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Review Article

# The Involvement of Metastasis in Immunosuppression: A New Frontier in Prostate Cancer Personalized Immunotherapy

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## Abstract

An individualized immunotherapeutic management of metastatic prostate cancer still remains a great challenge due to the existence of multifactorial complexities like immunosuppressive tumor microenvironment, influence of metastatic dissemination, lack of proper clinical responsiveness and its patient specific marked variations. The overall process of prostate cancer metastasis ranging from its epithelial mesenchymal transition (EMT) to distant metastatic colonization plays a leading role in development of immunosuppression in various ways. In this regard, different contributing factors in prostate cancer immunosuppression may include epithelial mesenchymal transition (EMT), metastatic signaling molecules, pre-metastatic colonization, tumor micro-environmental remodeling and cancer stem cells. Here, in this mini-review we particularly focus on important metastatic aspects, their mechanistic overviews and how they modulate acquisition of immunosuppressive phenotypes in advanced prostate cancer along with the key criteria needed to make the immunotherapy more individualized. Additionally, we also provides an in-depth assessment for most of the associated critical factors, like as pre-clinical model, predictive biomarker, clinical trial and patient selection criteria's required for personalization of prostate cancer immunotherapies based on different immunosuppressive signaling routes.

**Keywords:** Prostate Cancer; Personalized Treatment; Immunosuppression; Bone Metastasis; Epithelial Mesenchymal Transition (EMT)

## Introduction

It is generally believed that bone metastasis is the principal cause of mortality for patients with advanced metastatic castration resistant prostate cancer [1-3]. Radiological evidence based current report suggests that more than 90% of patients in advanced prostate cancer suffer from bone metastasis with lymph node involvement and a considerable number of cases also exhibits visceral metastasis [1,2]. Bone metastasis presents the most clinically challenging features of prostate cancer management that drastically affects patient's quality of life through several skeletal related events including severe bone pain, pathological fracture, spinal cord compression, bone marrow replacement, nerve impingement, and cachexia [1-3]. The prognosis of metastatic CRPC in majorities of cases remains unsatisfactory and that is why many clinical experts in India and throughout the globe still considers metastatic CRPC as an incurable disease [4,5]. As a matter of fact, current therapeutic strategies in metastatic CRPC including radiotherapy, chemotherapy and targeted therapies are mainly palliative in nature that only attempts to minimize prostate tumor mediated osteolysis [1-5]. Currently in many cases for management of metastatic prostate cancer, Bisphosphonates like Denosumab and Zoledronic Acid is frequently used to reduce bone metastasis associated clinical complications [5]. But, the reported survival benefit of bisphosphonates and other standard of care treatment is mainly poor and are also associated with serious side effects like osteonecrosis of the jaw (ONJ) which in most of the cases are unable to improve metastatic CRPC patients overall health related qualities of life [5].

The emerging concept of personalized immunotherapy provides an ultimate new hope to efficiently bypass currently observed challenges in metastatic CRPC treatment and to specifically address other important oncogenic feature like tumor heterogeneities. As a result, for more than last decades a significant pace of drug development has been observed for identification of novel immunotherapeutic modalities to effectively target metastatic CRPCs [6]. According to the currently available literature, most of the concerned clinical reports have specifically indicated for the existence of immunosuppression in association with an immunologically cold tumor microenvironment (TME) as the fundamental barrier for achieving the desired clinical success in personalized immunotherapeutic management of metastatic CRPC patients [6,7]. A considerable number of current studies have suggested for the potential role of bone metastasis in induction and establishment of prostate cancer associated immunosuppression like incidents and functional exhaustion of cytotoxic T lymphocytes [7,8]. Recently, a combination therapy based on dual inhibition of immune checkpoints through administration of Ipilimumab (anti-CTLA4) and Nivolumab (anti-PD-1) in the largest phase-II clinical trial for bone metastatic CRPC patients (CheckMate 650 trial) have demonstrated significant improvement in the objective response rate (ORR) in comparison with previous findings [9]. In addition, a pre-clinical study reported in 2022 by Zhi et al. has shown intermittent blocking of PI3K pathway in PTEN-null prostate cancer mouse model dramatically reduces tumor intrinsic immunosuppression in conjunction with an apparent increase in T cell driven anti-prostate tumor immune responses [10]. This impressive results along with a number of recent finding convincingly argues for the introduction of immunosuppression based therapeutic strategies for metastatic CRPC and therefore potentially recommends for identification of metastasis associated immunosuppressive routes in detail [6-10]. To principally address all of these major issues, Prostate Cancer Systems Medicine Initiative, the first Indian cancer precision medicine research movement attempts to explain the following, a) How prostate cancer metastasis modulates suppression of anti-prostate tumor immune responses? and b) Why prostate cancer immunosuppression should be given essential priorities in future personalized clinical management of metastatic prostate cancer patients?

## Prostate cancer metastasis and immunity: A hot destination in personalized immunotherapy

The current trends in prostate cancer patient specific immunotherapy development is mainly based on modulation of prostate cancer associated immune responses for activation of 'prostate cancer-immunity cycle' and inhibition of patient centric immunosuppressive mechanisms [7,8]. At the same time, majorities of studies in prostate cancer bone metastasis have pointed out for the persistence of low level of immunogenicity's in respect with primary prostate tumor. As a consequence, the clinical response of metastatic CRPCs towards most of the currently available immunotherapies are significantly poor and in majorities of cases is linked with lower survival benefits [6,9]. This poor immunogenic

nature in metastatic prostate cancer can be centrally characterized by observed phenomenon of immunological ignorance that mostly includes inherently poor levels of antigenic presentation, functional inactivation of cytotoxic T cells by numerous ways, prevalence of immune checkpoints and accumulation of immune modulatory cells along with different types of immunosuppressive molecules [8]. Basically, the facts of immunological ignorance in metastatic prostate cancer is mainly driven by lack of expression for most of the genes involve in 'prostate cancer-immunity cycle', which is inherently associated with the uniqueness present in prostate tumor microenvironment (TME), its tumor-immune contextures and influence of bone marrow microenvironments on systemic spreading of prostate cancer. In fact, metastatic progression in prostate cancer can be alternatively viewed as a microenvironment driven disease that involves complex reciprocal interactions between prostate tumor and bone microenvironment with immune system at different levels, namely tumor-stromal-immune cells, tumor-matrix-immune cells and tumor-vasculature-immune interactions [6,8,11,12]. As a result of these multifactorial interactions, both metastatic prostate tumor and immune system synergistically modulate each other activities in bone marrow microenvironment that ultimately leads to the development of immunosuppression and immune resistance like scenarios. The principal characteristics of immunosuppressive TME in metastatic prostate cancer are associated with direct suppression of anti-tumor immune response generation, induction of pro-tumorigenic molecules and active recruitment of immunomodulatory cell populations [7-12].

## Role of metastasis in prostate cancer immunosuppression

The development of prostate cancer metastasis, which may be accounted for nearly 90% of deaths from advanced prostate cancer patients, provides a strong stimulation for induction, maintenance and enrichment of immunosuppressive tumor microenvironments [8,12]. This stimulatory effect is mainly observed at two major steps in the process of prostate cancer metastatic progression-during the initial onset of epithelial mesenchymal transition (EMT) and finally the formation of pre-metastatic niches in distant bone and lymph node microenvironment [Figure 1 and Figure 2]. A very brief description of key molecular mediators and cellular determinants in metastatic PCa associated immunosuppression are given in Table 1.

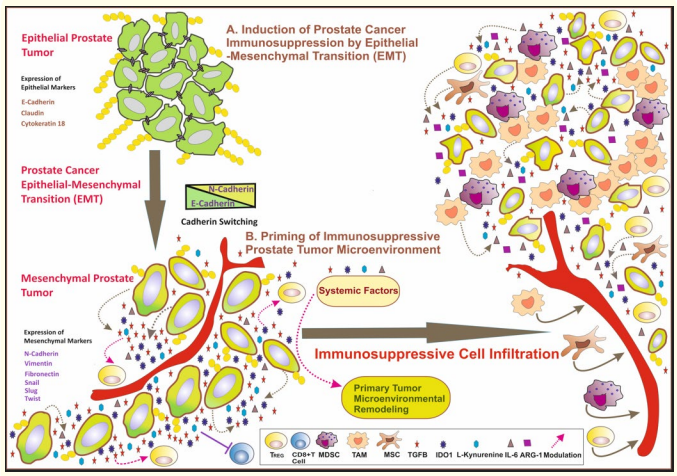


**Figure 1:** Role of epithelial mesenchymal transition (EMT) in prostate cancer immunosuppression.

Epithelial mesnchymal transition (EMT) can be considered as the most fundamental driving force for prostate cancer immunosuppression induction. A. During prostate cancer epithelial mesenchymal transition (EMT) initiation, cadherin switching mediated acquisition of N-Cadherin critically triggers the secretion of systemic factors like TGFB and IDO1 (Indoleamine 2,3-dioxygenase) from local prostate tumor, which is significantly associated with the infiltration of regulatory T cells (TRegs) in prostate tumor microenvironment. At the same time, these systemic factors also restricts entry of CD+T cell in those concerned areas and there by initiates suppression of adaptive immune response developments. B. Acquisition of mesenchymal phenotypes by prostate tumor cells along with gradual infiltrations of diverse immunosuppressive cell types particularly favors for priming of immunosuppression establishment around mesenchymal prostate tumor. These priming phase is mainly mediated through secretion of various systemic factors like PD-L1, TGFB, IL-6, IDO-1, ARG-1, L-Kynurenine from both morphologically altered prostate tumor cells and immunosuppression inducing immune associated cells like Tumor associated macrophages, myeloid derived suppressor cell (MDSC), regulatory T cells (TRRGs), and mesenchymal stem cells (MSC).

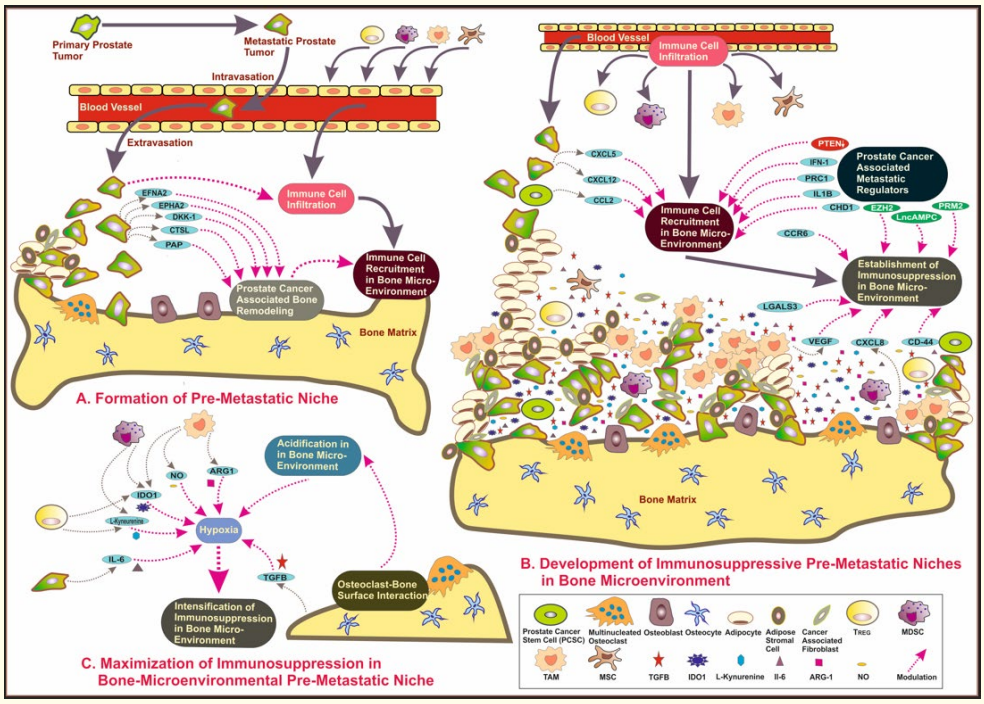


**Figure 2:** Role of bone metastasis in establishment of prostate cancer immunosuppression.

The process of bone metastasis finally shapes the overall magnitude of immunosuppression establishment in prostate cancer. A. In the late phase of prostate cancer metastasis, arrival of circulating tumor cells (CTC) in the bone microenvironment area leads to initiation of bone metastatic colonization, which is further reinforced by direct functional interactions between prostate tumor cells and bone marrow resident cells like osteoblasts and osteoclasts in context of prostate cancer bone remodeling. At the same time, infiltration of prostate cancer cells strongly modulate formation of these pre-metastatic niches in association with mobilization of immunosuppressive cells like regulatory T cells (Tregs), Tumor associated macrophages (TAM), myeloid derived suppressor cell (MDSC) and prostate cancer stem cells (PCSC) in bone microenvironment. Subsequently, prostate cancer associated bone remodeling dictates the recruitment of immunosuppressive cells in prostate cancer bone microenvironment. B. Progressive accumulation and recruitment of immunosuppressive cells in prostate cancer bone microenvironment is largely facilitated by CXCL-5, CXCL-12 secreted by disseminated prostate cancer cells, CCL2 released by prostate cancer stem cells and several prostate cancer associated metastatic regulators, like PRC1, CHD1, IL1B, IFN-1 and loss of PTEN tumor suppressor. Accumulations of these immunosuppressive cell types in prostate cancer bone marrow pre-metastatic niches greatly stimulates secretion of soluble immune-modulatory systemic factors (TGFB, IL-6, IDO1, NO, ARG-1, VEGF, CXCL-8), which accompanied by a number of metastatic regulators (EZH2, PRM2, LncAMPC, LGALS3, CD44, CCR6) and thereby strongly supports for establishment of immunosuppression. C. The release of soluble immune-modulatory systemic factors by immunosuppressive cell types along with bone micro-environmental remodeling firmly triggers generation of hypoxia and subsequently promotes maximization of immunosuppression in prostate cancer bone metastatic niches. The acidification in prostate cancer associated bone microenvironment by means of osteoclast-bone surface interaction accompanied by secretion of NO, ARG1 from tumor associated microphages (TAM), IDO1, L-Kynurenine from myeloid derived suppressor cells (MDSCs) and regulatory T cells (Tregs), IL-6 from disseminated prostate cancer cells and TGFB from destruction of bone constituents stimulate induction of hypoxic stress responses and thereby maintains a much more higher levels of immunosuppression than original prostate tumor microenvironment.

| Metastatic Driver | Cell Type Involvement | Characteristic Feature | Role in Prostate Cancer Immunosuppression | Reference |
|---|---|---|---|---|
| PRC1 (Polycomb Repressor Complex 1) | TAM, Regulatory T Cells | Stemness | PRC1 is significantly involved in prostate cancer bone metastasis and induces CCL2, which governs both stemness and immunosuppression through recruitment of TAM and TREGS in double negative prostate cancer. | Cancer Cell 36 (2019) 139–155.e10. |
| VEGF | M2 TAM | TAM | Critical mediator secreted by metastatic prostate tumor associated macrophage and significantly involved in immune suppression. | Cancers (Basel) 12 (2020) 2718. |
| CXCL8 | M2 TAM | TAM, PTEN loss | Critical mediator secreted by metastatic prostate tumor associated macrophages along with PTEN loss mediated increased expression and significantly involved in immune suppression. | Biomedicines 10(2022) 1778. Cancers (Basel)12 (2020) 2718. |
| IL6 | M2 TAM | TAM | IL-6 is secreted by metastatic prostate tumor associated M2 TAM and plays a role in PCa immune suppression and metastatic advancement. | Cancers (Basel)12 (2020) 2718. |
| IL1B | M2 TAM, MDSC | TAM, Immuno-Suppression Modulator | IL1B modulates prostate cancer immunosuppression by inducing accumulation of MDSC in PCa microenvironment. | Cancers (Basel)12 (2020) 2718. Adv Sci (Weinh)10 (2023) e2206889. |
| IFN (Type-1 Interferon) | Bone metastatic Prostate tumor cell | Dormancy, Immuno-Suppression Modulator | Alterations of tumor intrinsic type-1 signaling during dormancy regulation in bone metastatic prostate cancer significantly suppress a number of HLA genes which in turn mediates loss of prostate tumor immunogenecity and associated with the marked accumulation of immune suppressive cells. | EMBO Rep21 (2020) e50162. |
| LGALS3 (Galectin-3) | Cancer Stem Like Cells | Cancer Stem Like Cells | Both in primary prostate cancer and during its lymph node metastasis, Galectin-3 plays an unique role in prostate tumor immunosuppression. | Front Immunol11 (2020) 1820. |
| N-Cadherin | $T_{REG}$ | Epithelial Mesenchymal Transition (EMT) | Prostate cancer associated epithelial mesenchymal transition is accompanied by N-Cadherin, which essentially up-regulates immunosuppressive IDO1 expression in metastatic prostate cancer patients. | Front Immunol11 (2020) 1820. |
| TGFB1 | Prostate stromal & immune cells | Epithelial Mesenchymal Transition (EMT) | TGFB1, which is secreted by immune and stromal cells during late phase of prostate cancer development and plays a key mediator role in acquisition of EMT and immune suppression. | Cancer Res 78(2018) 4671-4679. |

| | | | | |
|---|---|---|---|---|
| CD44 | CSC marker, present in CD44+ Prostate Tumor Cell | EMT,Stemness, Tumor Burden | CD44 functions as CSC associated inducer of immunosuppression in prostate cancer in a manner that is dependent on tumor burden and IL-6 mediated signaling. | Cancers (Basel) 11(2019)99. |
| CHD1 | MDSC | TME Remodeling, PTEN loss, Immuno-Suppression Modulator | During PTEN loss in prostate cancer, CHD1 modulates the formation of immunosuppressive tumor microenvironment through recruitment of MDSCs by IL-6 dependent manner. | Cancer Discov 10(2020) 1374-1387. |
| Enhancer of Zeste Homolog-2 (EZH2) | TAM | EpigenomicRegulation | EZH2, an oncogene mainly involved in regulation of prostate cancer metastatic progression and also associated with formation of immunosuppressive prostate TME. | Nat Cancer 2(2021) 444-456. |
| RANKL | Regulatory T Cells, Dendritic Cell | Bone Remodeling, Immuno-Suppression Modulator | RANK along with its ligand RANKL mediated signaling in prostate tumor bone microenvironment plays a vital role in expansion of bone marrow regulatory T cells, which assists in the genesis of immunosuppressive niches and contributes in bone metastatic progression. | Oncoimmunology 1(2012) 152–161. |
| CCR6/CCL20 Axis | T Lymphocyte | T Cell Exhaustion | CCL20 and its receptor CCR6 on myeloid cells are involved in the formation of immunosuppressive tumor microenvironment in bone metastatic prostate by mediating an exhaustion of T cells. | Cancer Cell 39(2021) 1464–1478.e8. |
| PI3K | MDSC, Regulatory T Cells | PTEN loss, | PTEN loss or activation of PI3K signaling pathways are significantly involved in acquisition of prostate tumor cell intrinsic immune-suppression and immune-resistance. | Nat Commun 13(2022) 182. |
| PTEN | FoxP3+ regulatory T cells ($T_{REG}$s) | PTEN Deficiency | The loss of PTEN tumor suppressor is closely linked with genesis of immunosuppressive state in bone metastatic prostate cancer by an increase in subpopulation of FoxP3+ T regulatory cells ($T_{REG}$s). | Prostate 79(2019) 969-979. |
| RRM2 | Metastatic prostate tumor, Regulatory T Cells, M2 Macrophage | Prostate Cancer Aggressiveness | PRM2, an oncogene that is over-expressed in metastatic prostate cancer plays a role in promotion of immunosuppressive state in prostate tumor immune microenvironment. | Mol Oncol 14(2020) 1881–1897. |
| BHLHE22 | Neutrophils, Monocyte | Epigenomic Regulation | BHLHE22, a central mediator of prostate cancer bone metastasis progression is also actively involved in immunosuppression through mediating an infiltration of neutrophil and monocytes. | J Immunother Cancer 11(2023) e005532. |

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|---|---|---|---|---|
| LncAMPC | Metastatic prostate tumor | Long non coding RNA | LncAMPC plays an oncogenic role in activation of LIF/LIFR/JAK1/STAT3 mediated signaling, which stimulates metastatic progression along with immunosuppression. | Mol Ther28(2020) 2473-2487. |
| AR Antagonist during ADT | Metastatic Prostate Tumor Cell | Androgen deprivation therapy (ADT) | Androgen deprivation therapy (ADT) plays a critical role in prostate cancer associated immunosuppression mainly through de-regulation of T-cell activation and gamma interpherone production. | Sci Transl Med 8 (2016) 333ra47.<br><br>Front Endocrinol (Lausanne) 13 (2022) 1055826. |

**Table 1:** List of molecular mediators and metastatic regulators involved in prostate cancer bone metastasis associated immunosuppression.

## Epithelial mesenchymal transition

Epithelial mesenchymal transition (EMT) itself plays the central most role in initiation of prostate cancer invasion and metastatic through actively driving prostate cancer cell separation from primary tumor masses and reported to be intrinsically associated with acquisition of immunosuppressive clinical responses in prostate cancer patients [13,14]. The most basic step of prostate cancer EMT are mainly driven by the emergence of a mesenchymal phenotype by epithelial prostate tumor cells, which is mostly characterized by the gain of mesenchymal marker like N-cadherin accompanied by the loss of epithelial marker E-cadherin [Figure 1]. This EMT mediated cadherin switching stimulates up-regulation of immunosuppressive soluble mediators like IDO1 (Indoleamine 2,3-dioxygenase), L-Kynurenine and TGFB1 (Transforming growth factor beta 1) in local prostate tumor microenvironment. These EMT directed local increase in immunosuppressive mediators are significantly linked with the higher infiltration of FOXP3+CD4+ regulatory T cells ($T_{REG}$s) in prostate tumor microenvironment (TME) in association with marked inhibition of adaptive immune response development through reduction in number of CD8+ cytotoxic T cells [13]. In context of EMT induced immune evasion in prostate cancer, patient's tumor burden in some cases plays a modulatory role in promotion of EMT related processes towards formation of immunosuppressive TME [14]. A recent study based on development of transgenic adenocarcinoma of the mouse prostate (mod) tumor model and analysis of patients biopsy specimen, has mentioned that tumor burden is closely associated with clinically aggressive prostate cancer development along with its EMT associated metastatic progression and immunosuppressive behavior acquisition. Although, the detail mechanistic basis of this events are not well established, but it has profound potential to guide future personalized treatment associated decision making processes during clinical management of CRPC patients. Because tumor burden has been reported to be significantly linked with the latency period of castration resistant prostate cancer development, its median survival rate in CRPC patients and corresponding aggressive clinical feature acquirement [14,15].

## Metastasis associated signaling molecules

The hallmark characteristics of prostate cancer metastatic progression is largely contributed by several metastatic drivers and key signal transduction proteins that critically coordinate metastasis related events in association with modulation of prostate tumor immune microenvironment [Table 1]. Basically, the oncogenic activities of these metastatic drivers are substantially linked with suppression of prostate tumor targeting immune responses by a variety of means including recruitment, activation and proliferation of immunosuppressive cells in prostate TME, which in turn enhances metastatic advancement and therapeutic resistance. For example, in a recent pre-clinical model in double negative prostate cancer (DNPC) has indicated Polycomb Repressor Complex 1 (PRC1) triggers prostate cancer bone metastatic colonization with transcriptional up-regulation of several pro-metastatic inducers including CCL2 (Chemokine

ligand 2 or Monocyte chemo-attractant protein 1), which in-turn stimulates recruitment of FOXP3+ regulatory T cells ($T_{REG}$s) and M2 like tumor associated macrophages (TAM) in bone marrow pre-metastatic niches [16]. In another pre-clinical study involving intratibial-xenograft model reported in 2016, has shown that Tasquinimod (AR-215050) treatment markedly reduces pre-metastatic tumor growth in bone microenvironment in association with reduction of immunosuppression in castrated mice [17]. This somehow critically suggests that prostate cancer metastasis initiation and induction of immunosuppression are coordinated events and a fraction of metastasis regulatory intracellular signaling circuitries are significantly involved in maintenance of immunosuppressive state around prostate tumor and its distant pre-metastatic niches. For instance, oncogenic transcription factor STAT3 (Signal transducer and activator of transcription 3), which plays a potential regulatory role in different aspects of prostate cancer tumorigenesis and metastatic progression are also involved in the induction of immunosuppression in prostate cancer TME and its pre-metastatic niches in several distinct ways. First, through regulation of prostate cancer stem cell associated CD44 expression and its subsequent immunosuppression, second, by transcriptional activation of immunosuppressive PD-L1 (Programmed death-ligand 1) in prostate cancer stromal cells and third, by means of pathophysiological activation of PMN-MDSCs in pre-metastatic niches [14]. A significant number of pro-tumorigenic transcriptional regulators and metastatic drivers, such as NF-KB (Nuclear factor kappa B), SOCS3 (Suppressor of cytokine signaling 3), STAT6 (Signal transducer and activator of transcription 6), ETS (Erythroblast transformation specific) and FOXP3 (Forkhead Box Protein 3) are centrally involved in prostate cancer immunosuppression [Table 1]. A patient specific precise assessment of these metastatic regulators may provide a highly specific means to normalize local immunosuppressive mechanisms and formulation of effective combinatorial therapies according to patient's unique disease characteristics. For development of such types of individualized treatment strategies, it is necessary to identify the corresponding predictive biomarkers for those metastatic drivers in metastatic CRPC setting and their further detail functional characterization in respect of currently used immunotherapies.

## Pre-metastatic colonization and TME remodeling

A substantial number of evidences have indicated prostate cancer metastatic progression is markedly triggered by the formation of a supportive immunosuppressive microenvironment in distant bone marrow and lymph node regions [14,16,17]. Both prostate tumor microenvironment and its pre-metastatic niches are highly heterogeneous in nature and mainly composed of cancer associated fibroblasts (CAFs), myofibroblasts, adipose-stromal cells (ASCs), adipose tissues, extra-cellular matrix components (ECM), circulating prostate tumor cells, prostate tumor derived extracellular vesicles and cytokines, mesenchymal stem cells (MSC), macrophages, regulatory T cells ($T_{REG}$s), myeloid derived suppressor cells (MDSCs), mast cells and different bone forming cellular constituents [Figure 2.B]. These prostate tumor microenvironment and its pre-metastatic niches are essentially characterized by local accumulation of immunosuppressive soluble mediators and prevalence of hypoxic zones due to hyper-activation of ROS dependent signaling by stromal components and various immune-regulatory cells [13,16,18]. As a matter of facts, the local enrichment of various immunosuppressive molecules like TGFB (Transforming growth factor beta 1), IDO1(Indoleamine 2,3-dioxygenase), IL-6 (Interleukin 6), ARG-1 (Arginase 1), NO (Nitric oxide), L-Kynurenine, PD-L1 (Programmed death-ligand 1) individually triggers hypoxic stress responses, which along with over all repressive activities mediated by tumor-educated immune-modulatory cells are potentially responsible for acquisition and predominance of "Immunologically Cold" nature in prostate tumor microenvironment and its pre-metastatic niches [Figure 2.C]. Moreover, the inherent immunosuppressive characteristics of bone marrow, lymph node environments and higher levels of physical (adhesive) and functional interactions between different immune regulatory cells and bone marrow stromal constituents further reinforces their pathophysiological activation and immunosuppressive functioning [7,10,11,13,16-18]. Recently, various prostate tumor intrinsic factors have been identifiedas a key mediator for remodeling of its bone metastatic prostate tumor immune microenvironment towards establishment of an immunosuppressive state. The intrinsic factors are: i) loss of PTEN (Phosphatase and TENsin homolog deleted on chromosome 10) tumor suppressor, ii) hyper activation of PI3K mediated signaling pathways, iii) oncogenic activation of TGFB signaling axis and iv) dysregulation of interferon gamma (IFNG) associated signaling [Table 1]. In nearly 50% of primary prostate tumor and more than 70% of bone metastatic prostate cancers generally exhibits loss of PTEN tumor suppressive activities, which essentially modulates infiltration of immunosuppressive cells like FOXP3+ $T_{REG}$s in bone

microenvironment along with marked increase in IDO1 activities [10]. This PTEN loss mediated oncogenic activation of PI3K signaling in bone metastatic prostate cancer is crucially associated with $T_{REG}$s and MDSCs infiltration, repression of dendritic cell's maturation and inhibition of T cell mediated cytotoxic activities. TGFB can be considered as one of the major mediator of prostate cancer bone remodeling that arises due to massive destruction of bone components and significantly involved in further metastatic spread and immunosuppression [18].

The late formation of pre-metastatic niches in prostate cancer perhaps provides the strongest stimulation for accumulation and functional enrichment of different immunosuppressive cell populations like macrophages, regulatory T cells ($T_{REG}$s), polymorphonuclear MDSCs, which together forms a potential barrier for activities associated with all forms of anti-prostate tumor immune responses including CTL activation [13,16,18]. These potential immunosuppressive cells are manly attracted towards pre-metastatic niches in response of prostate tumor derived cytokines, bone marrow derived chemo attractants and hyper activation of different prostate cancer oncogenes and metastatic inducers [Figure 2.B]. Several prostate tumor extrinsic mechanisms are significantly involved in its TME remodeling for suppression of prostate tumor targeting immune response development. The extrinsic mechanisms are i) $T_{REG}$s mediated pathological remodeling of prostate cancer bone microenvironment, which is characterized by enhancements in osteoblastic bone resorption, distant metastatic spread and activation of multiple immunosuppressive mechanisms that modulates tumor infiltrating lymphocytes accumulation [18], ii) MDSCs and MSCs mediated stimulation of prostate cancer hallmarks activities that preferentially stabilizes immunosuppressive niches in bone and lymph node microenvironment through activation of prostate cancer cell growth, survival and proliferation [18,19], iii) polarization of macrophages into its M2 phenotype that substantially supports for prostate TME remodeling through secretion of immunosuppressive cytokine and chemokines, activation of CCL20-CCR6 driven signaling and recruitment of immune-modulatory cells [18], iv) formation of cancer associated fibroblasts (CAFs) through trans-differentiation of mesenchymal stem cells (MSCs) and v)TGFB mediated impairment in natural killer cells tumor suppressive activities [18,20]. The detail further clinical assessment for each of these prostate tumor related intrinsic and extrinsic mechanisms in respect with currently available immunotherapeutic strategies are largely required to use them in future personalized treatment of metastatic CRPCs.

## Cancer stem cell

Prostate cancer stem cells (PCSCs) play a key driver functions in disease advancement through mediating its metastatic dissemination, tumor growth, immunosuppression and disease recurrence. The principle characteristic feature of PCSCs mainly include its contribution for imparting resistance to all forms of standard of care therapies due to its exceptionally slow growth rate and unique abilities for tumor regeneration [21]. During prostate tumorigenesis, epithelial mesenchymal transition (EMT) plays a critical modulatory role for development and transformation of PCSCs with a higher metastatic potential that significantly supports for its involvement in CRPC progression and immunosuppressive TME formation [14,21]. In this major context, pro-inflammatory cytokine IL-6 exhibits a major regulatory role in coordination of PCSCs activation and selection related events including acquisition of its stemness and immunosuppression associated features. In order to coordinate PCSCs associated immunosuppression and aggressive behavior development, IL-6 mainly depends on STAT3 mediated transcriptional regulatory events and expression of aggressiveness associated marker CD44. A recent study based on a pre-clinical prostate cancer model has demonstrated significant levels of attenuation in the formation of immunosuppressive TME along with a marked decrease in CD44 during IL-6 inhibition. This IL-6 induced STAT3 mediated regulatory operational mode in PCSCs are also involved in PD-L1/PD-1 immune checkpoint activation and TME remodeling, both of which are intrinsically associated with prostate cancer immunosuppression. Patient specific therapeutic targeting of IL-6 trans signaling in CRPC associated immunosuppression may provide a potential resource for individualized treatment as IL-6 expression level exhibits significant correlation with prostate cancer clinical features like clinical stage, Gleason grade, serum PSA level, disease burden and its biochemical recurrence [14].

Although the exact regulatory mode of interaction between PCSCs and metastatic prostate tumor immune environment is mostly unknown but it has been reported that in some cases, PCSCs mainly depends on the epigenomic regulatory activities of

metastatic regulator Polycomb Repressor Complex1 (PRC1) for immunosuppression induction during metastatic progression. Particularly in pre-clinical setting of double negative prostate cancer [DNPC- AR pathway negative, neuroendocrine phenotype negative], the core oncogenic signaling circuitries of PRC1 has demonstrated a central most involvement in the coordination of prostate cancer associated stemness with micro environmental immunosuppression and bone metastatic disease development [16]. In context of prostate cancer associated lymph node metastasis, extracellular matrix binding protein Galectin-3 (GAL3) plays a very critical regulatory role in coordination of PCSCs metastatic and immunosuppressive activities. Detail pre-clinical investigation in TRAM model has indicated for the oncogenic role of Galectin-3 (GAL3) in cancer stem cell induced prostate cancer lymph node metastasis along with the suppression of immune response development. In the corresponding pre-clinical model, the specific immunomodulatory functioning of GAL3 has been explained by its over-expression patterns at the leading areas of prostate cancer lymph node metastasis where PCSCs and immune cells physically interacts each other [22].

## Personalized treatment perspectives and future directions

A rapid advancement in several precision medicine techniques like liquid biopsy, NGS based tumor genome profiling, quantitative proteomics, single cell sequencing, immune cell profiling and pre-clinical prostate cancer modeling provides a strong rational for formulation of individualized immunotherapies with a view to dramatically improve survival benefits and health related qualities of life for metastatic CRPC patients. But, majorities of current clinical trials involving a spectrum of immunotherapeutic approaches have critically suggested for the lack of necessary effectiveness in terms of therapeutic response development in metastatic prostate cancer [6,10]. Due to this universally accepted facts in prostate cancer clinical research along with the past insights obtained from several leading precision care platform based studies, it could be easily assumed that finding an appropriate treatment for advanced prostate cancer patients in connection with its proper selection and therapeutic sequencing is the most challenging tasks now a day's [5,23]. This grand challenges mainly arise because of biological complexities in prostate cancer metastasis and its extra ordinary levels of heterogeneities observed in different levels like genomic instabilities, post-translational modifications, pathological growth patterns, micro environmental remodeling and clinical manifestations [23,24]. Additionally from the immunological point of view, the typical characteristics of the metastatic prostate tumor associated immune microenvironment frequently includes existence of immune desert like appearances (also known as 'immunologically cold' microenvironment) during early phase and the presence of functional immunosuppressive networks in the late phase of metastatic spread [7,18,24]. In order to effectively minimize all of these challenging factors and to significantly improve both qualitative and quantitative aspects of clinical responses, the central aim of personalized immunotherapy in metastatic CRPC setting will mainly involve identification of patient specific local immunosuppressive mechanisms and development of combinatorial therapeutic strategies [18,23].

Most importantly, two specific feature of prostate cancer metastasis like exhaustion of antitumor cytotoxic T lymphocytes and accumulation of bone metastatic state specific immune-regulatory cells will provide critical support for precise identification and normalization of patient specific immunotherapeutic strategies to successfully overcome 'immunological cold' nature of prostate TME. This normalization of personalized immunotherapy will primarily involve identification of metastatic prostate tumor dependent intrinsic and other extrinsic mechanisms of immunosuppression and finding its appropriate combination with T cell oriented immunotherapies in a patient centric manner [7,18]. Single or dual agent based combinatorial therapeutic measures involving selective inhibition of metastatic CRPC patient specific immune repression mechanisms have enormous potential to significantly improve clinical effectiveness of various currently available immunotherapeutic strategies, like personalized vaccination, immune checkpoint inhibition and adoptive cell therapy (ACT) [10,23]. For an example, the recently reported CheckMate 650 trial with binary inhibition of immune checkpoint blockade have shown a pronounced increase in objective response rate (25%) accompanied by an overall improvement in clinical response in a subsets of pre-chemotherapy patients with asymptomatic and minimally symptomatic metastatic CRPC [9]. But, at the same time the reported observation of significant levels of toxicities (>42%), treatment discontinuation due to drug related adverse effects (38%) and death (4.4%) related incidents in the corresponding non-randomized phase-II clinical trial also pragmatically supports for identification of tailored treatment strategies to reasonably increase patient specific tolerability's of immunotherapy [25]. In

order to optimally balance the immunotherapeutic efficiencies with toxicity related issues in future individualized management of metastatic CRPC patients, the following factors may play an instrumental role.

## Pre-clinical model

For optimal tailoring of immunotherapies according to the patients' requirements, a comprehensive understanding of 'prostate cancer-immunity cycle' is urgently needed to critically assist accurate bench to beside translation of novel scientific insights [26]. In this crucial point, currently there exists a significant lack in proper pre-clinical characterization of available immunotherapies over complex phenomenon of prostate cancer associated bone metastasis in different aspects, including immunotherapy based clinical trial designing, bone metastasis associated pre-clinical model development, and functional understanding of reciprocal interactions between prostate tumor, its bone microenvironment and the immune system in bone metastatic condition [12,16]. Although, the conventional orthotopic and transgenic animal model can provide essential foundation for studying immunosuppressive prostate tumor microenvironment and its cellular interactions, but exact recapitulation of bone metastatic progression in immunosuppressed condition still remains difficult [27]. Similarly, currently used intra-cardiac and intra-osseous route of injection strategies in animal model can support detail functional characterization of bone marrow associated micro-environmental constituents and distant immunosuppressive metastatic niches; however they cannot efficiently rule out the basic process of metastasis associated dissemination [28]. In this critical context, multi-parametric liquid biopsy based invasive approaches may represent an ideal platform for revealing mechanistic complexities of epithelial mesenchymal transition (EMT), dissemination associated early events, micro-metastasis, oligometastasis and stem cell associated characteristic features in prostate cancer [29,30]. Through liquid biopsy based real time assessment of circulating tumor cells (CTC), disseminated tumor cells (DTC), extracellular vesicles (EVs), circulating tumor DNA (ct DNA) and circulating tumor RNA (ct RNA) in conjunction with single cell RNA analysis can provide an excellent opportunity for detail molecular and immunophenotypic characterization of patient specific immunosuppressive tumor immune microenvironment and distant metastatic sites [29,30].

It has been currently estimated that nearly 1% of the studies involving conventional immunotherapeutic approaches are based on bone metastatic CRPC associated clinical trials [12]. For these vital reason, future pre-clinical studies are largely needed to maximally explore the immunotherapeutic landscape of bone metastatic CRPCs in point of several bone metastasis related key events like metastatic dormancy, vicious cycle, osteoclastic bone resorption, osteolysis, osteogenic growth, prostate tumor-bone endothelial cell interaction and bone remodeling [27,28]. Apart from patient centric normalization of immunotherapeutic strategies, there also exists other potential research areas which can profoundly impacts efficiencies during personalized immunotherapeutic interventions and immunosuppression based targeting therapies. The concerned areas are patient centric metastasis specific response profiling during the course of immunotherapy, dynamic modeling of anti-prostate tumor specific immune responses and identification of key clinical determinants along with the mechanistic frame working for development of immunosuppression based personalized treatment regime [18,29-32]. As a matter of fact, liquid biopsy based precision medicine platform can only provide the necessary logistic supports for dynamic monitoring of patient specific immune responses through real time assessment of CTC count in several different contexts including genomic alterations, prostate tumor methylation, immunophenotyping, clonal selection, heterogeneity and disease status profiling [29,30].

## Predictive biomarker

Now a day's predictive biomarkers are considered as one of the most essential classical tool to effectively guide personalized therapeutic interventions in several major aspects of metastatic CRPCs including precise identification of patients tumor-immune interactions, better performances during clinical decision making processes, appropriate treatment selection and sequencing for a particular patient in a specific stage of the disease, greater assistance for personalized clinical trial designing and accurate profiling of treatment responses and resistance like incidents [23,26]. Development of predictive biomarker based treatment strategies in case of a complex heterogeneous disease like metastatic prostate cancer are of paramount importance for improvement in treatment efficacies through maximum exploration of current therapeutic landscape to critically determine which particular immunotherapy or therapeutic combination is preferentially

suitable for a specific patient. In agreement with this statement, many experts currently assumes that the substantial lack of proper predictive biomarker is largely responsible for observed lowest efficacies of immunotherapies in metastatic prostate cancer where clinical responses varies significantly from patient to patient [26,31,32]. In context of immunotherapy, this inter-patient variation of clinical responses are one of the unique characteristics of advanced prostate cancer which selectively arises due to functional inactivation for most of the components in 'prostate cancer-immunity cycle' and induction of local immunosuppressive mechanisms [7,18,24]. On the other hand, metastasis related key features like EMT, stemness, disease burden and the phenomenon of bone metastatic colonization strongly modulates genesis of patient specific immune imbalance in prostate cancer through modulation of anti-tumor immune response generation and immunosuppression [13,14,16-18]. In this context, oncoproteins like PRC1, IL-6, and GAL3 have been identified as key regulator and potential predictors for prostate cancer metastatic advancement. Most importantly, all of these metastatic regulators are intrinsically associated with prostate cancer immunosuppression, stem cell associated features and disease burden. Precise future application of this prostate cancer metastatic biomarker in connection with immunotherapeutic perspectives may provide a new dimension for identification of patient specific effective strategies to improve treatment efficacies [14,16,22]. To potentially explore these future promising areas in cancer precision medicine, a further detail investigation is urgently needed to accurately assess the predictive abilities of these metastatic biomarkers in response of prostate cancer targeting immunotherapeutic approaches and proper validation of their clinical utilities. Similar types of studies are also essentially needed for other cancer metastatic regulators like P1NP, PRDX4, LPC1 and OPG/RANKL, which can significantly predict bone metastatic recurrence in prostate cancer [33].

Recently, a number of predictive biomarkers in response of immune checkpoint inhibition therapy have been identified in a subsets metastatic CRPC patients and mainly includes tumor mutational burdens (TMB), microsatellite instabilities, mismatch repair defects, biallelic loss mediated functional inactivation of CDK12 and expression levels of PD-L1 checkpoint [9,34,35]. Majorities of these genomically unbalanced subtypes of metastatic prostate cancer harboring a specific genomic instabilities or mutation are inherently immunogenic in nature and particularly susceptible for immune checkpoint blockade therapies [9,32]. For an instance, metastatic castration resistant prostate tumor with either microsatellite instabilities or mismatch repair defects are critically characterized by an elevation in the number of tumor infiltrating lymphocytes along with an increase in immunosuppressive PD-L1 expression on their membrane andcan serve as a potential biomarker for PD-L1 checkpoint [36,37]. Several recent clinical trial based on metastatic CRPC patients treated with immune checkpoint inhibitor Nivolumab or Pembrolizumab have shown a range of durable clinical responses in case of high microsatellite instabilities or mismatch repair deficiencies [36,38,39]. Similarly, tumor mutational burden (TMB) in conjunction with an assessment of immunosuppression based marker has been found to exhibit effective predicting abilities for selection of appropriate immune checkpoint based therapies in advanced prostate cancer [36,40]. In these major context, three potential immunosuppressive checkpoint molecules- CTLA-4, PD-1/PD-L1 and VISTA have demonstrated remarkable activities for prediction of immunotherapeutic responses in metastatic CRPCs against several checkpoint blocking therapeutic agents like-Ipilimumab, [18,36,41]. These immune checkpoint molecules are basically involved in the process of immunoediting, which critically regulates the mode of immunosuppression through activation of tumor infiltrating immunosuppressive cell populations and repression of effector T cell mediated prostate tumor targeting immune responses [18]. Among the different checkpoint molecules, PD-L1 functions as a dynamic biomarker in metastatic prostate cancer patients and intrinsically involved in several vital clinical aspects associated with late-stage complications including immunosuppression, immune resistance, lymph node metastasis and Enzalutamide treatment resistance [36,37,42]. Currently, PD-L1 can be considered as the hub of prostate cancer associated immunosuppression related events due to its complex involvement with different routes of prostate cancer immunosuppression networks ranging from its immune checkpoint activities, prostate cancer stem cell signaling, master-regulator STAT3 mediated rewiring of transcriptional regulatory circuitries and tumor micro environmental remodeling [14]. Biomarker guided therapeutic targeting of these immune checkpoints by either mono-therapy or combination based applications will significantly improves clinical response development and survival benefits in only a small fraction of metastatic prostate cancer patients. But, the majorities

of patient's remains mostly unresponsive to immune checkpoint blocking therapies due to redundant activations of checkpoints, diverse mode of immunosuppression and lack of necessary adaptive immune response development [9,25,36,42]. By considering these practical facts, additional measures are urgently required for development of multi-panel biomarkers for robust prediction of clinical responses and associated treatment toxicities across a range of currently available immunotherapeutic approaches and different contexts of patient specific immunosuppression [29,30,43].

## Clinical trial and patient selection criteria

For development of personalized immunotherapies and maximization of its treatment efficacies in advanced prostate cancer, identification of basic molecular framework for biomarker based patient stratification along with patient selection criteria enriched clinical trial designing is essentially required. The recently completed and currently undergoing prospective and retrospective clinical trials under different therapeutic platforms including immunotherapies have repeatedly suggested for the significant variation in clinical response rates and therapeutic efficiencies from patients to patients in metastatic CRPC setting [26,34]. In addition, multiple lines of evidences have clearly indicated for the role of immunosuppression and associated events like infiltration of immunoregulatory cells in acquisition of prostate cancer heterogeneities, metastatic complications and treatment failure [44,46]. To critically overcome these major challenges, further research priorities are largely needed in the area of immunosuppressive and predictive biomarker based clinical trial designing and their appropriate tailoring on account of patient's comorbidity, tumor recurrence, metastatic condition and treatment side effects [26,34,43-45]. As the number of immunosuppression based predictive biomarkers are gradually rising with the identification of novel immunosuppressive pathways in prostate cancer, the future of biomarker driven clinical trial will largely depend on the proper evaluation of multi-panel biomarker, validation of their corresponding multiplex assay systems and identification of adaptive strategies for development of platform trials [43-45].

In order to match right immunotherapeutic approaches for different clinical subtypes of metastatic CRPCs in association with biomarker based robust prediction of immunotherapeutic efficacies, several characteristic pathophysiological and genomic feature can be considered for patient selection- such as tumor intrinsic immunosuppressive mechanisms, infiltration levels of immunosuppressive cells, phenotyping of tumor immune microenvironment, microsatellite instabilities, status of PTEN tumor suppressor and tumor mutational burden [18,31,38-40,44,46,47]. But, the clinical efficiencies of immunotherapy based corresponding clinical response predictors are largely challenged by the low level of occurrences of corresponding genomic instabilities in metastatic CRPCs, such as microsatellite instabilities in only 3%, mismatch repair deficient comprises nearly 12% and tumor mutational burden varies between 0.5-10% of patient populations [38-40]. At the same time, it has been demonstrated in several studies that in setting of metastatic CRPCs, immunotherapies work much better with an exceptional clinical responses in only a number of few patients [9,18,48]. As a consequence, the trends in current clinical research strongly recommends for patients selection criteria enriched clinical trial designing in order to reach efficient endpoints, for optimization of proper doses and minimization of treatment associated toxicities [18,47]. For generalization and accurate drafting of data driven patient selection criteria in advanced prostate cancer related immunotherapies, different immune subtype based patient stratification may provide an additional advantage for efficient management of metastatic cases over other conventionally used approaches. Now a days, immune sub-typing of metastatic CRPC patients are mainly done through either immune gene signature based classification or fine phenotypic characterization of immunosuppressive mechanisms [18,44,31,49]. Interestingly, a gene expression based recent study by Jiawei Zhou et.al has introduced the concept of immunosuppression based immune sub-typing for classification of metastatic prostate cancer patient with a view to accurately predict treatment response and associated clinical prognosis [31,49]. According to the tumor infiltrating immune cell densities and route of immunosuppression, it has been possible to clinically meaningfully stratify advanced prostate cancer patients into three major immune subtypes- highly infiltrated cytolytic functioning, M2-TRAM accumulation and $T_{REG}$ abundance. In this immune phenotype based sub-typing, infiltration levels of M2-TAM have found to be critically linked with the worst clinical prognosis for metastatic CRPCs and highest levels of resistance for immune checkpoint blocking therapies [31]. In addition,

several other characteristic features of metastatic prostate tumor and its disease recurrence have been proposed as a predictor of therapeutic responses in metastatic CRPCs and include oncogenic and metastatic drivers, mutant proteins, inflammatory mediators, angiogenic regulators, extracellular matrix constituents, pro-tumorigenic growth factors, micro RNAs and oncogenic cytokines/chemokines [29,48]. All of these clinical response predictors should be systematically incorporated into the proposed molecular framework to identify the spectrum of patient selection criteria with a view to effectively guide biomarker based stratification of advanced prostate cancer, its personalized treatment selection and development of required adaptive strategies for designing of novel clinical trials.

## Conclusion

The issue of immunosuppression can be regarded as the most basic challenge for achieving clinical success in prostate cancer immunotherapies. Majorities of the intrinsic and extrinsic mechanisms of immunosuppression are deeply rooted in various aspects of prostate cancer metastatic advancement. The complex multifactorial interactions between prostate tumor, its pre-metastatic niches and the immune system plays the predominant modulatory role in governing the status of tumor infiltrating immune regulatory cell populations and formation of immunosuppressive networks. The future application of immunotherapies for personalized treatment of metastatic CRPC patients will significantly depends on accurate therapeutic translations of these immunosuppressive pathways for improving the performance of anti-tumor immune responses. But, majorities of pathophysiological events and corresponding oncogenic mediators related with prostate cancer bone metastasis remains pre-clinically and clinically uncharacterized in terms of currently approved immunotherapeutic approaches. An integrative research initiative is urgently needed to develop biomarker based effective immunotherapeutic strategies for patient specific interventions of these metastasis associated immunomodulatory events and corresponding drivers.

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## Declaration of Competing Interest

The authors declare no potential conflict of interest.

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