Multiple Roles of Chemokine (C-C Motif) Ligand 2 in Promoting Prostate Cancer Growth

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Prostate cancer continues to be the most common nonskin cancer diagnosed and the second leading cause of cancer death in men in the United States. Prostate cancer that has metastasized to bone remains incurable. The interactions between prostate cancer cells and the various cells of the host microenvironment result in enhanced growth of tumor cells and activation of host cells that together culminate in osteoblastic bone metastases. These dynamic tumor–host interactions are mediated by cancer and host-produced cytokines and chemokines. Among them, chemokine (C-C motif) ligand 2 (CCL2) has been identified as a prominent modulator of metastatic growth in the bone microenvironment. CCL2 is produced by bone marrow osteoblasts, endothelial cells, stromal cells, and prostate cancer cells. It has been demonstrated to modulate tumor-associated macrophage migration and promote osteoclast maturation. In addition, CCL2 functions through binding to its receptor CCR2 to induce prostate cell proliferation, migration, and invasion in both autocrine and paracrine manners. CCL2 protects prostate cancer cells from autophagic death by activating survivin through a PI3K/AKT (phosphatidylinositol 3-kinase/protein kinase B)–dependent mechanism. Inhibition of CCL2 substantially decreases macrophage infiltration, decreases osteoclast function, and inhibits prostate cancer growth in bone in preclinical animal models. The multiple roles of CCL2 in the tumor microenvironment make it an attractive therapeutic target in metastatic prostate cancer as well as in other cancers.


An estimated 192,280 new cases of prostate cancer were diagnosed in the United States during 2009. Unfortunately, metastatic prostate cancer continues to be an incurable disease, resulting in an estimated 27,360 deaths in 2009 (1). For the past decade, novel therapeutic strategies have targeted not only the tumor cells but also the surrounding host microenvironment that has been demonstrated to interact with malignant cells in a cycle that perpetuates cancer cell survival and progression while promoting bone destruction [as reviewed in (2)]. Based on an emerging understanding of tumor cell–microenvironment interactions, these developments have changed the treatment model of advanced prostate cancer.

Chemokines play a central role in the bone–tumor ecosystem (3). They activate seven transmembrane G protein–coupled receptors and are classified based on the relative position of cysteine residues near the N terminus into four major families: CC, CXC, C, and CX3C (4). Chemokines have substantial effects as chemotactic factors on normal development, inflammation, atherosclerosis, and angiogenesis (4). Chemokines have been implicated in many aspects of tumorigenesis cell biology, including roles in the regulation of cancer cell growth, angiogenesis, metastasis, and host immune response (5).

Chemokine (C-C motif) ligand 2 (CCL2, also known as monocyte chemoattractant protein-1) recruits and activates monocytes during the inflammatory response. CCL2 has been implicated in the development of multiple inflammatory disorders and is being explored as a potential target for the treatment of these diseases. In prostate cancer, understanding of the role of CCL2 in the promotion of malignancy has led to its identification as a therapeutic target (Table 1).

Identification of CCL2 Expression in Prostate Cancer Tissues and Cell Lines

To better understand the role of growth factors and cytokines in the development of advanced prostate cancer, Loberg et al. (7) isolated and analyzed metastases from patients who had died of prostate cancer. CCL2 expression was four times higher in the tumor–bone microenvironment compared with that in bone marrow adjacent to the tumor as measured by cytokine arrays (7). Lu et al. (8) found that in primary tumors, CCL2 was overexpressed as determined by immunohistochemistry (8). Sixty-four percent (53/83) of prostate cancer tissue specimens expressed various amounts of CCL2 in the stromal and epithelial compartments, whereas 38% (16/42) of noncancer tissue specimens expressed low levels of CCL2 (8).

Many different cell types present in the primary and metastatic tumor microenvironments produce CCL2. Prostate cancer cells LNCaP, C4-2B, PC-3, and VCaP produce higher amounts of CCL2 than primary prostate epithelial cells (7,8). Whereas human aortic endothelial cells and human dermal microvascular endothelial cells secrete low levels of CCL2, it has been demonstrated that...
human bone marrow endothelial cells secrete substantially higher levels of CCL2 (7). CCL2 has also been reported to be produced by osteoblasts (11,19). In a cell coculture system, CCL2 was over-expressed in endothelial cells and osteoblasts by prostate cancer cell–conditioned medium (11). In osteoblasts, this secretion is mediated in part by parathyroid hormone–related protein (PTHrP). CCL2 expression by osteoblastic cells was inhibited by a PTHrP antagonist, demonstrating a potential mechanism by which PTHrP produced by prostate cancer could stimulate production of CCL2 by osteoblasts (11,19).

**Association of CCR2 Expression With Pathological Stages and Tumor Grade**

The pleiotropic roles of CCL2 in the development of cancer are mediated through its receptor, CCR2 (20). It has been demonstrated that prostate cancer cells themselves express CCR2 mRNA and protein (20). The aggressive cell lines PC-3, DU145, and C4-2B express higher levels of receptor compared with cell lines of LNCaP and non-neoplastic prostate epithelial cell. Analysis revealed a positive association between prostate cancer progression and CCR2 expression, which was confirmed by real-time polymerase chain reaction and immunohistochemical staining on tissue microarray specimens (20). CCR2 mRNA and protein were expressed at higher levels within prostate cancer metastatic tissues as compared with localized prostate cancer and benign prostate tissue (17,20). Higher CCR2 expression was also associated with higher Gleason score and higher clinical pathologic stages, suggesting that CCR2 may be associated with prostate cancer progression.

**CCL2 Acts in a Paracrine and Autocrine Manner to Stimulate Prostate Cancer Cell Proliferation and Migration**

CCL2 induces PC-3 and VCaP cancer cell proliferation via activation of the phosphatidylinositol 3-kinase/protein kinase B (PI3K/AKT) pathway (7,8). Furthermore, CCL2-induced chemotaxis was abolished by a CCR2 antagonist, indicating that the functional receptor is CCR2 (8). Migration is also mediated through the AKT pathway because p70-S6 kinase, a downstream target of AKT, was phosphorylated and actin rearrangement induced as a result of CCL2 stimulation. A role for CCL2-mediated prostate cancer cell extravasation into the bone is suggested by the report that CCL2 stimulated prostate cancer cell migration through a layer of bone marrow endothelial cells, which appears to be mediated, in part, by activation of the small GTPase Rac, through the actin-associated protein PCNT1 (10). Furthermore, the thrombin receptor PAR1 facilitated CCL2-induced PC-3 cells by activating RhoA (9). Thrombin stimulation appears to place the cells in a “ready state” for a migratory response to a chemoattractant.

**Induction of Survivin by CCL2 and Protection of Prostate Cancer Cells from Autophagic Death**

The interaction between cancer cells and the host environment is a vital component of tumorigenesis (3). Cancer cells hijack molecules normally associated with host response, resulting in tumor promotion. For example, tumor cells take advantage of signaling molecules of the immune system such as CCL2 to proliferate, survive, and invade other tissues. CCL2 elicits a strong survival advantage in prostate cancer PC-3 cells through PI3K/AKT–dependent regulation of autophagy via the mammalian target of rapamycin pathway and increased survivin expression (6). CCL2 protects prostate cancer cells from autophagic death, a survival mechanism that is activated by lack of nutrients. Autophagy promotes cell survival through controlled breakdown of the cell; however, excessive autophagy results in rapid nonapoptotic cell death. CCL2 induced survivin in PC-3, DU145, and C4-2B prostate cancer cells exposed to low serum levels. The CCL2 stimulation of survivin, reduction of light chain 3-II, and resultant PC-3 cell survival were all inhibited by blocking the PI3K/AKT pathway by the PI3K inhibitor LY294002 or the AKT-specific inhibitor X (Akti-X). Downstream of AKT, CCL2 stimulates sustained mTORC1 (mammalian target of rapamycin complex 1) activity.

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**Table 1. Chemokine (C-C motif) ligand 2 (CCL2) as a potential therapeutic target for prostate cancer*  
In vitro**

<table>
<thead>
<tr>
<th>Studies</th>
<th>Cells/models used</th>
<th>Findings</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>In vitro</td>
<td>PC-3, LNCaP, PC-3, VCaP</td>
<td>CCL2 protects cancer cell survival via PI3K/AKT pathway</td>
<td>(6)</td>
</tr>
<tr>
<td></td>
<td>HBME, PC-3</td>
<td>CCL2 induces cancer cell proliferation, migration, and invasion in both autocrine and paracrine manners</td>
<td>(7–9)</td>
</tr>
<tr>
<td></td>
<td>hFOB, osteoblasts</td>
<td>PTHrP increases CCL2 expression in osteoblasts</td>
<td>(10)</td>
</tr>
<tr>
<td></td>
<td>VCaP, PC-3, LNCaP, HBME</td>
<td>CCL2 indirectly induces angiogenesis via VEGFA production from prostate cancer cells</td>
<td>(11)</td>
</tr>
<tr>
<td></td>
<td>Osteoclasts</td>
<td>Osteoclastogenesis from both human and mouse bone marrow monocytes, and CD11b+ human peripheral monocytes</td>
<td>(12–14)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(14–16)</td>
</tr>
<tr>
<td>In vivo</td>
<td>Intratibial or intracardiac injection</td>
<td>Neutralizing antibody against CCL2 inhibits prostate cancer PC-3 and VCaP growth in bone</td>
<td>(13,17)</td>
</tr>
<tr>
<td></td>
<td>Intratibial injection</td>
<td>CCL2 knockdown in PC-3 cells inhibits the tumor cell growth in bone</td>
<td>(18)</td>
</tr>
<tr>
<td></td>
<td>Subcutaneous implant</td>
<td>CCL2 promotes prostate cancer growth through the regulation of macrophage infiltration</td>
<td>(12)</td>
</tr>
</tbody>
</table>

* HBME = human bone marrow endothelial cells; hFOB = human fetal osteoblast; LNCaP = lymph node cancer of the prostate; PC-3 = prostate cancer 3; PTHrP = parathyroid hormone related peptide; VCaP = vertebral cancer of the prostate; VEGFA = vascular endothelial growth factor A.
through increased phospho-p70S6 kinase (6). Treatment of PC-3 cells with rapamycin, a known inducer of autophagic death, decreases survivin expression, which results in a decrease in cell viability in serum-starved conditions. CCL2 rescues PC-3 cells under this stress by increasing survivin expression, resulting in a partial resistance to rapamycin-induced death (6). These data suggest that CCL2 signaling limits autophagy in prostate cancer cells and provides them with a powerful survival mechanism in nutrient-starved conditions that might be seen by tumor cells that are outgrowing their blood supply.

Mediation of Prostate Cancer Cell–Induced Bone Lesions by CCL2

It is commonly accepted that osteoclastogenesis and bone resorption are critical steps leading to the development of skeletal metastases and are essential for prostate cancer establishment in the bone (2,3). Multiple factors, including receptor activator of nuclear factor kappaB ligand (RANKL), are important mediators of bone remodeling (21–23). Because RANKL expression by prostate cancer cell lines is generally low, Lu et al. (15) used a human cytokine antibody array to analyze the conditioned media of prostate epithelial cells and LNCaP, C4-2B, and PC-3 cells for soluble factors that could contribute to cancer-induced osteoclast activity. CCL2 was produced in high amounts by prostate cancer cells as compared with prostate epithelial cells. PC-3-conditioned media treatment of human bone marrow mononuclear cells in vitro resulted in markedly elevated IL-6 and IL-8, and further culture studies revealed a potential RANKL-independent mechanism for osteoclast maturation that appears to be dependent, in part, on the presence of CCL2 and interleukin (IL)-8. Resorption of dentin slices was inhibited by both CCL2 and IL-8 antibody treatment. Data from multiple studies suggest that CCL2 promotes preosteoclast cell fusion with resultant formation of multinucleated tartrate-resistant acid phosphatase-positive (TRAP) osteoclasts (15,23). These cells, however, require the addition of another factor, such as RANKL, to lyse bone matrix. Consistent with these findings, Mizutani et al. (16) reported that conditioned medium from PC-3 cells induced human peripheral blood CD11b+ cells to differentiate into osteoclasts capable of bone resorption. Analysis of the conditioned media demonstrated elevated IL-6 and IL-8, and further culture studies revealed a potential RANKL-independent mechanism for osteoclast maturation through these cytokines (16). Clearly, the bone microenvironment is filled with multiple factors that contribute to osteoblastic metastases. For example, the endothelin-1 signaling axis has been shown to play a role in the generation of bone metastases through stimulation of osteoblast activity [see review in (24)]. Specifically, Yin et al. (25) reported that endothelin-1 produced by cancer cells can interact with the endothelin A receptor on osteoblasts to stimulate new bone formation in vitro and osteoblastic metastases in vivo. Currently, the relationship of CCL2 with the endothelin signaling axis is unknown.

CCL2 as a Therapeutic Agent to Treat Prostate Cancer

CCL2 promotes prostate cancer tumorigenesis and metastasis through distinct mechanisms: 1) directly, by promoting cancer cell growth, survival, invasion, and migration; and 2) indirectly, through the regulation of monocytic lineage cells that include macrophages and osteoclasts within the tumor microenvironment [see review in (5)]. In one study using subcutaneously implanted VCaP cells, Loberg et al. (12) found that administration of systemic anti-CCL2 antibody substantially inhibited tumor xenograft growth. This inhibition was accompanied by decreased CD68(+)-macrophage infiltration, as well as substantially decreased microvascular density.

In a second preclinical animal model using severe combined immunodeficiency mice, PC-3 cells were injected via the intracardiac route to simulate metastatic seeding (17). Neutralizing antibodies against CCL2 (anti-human CNT0888 and anti-mouse C1142) attenuated prostate cancer cell–mediated overall tumor burden by 96%. The addition of CCL2 inhibition to cytotoxic therapy with docetaxel induced tumor regression and significantly decreased tumor burden as compared with docetaxel treatment alone. The use of dual human and mouse antibodies allowed the investigators to differentiate between the effects of host vs cancer cell–derived CCL2 and demonstrated that the majority of CCL2 was supplied by the host microenvironment (17).

In a third preclinical model study using severe combined immunodeficiency mice, Li et al. (13) injected prostate cancer cells intratibially to simulate prostate cancer bone metastasis. Systemic treatment with neutralizing antibodies to CCL2 substantially decreased tumor burden, blood vessel density, and the number of active osteoclasts at the tumor–bone interface. The decreased number of osteoclasts was mirrored by a decrease in bone resorption markers. Serum TRAP5b levels were decreased by 50%–60% in mice bearing VCaP or PC-3 cells that were treated with anti-CCL2 antibodies (13).

In a fourth study, Lu et al. (18) stably transfected prostate cancer PC-3 cells with CCL2 short hairpin RNA to knock down CCL2 expression and implanted them into the tibia of severe combined immunodeficiency mice (18). Examination of these tumors revealed substantially decreased osteoclast numbers and tumor volume, as determined by bone histomorphometry (18).

Taken together, these studies (12,13,17–19,26) provide compelling evidence that CCL2 facilitates and promotes cancer cell survival, the attraction and education of tumor-associated macrophages with subsequent stimulation of angiogenesis, and osteoclastogenesis. Further evidence can be provided by looking at the phenotype of knockout mice; CCL2 and CCR2 knockout mice have no obvious endocrine or sexual function phenotype, showing mostly defects in leukocyte adhesion and in induced immunity (27–31). Current investigations involving these mice will be valuable in studying the role of CCL2 in tumorigenesis. For example, mouse model experiments crossing CCL2 or CCR2 knockouts with transgenic mice that develop primary prostate cancers are ongoing (32).

CCL2 in Other Cancer Types

Chemokines contribute to the development of malignancy through roles in progression, migration, angiogenesis, and metastases in multiple cancer types [reviewed in (33)]. CCL2 was first identified and purified in 1989 from human glioma and myelomonocytic cell
lines (34). CCL2 is located on chromosome 17q11.2-q12. It encodes a 99-amino acid precursor protein that undergoes post-translational processing and is ultimately secreted as a 76-amino acid protein. Since its discovery, studies have demonstrated the overexpression and resultant promotion of tumor growth of CCL2 in melanoma (35) and ovarian (36), breast (37–40), esophageal (41,42), gastric (43), renal cell (44), lung (45–47), colon (48), and papillary thyroid carcinomas (49) (Table 2). CCL2 is produced by tumor cells and multiple different host cells, including stromal cells, leukocytes, and endothelial cells [see review in (39)].

In breast tumors, CCL2 expression is associated with advanced disease state, tumor progression, and angiogenesis (37,52–55,68). The level of CCL2 expression predicts recurrence (54). CCL2 induces angiogenesis through multiple mechanisms including directed induction of vascular endothelial growth factor A (54) and hypoxia-inducible factor-1 (69). CCL2 also stimulates angiogenesis through the attraction of tumor-associated macrophages that, in turn, produce vascular endothelial growth factor (69). In breast cancer bone metastases, CCL2 leads to enhanced osteolysis, resulting in release of bone matrix–bound angiogenic factors, including platelet-derived growth factor, fibroblast growth factors-1, and transforming growth factor β (70). CCL2 directly stimulates breast cancer cells to promote tumorigenesis (71–73). For example, CCL2 exerts prometastatic effects by regulating the membrane glycoprotein dysadherin and Duffy antigen in breast cancer cells, supporting a potential therapeutic role for CCL2 blockade. In a human breast cancer preclinical mouse model, treatment with antibodies to CCL2 prolonged survival and suppressed lung metastases (56).

Multiple myeloma (MM) is a cancer of plasma cells characterized by osteolytic bone lesions [see review in (74)]. MM cells secrete CCL2 in response to stimulation by IL-6 (63,75) and tumor necrosis factor alpha (64). Migration of MM cells across endothelial cells is increased by treatment with tumor necrosis factor alpha via TNF receptor 2 and apparent stimulation of autocrine CCL2 (64). Other studies have demonstrated that marrow stromal cells produce CCL2 in both normal and MM patients (65,76). Conditioned media from these stromal cells were chemotactic to MM cells, and this effect could be inhibited by neutralizing antibodies against CCL2 (65,76). Bone marrow endothelial cells derived from MM patients express higher amounts of CCL2 as compared with human umbilical vein endothelial cells (HUVECs), and CCL2 has been shown to directly induce the formation of blood vessels in vivo (56,66,77). Altogether, these reports suggest that CCL2 has a central role in MM cell homing and tumorigenesis (63–66,74–77).

### Serum CCL2 as a Cancer Biomarker

Several small studies have demonstrated that serum CCL2 is elevated in cancer patients (18,45,78,79). In a study of 39 prostate cancer patients at various stages, Lu et al. (18) reported that elevated serum CCL2 was associated with bone disease, suggesting the possibility of using serum CCL2 as a prognostic biomarker (18). In addition, serum CCL2 levels have been reported to be elevated and associated with tumor stage in patients with breast (78), ovarian (79), and lung cancers (45). Larger studies are required to determine whether CCL2 has potential as a diagnostic or prognostic biomarker in all of these cancers.

### Table 2. Current findings on the roles of chemokine (C-C motif) ligand 2 (CCL2) in cancers other than prostate cancer

<table>
<thead>
<tr>
<th>Cancer types</th>
<th>Findings</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Melanoma</td>
<td>Expresses in malignant melanoma</td>
<td>(35)</td>
</tr>
<tr>
<td></td>
<td>Enhances tumor angiogenesis</td>
<td>(50)</td>
</tr>
<tr>
<td></td>
<td>Decreases T-cell chemotaxis</td>
<td>(51)</td>
</tr>
<tr>
<td></td>
<td>Increases expression in malignancy</td>
<td>(36)</td>
</tr>
<tr>
<td>Ovarian adenocarcinoma</td>
<td>Expresses in tumor cells</td>
<td>(37–40,52,53)</td>
</tr>
<tr>
<td></td>
<td>Expression correlates with invasion</td>
<td>(37,39,54,55)</td>
</tr>
<tr>
<td>Gastric cancer</td>
<td>Expresses in tumor cells</td>
<td>(41,42)</td>
</tr>
<tr>
<td></td>
<td>Promotes angiogenesis</td>
<td>(39,54,56)</td>
</tr>
<tr>
<td></td>
<td>Promotes metastasis</td>
<td>(39)</td>
</tr>
<tr>
<td>Esophageal cancer</td>
<td>Expresses in tumor cells</td>
<td>(41,42)</td>
</tr>
<tr>
<td></td>
<td>Promotes angiogenesis</td>
<td>(43)</td>
</tr>
<tr>
<td></td>
<td>Promotes invasion</td>
<td>(43)</td>
</tr>
<tr>
<td></td>
<td>Promotes lymph node metastasis</td>
<td>(58)</td>
</tr>
<tr>
<td>Renal cell carcinoma</td>
<td>Expresses in tumor cells and promotes angiogenesis</td>
<td>(59)</td>
</tr>
<tr>
<td>Lung cancer</td>
<td>Expresses in tumor cells</td>
<td>(45–47)</td>
</tr>
<tr>
<td></td>
<td>Promotes invasion</td>
<td>(45)</td>
</tr>
<tr>
<td></td>
<td>Mediates bone resorptive lesions</td>
<td>(45)</td>
</tr>
<tr>
<td>Colon carcinoma</td>
<td>Expression increases with tumor stage</td>
<td>(60)</td>
</tr>
<tr>
<td>Papillary thyroid cancer</td>
<td>Expresses in tumor cells</td>
<td>(49)</td>
</tr>
<tr>
<td></td>
<td>Promotes lymph node metastasis</td>
<td>(49)</td>
</tr>
<tr>
<td></td>
<td>Expression correlates with recurrence</td>
<td>(49)</td>
</tr>
<tr>
<td>Leukemia</td>
<td>Expresses in lymphoblastic leukemia</td>
<td>(61)</td>
</tr>
<tr>
<td></td>
<td>Increased CCL2 serum level in acute myeloid leukemia</td>
<td>(62)</td>
</tr>
<tr>
<td>Multiple myeloma</td>
<td>Expresses in tumor cells and promotes migration</td>
<td>(63–66)</td>
</tr>
<tr>
<td></td>
<td>Promotes tumor cell chemotaxis</td>
<td>(66,67)</td>
</tr>
</tbody>
</table>

### Conclusions

Prostate cancer preferentially metastasizes to bone, and nearly 100% of men have evidence of osseous disease at the time of death (80,81). For skeletal metastases, therapeutic targets are present in tumor cells and in the cellular and noncellular components of the bone microenvironment. Targeting CCL2 as a treatment for cancer serves as an example of development of combination cancer treatments based on the realization that tumors function as ecological communities (82). Ecosystems are a recognized framework for understanding how the various physical elements and species of a given environment interact spatially and temporally (83,84). Cancer and host cells can be viewed as species interacting with the inorganic parts of the tumor microenvironment as an ecosystem.
endothelial growth factor.

kappaB ligand; TGF-

chemokines and cytokines, which act together to promote metastatic

between tumor-derived factors, such as PTHrP, and host-derived

expression from osteoblasts. CCL2 appears to mediate the interactions

parathyroid hormone related peptide (PTHrP), which stimulates CCL2

and activation of osteoclastogenesis. Prostate cancer cells produce

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cer cell proliferation, survival, and migration. In addition, CCL2 contrib

CCL2, by binding to its receptor CCR2, directly stimulates prostate can

Figure 1. Roles of chemokine (C-C motif) ligand 2 (CCL2) in prostate
cancer cells and the bone microenvironment. The CCL2/CCR2 axis has
been identified as an important contributor to prostate tumorigenesis.

endometrial cancer cell proliferation, survival, and migration. In addition, CCL2 contrib-
utes to the development of metastases in the bone microenvironment
by stimulating macrophage recruitment and education, angiogenesis,
and activation of osteoclastogenesis. Prostate cancer cells produce
parathyroid hormone related peptide (PTHrP), which stimulates CCL2
expression from osteoblasts. CCL2 appears to mediate the interactions
between tumor-derived factors, such as PTHrP, and host-derived
chemokines and cytokines, which act together to promote metastatic
tumor growth in bone. IGFI = insulin-like growth factor; IL-8 = interleukin
8; PCa = prostate cancer; RANKL = receptor activator of nuclear factor
kappaB ligand; TGF-β = transforming growth factor β; VEGF = vascular
endothelial growth factor.

(82), CCL2 clearly facilitates the development of a prostate cancer
ecosystem within bone in a dynamic spatial and temporal fashion,
and its production by stromal and endothelial cells and osteoblasts
stimulates cancer cell survival and proliferation, osteoclast maturation,
and macrophage attraction and education (Figure 1).

It has been well demonstrated in ecology that altering the envi-
ronment or removing a species from its ecological niche can
have profound effects on other species within the habitat (85).
The goal of developing ecological therapy for cancer is to pro-
foundly alter the niche within which the cancer cells exist, render-
ing it uninhabitable for tumor cells but without destroying the
ability of the normal host cells to reestablish a normal homeostatic
bone marrow microenvironment. High-dose radiation, for ex-
ample, may destroy a specific cancer metastasis but can also lead
to the loss of the normal bone physiology and architecture, result-
ing in a structure that can no longer provide normal hematopoi-
etic function. The high and abnormal amounts of CCL2 produced
by the tumor microenvironment are not required for normal
function. Inhibiting CCL2 function, therefore, should not have
profound or lasting effects on normal marrow function. As dem-
onstrated in multiple preclinical models throughout this review,
inhibition of CCL2 blocks cancer cell survival, inhibits the func-
tion of tumor-associated macrophages, and interferes with osteo-
clast maturation. Tumor-associated macrophages exist as an
invasive species within the bone metastasis habitat, promoting
growth of the cancer cells by providing needed growth factors and
matrix-altering enzymes. The inhibition of their chemotraction
and education should not affect normal marrow function. Similarly,
osteoclast function is inappropriately activated in bone meta-
stases. The loss of this inappropriate activation should not affect
normal marrow function. Early toxicity studies appear to support
these observations, as treatment with anti-CCL2 antibodies
appears to have few side effects (6).

Based on these collective data (12,13,17,18), therapy to inhibit
CCL2 activity with the use of a monoclonal antibody (CNT0888)
has entered into the clinic and is currently the subject of phase I
and phase II testing. Because of effects on multiple cell types, the
inhibition of CCL2 should have therapeutic activity in prostate
cancer as well as in multiple other cancers, especially those that
develop in, or metastasize to, bone. It should be possible to identify
patients with osteoclast-mediated bone destruction by bone turn-
over markers such as urinary N-telopeptide of collagen cross links
(NTX) and to follow these serially in patients as surrogate
measures of activity. Anti-CCL2 therapy in these patients should
decrease skeletal-related events. Patients with primary bone sar-
comas and MM would also be excellent candidates to consider for
therapies blocking this cytokine. Diseases that are associated with
macrophage infiltration, such as the peritoneal seeding associated
with metastatic ovarian cancer, should also be evaluated. In addi-
tion, based on mechanisms of action and the preclinical data, anti-
CCL2 therapy, in conjunction with standard cytoreductive
therapies for these diseases, will likely provide the most clinical
benefit to patients. Inhibition of CCL2 may serve as a model for
several therapies now in clinical development, which interrupt
cytokine networks.

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