



Multifaceted Roles for Macrophages in Prostate Cancer Skeletal Metastasis

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Bone-metastatic prostate cancer is common in men with recurrent castrate-resistant disease. To date, therapeutic focus has largely revolved around androgen deprivation therapy (ADT) and chemotherapy. While second-generation ADTs and combination ADT/chemotherapy approaches have been successful in extending overall survival, the disease remains incurable. It is clear that molecular and cellular components of the cancer-bone microenvironment contribute to the disease progression and potentially to the emergence of therapy resistance. In bone, metastatic prostate cancer cells manipulate bone-forming osteoblasts and bone-resorbing osteoclasts to produce growth and survival factors. While osteoclast-targeted therapies such as bisphosphonates have improved quality of life, emerging data have defined important roles for additional cells of the bone microenvironment, including macrophages and T cells. Disappointingly, early clinical trials with checkpoint blockade inhibitors geared at promoting cytotoxic T cell response have not proved as promising for prostate cancer compared to other solid malignancies. Macrophages, including bone-resident osteomacs, are a major component of the bone marrow and play key roles in coordinating normal bone remodeling and injury repair. The role for anti-inflammatory macrophages in the progression of primary prostate cancer is well established yet relatively little is known about macrophages in the context of bone-metastatic prostate cancer. The focus of the current review is to summarize our knowledge of macrophage contribution to normal bone remodeling and prostate-to-bone metastasis, while also considering the impact of standard of care and targeted therapies on macrophage behavior in the tumor-bone microenvironment.

Keywords: bone, prostate cancer, metastasis, macrophage, polarization, therapy

INTRODUCTION

In 2018 alone, approximately 28,000 deaths from prostate cancer are predicted (1). While early stage disease is often treated successfully with surgery, radiation, and/or androgen deprivation therapy (ADT), advanced prostate cancer remains a moving target. Advanced disease typically manifests in the skeleton where metastases are often sensitive to first- and second-generation ADT. However, in a short period, the cancer becomes castrate resistant. In bone, prostate cancer causes extensive bone remodeling and formation that result in intense pain and heightened risk of pathologic fracture (2). These symptoms drastically reduce the patients' quality of life and contribute substantially to disease morbidity and mortality. Bone-metastatic castrate-resistant prostate cancer (mCRPC) is currently incurable and appears to be refractory to recent advances in immunotherapy, such as checkpoint inhibitors (3–5). However, immune-based therapies such as Sipuleucel-T have been beneficial for some patients indicating that there may be room for alternative strategies in targeting the immune

microenvironment of bone mCRPC. Despite macrophages constituting 8–15% of healthy adult male bone marrow, their role in the context of the bone-metastatic CRPC remains relatively underexplored.

MACROPHAGE FUNCTION IN TISSUE HOMEOSTASIS

Macrophages are phagocytic cells of the innate immune system responsible for maintenance of tissue homeostasis. Myeloid in nature and originating from hematopoietic stem cells that mature and differentiate into myeloblasts and monocytes, macrophages are noted for their diverse morphology and function across various tissues (6–8). For example, microglia are residential macrophages of the brain and play an important role in regulating synapse behavior (9). These cells have further demonstrated roles in immune modulation of inflammatory response to brain trauma at the blood–brain barrier (10). Other organ-specific macrophages include kupffer cells which turnover heme molecules through phagocytosis and degradation of hemoglobin in the liver (11, 12), and alveolar macrophages which engulf and eliminate dust particulates and microbes from the air on the luminal side of the mucosal epithelium lining in the lung (13, 14). Precursor and mature macrophages derived from the bone marrow also circulate the body, surveying and infiltrating sites of injury and infection to regulate local responses. Macrophages are known for their plasticity, and depending on signaling cues, can polarize into pro- or anti-inflammatory phenotypes. Traditionally, these phenotypes have been referred to as M1 and M2, but more recently it has been recognized that there are a spectrum of phenotypes across the M1/M2 continuum. Inflammatory stimuli released by necrotic or damaged tissue, such as interferon-gamma (IFN γ), interleukin-12 (IL-12), and reactive oxygen species (ROS) promote polarization into a pro-inflammatory phenotype (15–19), leading to the secretion of pro-apoptotic cytokines such as tumor necrosis factor (TNF) to induce apoptosis of neighboring cells. Pro-inflammatory macrophages can remove apoptotic neutrophils and cellular debris through phagocytosis and efferocytosis (20–24) and participate in the adaptive immune response by presenting disease-associated antigens to T and B cells that specifically target infectious agents or diseased cells (25–27). Following injury/infection resolution, secretion of factors including interleukin-10 (IL-10) and transforming growth factor beta (TGF β) by fibroblasts and platelets promote the polarization of anti-inflammatory macrophages (28). Anti-inflammatory macrophages suppress further inflammation by secreting TGF β , vascular endothelial growth factor (VEGF), and ROS that will deactivate T cells and promote T_H2 response (29–32). These factors will also stimulate expansion of fibroblasts, endothelial cells, and other cell types for tissue repair (33, 34).

MACROPHAGE ROLES IN BONE REMODELING AND INJURY REPAIR

In the bone marrow, osteoclasts and osteoblasts are bone-specific cell populations that serve to resorb and mineralize the bone,

respectively. The activities of these two populations are tightly coupled to ensure balanced bone turnover as well as returning the bone to homeostasis subsequent to injury. Osteoclasts are found residing on osteal surfaces and are histologically characterized as tartrate-resistant acid phosphatase (TRAP) positive and multi-nucleated (35, 36). Osteoclasts migrate to sites of active bone remodeling by chemotaxis, where they are involved in demineralization and resorption of the bone matrix (37–39). Upon apoptosis of the osteoclast, mesenchymal stem cell-derived osteoblasts rebuild the bone matrix *via* the deposition of type I collagen and hydroxyapatite (40). Traditionally, due to their myeloid origins and bone-specific functions, osteoclasts are considered the bone-resident macrophage population. However, roles for pro- and anti-inflammatory macrophages in controlling and coordinating osteoclast and osteoblast bone remodeling have been described. For example, IFN γ - and IL-12-stimulated NOS2 and TNF positive pro-inflammatory macrophages can promote osteoclast formation and bone resorption (41, 42). Conversely, anti-inflammatory macrophages are thought to contribute to bone formation (43).

A distinct population of bone-resident macrophages, osteomacs, has been described, and recent studies have shown important roles for these cells in modulating osteoblast activity in both bone homeostasis and injury repair (44). Osteomacs are morphologically characterized as mononuclear cells that form canopy-like structures around osteoblasts and can occupy as much as 75% of both murine and human endosteal and trabecular bone surfaces that are under active remodeling (45–48). Histologically, osteomacs are distinct from osteoclasts and are F4/80 positive but TRAP negative. Additionally, other groups have shown osteomacs to express common macrophage markers such as CD68, and also more specific markers, such as Mac-3 and CD169 (45, 46, 49). While osteomacs can be stimulated by receptor activator of nuclear kappa B ligand (RANKL) and colony stimulating factor-1 (CSF-1/M-CSF) to become osteoclasts *in vitro*, monocytes and other myeloid precursors were found to be more efficient osteoclast precursors (45). These data indicate that osteomacs are a plastic, yet distinct cell type, with specific functions in the bone marrow microenvironment. Indeed, further studies have revealed that osteomacs have diverse roles in regulating osteogenesis and osteolysis. Osteoblasts become inefficient as they age and need to be replenished to ensure proper homeostatic bone turnover (46). During normal bone turnover, osteomacs engulf apoptotic osteoblasts in a process called efferocytosis, which induces the secretion of TGF β , TNF, and oncostatin M that facilitate osteoblastogenesis and bone formation (45, 46, 48). This mechanism has been confirmed in various *in vitro* and *in vivo* contexts. For example, removal of osteomacs from bone marrow-derived osteogenic co-cultures reduced osteoblast number and osteoblastic mineralization (47). The MAFIA murine model is one in which administration of ligand AP20187 can systemically suppresses macrophage differentiation. Reduced osteoblast occupancy of the endosteal bone surfaces was observed in maturing MAFIA mice following AP20187 administration (47, 50). Congruently, parathyroid hormone-induced bone anabolism in the MAFIA model was suppressed upon macrophage ablation (51). Interestingly, when murine macrophages

were depleted by clodronate liposome-induced apoptosis, osteoblast numbers remained stable (47, 50). Further comparison between two methods of macrophage depletion showed that transient macrophage apoptosis induced osteomac expansion and efferocytosis, which further enhanced osteoblast activity (46, 51, 52). Additionally, C57BL/6 mice bone marrow treated with trabectedin, a chemotherapy antagonist of macrophages, showed diminished phagocytic genetic signature, efferocytotic osteomac-induced RUNX2 positive osteoblastogenesis, and associated BV/TV status (53). During bone fracture repair, osteomacs can also sense apoptotic damaged cells and in response, initiate inflammation and immune recruitment through secretion of immune attractant factors, such as chemokine (C-C motif) ligand 2 (CCL2) and M-CSF (48). Additionally, LPS-stimulated osteomacs express TNF and NOS2, and suppress osteoblast activity *in vitro* (45). *In vivo*, bone fracture induced pro-inflammatory polarization of immune macrophages and osteomacs to secrete TNF and IFN β , driving osteoclastogenesis and osteolysis (45). In fact, osteomacs have been shown to associate with osteoclasts at catabolic sites, substantiating their distinction from osteoclasts, and supporting their additional roles in regulating osteolysis (48). These studies indicate that osteomacs can direct the transition between osteolysis and osteogenesis by directly modulating the expansion and activity of osteoclasts and osteoblasts for repair in the event of bone injury (46). Taken together, these studies demonstrate the complex roles of bone-resident macrophages in bone remodeling (54, 55). How they contribute to the progression of bone-metastatic prostate cancer and respond to applied therapies has not been fully elucidated at this juncture.

MACROPHAGES PROMOTE PRIMARY PROSTATE CANCER PROGRESSION

Just as in other cancers, chronic inflammation in prostate cancer is thought to serve as a prelude to tumorigenesis (56). In fact, in cases of premalignant prostatic inflammatory atrophy, macrophages were observed coalescing at sites where inflammation-driven neoplasia caused disruptions in the epithelial lining of the prostate (57). In primary prostate cancer, pro- and anti-inflammatory tumor-associated macrophages (TAMs) have been found to comprise a significant portion of the immune cells infiltrating the tumor microenvironment with studies beginning to dissect roles for each population with regards to progression of the disease (58, 59). The exact pro- and anti-inflammatory constitution of TAMs vary across cancer types, but protective roles for TAMs have been described in prostate cancer. For example, macrophages located in the tumor-peripheral stroma correlated with increased recurrence-free survival (60), while macrophages expressing CD204, a marker associated with activation of antigen presentation in dendritic cells, correlate with better overall survival and prognosis (60–62). However, for the most part, macrophages have been found to contribute to, or directly promote, primary prostate cancer progression with individual patient cohort and meta-analysis studies identifying that macrophage infiltration correlates with disease aggressiveness and poor prognosis in prostate cancer (63–67). With respect to therapy, the density of

anti-inflammatory macrophages in the primary disease correlates with extracapsular and biochemical recurrence following radical prostatectomy and/or ADT (63, 65, 66, 68).

The tumor-promoting roles of anti-inflammatory macrophages are thought to revolve around their immune-suppressive and angiogenic effects, both of which are important hallmarks of prostate cancer progression (68–71). Prostate cancer cells have been shown to secrete factors such as CSF-1 and CCL2 that lead to the recruitment of monocytes and macrophages that facilitate these processes (68, 72–78). Once recruited to the microenvironment, macrophages are exposed to a milieu of environmental cues that can drive their polarization into pro- or anti-inflammatory states (58). For example, exposure to tumor-derived IL-10 and -13 promotes macrophage polarization into an anti-inflammatory state. Subsequently, macrophages secrete factors, such as epidermal growth factor (EGF), platelet derived growth factors, and VEGF that promote cancer cell proliferation and angiogenesis of the tumor microenvironment (69, 79–83). Furthermore, ARG1 and TGF β positive anti-inflammatory macrophages, along with myeloid-derived suppressor cells and regulatory T cells, collectively suppress inflammation and immune response within the tumor microenvironment (84–88). Both pro- and anti-inflammatory macrophages can also modulate T cell expansion and cytotoxicity by regulating the bioavailability of L-arginine, an important amino acid for T cell activity and survival (89). In addition, NOS2 positive pro-inflammatory macrophages synthesize nitric oxide that can promote T cell T_H1 expansion (90, 91). Conversely, anti-inflammatory macrophages expand during T_H2 response and additionally suppress T cell proliferation through expression of co-inhibitory molecule PD-L2 (30). Importantly, macrophages can also contribute to the activity of non-immune cells in the tumor microenvironment, such as cancer-associated fibroblasts (CAFs). Macrophage-secreted factors such as TGF β are known potent regulators of CAFs that also promote tumor growth and invasion into the peripheral tissue to facilitate metastasis (68, 71, 92, 93).

MACROPHAGE ROLES IN ESTABLISHING THE PRE-METASTATIC BONE MARROW NICHE?

While much is known about the role of macrophages in primary prostate cancer progression, less is known about how their polarization states in the bone marrow contribute to, or protect against prostate cancer metastasis to the bone and subsequent establishment. TNF, TGF β , and VEGFA can be secreted by primary prostate cancer cells into circulation (94), which can activate marrow cell populations including bone-resident macrophages and hematopoietic progenitor cells. Furthermore, these tumor-derived factors have been shown to induce the recruitment of immunosuppressive myeloid populations into the bone that support immune evasion and ease the establishment of circulating tumor cells (95).

Emerging evidence has also defined important roles for prostate cancer-derived exosomes in the genesis of receptive pre-metastatic niches (96, 97). Exosomes are nanometer-sized

vesicles that can be shed in large numbers by cancer cells. The cargo contents of cancer cell-derived exosomes vary greatly, but can contain cell-adhesion molecules, receptor tyrosine kinases, proteases, miRNAs and miRNA processing machinery, mRNA, and DNA (98, 99). Injection of mice with exosomes derived from human prostate cancer peripheral blood or murine prostate cancer cells lines (TRAMPc1) demonstrated impaired murine osteoclast formation and enhanced osteoblast differentiation suggesting that prostate cancer-derived exosomes play a role in tipping the balance toward bone formation, a common hallmark of bone-metastatic prostate cancer (100, 101). Milk fat globule-EGF factor 8 protein (MFG-E8) was found in human prostate cancer patient exosomes, and tissue biopsies. MFG-E8 has been shown to mediate macrophage efferocytosis of apoptotic osteoblasts and cancer cells; these macrophages then exhibit an anti-inflammatory phenotype and in turn promote immune suppression through expression of TGF β and ARG1 (102, 103). Characterization of prostate cancer-derived exosomes has identified various proteins and miRNA that can promote metastasis. Among the miRNA identified, miRNA-21 is particularly interesting given that it is upregulated in bone-metastatic prostate cancer and has known roles in regulating osteoclasto- and osteoblastogenesis (23, 97, 104, 105). Additionally, miRNA-21 is known to regulate macrophage phagocytosis of necrotic or diseased tissue in the context of wounding (23). Other miRNA identified in prostate cancer-derived exosomes that can influence osteoclast and osteoblast differentiation include miRNA-128 and -183 (95, 97).

Collectively, these studies show that bone marrow macrophages contribute to bone-metastatic outgrowth of disseminated prostate cancers, whereby cancer-derived signals or exosomes significantly influence macrophage activity in the pre-metastatic niche. In turn, these changes appear to be permissive for prostate cancer cell colonization of bone.

TAMs IN METASTATIC CASCADE OF PROSTATE CANCER

The role of TAM in the metastatic dissemination of primary prostate cancer has been extensively studied and reviewed. Here, we reference seminal review articles that outline the molecular and cellular communication between TAMs and primary prostate cancers resulting in tumor vascularization, epithelial-to-mesenchymal transition, intravasation, and eventual colonization of distal sites, including, specifically, the skeletal bone marrow (58, 83, 106–109).

MACROPHAGES IN THE PROGRESSION OF ESTABLISHED PROSTATE TO BONE METASTASES

Once actively growing in the skeleton, prostate cancer cells manipulate the cells of the bone microenvironment to promote areas of extensive osteolysis and osteogenesis. Osteoclasts have traditionally been regarded as a specialized bone-resident macrophage population due to their myeloid lineage and phagocytic nature in bone resorption, which leads to the release of bone

matrix-sequestered factors that feed the metastatic prostate cancer cells (110–112). While macrophages can fuse and form into osteoclasts in response to RANKL (113, 114), the role of individual macrophage populations in controlling prostate cancer bone interaction remains relatively underexplored. Recent observations in patient biopsies have implicated the role of osteal macrophages in established bone-metastatic prostate cancer (115). CD68 positive macrophages were detectable at high density within the tumor, whereas osteoclasts and osteomacs were found at the tumor-bone interface, suggesting potentially differential functions for each population in the growing lesions (115). Studies have also defined causal roles for macrophage populations in the growth of prostate cancer in bone. For example, intratibial inoculation of RM1 prostate cancer cells into macrophage-depleted bone marrow of MAFIA mice resulted in decreased pathologic osteolysis (107, 116). Additionally, depleting macrophages using clodronate liposome prior to tumor inoculation significantly limited cancer growth in bone (116). Further evidence supporting contributory roles for macrophages in the progression of bone-metastatic prostate cancer lesions has been provided using similar total macrophage depletion approaches (107, 115). Additionally, roles for osteomacs in the cancer-bone microenvironment have also been described, where CD169 positive tumor-associated osteomacs were found to facilitate tumor-induced pathologic osteogenesis. Interestingly, CD169 negative macrophages have been shown to promote tumor growth (115) and phenotypically resemble CD206 positive anti-inflammatory macrophages found in primary prostate cancer (109, 117). Taken together, these studies suggest that macrophages contribute to prostate cancer metastasis and growth in the bone microenvironment (**Figure 1**). However, deeper investigations into the precise roles of pro- and anti-inflammatory macrophages and osteomacs in the process are warranted.

MACROPHAGE RESPONSE TO STANDARD OF CARE TREATMENTS/THERAPIES

As discussed, macrophage polarization can have protective or contributory roles; however, the impact of standard of care approaches on macrophage behavior has not been explored in depth thus far. For men with bone-metastatic CRPC, treatment options largely focus on radiation therapy to alleviate pain and reduce tumor burden, or therapeutics that target the cancer cells, such as chemotherapy and ADT. Although castrate-resistant, CRPC prostate cancer cells remain dependent on androgen signaling *via* the expression of constitutively active androgen receptor splice variants, and/or autocrine expression of their own androgen (118–120). Underscoring this dependency on androgens or the AR receptor for survival, second-generation ADTs (enzalutamide and abiraterone) have been shown to significantly improve overall survival. In murine xenograft models, enzalutamide treatment of C4-2B and TRAMPc1 prostate tumors induced STAT3-mediated CCL2 expression and recruitment of CCR2 positive macrophages, enhancing angiogenesis and tumor invasion (121–123). Other second-generation ADTs, such as

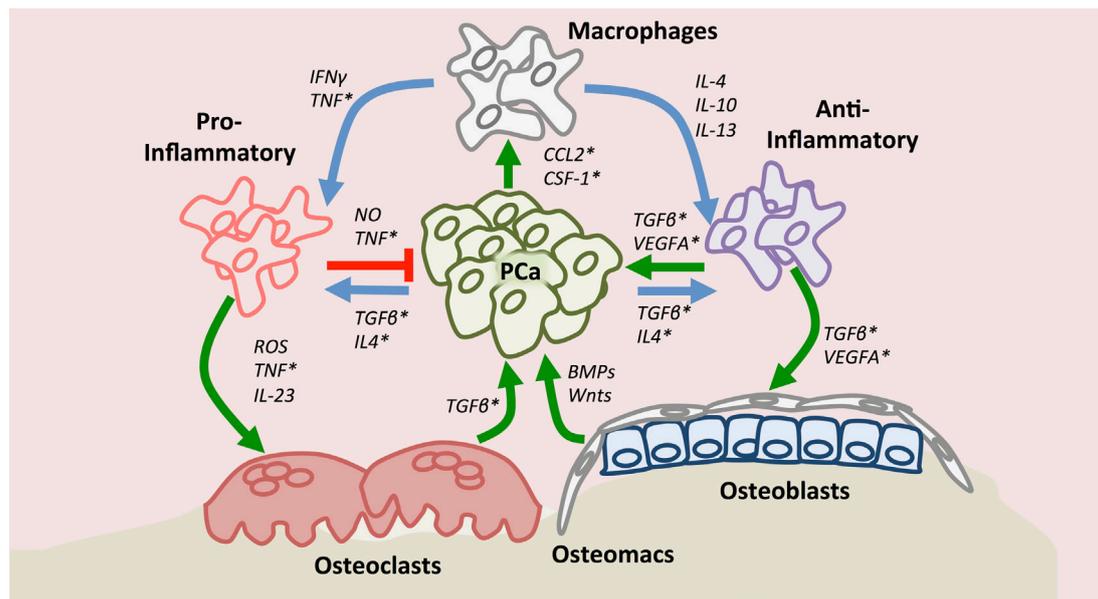


FIGURE 1 | Macrophage roles in the context of the bone-metastatic prostate cancer. Upon recruitment to the site of metastasis by chemokine (C-C motif) ligand 2 and or colony stimulating factor-1, macrophages may polarize (blue arrows) into pro- or anti-inflammatory states depending on environmental cues. Tumor-associated macrophages have protective (red arrow) or contributory effects (green arrow) directly on prostate cancer. Importantly, macrophages, including bone-resident osteomacs, impact osteoclast and osteoblast function (green arrows) thereby also indirectly regulating prostate cancer progression in bone. Asterisks denote factors to which small molecule or biological inhibitors have been developed.

abiraterone, have also been shown to upregulate cancer cell CSF1 expression to promote macrophage infiltration, wound healing and, subsequently, tumor proliferation (75). Additionally, ADT drives tumor secretion of IL-10 and -13 that contribute to the polarization of macrophages into an anti-inflammatory phenotype (75). While in-depth studies have not examined the precise effects of second-generation ADT on macrophage behavior in bone-metastatic disease, it is plausible that the drugs may have actions similar to those noted at the primary site by promoting an anti-inflammatory phenotype. Critically, little work has been done to explore the role of ADT on bone-resident macrophages. As discussed, osteomacs appear to be key regulators of bone formation, and androgen depletion may impact the ability of osteomacs and osteoblasts to generate bone. This would be beneficial in reducing the aberrant osteogenesis associated with bone-metastatic prostate cancer, although it could promote systemic osteoporosis, a phenomenon noted in men undergoing chronic ADT treatment (124).

Taxane chemotherapies such as docetaxel and cabazitaxel are also used for the treatment of advanced prostate cancer patients. These drugs inhibit microtubule disassembly during mitotic chromosome segregation and induce apoptosis in neoplastic cells, and they are commonly given to patients with mCRPC who have failed ADT (125–128). Interestingly, for chemotherapy-sensitive CRPC, docetaxel has immune-stimulatory effects and can inhibit myeloid-derived suppressor cells, while promoting a switch in macrophages from an anti- to pro-inflammatory phenotype (129, 130). However, bone-metastatic CRPCs eventually become resistant to docetaxel, at which point they progress. In the

case of chemotherapy-resistant cancer, the cancer cells can now secrete inflammatory cytokines such as IL-6 and -8, to recruit and differentiate monocytes and endothelial cells, for immune suppression and angiogenesis, respectively (131–134). Specifically, IL-6-induced mature macrophages are subsequently driven by other secreted cytokines such as IL-4 to anti-inflammatory states to induce immune suppression (131). IL-6 also induces prostate cancer survival by inducing Bcl/Stat-mediated survival signaling (131). Docetaxel can also induce CCL2 expression in cancer cells, a potent factor that not only induces prostate cancer growth and is correlated with disease progression but also recruits anti-inflammatory macrophages that drive tumor progression (74, 131, 135–139). Anti-inflammatory macrophages may also promote bone formation, but studies have shown that docetaxel impacts bone remodeling by suppressing osteoclast formation and osteoblast expansion, therefore, potentially off-setting the contribution of anti-inflammatory macrophages to cancer-induced bone disease (140).

Newer therapies being employed in the clinic may also have important effects on macrophage behavior in bone. For example, radium-223 is an alpha-emitting radionuclide that binds to calcium and promotes prostate cancer cell death in the neighboring vicinity. The treatment has been successful in extending the overall survival of men with bone-metastatic CRPC. The apoptosis induced by radium-223 may increase the bioavailability of tumor antigen in a cytotoxic microenvironment. Since macrophages are strong antigen presenting cells that mediate T cell antigenicity, it will be interesting to explore whether peripheral macrophages become pro-inflammatory and immune-stimulatory (141).

However, the effects of radiation therapy can be double edged. In humans, myelosuppression, leucopenia, and lymphopenia, were noted in radium-223 patients (142, 143). Further, in multiple cancer models, radiotherapy has been demonstrated to enhance macrophage infiltration, and over time, the polarization of macrophages into an anti-inflammatory phenotype may promote angiogenesis and cancer cell survival/recurrence (144–146). Taken together, these studies indicate that while applied therapies are initially successful in limiting disease progression, the emergence of resistant disease is often coupled/correlated with changes in macrophage polarization. Whether chronic exposure to standard of care therapies alters the microenvironment which in turn facilitates the emergence of resistant cancer cells remains to be determined. Conversely, little is known as to whether the evolution of resistant cancer cells in response to therapy impacts the behavior of the surrounding microenvironment.

CAN MACROPHAGE-BASED THERAPIES BE IMPACTFUL FOR THE TREATMENT OF BONE-METASTATIC PROSTATE CANCER?

The addition of therapies geared at blocking macrophage function, in particular anti-inflammatory function, in combination with standard of care treatments may yield more effective and durable responses in addition to preventing the recurrence of resistant disease. The role of macrophages in promoting the progression of numerous solid malignancies has been described, and as a consequence, translational studies have been geared toward the development of targeted therapies that can either deplete myeloid populations and/or alter the polarization status of macrophages. Numerous factors control macrophage infiltration and polarization but can have dual tumor-promoting and -protective effects. For example, correlative and causal roles for TNF in the progression and metastasis of prostate cancer have been described (147–153). Given the roles of TNF in inflammatory diseases, such as arthritis, it is unsurprising that biologicals targeting either the ligand or the receptor have been an active area of research. In the cancer setting, TNF can promote tumor growth and angiogenesis with preclinical trials demonstrating efficacy for TNF blocking reagents (154). Conversely, however, tumor-protective roles have been described in addition to potential risks for the development of cancers such as soft tissue sarcoma. This, combined with the potential for adverse toxicity associated with TNF inhibition, has diminished enthusiasm for the application of TNF inhibitors in the cancer setting. However, more encouraging results for other targets that impact macrophage behavior have been noted including, CCL-2/CCR-2, IL-4, and CSF1 receptor (CSF1R).

CCL-2

Chemokine (C–C motif) ligand 2 is expressed by prostate cancer cells, and while it can promote cell growth and invasion in an autocrine manner, it has also been shown to be a key driver of CCR2-expressing (CCL2 receptor) macrophages and monocyte recruitment (73, 155, 156). Moreover, the role of CCL2 seems particularly relevant in the context of bone-metastatic disease,

where CCL2-expressing prostate cancers recruit endothelial cells and osteoblasts to drive angiogenesis and osteogenesis, respectively, both of which enhance the progression of the disease (73, 157). Underscoring the importance of CCL2 in the tumor-bone microenvironment, studies demonstrated that neutralization of CCL2 with a monoclonal antibody (C1142) was successful in both attenuating tumor growth as well as bone pathology in various preclinical models (156, 158). As a result, the humanized version, CNTO 888 (Carlumab), was developed to neutralize CCL2 signaling function in advanced prostate cancer. While the drug was well-tolerated in clinical trials, no anti-tumor activity was noted as a single agent for the treatment of metastatic CRPC (158–161). Given that targeting CCL2, or the receptor CCR2, in other diseases has been shown to be impactful in reducing inflammatory responses, it is possible that combination with standard of care treatments may result in more profound effects. Interestingly, heightened levels of CCL2 were noted in patients that developed resistance to docetaxel, and pre-clinical studies in which docetaxel and C1142 were combined demonstrated significant inhibition of bone-metastatic cancer growth and associated bone disease (139, 162, 163). Surprisingly, a phase I clinical trial combining docetaxel with CNTO 888 demonstrated tolerability but not a suppression of serum CCL2 levels or tumor response. This may indicate that higher dosing is required to block the CCL2–CCR2 axis or a combination of CNTO 888 with CCR2-specific antibodies such as MLN1202 is needed to achieve effective responses in humans (164). In addition to potentially depleting macrophages from the bone-tumor microenvironment, CCL2/CCR2 therapies can also reduce osteoclast recruitment and formation thereby protecting the patient from skeletal-related events such as pathologic fracture (157, 165).

Interleukin 4 (IL-4)/IL-4R

Interleukin 4 is an anti-inflammatory cytokine found upregulated in various solid malignancies that can promote tumor growth by driving anti-inflammatory macrophage polarization which in turn facilitates tumor proliferation, angiogenesis, and metastasis (166–168). This effect may be concentration dependent as high levels of IL-4 have an anti-tumor effect (169–171). In prostate cancer, IL-4 trends with PSA expression and can stimulate IL-4 receptor (IL-4R) positive prostate cancer cells to grow and metastasize *via* downstream activation of JAK/STAT6 pathway (172). IL-4 can also promote anti-tumor immunity. While IL-4 supports proliferation of T cells, it converts mature CD8 T cells from T_H1 to T_H2 response; this transition suppresses their cytolytic potential and leads to immune evasion and tolerance (168). IL-4 expression is especially heightened in hormone-refractory versus hormone-sensitive prostate cancer (169, 172). In the context of ADT, studies have shown that IL-4 can induce AR signaling reactivation, independent of androgen, suggesting IL-4 over expression as a resistance mechanism to restore cancer growth in androgen-depleted prostate cancer (172, 173). Combination of anti-IL-4 agents with ADT may, therefore, extend tumor ADT sensitivity. To this end, IL-4-targeted therapies are in development for the treatment of asthma and allergic responses. However, the anti-cancer effects of the therapy could be lessened due to the potential impact of IL-4 blockade on the activity of cytotoxic

immune cells. Adverse systemic effects may also be an issue, but strategies that focus on cancer cell- or TAM-specific delivery may be of use. Furthermore, IL-4 has been shown to limit osteoblast proliferation and induce the expression of IL-6 while inhibiting osteoclastogenesis (174, 175). Therefore, while inhibiting IL-4 may exacerbate the cancer-associated bone disease, it would also inhibit IL-6 expression by osteoblasts which in turn may prevent macrophage-mediated resistance to chemotherapy (176).

CSF1/CSF1R

Prostate cancer-derived colony stimulatory factor 1 (CSF1) can lead to the recruitment of CSF1R positive macrophages. In the tumor, CSF1 signaling promotes macrophage survival and polarization into an ARG1, CD206, and IL-10 positive anti-inflammatory phenotype, while simultaneously inhibiting a NOS2 and IL-12 positive pro-inflammatory phenotype (75–77). Additionally, tumor-derived CSF1 recruits MDSC, and these immunosuppressive myeloid infiltrates are particularly important in tumor survival and progression (76). Interestingly, standard of care therapies such as radiation and ADT promote CSF1 expression by prostate cancer cells leading to increased infiltration of macrophages (75, 77). The CSF1/CSF1R axis is known to play a role in macrophage infiltration and anti-inflammatory polarization in other cancers and several anti-CSF1R agents have been developed, including GW2580 and PLX3397. These agents have demonstrated significant success in abrogating therapy-induced CSF1R positive macrophage infiltration using animal models of cancer progression, including prostate cancer (75, 77, 177–179). Further, treatment with ADT and PLX3397 or GW2580 reduced macrophage infiltration compared to either therapy as a single agent (75). This indicates that combination of ADT and anti-CSF1R therapy would be clinically beneficial. Currently, several clinical trials are ongoing that will test the efficacy and impact of CSF1/CSF1R inhibitors. For prostate cancer, recent studies have shown that PLX3397 delays the emergence of CRPC by reducing the number of infiltrating TAM, and a phase II clinical trial was performed in a small cohort of bone-metastatic CRPC patients with results pending (NCT 01499043). Various other combination therapy studies for prostate cancer using ADT with PLX3397 and other anti-CSF1R agents are underway and it will

be interesting to see how well they perform relative to when used as single agents (78). Of note, blockade of CSF1R signaling in mice significantly reduced osteoclast number, leading to increased bone mass that may be useful in offsetting ADT-associated osteoporosis (180).

CONCLUSION

Bone-metastatic CRPC is currently incurable and will be present in over 90% of the men who succumb to the disease. While ADTs and chemotherapy have improved overall survival rates, more work is required to help in controlling and/or eradicating the disease. This can be achieved by understanding the cellular and molecular mechanisms involved. To this end, clear roles for the stromal and immune components of the tumor microenvironment have been described. Macrophages represent a large component of the immune infiltrate, and depending on their polarization state, can contribute to the progression of the disease. Many standard of care therapies focus on elimination of the cancer cell but indirectly, these therapies also impact the behavior of the surrounding macrophage population and lessen therapeutic efficacy. The factors controlling macrophage infiltration and polarization are the focus of translational efforts with several reagents in clinical trials. Combination therapies such as ADT with anti-CCL2/CCR2 or anti-CSF1R inhibitors may prove to significantly extend the overall survival of men with bone-metastatic CRPC. Further, given the role of macrophages in controlling bone remodeling, dampening macrophage activity may reduce prostate cancer-induced osteogenesis, thereby directly improving patient quality of life.

AUTHOR CONTRIBUTIONS

CHL and CCL wrote and edited the review.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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