IN PERSPECTIVE

Integrating the immune microenvironment of prostate cancer induced bone disease

Claire L. Ihle1 | Philip Owens1,2

1Department of Pathology, University of Colorado Anschutz Medical Campus, Aurora, Colorado
2Department of Veterans Affairs, Research Service, Eastern Colorado Health Care System, Aurora, Colorado

Correspondence
Philip Owens, Department of Pathology, University of Colorado Anschutz Medical Campus, P18-5122 12800 East 19th Ave, Aurora, CO 80045-0611.
Email: philip.owens@cuanschutz.edu

Funding information
U.S. Department of Veterans Affairs.
Grant/Award Number: 1KBX00002929

Abstract
Prostate cancer (PCa) is the most frequently diagnosed cancer for men in the U.S. but does not impede patient survival until the disease is metastatic. Metastatic lesions most frequently occur in the bone, which exhibits a distinct microenvironment of immune and bone cell populations. Advances in the diagnosis and treatment of primary PCa allow for the use of tailored therapeutic approaches based on biomarkers, protein expression, and histopathology. Understanding the molecular and cellular characteristics of primary tumors has advanced therapeutic development and survival for patients with PCa. Personalized medicine has only recently emerged for the treatment of metastatic bone lesions. Tumor induced bone disease (TIBD) in patients with PCa can be classified into lytic, blastic, or mixed pathologies, with most patients exhibiting the blastic phenotype. Progress has been made in treating TIBD, but metastatic PCa has yet to be cured. Immune checkpoint inhibitors have exhibited limited responses in immunosuppressive PCa tumors, but have yet to be assessed in metastatic sites which may be susceptible to an increased inflammatory response. Recent discoveries have uncovered distinct tumor microenvironments (TMEs) of blastic and lytic bone metastases from patients with PCa, identifying actionable targets for therapeutic applications, including immune checkpoint inhibitors and targeted therapeutics. Enrichment for macrophages and T cells in patient samples suggests metastatic sites may be reappraised as immunologically targetable, despite their immunologically "cold" primary tumors. The practice of performing bone biopsies will help identify unique cellular and protein targets in the bone TME that can guide therapy decisions.

KEYWORDS
bone metastasis, immunotherapy, prostate cancer, tumor microenvironment

1 | PROSTATE CANCER

1.1 | Scope of prostate cancer

Prostate cancer (PCa) is the most commonly diagnosed cancer for men in the U.S., accounting for more than one in five new cancer diagnoses.1 Thanks to early diagnosis and effective treatment options, survival is extremely high at >99% for localized and regional tumors.2 Of the 191,930 estimated new cases of PCa in the U.S. for 2020, only 33,330 patients are expected to succumb to the disease.3 However, once distant metastases are present patient with PCa survival drops to 30%, as patients face fewer therapeutic
opportunities tailored to their disease. Great advances have been made in diagnosing and treating PCa, but opportunities remain for promoting patient quality of life and survival through diagnostic and therapeutic approaches for metastatic disease.

1.2 | Diagnosis of prostate cancer

Advances in diagnosing PCa have improved patient survival through precision medicine approaches. Along with digital rectal examination, prostate-specific antigen (PSA) serum screening can identify PCa. However, thoughtful consideration needs to balance the benefit of early detection with the risk of potential harm physically from a follow up prostate biopsy. Additionally, psychological trauma can result from false-positive tests.

PCa incidence initially surged and then dropped as screening practices for PSA changed. Screening is now considered a patient’s choice. Before proceeding to biopsy, patients have the option to test for a range of other PCa screens beyond PSA, including a 4K score test, a prostate health index, Apifin, PCA3, the Michigan prostate score, and Select MDx to avoid undergoing an unnecessary biopsy.

In the event of a positive biopsy, biomarkers have become available to define a patient’s prognosis based on molecular and image-based analysis of the prostate tumor, and aid in steering treatment approaches for primary tumors. Genomic biomarker tests are being used including the ProLaris cell cycle progression test and ProMark test for tumor aggressiveness. Primary PCa is diagnosed by prostate biopsy then stratified into risk level based on gleason score, PSA level, and clinical stage. Histopathology of the prostate biopsy aids in classifying the tumor as an adenocarcinoma or neuroendocrine carcinoma. Patients with PCa normally exhibit prostate adenocarcinoma, which is driven by androgen and frequently has high PSA levels. A rare group of patients with PCa display a neuroendocrine carcinoma with lower PSA levels and visceral metastases, which often occur as a mechanism of resistance, resulting in more aggressive tumors.

1.3 | Treatments for prostate cancer

Patients stratified to the very low risk group with localized PCa are often monitored without taking clinical action. Active surveillance including PSA screening, physical exams and prostate biopsies are performed to watch for disease progression. Younger men with localized PCa or increased primary tumor progression receive local radiotherapy and may undergo a radical prostatectomy. Once PCa is no longer localized to the prostate of advanced or high risk patients, chemical, or surgical castration as part of androgen deprivation therapy (ADT) reduces testosterone production, diminishing tumor progression. PCa adenocarcinomas are normally responsive to radical prostatectomy or radiation followed by ADT for more advanced cases, while neuroendocrine patients are less responsive to ADT. Unfortunately, castration-resistant prostate cancer (CRPC) can occur after 14 to 20 months of ADT response. CRPC is characterized by high PSA levels and dysregulated androgen receptor signaling, leading to recurrence of primary or metastatic tumor progression.

2 | PROSTATE CANCER METASTASIS

2.1 | Visceral and bone metastases

Despite meaningful advances made in the treatment of primary PCa tumors, metastases still provide an obstacle to patient wellbeing and survival. The site of the metastasis dictates the length of survival for metastatic patients with PCa. Visceral metastases exhibit the poorest overall survival time, with an average of 13.5 months for liver metastases and 19.4 months for lung metastases. Visceral metastases are more rare, with only 20% of patients with metastatic castration-resistant prostate cancer (mCRPC) exhibiting soft tissue metastases at the start of clinical studies. Bone is the most common metastatic site for PCa but has a longer patient with mCRPC survival of 21.3 months.

The bone is a distinct metastatic site due to its constant mineral resorption and replacement carried out by bone cells. Osteoclasts execute bone resorption while osteoblasts form bone, with crosstalk between both cell types in the bone remodeling compartment. Bone metastases display tumor induced bone disease (TIBD) which can be lytic, blastic or a mixture of both pathologies. Lytic TIBD results in the destruction of normal bone by osteoclasts, while blastic (also referred to as sclerotic) TIBD mediates deposition of new bone scleroses by osteoblasts. PCa bone metastasis TIBD pathologies are predominantly blastic, with a smaller proportion of lytic or mixed pathology. TIBD frequently results in skeletal-related events (SREs) due to scleroses including debilitating bone pain, fractures, and spinal cord compressions that reduce patient quality of life and survival outcomes. Beyond the clinical effects of SREs, there are adverse economic effects for the 53% of metastatic patients with PCa who experience at least one SRE. In a cohort of Medicare enrolled patients, experiencing one or more SREs lead to an attributable cost of $21 191, with increased emergency room visits, hospitalizations, and death. The prevalence of blastic metastases is unique to PCa, as breast cancer also frequently metastasizes to the bone but predominantly exhibits a lytic or mixed phenotype.

2.2 | Diagnosis of bone metastasis

Metastatic disease is defined by imaging protocols. These have recently undergone technological advancements leading to more sensitive detection. The result is earlier and more precise detection of small lesions. Metastatic patients with PCa often experience a SRE, primarily bone pain, which triggers the assessment of a bone metastasis diagnosis. The old approach of radionuclide bone
scanning and computed tomography (CT) is being replaced with more sensitive imaging methods.\textsuperscript{27} Compared with radionuclide and CT scanning alone, clinicians are now able to better detect PCa metastases using positron-emission tomography with prostate-specific membrane antigen (PSMA) labelling on the surface of PCa cells even when PSA levels are low.\textsuperscript{28} Whole-body magnetic resonance imaging now provides a one-step screening test for detection of PCa metastases that outperforms the conventional two-step protocol of radionuclide bone scanning followed by CT or magnetic resonance imaging.\textsuperscript{29} With earlier metastatic PCa diagnosis, patients with PCa may benefit from more personalized treatment options that can enhance their quality of life and survival.

### 2.3 Treatment of bone metastasis

Metastatic bone therapeutic options have improved over the past decade, with patients with PCa receiving novel chemotherapies, hormone therapies, bisphosphonates, and immunotherapy.\textsuperscript{27} Resistance to first-line docetaxel chemotherapy regimens can now be alleviated with cabazitaxel and prednisone treatment.\textsuperscript{30} Enzalutamide is a second-generation ADT that retains its activity in CRPC, and has enhanced progression-free survival as well as overall survival of patients.\textsuperscript{27,31} A new therapeutic approach to treating mCRPC is bipolar androgen therapy, in which patients are treated with testosterone to resensitize them to enzalutamide treatment.\textsuperscript{32} Metastatic castration-sensitive patients with PCa have also shown benefit from enzalutamide treatment combined with ADT, receiving recent FDA approval.\textsuperscript{33} The expansion of chemotherapy and ADT options available to PCa patients with TIBD have extended patient survival time by delaying development of therapy resistance.

Standard of care treatment includes ADT for advanced and metastatic PCa which inadvertently increases bone loss, mediated by enhanced osteoclast activity.\textsuperscript{34} To alleviate the loss of bone mineral density during ADT treatment, antiresorptive bisphosphonates are used to inhibit osteoclast activity, such as pamidronate and zoledronic acid.\textsuperscript{35,36} The anti-RANKL biologic denosumab, which binds to the osteoclast activity promoting cytokine RANKL, has also been shown to reduce bone turnover in ADT patients with PCa.\textsuperscript{37,39} Antiresorptive therapies have previously focused on the inhibition of osteoclast mediated bone loss. A new bone targeting therapy is promoting osteoblasts to generate new healthy bone by blocking the activity of a bone formation inhibitor. Sclerostin (SOST) is a bone formation inhibitor that has been a popular target for new studies into TIBD therapeutics.\textsuperscript{40} Promising preclinical data found that inhibition of SOST in a breast cancer bone metastasis model reduced metastatic burden and bone destruction.\textsuperscript{41} An anti-SOST and DKK-1 bispecific antibody has also shown promise in animal models for building bone mass, providing another potential therapeutic approach for maintaining metastatic PCa bone health.\textsuperscript{42} Reversing bone density loss by inhibiting osteoclast activity and promoting osteoblast bone formation is improving metastatic patient bone health.

Metastatic bone lesions frequently cause patients to experience moderate to severe bone pain.\textsuperscript{43} To treat TIBD pain, external beam radiation therapy has been used in the clinic for many years.\textsuperscript{44} More recently, radium-223 binds blastic lesions of metastatic patients with PCa to prolong overall survival by 3.6 months.\textsuperscript{45} Despite numerous advances, treatments for PCa bone metastases are not yet curative, as treatments are limited to reducing metastasis growth and supporting bone health.\textsuperscript{20} Patients with mCRPC have a median overall survival of only 13 months, highlighting the need for improving metastatic PCa therapies.\textsuperscript{46} Studying the immune microenvironment of bone will increase the likelihood of discovering therapeutic cures for TIBD in patients with PCa.

### 3 IMMUNE MICROENVIRONMENT OF PROSTATE CANCER METASTASIS

#### 3.1 Immunotherapy of metastatic cancer

The breakthrough of immunotherapies has allowed for the rewiring of the immune cells in the tumor microenvironment (TME) to carry out antitumor functions and prolong survival. Immunotherapies have transformed the treatment for many solid tumors, including metastatic breast cancer, lung cancer and melanoma.\textsuperscript{27,40} These immune checkpoint inhibitors target CTLA-4, PD-1, or PD-L1 to reactivate the immune system, which carries out a cytotoxic immune response.\textsuperscript{49} CTLA-4 and PD-1 are negative regulators of T cells which attenuate T cell activity in the TME.\textsuperscript{50} While PD-L1, the ligand to PD-1, is expressed on many cell types, including cancer cells to impede antitumor immune responses.\textsuperscript{50} Response to immune checkpoint blockade is dependent on a patient’s ability to elicit antitumor immunity based on their system immunity, environmental factors, TME as well as the tumor genome and epigenome.\textsuperscript{51} The success of immunotherapies in these cancers is dependent on a high tumor mutation burden, resulting in neoantigen expression as well as the presence of tumor-infiltrating lymphocytes.\textsuperscript{49} When tumors highly express immune checkpoint markers and reflect a high immune infiltration, they are predicted to have better response to immune checkpoint blockade.\textsuperscript{52}

Metastatic PCa is characterized by an immune-suppressive TME, resulting in limited immunotherapy response.\textsuperscript{53} The only immunotherapy approved for minimally symptomatic mCRPC is sipuleucel-T (Sip-T), a therapy that sensitizes a patient’s T cells to identify and kill PCa cells.\textsuperscript{54} When patients undergo Sip-T treatment, antigen presenting cells (APCs) are harvested from their blood and cultured with a fusion protein consisting of prostate acid phosphatase (PAP) and granulocyte-macrophage colony-stimulating factor (GM-CSF).\textsuperscript{55} Once these activated APCs are delivered back to patients, their immune systems can then carry out a cytotoxic T cell mediated response to the PAP expressing PCa cells.\textsuperscript{54,55} In early phase III clinical trials, variation in the number of bone metastases in the patients enrolled dictated differing response rates.\textsuperscript{55} Sip-T results were initially not as promising due to treating more advanced patients with extensive bone metastases at baseline compared
with the placebo group. Later clinical trials of Sip-T reduced the relative risk of death by 22% for patients with mCRPC, but the time to disease progression was still the same between treatment and placebo groups. Despite the benefits of Sip-T, the cost of treatment is still exceedingly high, with a cost of $104,536 estimated in 2014 for a gain of 0.37 quality-adjusted life years.

### 3.2 | Emerging immunotherapies for metastasis

With the success of immunotherapies in other metastatic solid tumor settings, a great effort has been put into developing immunotherapies for metastatic PCa. Vaccine therapies are being studied to elicit the patient’s antitumor immune response with DNA-based, cell-based, peptide-based and viral vector-based vaccines. Numerous tumor-associated antigens have been identified as potential immunotherapeutic targets against PCa including PSA, PSMA, PAP, and prostate stem cell antigen. Current clinical trials are working on developing a vaccine encoding PAP with GM-CSF, which may elicit an antigen-specific T cell response and is being investigated as a combination treatment with Sip-T and nivolumab (NCT03600350). Cell-based vaccines attempting to expand upon the success of Sip-T to different PCa targets have shown mixed results. The allogenic PCa cell lines LNCaP and PC3 were engineered to express GM-CSF and activate an antitumor response in patients, which showed early clinical trial success, but failed in phase III trials to benefit patient outcomes. Chimeric antigen receptor T cells, which have succeeded in the clinic for B cell malignancies, are now in clinical trials for PCa antigen targets.

Since PCa tumors are a “cold” immune desert with low immune infiltration, low tumor mutation burden and low antigen presentation, the response to monotherapy immune checkpoint inhibitors will never be as strong as in highly inflamed non-small cell lung cancer, melanoma, or renal cell carcinoma. The immunosuppressive cell populations in metastatic PCa may contribute to the lack of response to immune checkpoint inhibitors for metastatic patients. Two components of the immunosuppressive microenvironment, monocytic myeloid-derived suppressor cells and T regulatory cells, have been found to correlate with negative prognostics markers for mCRPC patients. Despite the failure of immune checkpoint inhibitors being used as monotherapies in metastatic PCa, CTLA-4, PD-1, and PD-L1 clinical trials are continuing, focusing on combination therapy approaches. Early clinic studies for PD-1 and PD-L1 blockade have not produced significant patient benefit, and are now being tested in combination with other immunotherapy, ADT, chemotherapy, and radiation therapy approaches. A phase I/II trial is currently testing a combination of up to four different immunotherapies in mCRPC to determine if combined therapy approaches facilitate antitumor activity (NCT03493945). To generate successful combinational therapies, immune desert and immune excluded PCa tumors will need to be converted to inflamed tumors, which will require identification of the correct patient population.

### 3.3 | Distinct microenvironments of bone metastases

Therapeutic successes for metastatic bone patients with PCa have been limited in part due to the unique microenvironment of bone. In our previous work, we identified distinct immune microenvironments in PCa bone metastases from patients exhibiting lytic and blastic TIBD (Figure 1). Formalin fixed and paraffin embedded (FFPE) decalcified patient biopsies were analyzed for protein and gene expression to identify unique signaling and cellular components in lytic and blastic bone microenvironments (Figure 1). In lytic patient samples, increased immune infiltration was observed, with a strong macrophage and T cell presence. The phosphatidylinositol-3-kinase-protein kinase B (PI3K-AKT) signaling pathway was enhanced in lytic metastases, with high pAKT protein staining in the PCa cells, T cells, and macrophages. The inflammatory drivers S100A8, S100A9, CCL5, and WNT5A were enriched in lytic patient samples, along with the B7-H3 immune checkpoint target, which was upregulated in the T cell and macrophage compartments. A separate expression signature was observed in the biopsies from blastic metastatic PCa bone samples. Reduced immune cell infiltration was observed in blastic biopsies, but tumor cells, macrophages, and T cells were enriched for PD-L1 and IDO-1. Enhanced Janus kinase-signal transducers and activators of transcription (JAK-STAT) signaling resulted in high pSTAT3 expression in blastic patient samples. Gene expression profiling of blastic samples identified greater MMP7, LAMC2, and SHC2 expression. These findings depict a TIBD microenvironment that is unique in composition and molecular signatures, which can be translated to therapeutic targets.

Primary PCa tumors are classified as immunologically “cold,” with low neoantigen expression and immune cell involvement, supporting their failure to respond to immune checkpoint inhibitors. Due to the heterogeneity of metastatic PCa with unique genetic and immunogenic characteristics, metastases should be considered distinct tumors from their primary origin. The conventional assessment of immune infiltration and tumor mutation burden for immunogenicity scoring should take into consideration protein expression, which has proven to be a successful predictor of immune checkpoint inhibitor response. Based on our detection of macrophages and T cells in the microenvironment and immune checkpoint inhibitor expression, metastatic bone may be re-examined as immunologically “hot.” These findings highlight the need to reassess therapeutic approaches for PCa patients with metastatic bone to implement a more personalized medicinal methodology.

### 4 | NEW HORIZONS FOR METASTATIC BONE

#### 4.1 | Biopsy and molecular profiling of bone metastases

Bone biopsies can allow clinicians to detect expression alterations and mechanisms of resistance that better inform therapeutic
Unfortunately, bone biopsies are still not universally performed, and if they are it is only to assess hormone status and pathology. The technical challenges of obtaining usable protein and RNA for molecular characterization from bone biopsies also pose as a hurdle to endorsing biopsies. Patient bone biopsies are not normally processed as fresh frozen samples for precision medicine analysis. This is problematic since FFPE and decalcification processing of bone degrades nucleic acids. Additionally, the biopsy procedure can be difficult and painful for patients. However, the wealth of molecular information obtained from a bone biopsy outweighs the procedural and technical risks. In a multiple myeloma mouse model, transcriptomic profiling of the bone microenvironment identified bone morphogenetic protein signaling as a targetable approach for treatment of myeloma TIBD. Based on the cellular and molecular composition of an individual patient’s TIBD, appropriate therapeutics can be selected to improve patient outcomes.

Current therapeutic guidelines for metastatic PCa do not differentiate between lytic or blastic bone phenotypes. Based on our findings that lytic and blastic PCa metastases exhibit distinct microenvironments and signaling pathways, we propose using more biopsy guided approaches for therapy selection. The signaling markers and immune checkpoints identified in bone samples corroborate the approach of repurposing clinically validated targeted therapies for metastatic PCa patients with TIBD (Figure 1). Therapeutics targeting STAT3 or AKT signaling, as well as immune checkpoint inhibitors could pave the way for new approaches to treating PCa bone metastases. Targeting the macrophages or T cells in TIBD could aid in switching the microenvironment from pro-tumorigenic to anti-tumorigenic and provide a benefit for difficult to target metastases. Further understanding the distinct TIBD of metastatic PCa could help patients enroll in immunotherapies and use targeted agents based on the nature of their bone metastases rather than relying only on palliative and hospice care.

**FIGURE 1** Cellular and molecular features in bone with metastatic prostate cancer that displays unique immune microenvironments of lytic or blastic disease. Lytic disease exhibits a loss of bone driven by osteoclast activity, with robust infiltration of immune cells including macrophages and T cells. Enhanced AKT signaling is expressed throughout the tumor microenvironment, and the immune checkpoint target B7-H3 is enhanced in macrophages and T cells. Gene expression analysis revealed enrichment for S100A8, S100A9, WNT5A, and CCL5 in lytic bone biopsies. Patient samples with blastic tumor induced bone disease featured sclerotic bone lesions. Reduced immune cell infiltration was observed, but immune checkpoint markers IDO-1 and PD-L1 were expressed on tumor cells, macrophages and T cells. Strong STAT3 signaling and expression of MMP7, LAMC2, and SHC2 were widespread in the blastic samples. AKT, protein kinase B; STAT3, signal transducer and activator of transcription 3.

**5 | CONCLUSIONS**

The impact of immunotherapy on the bone of patients with PCa has only begun to be explored. It is well established that immune checkpoint inhibitors result in immune-related rheumatic and musculoskeletal adverse events, such as inflammatory arthritis, arthralgia, and myalgia. Yet only recently has a study found...
that immunotherapies result in SREs. In a small group of patients with melanoma, non-small cell lung cancer and renal cell carcinoma who received immune checkpoint inhibitors, a median of 8 months (ranging from 1 to 18 months) of treatment occurred before new fractures or lytic bone lesions were observed not related to any bone metastases. This study suggests further investigation into the changes bone undergoes during immunotherapy treatment is necessary to fully understand the risk of SREs. Since PCa TIBD is predominantly blastic rather than lytic, we anticipate a distinct response to immune checkpoint inhibitor therapy may occur in the bone microenvironment. Due to the enrichment of myeloid immune cells in bone marrow, it is plausible that changes to the immune microenvironment in the bone occur under specific immunotherapies to then alter the homeostasis of healthy bone remodeling. Additional disparities in the composition of lytic and blastic bone metastases could influence the characteristics of skeletal changes under immune checkpoint inhibitor treatment. This is an exciting time for the research and treatment of TIBD, with increasingly nuanced understanding of an individual patients' disease to help select the optimal treatment for both their PCa and TIBD.

ACKNOWLEDGMENTS
We apologize for not being able to cite all relevant references. We would like to thank Meredith D. Provera and Desiree M. Straign for reviewing the manuscript. This work was supported by VA Grant 1KBX00002929 (PO).

CONFLICT OF INTERESTS
The authors declare that there are no conflict of interests.

AUTHOR CONTRIBUTION
CLI wrote and PO edited the manuscript.

ORCID
Philip Owens
http://orcid.org/0000-0002-1452-9285

REFERENCES


**How to cite this article:** Ihle CL, Owens P. Integrating the immune microenvironment of prostate cancer induced bone disease. *Molecular Carcinogenesis.* 2020;59:822-829. https://doi.org/10.1002/mc.23192