A marrow-minded look at immune checkpoint blockade resistance in metastatic castration resistant prostate cancer

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Prostate cancer is the second most common malignancy diagnosed in men and the sixth highest contributor to cancer-related mortality worldwide (1). Advanced prostate cancer initially responds to androgen deprivation therapy before progression to an incurable state termed metastatic castration resistant prostate cancer (mCRPC). mCRPC is typically treated with taxanes or androgen receptor (AR) pathway inhibitors, but the survival benefit is variable and often brief (2). In recent years, focus has turned to the development of therapies outside of the AR signaling pathway. PARP inhibitors and pembrolizumab are effective in the subset of mCRPC with homologous recombination repair and mismatch repair defects, respectively (3,4). Further agents including PSMA-lutetium-177, DNA peptide-based vaccines, and oncolytic viruses are in various stages of clinical development (3). While single agent immune checkpoint blockade (ICB) has proved relatively ineffective in unselected mCRPC, in this commentary, we highlight its potential for eliciting potent anti-tumor responses when combined with agents that target the uniquely immunosuppressive tumor microenvironment (TME) of mCRPC.

In recent years, ICB has shown remarkable success in the treatment of some metastatic cancers including melanoma, lung, and urothelial carcinoma. Approved ICBs target either CTLA-4 or the PD-1/PD-L1 axis to promote tumor cell elimination by enhancing T cell activation and invigorating exhausted T cells in the TME. In metastatic melanoma, long-term disease control is possible via the generation of T cell memory in response to CTLA-4 blockade (5,6). In prostate cancer, targeting the immune system initially gained traction with the FDA approval of Sipuleucel-T, an autologous cellular vaccine, for patients with mCRPC (7). However, subsequent phase III clinical trials employing ICB with ipilimumab (anti-CTLA-4), atezolizumab (anti-PD-L1), autologous cellular (PROSTVAC) or viral vaccine (GVAX), and phase II trials with pembrolizumab (anti-PD1) have shown no significant survival benefit (8,9). Early results from CheckMate 650, an on-going phase II clinical trial employing a combination of nivolumab (anti-PD1) and ipilimumab (anti-CTLA-4), showed promising objective response rates of 26% in chemotherapy-naive patients, and 10% in chemotherapy-exposed patients. However, about half of patients experienced grade 3–5 adverse events, and a third discontinued treatment (10). Collectively, ICB clinical trial results and correlative analyses have indicated that most mCRPC is immunologically 'cold' and resistant to current immunotherapeutic approaches.

The poor response to ICB in prostate cancer is attributable to a combination of extrinsic and intrinsic resistance mechanisms including low mutational and neoantigen burden, defective MHC-I restricted antigen processing and presentation, paucity of inflammatory gene signatures, and the presence of immunosuppressive cell types (11,12). In particular, myeloid derived suppressor cells (MDSCs) appear to have an important role in mCRPC progression as they secrete cytokines that suppress T cells, promote M2 macrophage polarization, and can also directly

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promote AR-mediated tumor growth through IL-23/IL-23R signaling (13). Treatment exposure likely further alters the immune microenvironment and contributes to ICB resistance. For example, increased M2 macrophages and Tregs have been observed in tumors or tumor draining lymph nodes of mice exposed to androgen deprivation therapy (14-16). Of course, prostate tumors are notoriously heterogeneous, and in a recent phase II trial of ipilimumab in mCRPC, elevated CD8^{+} T cell infiltration and expression of an IFNγ gene signature in pretreatment tumors were associated with disease control and survival benefit (17). Importantly, this suggests that there may be an opportunity to improve the efficacy of ICB in prostate cancer through a combination approach that targets the immunosuppressive TME and invigorates infiltrating T cells. One such approach, with demonstrated efficacy over single agent use in a preclinical mouse model, was the pairing of ICB with phosphokinase inhibitors which cooperatively decreased mCRPC tumor growth, by selectively targeting tumor infiltrating T cell-inhibitory MDSCs, and synergistically enhancing ICB induced CD8^{+} T cell expansion (18).

Bone is the favored site of metastatic lesions in mCRPC and appears to be uniquely suited to promoting prostate cancer growth. Indeed, the establishment of bone metastases fits with Paget's 'seed and soil' hypothesis as prostate cancer cells migrate by hijacking bone tropic chemokine cues meant for hematopoietic cells, invade the bone endosteum, and eventually colonize the bone through paracrine signaling processes involving tumor cells and bone-resident osteoblasts, osteoclasts, and other stromal cells (19). Bone metastases frequently form in the axial skeleton including the spine, pelvis and ribs. These skeletal locations also house the bone marrow which functions as an essential lymphoid organ containing hematopoietic stem and progenitor cells, as well as mature adaptive and innate immune cell lineages (20). Therefore, for successful metastatic colonization, prostate tumor cells must first establish a foothold in the marrow and alter the bone microenvironment to a pro-tumorigenic state. The presence of a variety of immune cells suggests that tumor-immune crosstalk in the bone microenvironment is an important factor in metastatic disease progression. In this regard, TGF-β1 released by dysregulated osteoclastic bone resorption, stands out as a critical immunomodulator that can exert suppressive effects on antitumor immunity by inhibiting Th1 cells and cytotoxic CD8 T cells (21). Other examples of tumor-promoting interactions between bone metastases and immune cells include the recruitment of

immunosuppressive Tregs, MDSCs and macrophages via tumor mediated chemokine signaling, and expression of osteoclast activating molecules by plasmacytoid dendritic cells (22). Taken together, it is plausible that tumor-immune cell crosstalk within the bone microenvironment contributes to immunotherapy resistance however this remains a relatively underexplored area.

The value of dissecting the bone immune microenvironment in mCRPC was thrown into the limelight by a recent groundbreaking study from Jiao and colleagues that identified and exploited an ICB resistance mechanism within the bone marrow of men with mCRPC (23). The work was predicated upon the observation that patients with bone metastases have worse outcomes in ICB trials than those without bone metastases (10,24). Examination of immune signatures in the bone and soft tissue metastases of ICB-treated patients revealed site-specific differences in the tumor-associated T cell compartment. In bone metastases, ipilimumab treatment led to an expansion of Th17 cells while visceral metastases exhibited a skew towards Th1 responses. This distinction in immune response is important because Th1 cells are thought to be better at generating and maintaining cytotoxic CD8^{+} T responses necessary for tumor clearance compared with Th17 cells. To validate these site-specific differences in T helper response, the authors generated osseous and subcutaneous mCRPC animal models using a syngeneic Myc-CaP cell line and degarelix-induced castration. Consistent with the human data, when treated with a combination of anti-CTLA-4 and anti-PD1, animals with only subcutaneous tumors displayed increased survival and tumor control compared to mice with osseous tumors. Furthermore, single cell analysis of the immune infiltrates in osseous and subcutaneous tumors suggested that although ICB resulted in expanded CD4^{+} T cells, CD8^{+} T cells, and dendritic cells in both metastatic niches, the quality of the CD4^{+} T cell response differed considerably. Again mirroring human metastatic sites, Th17 and Treg cells dominated in the bone while Th1 cells expanded in subcutaneous tumors (23). Interestingly, this pattern of Th17/Treg *vs.* Th1 polarization also appeared to be present prior to ICB treatment, suggesting that ICB expands T cells in a lineage-constrained manner.

To investigate further, the authors interrogated the cytokine, chemokine, and growth factor profile of bone marrow in their model system. Bone metastases showed an increase in TGF-β1 expression relative to normal contralateral bone from the same animal or relative to bone from healthy control animals. IL-6 was also altered in bone

marrow but the elevated expression pattern was similar in both healthy and diseased bone compared to serum indicating that increased IL-6 is merely a feature of the osteogenic bone environment. Nevertheless, TGF-β1 and IL-6 are well-established synergistic promoters of Th17 polarization (25), and TGF-β1 can also limit Th1 responses; thus providing a potential explanation for the inappropriate Th17 response observed in the ICB-exposed bone metastases of both animal models and humans. Elevated TGF-β1 expression was tied to RANKL dependent bone resorption by osteoclasts, as RANKL blockade reduced osteoclastic activity and decreased TGF-β1 expression. The increased TGF-β1 expression observed in murine tumor bearing femurs was also confirmed in human mCRPC bone lesions (23).

The identification of elevated RANKL, IL-6 and TGF-β1 nominated several options for evaluation in combination with ICB to improve treatment outcomes. However, Jiao and colleagues found that anti-TGF-β blockade synergized best with anti-CTLA-4 and anti-PD1 to control tumor growth and improve survival. Importantly, anti-TGF-β could be combined with anti-CTLA-4 alone to provide similar therapeutic benefit, thereby highlighting a clinically-relevant pairing which may reduce adverse effects compared to dual ICB. Examination of immune cell composition in these single ICB and anti-TGF-β treated osseous mCRPC tumors showed an expansion of Th1 cells and depletion of Tregs. Surprisingly, the Th17 compartment was still present suggesting that pre-existing Th17 cells were able to expand in a TGF-β independent manner in response to treatment. IL-6 and IL-23 are known to promote and maintain the Th17 lineage (26) and it is possible that osteoblasts or MDSCs secreting these cytokines in CRPC bone mets may continue to support Th17 cell development. Since cytotoxic T cells are the main tumor eliminating immune cell type, the authors investigated the effects of anti-TGF-β and anti-CTLA-4 alone or in combination on intratumoral CD8 T cells. T cell receptor diversity analysis showed that the proportion of expanded clones was highest in the context of combined anti-TGF-β and anti-CTLA-4 blockade (although only a few T cell clones showed evidence of expansion). These CD8 T cell clones possessed an effector/memory signature and expressed genes associated with T cell cytotoxicity and tissue retention (23). In summary, this study provided detailed insight into a TGF-β linked bone-specific mechanism of mCRPC resistance to ICB, and demonstrated that targeting this immunosuppressive cytokine could move the needle from 'cold' to 'hot' in recalcitrant bone metastases.

TGF-β signaling also appears to be a core pathway contributing ICB resistance in 'immune excluded' metastatic urothelial carcinomas and colorectal cancer (27,28). Encouragingly, in preclinical studies, the TGF-β signaling pathway can be targeted by anti-TGF-β mediated depletion or by TGF-β inhibitors such as galunisertib (27,28). Together, these findings set the stage for clinical trials testing ICB in combination with TGF-β inhibition in mCRPC and other cancers with immunosuppressive microenvironments. Of course, given the heterogeneity of prostate cancer, clinical implementation of this combination will likely require development of predictive biomarkers. Importantly, while Jiao *et al.* utilized a MYC-expressing mCRPC model system (23), human mCRPC comprises several distinct molecular subtypes, each with differential biological and clinical impact (29). As such, there is a need to understand whether underlying tumor genomic and transcriptomic subtypes are associated with the degree of immunosuppression in bone metastases. This may be particularly relevant in the context of AR expression heterogeneity, since about 20% of bone metastases appear to exhibit low AR activity, and higher concomitant immune cell infiltration and MHC-I expression (30).

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Footnote

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