations, is dependent on three growth factors: M-CSF/CSF1, GM-CSF/CSF2 and G-CSF/CSF3. Targeting these growth factors by either blocking agents or genetic ablation reduced TAM accumulation leading to delayed tumor progression in models of breast and pancreatic neuroendocrine cancer.[39–43] Not surprisingly, blocking GM-CSF or G-CSF led to a preferential decrease in $CD11b^{+}Gr1^{+}$ and $Ly6G^{+}$ whereas CSF-1 blockade appears to have broader depletion of both $CD11b^{+}Gr\text{-}1^{+}$ and $CD11b^{+}Gr\text{-}1^{-}$.[38,40] In addition

to preventing the recruitment of monocytes, inhibition of the CSF1/CSF1R also blocks the polarization of TAMs into the pro-tumoral phenotype.[44] Qian et al further similarly showed that targeting CCL2 in a murine model of breast cancer mirrors CSF-1 ablation with significantly reduced metastatic disease.[45]

Clinical studies

Based on the preclinical data, clinical trials using agents that block CSF1/CSF1R and CCL2 target myeloid cells have been examined. While these therapies have largely been deemed safe, their anti-tumor efficacy has been mixed. Clinical trials of CSF1/CSF1R inhibitors including monoclonal antibodies and tyrosine kinase inhibitors are ongoing in multiple different malignancies with variable results.[46–48] In a phase 1 clinical trial, application of the monoclonal antibody emactuzumab (RG7155) inhibiting CSF1R activation achieved an objective response for 86% of patients with diffuse-type tenosynovial giant cell tumor with modest toxicity. One caveat, however, is that CSF-1R is overexpressed in this disease and thus the response may be due to direct activity on the receptor and not its effects on macrophages.[48] Other studies with CSF-1/CSF-1R directed agents in other disease histologies have been less successful with most showing limited responses.[49] Interestingly, in a study which sought to identify additional factors that mediate resistance to CSF-1R antibody, IL-4 treatment restored viability of emactuzumab-treated macrophages in vitro with this population of macrophages showing increased expression of CD206.[50] This in vitro data was mirrored by data from melanomas with high levels of IL-4 expression which show more $CD206^{+}$ macrophages infiltration upon emactuzumab treatment suggesting that the IL-4 pathway may be an important target in conjunction with CSF-1R directed agents.[50] The phase 1 study with the CCL2-blocking agent (carlumab) in patients with advanced solid malignancies, showed evidence of transient free CCL2 suppression and preliminary anti-tumor activity with minimal toxicity.[51] However, the follow-up phase 2 clinical trial in patients with metastatic prostate cancer, failed to show anti-tumor activity in part because cessation of carlumab resulted in rebound elevation of serum CCL2 levels likely due to compensatory increases in CCL2 production with antibody administration.[52] Another potential explanation for the lack of anti-tumor activity with carlumab is the weak binding affinity of of the antibody which may have allowed for continued CCL2 signaling.[52]

Another strategy to target macrophages has been to target inflammatory cytokines that attract myeloid cells to sites of inflammation. One example of this is the anti-IL-6 antibody (siltuximab), which was shown to decrease circulating CCL-2 and CXCL-12 leading to reduced TAM infiltration in ovarian xenografts.[53] Consistent with this preclinical data, siltuximab has demonstrated modest anti-tumor efficacy in patients with prostate[54] and renal cancer[55] though it has been less successful in other solid tumors.[56] Thus, drugs targeting macrophage infiltration into tumors as single agents have had excellent safety and some modest responses. Given the limited clinical responses to agents directed at the entire macrophage population, other groups have pursued promising strategies for tumors that target macrophage functional phenotypes instead.

Targeting macrophage polarization in cancer

Experimental studies

As TAMs often express M2-like characteristics, several groups have pursued a therapeutic strategy of reprograming TAMs towards more cytotoxic, anti-tumor M1 phenotypes.[57] To that end, two primary strategies to re-educate macrophages have been employed successfully: enhancing M1 polarization directly and preventing M2-polarization (Table 2). Therapies designed to increase M1 polarization are typically directed at activating the pathways used to clear damaged or infected cells. For example, bacterial products such as *Pseudomonas aeruginosa* mannose-sensitive hemagglutinin which activates toll-like receptors[58] and nanoparticles like ferumoxytol, a bioconjugated manganese dioxide which stimulates production of reactive oxygen species have been used in murine models to target macrophages. Both treatments demonstrated reduced tumor growth and progression to metastases in models of lung and breast cancer.[58–60] Alternatively, preventing M2 polarization has also demonstrated anti-tumor efficacy in several different models. Two of the most promising approaches to target the M2 phenotype in macrophages include elimination the DICER protein in macrophages which leads to overexpression of microRNAs miR-511-3p or miR-26a both of which inhibit signaling required for M2 polarization and CSF-1/CSF-1R blockade.[61–63] Interestingly, CSF-1/CSF-1R blockade in murine models of pancreatic cancer and glioma led to selective killing of the M2 macrophages[64] and repolarization of the remaining TAMs into the M1 phenotype.[44] Overall, re-educating macrophages to an anti-tumor phenotype in murine models of cancer has been very effective and thus many of these strategies are starting to be explored in the clinical setting.

Clinical studies

The strategy of enhancing M1 macrophage activation has shown some clinical success. Two examples, CD40 agonist and β-glucan administration, whose primary mechanisms of action involve macrophages, have demonstrated early activity in both hematologic and solid malignancies. Anti-CD40 antibody triggers an anti-tumor immune response by signaling through CD40, a receptor of the TNF-α family widely expressed by antigen-presenting cells particularly macrophages. Trials with humanized anti-CD40 antibodies have demonstrated the ability to trigger T cell specific anti-tumor immune responses against diffuse large B cell lymphomas, melanoma and pancreatic cancer.[65–70] β-glucan, a yeast-derived polysaccharide, that can differentiate TAMs into an M1 phenotype has also been shown to have modest activity in a phase II multi-cancer study.[71]

One agent with potent anti-tumor activity that involves macrophages currently in clinical use for the treatment of sarcomas is Trabectedin (ET743, Yondelis), a natural product derived from the marine tunicate *Ecteinascidia turbinate*.[72–75] Though primarily thought of as a DNA-damaging agent,[76] recent data has revealed that administration also leads to specific apoptosis of macrophages by activating the caspase-8 signaling pathway in macrophages[77] and further that it inhibits in vitro differentiation of macrophages and the production of IL-6 and CCL2.[77] Thus, many effective therapies, like trabectin, may have unappreciated effects on macrophages as part of their mechanism of action. The experimental and clinical studies

highlighted here revel some of the complex role that macrophages play in tumor biology. Increasingly it has also been recognized that macrophages have the capacity to regulate not only the development and progression of tumors, but also the response to therapies particularly radiation.

Radiation and the Immune System

Radiation (RT) has been used for the treatment of cancer for over a century following its discovery by Wilhelm Roentgen in 1895.[78] The term radiation can refer to multiple types of energy along the electromagnetic spectrum, however therapeutic radiation typically refers to ionizing radiation with energies from the kilovoltage to megavoltage range. In this range of energies, radiation creates free radicals which can damage DNA which is one of the main cell intrinsic mechanisms by which RT is thought to kill cancer cells.[79] Advances in the delivery of RT over the last decade has made RT one of the mainstays of treatment for virtually every cancer type with approximately 60% of all cancer patients receiving RT at some point during their course of treatment.[80] While the direct effects of RT on tumors cells has been well studied, the consequences of the cell damage induced by RT on the tumor stroma, particularly the tumor-associated immune cells, remains largely unexplored.

Radiation-induced inflammatory response and macrophages

Mechanistic studies about RT have long focused on the tumor cell intrinsic mechanisms and only recently has it been recognized that tumor cell extrinsic factors including the immune microenvironment play an equally important role in determining the overall response of a tumor to RT.[81] Recent experimental evidence has demonstrated that RT can trigger an anti-tumor immune response and that an optimal response depends on the ability of RT to generate a productive anti-tumor immune response. Several groups including our own have shown that there is a characteristic inflammatory-response induced by RT (Fig 1). Akin to other immune reactions, the RT-induced inflammatory response consists of five phases: innate recognition, initiation of inflammation, antigen presentation, effector response and resolution. Macrophages play an important role in all phases of the RT-induced inflammatory response.

The initial response to RT involves the release of innate danger signals known as damage-associated molecular patterns (DAMPs) from irradiated cells in response to the damage induced by RT.[82] This immunogenic cell death process is characterized in part by release of high mobility group protein box 1 (HMGB1),[83] the expression of calreticulin (CRT) on the surface of the tumor cells, [84] release of ATP into the extracellular space,[85] production of heat-shock proteins (HSPs)[86] and leakage of double strand DNA into the cytosol.[87] Macrophages can sense many of these innate inflammatory molecules through their expression of TLR4 for HMGB1, NOD-like protein receptor 3 (NLRP3) for ATP, low-density lipoprotein-receptor-related protein (LRP) for calreticulin and cyclic GMP-AMP (cGAMP) synthase (cGAS) and its downstream adaptor stimulator of interferon genes (STING) for cytosolic DNA.[88] Downstream signaling from these receptors leads to release of pro-inflammatory cytokines that initiate the inflammatory cascade. Unlike the other damage associated molecules, early CRT expression allows tumor cells to be efficiently

engulfed by macrophages and dendritic cells (DCs), thereby setting the stage for efficient presentation of tumor specific antigen to CTLs.[84]

Following innate recognition of the DAMPs, downstream signaling from the sensing of DAMPs converges upon activation of the NF-kB pathway leading to the production of pro-inflammatory cytokines to initiate inflammation.[89] Much of this signaling occurs in innate cells such as macrophages, dendritic cells and, to a lesser extent, in the tumor cells themselves. Analysis of the tumor stroma following RT revealed an increased number of macrophages due to their resistance to RT-mediated death and enhanced recruitment.[38,90,91] In addition to release of cytokines from innate immune cells following RT, several different cancer cell lines have demonstrated increased production of the cytokines IL-1α, IL-6, GM-CSF and IL-8 following exposure to RT compared to unirradiated controls.[92,93] Tumor cells also release other pro-inflammatory molecules including chemokines following RT. For example, in a murine mammary carcinoma model, the RT-induced chemokine CXCL16 recruits tumor infiltrating lymphocytes, amplifying the immune response.[94] The combination of these cytokines and chemokines acts as a positive feedback loop recruiting more immune cells chief among them are myeloid-macrophage cells which then release more pro-inflammatory molecules.

Once a response has been initiated, macrophages and DCs migrate to the lymphoid tissues carrying tumor antigens for presentation to T cells to generate an anti-tumor immune response. RT strongly enhances antigen presentation by inducing GM-CSF and expression of costimulatory molecules. Increased GM-CSF secretion following RT results in increased differentiation of DCs which augments tumor recognition by the host immune system.[95] Furthermore, T cell costimulatory molecules including ICAM-1 and B7.1 and 2 necessary for T cell activation can be induced within in the tumor following RT, which enhances the development of an anti-tumor immune response.[96,97] Once the T cell response is underway, release of IFN-γ from T cells as a result of RT further upregulates the expression of MHC I molecules on both tumor and stromal cells, leading to better antigen presentation.[98,99] Myeloid cells including macrophages play a critical role in the presentation of antigen and costimulation to T cells within the tumor microenvironment allowing for the development of an anti-tumor immune response.

As T cells become activated, they migrate back into tumors in response to factors such as CXCL16 released by macrophages, other immune cells and tumor cells as described above. These effector T cells produce a potent anti-tumor immune response that is one of the key mechanisms by which RT works therapeutically. Mediated primarily by IFN-γ producing $CD8^+$ T cells,[100] several recent studies have revealed that macrophages also play a role in supporting the initial anti-tumor immune response through both direct tumor cytotoxicity and the production of inflammatory cytokines.[101] However, even though RT produces a potent anti-tumor immune response in most patients, the magnitude and durability of the response to RT is highly variable. This occurs in part because simultaneously with the initial RT-induced anti-tumor response, RT also triggers potent immunosuppressive and healing mechanisms that support tumor regrowth and/or resistance (Fig 1). Consistent with this hypothesis, experimental data from several murine tumor models have shown that RT recruits both M1 and M2 macrophages from bone marrow derived myeloid cells.[102–104] For

example, in a murine prostate cancer model, both single dose (25 Grays/Gys) and fractionated irradiation (15 X 4 Gys) resulted in intratumoral macrophages with both higher expression of both M1 markers such as COX-2 and iNOS as well as M2 markers including Arg-1.[105] Interestingly, the balance of M1 versus M2 macrophages produced following RT may depend on the radiation dose. In a model of pancreatic adenocarcinoma, low-dose γ irradiation led to the differentiation of $iNOS^+$ M1 macrophages which promoted efficient recruitment of tumor-specific T cells by helping normalize the tumor vasculature.[106] Due to their plasticity and potent regulatory potential, existing macrophages and those subsequently recruited following RT play an important role in both the initial anti-tumor immune response and the later creation of an immunosuppressive pro-tumoral microenvironment.

Similar to other immune responses, the RT-induced effector phase is then followed by a resolution phase in which tissue homeostasis is restored by suppressing any ongoing immune responses and repairing the tissue by restoring the matrix integrity and blood supply through angiogenesis. Myeloid cells and tissue macrophages dominate this phase of immune responses having both immunosuppressive and tissue repair activity. RT-mediated inflammation also induces the pathways used for the resolution of immune responses in which macrophages play the major role. For example, RT induces the transcription of HIF-1α which leads to increased expression of CXCL-12, CCL-2, CSF1 and VEGF which support angiogenesis, recruit macrophages and promote their immunosuppressive function.[107–110] HIF-1α and IFN-γ signaling also induces the expression of PD-L1 in TAMs and tumor cells which suppresses the anti-tumor immune response.[111] Additionally, RT causes cancer cell death partially via apoptosis which is known to induce immunosuppressive and anti-inflammatory phenotypes in macrophages as they clear dying cells and antigens.[112] Apoptotic cells drive differentiation of macrophages into the M2 phenotype with enhanced secretion of anti-inflammatory cytokines such as TGF-β and IL-10 and upregulation of Arg-1.[113,114] In fact, in non-tumor settings, systemic administration of apoptotic cells is an efficient means to generate antigen-specific tolerance.[115] Thus, RT induces a complex immune response that includes strong immune stimulatory effects but also many immune suppressive pathways in the tumor microenvironment both of which depend on RT's effects on myeloid cells including macrophages.

Combining macrophage targeting with radiation therapy

As we outlined above, radiation recruits large numbers of myeloid cells to tumors in response to both immunogenic cell death and the ensuing hypoxia from microvessel apoptosis. Given the increased recruitment of myeloid cells post-RT and the limited efficacy of macrophage targeting alone,[44,116] the myeloid-macrophage compartment makes an ideal target for combining with RT to enhance its anti-tumor efficacy.

Targeting macrophage infiltration in combination with RT

Experimental studies

While macrophages play an important part in the initiation of the anti-tumor immune response following RT, they have a much more diverse and extensive role in suppressing post-RT anti-tumor immunity and supporting tumor regrowth. Both in vitro and in vivo

TAMs isolated from irradiated tumors support tumor growth[105] and resistance to RT.[117] This resistance may be attributed in part to the “pro-tumor” M2-like phenotype of macrophages following RT with increased expression of PD-L1 and Arginase-1 as well as secretion of VEGF[118] and MMP-9.[119] Thus, strategies eliminating or inhibiting macrophages in combination with RT have demonstrated enhanced anti-tumor efficacy.[120,121] Two methods for targeting macrophages in conjunction with RT have shown efficacy experimentally, depleting macrophages and preventing their migration to tumors (Table 3).

Early evidence that macrophages impact the efficacy of RT came from depletion of bone marrow-derived cells from tumors (consisting largely of macrophages) by whole body radiation which delayed lung tumor regrowth after local irradiation.[122] Subsequently, another group reported that elimination of TAMs by the macrophage-depleting agent liposomal clodronate increased the response to RT in a murine melanoma model.[118] These early studies suggested that macrophages in general limit the tumor response to RT and thus other groups have developed other strategies to target macrophages as whole-body RT and liposomal clodronate have limited clinical utility.

Elimination of macrophages in tumors remains challenging as there are limited agents with adequate depleting capabilities that can be used outside the experimental setting, thus most of the current agents targeting macrophages with RT have focused on inhibiting macrophage migration into tumors. Several pathways that have been effectively targeted in combination with RT include the adhesion molecule CD11b, the macrophage cytokine and its receptor CSF-1/CSF-1R, and the chemokines CCL2 and CXCL12 and their respective receptors CCR2 and CXCR4.

CD11b, the α-subunit of the CD18 integrin, is expressed primarily on monocytes and macrophages[123] and administration of a CD11b-neutralizing antibody resulted in an improved response to RT in a murine squamous cell carcinoma xenografts model.[90] Similarly, in CD18-deficient mice, implanted tumors are more sensitive to irradiation compared to their wild type littermates,[90] though interestingly mice genetically-deficient in CD11b have a similar level of radiosensitivity compared to wild type mice.

In irradiated tumors, the production of the macrophage cytokine CSF1 increases by around two-fold compared to non-irradiated tumors, likely due to the recruitment of the DNA damage-induced kinase ABL1 into the cell nucleus where it binds with the promoter of CSF1 to enhance its expression.[49] PLX3397 (Plexxicon) is a small molecule that selectively inhibits the tyrosine kinase activity of CSF1R. In murine models of prostate cancer, breast cancer and glioblastoma, application of PLX3397 with RT suppress tumor growth more effectively than RT alone.[49,101,107] In each of these models, blockade of CSF1R signaling with either a small-molecule inhibitor or a CSF-1 neutralizing antibody dramatically decreased the mobilization of TAMs and improved the therapeutic efficacy in a $CD8^+$ T-cell dependent manner.[101] Interestingly, CSF-1 inhibition may not only affect the presence of TAMs, but also their suppressive activity. A neutralizing antibody against CSF1 prevented RT from altering the phenotype of macrophages in tumors to M2 macrophages and increased the efficacy of RT in a murine model of pancreatic cancer.[124]

Similar to blocking CSF-1/CSF-1R, several agents have been developed to target the CCL2-CCR2 axis to inhibit the migration of monocytes into tumors. In several different mouse models of cancers, inhibition of CCL2 either by siRNA or monoclonal antibodies markedly reduced infiltration of macrophages leading to increased survival in tumor-bearing animals.[45,125–127] For example, using a syngeneic murine model of pancreatic ductal adenocarcinoma, Kalbasi et al found that RT induced a significant increase in CCL2 production in tumors with subsequent recruitment of $CCR2^+$ inflammatory monocytes.[128] Administration of an anti-CCL2 antibody inhibited RT-induced monocyte recruitment and delayed tumor growth but only when given in combination with RT.[128] Surprisingly, another study with a similar antibody found that interruption of CCL2 blockade led to enhanced metastases and reduced survival. This effect was attributed in part to enhanced angiogenesis from excess VEGF-A production and increased proliferation of metastatic cells from elevated IL-6 signaling both of which occurred as a result of rebound macrophage infiltration following cessation of CCL2 inhibition.[129] This highlights a need for caution when utilizing anti-CCL2 agents and perhaps all agents targeting macrophages as the monotherapy in metastatic cancer.

In a glioblastoma multiforme (GBM) xenograft model, pharmacologic inhibition of the CXCL12/CXCR4 interaction blocked the recruitment of $CD11b^+$ monocytes to the tumor and significantly slowed tumor regrowth.[102,130] In a autochthonous rat brain tumor model, treatment with the CXCL12 inhibitor Ola-peg with RT significantly prolonged survival compared to irradiation alone.[131] Treatment with AMD3100, a small bicyclam molecule that inhibits the binding between CXCL12/CXCR4, in combination with RT also significantly delayed tumor regrowth in xenograft and syngeneic models of breast, prostate and lung carcinoma.[122,132–134] Interestingly, the enhanced efficacy observed with AMD3100 is lost when it was administered 5 days after radiation, suggesting that the CXCR4 signal is responsible for macrophage recruitment only in the period shortly following RT.[122] In cervical cancer, analysis of human surgical specimens found that CXCR4 expression correlates with cancer severity. In orthotopic cervical cancer xenografts, addition of AMD3100 with standard radiation therapy and chemotherapy improved anti-tumor immune responses and reduced metastases without increased toxicity.[135] The importance of CXCL12 following RT is further underscored by studies targeting CXCR7, the high-affinity receptor for CXCL12. In murine and human GBM xenograft models, inhibition of CXCR7 by a specific antagonist (CCX771) post-RT prevented tumor recurrence and significantly prolonged survival.[136]

In sum, multiple agents targeting macrophages have shown synergy when administered with RT. While many of these agents have limited efficacy alone, more clinical trials to further explore the interaction between RT and macrophages are warranted.

Clinical studies

No clinical outcomes using macrophage targeting agents with RT have been reported to date, however several trials are underway in glioblastoma and other histologies testing the efficacy of adding agents that target macrophages to standard courses of RT.

Targeting macrophage polarization in combination with RT

Experimental studies

As targeting macrophages in combination with RT has shown early promise, other strategies, particularly those targeting macrophage polarization, have been explored to understand whether it might be possible to enhance the synergy with RT even further by promoting the anti-tumor activity of macrophages while limiting their tumor promoting functions. Similar to the studies targeting macrophage polarization alone described previously, the two main strategies of enhancing the anti-tumor (M1) macrophages or reducing pro-tumor (M2) macrophages have been employed in combination with RT (Table 3).

TLR agonists represent a novel approach to stimulate an anti-tumor immune response by activating cytotoxic activity in macrophages and thereby enhancing the subsequent innate and adaptive anti-tumor immune response.[137] In a murine model of skin-involving breast cancer, topical treatment with the TLR7 agonist imiquimod significantly slowed tumor growth when administered with RT versus imiquimod alone with responding tumors showing increased tumor infiltration by $CD11c^+$ dendritic cells, $CD4^+$, and $CD8^+$ T cells.[138] Moreover, low-dose chemotherapy cyclophosphamide which is thought to deplete myeloid-derived suppressor cells (MDSCs) given before start of treatment with imiquimod and RT further improved tumor inhibition.[138] Other chemotherapies have also been found to enhance the efficacy of RT in part through their effects on macrophages. For example, in a murine mammary carcinoma model, paclitaxel enhanced the effect of RT and was found to promote IL-12 production from TAMs to inhibit their suppression of T cell cytotoxic activity.[139,140]

Innate immune cells can also detect the presence of cancer cells and trigger an adaptive anti-tumor response. The STING pathway which senses cytosolic tumor-derived DNA promotes type I IFNs production and boost the anti-tumor immune response. Recent studies revealed that the therapeutic effect of RT depends on the STING pathway, through the production of type I IFNs and the downstream T cell response.[87] Importantly, treatment with a STING agonist and RT synergistically amplify the anti-tumor immune response in several tumor-bearing murine models.[87,88]

In addition to augmenting the anti-tumor activity, it has become increasingly recognized that targeting the immunosuppressive microenvironment in tumors is crucial to producing a successful anti-tumor immune response. Macrophages have a large role in establishing the immunosuppressive microenvironment in tumors. They acquire this immunosuppressive capability through a number of pathways including IL-4, TGF-β and Axl/MerTK. Multiple recent studies have demonstrated improved responses to RT when combined with agents that target these pathways.

In a syngeneic orthotopic murine model of breast cancer IL-4 blockade in combination with RT significantly delayed tumor regrowth compared to RT alone. This enhanced response to RT was associated with an increased number of $CD8^+$ T cells and reduced number of $CD4^+$ T cells.[101] Indeed, neutralization of IL-4 led to a significant enhancement of anti-tumor immunity while limiting the development of immunosuppressive macrophage phenotypes.

[101] This study suggests that therapeutic targeting of Th2 cytokines enhances the efficacy of RT by altering the macrophage phenotype to favor anti-tumor activity following RT.

Overexpression of TGF-β in tumors is associated with early metastatic recurrences and poor patient outcome.[141] In the MMTV-PyMT (expression of the polyoma middle T antigen under the control of the murine mammary tumor virus) transgenic model of metastatic breast cancer, both RT and chemotherapy increase the levels of circulating TGF-β which promotes lung metastasis during tumor regrowth.[142] Focal tumor radiation also sharply increases intratumoral TGF-β in a dose dependent manner.[143] Application of TGF-β neutralizing antibody enhances the local response to RT and abrogates the RT-induced increase in lung metastases in part through its effects on macrophages and the formation of regulatory T cells.[142,143] Macrophages express the full complement of TGF-β receptors and when exposed to TGF-β, macrophages adopt a strongly immunosuppressive, regulatory phenotype.[144] TGF-β has also long been known to be critical for the formation of regulatory T cells.[145] Thus, given the upregulation of TGF-β following RT, targeting TGF-β in combination with RT may have synergy because of the combination's potential to target multiple suppressive pathways in tumors.

The Tyro3/Axl/Mer family of tyrosine kinase receptors are strongly expressed on myeloid cells and associated with tumor invasion and metastasis.[146] These tyrosine kinases regulate the function of mature macrophages through their control of macrophage mediated apoptosis.[147] The receptor Axl is highly expressed in the radioresistant tumors but not in radiosensitive tumors. Genetic ablation of Axl enhanced antigen presentation, altered cytokine secretion and restored radiosensitivity.[148] Another receptor, Mertk, in the Tyro3/Axl/Mer family also mediates the development of a suppressive macrophage phenotype following RT. Ligation of Mertk on macrophages resulted in suppressive cytokine release via NF-κB p50 upregulation, which in turn limited tumor control following RT.[149] Elimination of this pathway led to enhanced anti-tumor immune responses following RT.

Metabolic reprogramming of the tumor has also been shown to alter the immune composition of the tumor microenvironment primarily through its effects on macrophages. Administration of a glycolysis inhibitor, 2-Deoxy-D-Glucose (2-DG), improved the radiosensitivity by increasing the RT-induced anti-tumor immunity in a murine model of Ehrlich ascites tumor.[150] A follow up study showed that the combined treatment of RT and 2-DG not only enhanced the functional activation of macrophages but also skews the macrophages towards an M1 phenotype. [151]

Thus, multiple lines of experimental evidence indicate that targeting macrophage phenotypes in combination with RT can dramatically enhance the response of tumors to RT. Strategies that augment the cytotoxic, classically activated M1 phenotype or reduce the immunosuppressive, alternatively activated M2 phenotype have all shown success in preclinical models. Thus, these strategies which directly or indirectly target myeloid cells and their functional activity are currently being explored in the clinical setting.

Clinical studies

Though no agents specifically targeting macrophage phenotype have been tested in combination with RT, several agents that strongly impact myeloid functional phenotypes have been successfully employed in clinical trials with RT. These agents include indoleamine-2,2-dioxygenase (IDO) inhibitors, TLR9 agonists and the tyrosine kinase inhibitor sunitinib.

TAMs and MDSCs highly express the tryptophan catabolic enzyme IDO, which induces T-cell dysfunction by depletion of tryptophan and accumulation of toxic catabolites. As IDO is in part responsible for tumor resistance to anti-CTLA-4 and anti-PD-1 therapy,[152] administration of an IDO inhibitor in combination with anti-CTLA-4 or anti-PD-1 has been tested in combination with dual checkpoint blockade. Indeed, similar to the preclinical data[153], the combination of an IDO inhibitor and an anti-PD-1 antibody demonstrated excellent clinical responses with the objective response rates reaching to 53% in late-stage melanoma patients.[154]

Several preclinical and clinical studies have also shown that TLR9 agonists such as CpG reduce the number and suppressive activity of tumor-infiltrating MDSCs and induces their maturation into M1 macrophages.[155] In patients with mycosis fungoides, a subtype of cutaneous T-cell lymphoma, combining RT and in situ vaccination with CpG showed a 33% response rate in a small phase I trial.[156] Preclinical data in mice and dogs combining CpG, RT and IDO inhibition suggest that optimal responses may require combinations of immunotherapies that both augment cytotoxic responses and inhibit immunosuppressive activity with RT.[153,157]

Several tyrosine kinase inhibitors are currently approved for the treatment of multiple cancer types. Mechanistically, they inhibit cellular signaling by targeting multiple receptor tyrosine kinases such as platelet-derived growth factor (PDGF), VEGFRs and c-Kit. [158] With respect to macrophages, sunitinib has been shown to limit macrophage M2 polarization by inhibiting STAT3 signaling which allows more macrophages to retain their M1 phenotype despite suppressive signaling. In a murine model of pancreatic cancer, sunitinib sensitized pancreatic tumors making them more susceptible to RT.[159] In a phase 2 trial of patients with localized high-risk prostate cancer, prolonged disease control was observed with the combined application of sunitinib, chemotherapy and RT.[160] Pathologic analysis of the tissue suggested that the combination of RT, chemotherapy and a similar tyrosine kinase inhibitor, sorafenib, can reverse TAMs to an anti-tumor phenotype.[161] Similar enhanced efficacy was seen in metastatic renal cell carcinoma[161] and unresectable hepatocellular carcinoma.[162]

In summary, while these agents all undoubtedly have effects outside of their impact on myeloid-macrophage cells, some of their bioactivity is likely attributable to their ability to influence the functional phenotype of macrophages. As macrophages appear to have an outsized role in regulating the tumor response to RT, therapies directed at pathways that increase the number of cytotoxic macrophages and reduce the formation of immunosuppressive, pro-tumor macrophages may prove to have potent synergy with RT.

Future macrophage-directed drug targets in combination with RT

Macrophages play a critical role in regulating the RT-induced inflammatory response. Thus, immunotherapies targeting macrophages and/or their functional polarization represent logical targets to be given in conjunction with RT and multiple preclinical studies and early clinical data supports this approach (Fig 2). Again, strategies to augment the anti-tumor immune response and prevent the late tumor and RT mediated M2 polarization of the myeloid-macrophage cells are the most promising targets to combine with RT. One of the most promising agent that augments the immune response to RT in preclinical models is STING agonists. As macrophages produce type I IFNs in a STING-dependent manner in response to cytosolic DNA produced by RT,[88] application of a STING agonist with RT may augment the RT-induced inflammation leading to enhanced anti-tumor immunity.

With the success of PD-1/PD-L1 directed therapies, it has become increasingly evident that reducing the immunosuppressive tumor microenvironment is necessary for the development of anti-tumor immunity. Macrophages play a key role in establishing this immunosuppression and thus agents that target the immunosuppressive function of macrophages will likely prove to be important targets in future trials. Two such pathways critical for establishing the immunosuppressive macrophage phenotype are the IL-10 and IL-4 pathways. IL-10 is a key immunosuppressive cytokine that promotes the regulatory functions of macrophages. Blockade of IL-10 in multiple animal models has led to delayed tumor growth and improved overall survival,[163,164] therefore targeting IL-10 in combination with RT may lead to better control of tumor progression via modulation of TAM-mediated immunosuppression. IL-4 is best known for its role in mediating Th2 immunity, however it has also long been associated with M2 polarization of macrophages as well. Binding of IL-4 to the IL-4 receptor results in the phosphorylation and dimerization of STAT6. STAT6 dimers translocate into the nucleus where they promote expression of the Th2 master regulator GATA-binding protein 3 (GATA3) which leads to transcription of the M2 program in macrophages.[165] Targeting this pathway through IL-4 or IL-4 receptor blockade and/or inhibition of downstream signaling has been shown in preclinical data to lead to significant enhancement of the efficacy of RT. Given the critical role of the IL-4 pathway in mediating the M2 macrophage phenotype, this remains an excellent target for future combinatorial clinical studies.

Conclusions

Macrophages participate and regulate nearly every aspect of tumor development from the initial establishment of the premalignant niche to the angiogenesis and immunosuppression needed for tumor progression and the development of metastases. They exhibit tremendous plasticity and can be polarized into phenotypes that range from the pro-inflammatory, anti-tumor M1 phenotype to the immunosuppressive, pro-tumor M2 phenotype in response to the different environmental stimuli encountered in tumors. Radiation therapy can affect the development of both the M1 and M2 activation pathways. The initial cell damage created by RT initiates a cytotoxic program characterized by Th1 cytokine secretion and $CD8^+$ T cells that favors the development of anti-tumor macrophage phenotypes. However, simultaneously RT activates immunosuppressive pathways including IL-4, IL-10 and TGF-β that lead to the

formation of immunosuppressive, pro-tumor M2 macrophages. Targeting macrophage infiltration and polarization in combination with RT has demonstrated tremendous synergy in preclinical models and early phase clinical trials. Further clinical trials are currently underway exploring combinations of RT with therapeutics directed at macrophages to produce enhanced cytotoxic activity while reducing or reversing the development of immunosuppressive macrophage phenotypes.

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Abbreviations

RT	radiation therapy
IFN-γ	interferon-γ
LPS	lipopolysaccharide
TLR	toll-like receptor
STAT	signal transducer and activator of transcription
NF-kB	nuclear factor-kB
CXCLs	C-X-C motif ligands
CCLs	C-C motif ligands
INOS	inducible nitric oxide synthase
IL	interleukin
TNF	tumor necrosis factor
Arg-1	arginase-1
VEGF	vascular endothelial growth factor
CSF1	colony stimulating factor 1
TAMs	tumor-associated macrophages
M-CSF	macrophage colony-stimulating factor
PD-1	programed death-1
HIF-1α	hypoxia-inducible factor-1α
MMP	matrix metalloproteinase

DAMP	damage-associated molecular pattern
HMGB1	high mobility group protein box 1
CRT	calreticulin
HSPs	heat-shock proteins
NLRP3	NOD-like protein receptor 3
LRP	low-density lipoprotein-receptor-related protein
CGAS	cyclic GMP-AMP synthase
STING	stimulator of interferon genes
DCs	dendritic cells
Gy	Gray
MiRNA	microRNA
GBM	glioblastoma multiforme
MDSCs	myeloid-derived suppressor cells
IDO	indoleamine-2,2-dioxygenase
PDGF	platelet-derived growth factor
GATA3	GATA-binding protein 3

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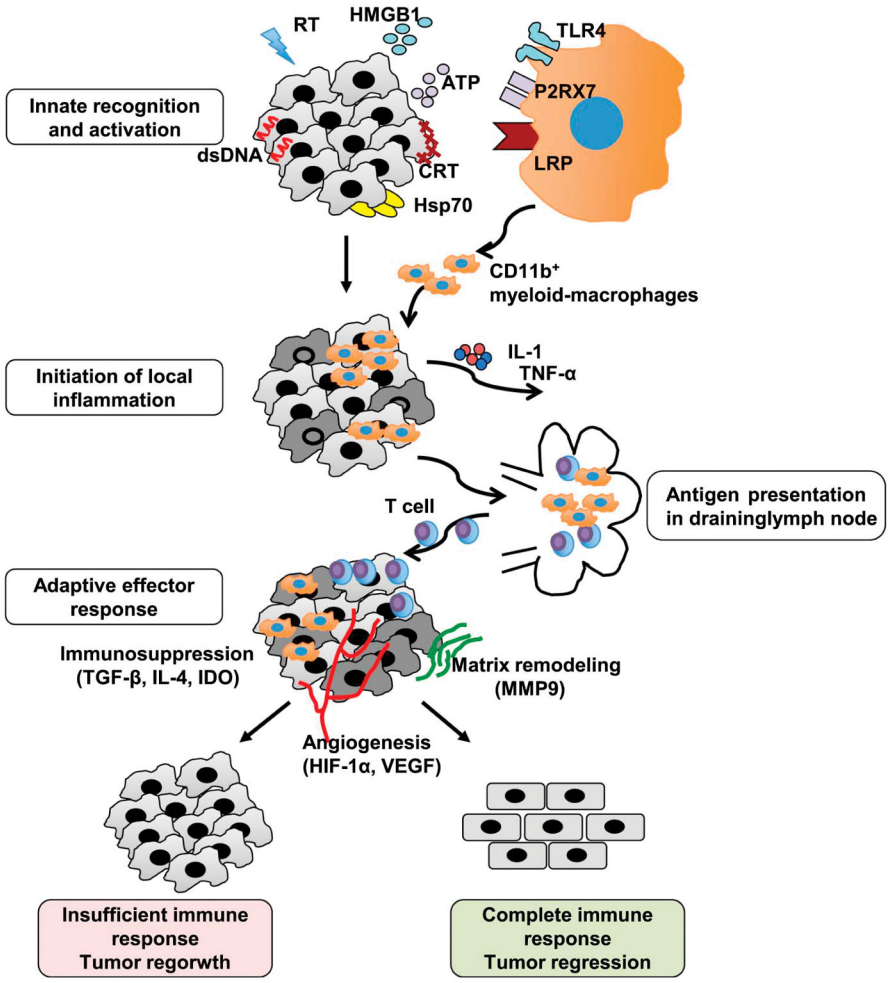

Fig. 1. Macrophages and the radiation-induced immune response

Ionizing radiation (RT) induces an anti-tumor immune response within the tumor through the generation of inflammatory mediators including cytosolic dsDNA, HMGB1, ATP, calreticulin (CRT) and Hsp70 within the tumor cells. These molecules activate the resident immune cells such as macrophages to secrete a series of cytokines/chemokines including IL-1 and TNF-α, which further recruits more macrophages to the tumor site. Activated macrophages and DCs migrate to the lymphoid tissues bearing tumor antigens, where they present them to T cells. Activated T cells then re-enter the circulation and return to the tumor where they target malignant cells. However, the outcome of the response is in part determined by the ability of the T cell response to the microenvironment. If the malignant cells are completely eradicated, macrophages help restore normal tissue homeostasis by supporting angiogenesis and matrix remodeling. If there is an insufficient immune response, macrophages still attempt to restore tissue to its normal state but in so doing inadvertently support tumor regrowth.

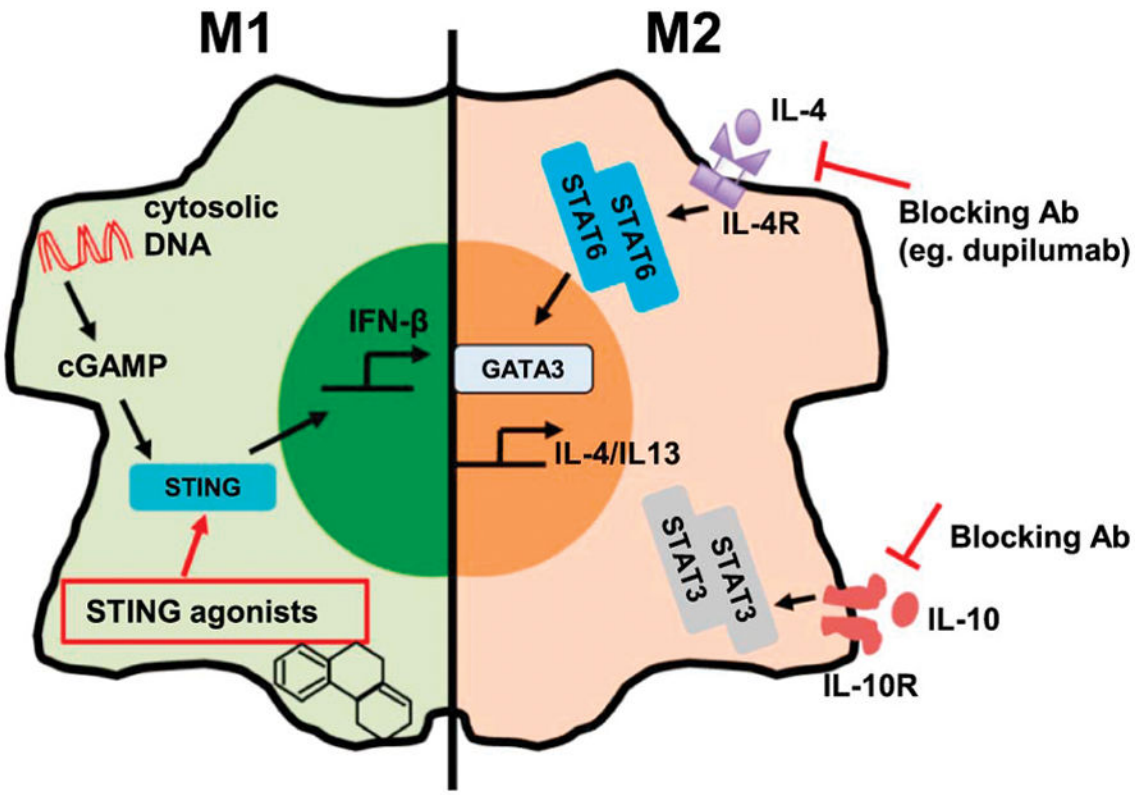

Fig. 2. Potential immune targets to combine with radiation therapy

The cGAS-STING cytosolic DNA sensing pathway is essential for production of type I IFNs such as IFN-β. An RT-induced DNA exonuclease Trex1 degrades cytosolic DNA to dampen the production of type I IFNs in response to RT. However, STING agonists can bypass this exonuclease and thus may directly activate macrophages to augment the response to RT. Ligation of IL-10 receptor activates STAT3, which is critical for the expression of its own cytokine IL-10. Blockade of the IL-10 pathway may disrupt this feedback and combined with RT to boost anti-tumor immunity. Upon IL-4 stimulation, the transcriptional regulator GATA3 induces a program that polarizes macrophages into an M2 phenotype. Agents that target the IL-4 pathway may improve the efficacy of RT by preventing formation of the M2 phenotype in macrophages in response to RT-induced IL-4 production.

Table 1

A simplified summary of the functions of the cytokines, chemokines and growth factors that produced by TAMs in tumor development.

Factors	Functions	Reference
PD-L1, PD-L2	immunosuppression by inhibiting T cell functions	26
TGF-β	immunosuppression by inducing Tregs, metastasis	166
IL-10	immunosuppression by suppressing antigen presentation	167
Prostaglandin E2	immunosuppression by suppressing DC activation	168
Arginase-1	immunosuppression by inhibiting T cell functions	27
CCL18	Metastasis	169
MMPs	metastasis, angiogenesis, matrix remodeling	170
TNF-α	metastasis, angiogenesis	171
VEGF	metastasis, angiogenesis	172
Fibroblast growth factor 2	Angiogenesis	173

Table 2

Single-reagents that target TAMs for cancer treatments.

Treatment	Type of study	References	
Targeting macrophage infiltration			
Anti-CSF1/CSF1R antibody	In vivo; mice; breast, osteosarcoma, pancreatic, glioma cancer	42–44,46,64	
PLX3397 (CSF1R inhibitor)	Phase 1 clinical trial; solid, pancreatic cancers	47,64	
Emactuzumab (anti-CSF1R antibody)	Phase 1 clinical trial; soft tissue tumors	48	
Siltuximab (anti-IL-6 antibody)	Phase 1, 2 clinical trials; ovarian, prostate, renal cell, advanced solid cancer	53–56	
Carlumab (anti-CCL2 antibody)	Phase 1, 2 clinical trials; solid, prostate cancer	51,52	
Anti-CCL2 antibody	In vivo; mice; breast, glioma, prostate cancer	45,126,127	
CCL2 siRNA	In vivo; mice; breast cancer	125	
Treatment	Type of study	Functions	References
Targeting macrophage polarization/activation			
P. aeruginosa mannose-sensitive hemagglutinin	Ex vivo	M1 promotion	58
Iron oxide nanoparticles	In vivo; mice; adenocarcinoma	M1 promotion	59
miRNAs	In vivo; mice; lung, liver cancer	M2 inhibition	61,62
STAT3 inhibition	Ex vivo; in vivo; mice; sarcoma	M2 inhibition	174,175
CD40 agonist	Phase 1 clinical trials; solid tumors and diffuse large B-cell lymphoma	M1 promotion	66,176
Dacetuzumab (anti-CD40 antibody)	Phase 1 clinical trial; non-Hodgkin's lymphoma	M1 promotion	67
CD870,873 (anti-CD40 antibody)	Phase 1 clinical trials; pancreatic ductal adenocarcinoma; solid tumors	M1 promotion	68–70
β-glucan	Phase 1 clinical trials; pancreatic ductal adenocarcinoma; solid tumors	M1 promotion	177
Trabectedin (anti-tumor reagent)	In vivo; mice; sarcoma, ovarian cancer Phase 3 clinical trial; soft tissue sacorma	M2 inhibition	73–75

Table 3

Studies that combine radiation therapy with macrophage manipulation for cancer treatment.

Treatment	Type of study	Reference	
Targeting macrophage infiltration			
Anti-CD11b antibody	In vivo; mice; squamous cell carcinoma	90	
PLX3397 (CSF1 inhibitor)	In vivo; mice; prostate, glioblastoma, breast cancer	38,49,101,107	
HIF-1 inhibitor	In vivo; mice; glioblastoma	102	
Ola-peg (CXCL12 inhibitor)	In vivo; rat; brain tumor	131	
AMD3100 (CXCL12/CCR4 inhibition)	In vivo; mice; prostate, cervical, lung cancer	122,133–135	
CCX771 (CXCR antagonist)	In vivo; mice, rat; brain tumor	136	
CXCL12 siRNA	In vivo; mice; astrocytoma	178	
Anti-CCL2 antibody	In vivo; mice; pancreatic ductal adenocarcinoma	128	
Treatment	Type of study	Functions	Reference
Targeting macrophage polarization/activation			
Anti-CSF1 antibody	In vivo; mice; pancreatic ductal adenocarcinoma	M2 inhibition	124
Anti-VEGF/VEGFR	In vivo; mice; melanoma, ovarian, breast,	M2 inhibition	118,179–181
Anti-TNFR antibody	In vivo; mice; melanoma	M2 inhibition	118
Paclitaxel (cell cycle inhibitor)	In vivo; mice; breast cancer	M1 promotion	140
CTLA-4 blockade	In vivo; mice; breast, melanoma cancer; Case reports, phase 1 clinical trial; melanoma, non-small cell lung carcinoma	M2 inhibition	148,182,183 183–185
TLR7 agonist imiquimod	In vivo; mice; breast cancer	M1 promotion	138
TLR9 agonist CpG ODN	Phase 1/2 clinical trial; mycosis fungoides	M1 promotion	156
IDO inhibitor	Phase 1b/2 clinical trial; melanoma	M2 inhibition	154
IL-2	In vivo; mice; sarcoma, melanoma Phase 1 clinical trial; renal cell carcinoma	M2 inhibition	186,187
Sunitinib (Tyrosine kinase inhibitor)	In vivo; mice; pancreatic adenocarcinoma; Phase 2 clinical trial; prostate cancer	M2 inhibition	159,160
Sorafenib (Tyrosine kinase inhibitor)	Patient case report; renal cell carcinoma; Phase 2 clinical trial; hepatocellular carcinoma	M2 inhibition	161,162