The Tumor Microenvironment and Immunotherapy in Prostate and Bladder Cancer

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KEYWORDS

• Immunotherapy • Prostate cancer • Bladder cancer • Immunoediting • Tumor microenvironment • Vaccines • Neoadjuvant treatment

KEY POINTS

- The immunoediting hypothesis posits that the immune system can either prevent or promote tumor development through 3 phases: elimination, equilibrium, and escape.
- Prostate cancer, a slow-growing cancer with a complex immunosuppressive tumor microenvironment, has responded poorly thus far to immune-related therapies. Understanding the prostate tumor microenvironment will be critical in the development of effective immunotherapies for prostate cancer.
- As compared with prostate cancer, bladder cancer has been successfully treated with immunotherapy, bladder cancer and its tumor microenvironment serve as useful models for improving our understanding of immunoediting and for testing novel immunotherapy approaches.
- Immunotherapy may be most effective in the neoadjuvant setting and/or in combination with other therapies rather than as a single therapy. Numerous studies are under way testing combination therapy approaches.

INTRODUCTION

Prostate cancer and bladder cancer, although both genitourinary cancers, mark opposite ends of the immunotherapy spectrum. Bladder cancer is an example of disease that has been successfully treated with immunotherapy and serves as an excellent model for understanding the immune system's complex role during cancer.[1–3] Conversely, prostate cancer is known to be a "cold" tumor, and has shown little success in responding to various immune-related treatments.[4–6] A greater understanding of the tumor microenvironment and of methods for harnessing the immune system to address tumor growth is needed to improve immunotherapies for both bladder and prostate cancer, as well as for cancer more generally.

Although bladder and prostate cancer are composed of immunologically distinct microenvironments, both possess tumor-promoting and tumor-suppressing cells. A significant challenge lies in maximizing the effects of tumor-suppressing cells while simultaneously knocking out tumor-promoting cells to prevent them from

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overwhelming the immune system. The prostate microenvironment defends against tumor-infiltrating cells and exhibits many immunosuppressive qualities contributing to its lack of response to immune checkpoint inhibitors and immunotherapy in general.[7,8] Bladder cancer exhibits an enriched immune gene expression pattern and enhancement of immune checkpoint genes in tumors and responds well to various immunotherapy regimens.

A better understanding of the tumor microenvironment (TME) is critical for the successful development of neoadjuvant immunotherapy approaches as these therapies target early cancer clones. Ideally, such an intervention can lead to a reduction in primary tumor size and prevent the development of low immunogenic cancer clones that can escape and metastasize. Targeting the TME in its earliest stages provides an opportunity to suppress malignancies before they become untreatable. At the same time, early-stage intervention will enable us to learn about the intricacies of these environments to develop more effective and targeted therapies.

Here, we provide a comprehensive overview of prostate and bladder cancer, including basic aspects of the disease and treatment, the elaborate cellular makeup of the prostate tumor microenvironment, and methods for exploiting various relevant pathways for the most effective treatment. First, we discuss the cancer immunoediting hypothesis and the complexities of host-tumor interaction, specifically the buildup of immunologically insensitive tumor variants that facilitate immune evasion and contribute to the growth of malignancies. We discuss the evolving therapeutic options for prostate and bladder cancer, focusing on some of the most promising research areas in both prostate and bladder cancer therapies, including immunotherapeutics. Finally, we highlight how intratumoral neoadjuvant immunotherapy approaches and combination modalities may improve immuno-oncological techniques.

THE 3 E'S OF IMMUNOEDITING

Research over the past decade has provided significant evidence of a critical role for the immune system in halting tumor growth.[9,10] Our understanding of immuno-oncology, based on preclinical animal models and human clinical trials, has revealed the immune system's inability to recognize tumor variants with reduced immunogenicity, resulting in successful immune evasion.[11] The more recent cancer immunoediting hypothesis suggests a dual role for the immune system, with the capability to protect against or promote cancer, specifically through modulation of the tumor microenvironment (TME), which plays a role both in tumor degradation and development.[12,13] The 3 E's, elimination, equilibrium, and escape, contribute to cancer immunoediting (**Fig. 1**)[12,14–17] Cancer immunoediting encompasses processes that promote the complete elimination of some tumors while enabling immunologic tolerance or indifference toward others.[15,18] Several abnormalities, including genetic mutations, chronic viral infections, and exposure to carcinogens, may induce the transformation of normal cells into cancer cells. In solid tumors, the transformation process triggers stromal changes in the tissue which alert the immune system to trigger elimination of transformed cells. Both innate and adaptive immune cells contribute to immunosurveillance with a major role for innate immune cells in cancer cell clearance. Transformed cells express cell-surface proteins that often serve as "eat me" signals for innate immune cells such as macrophages and dendritic cells (DCs). For example, calreticulin expressed on the cell surface of transformed cells aids in the phagocytosis of cancer cells.[19] Immunogenic cell death of tumor cells is accompanied by macrophage activation that releases proinflammatory cytokines such as interleukin (IL)-6, IL-1, tumor necrosis factor (TNF)-α, IL-12, which establish an antitumor environment. Activated macrophages also secrete chemokines such as CXCL9 and CXCL10, which recruit more immune cells to the tissue environment.[20]

Through phagocytosis, macrophages devour and eliminate cancer cells, whereas another class of innate immune cells, natural killer (NK) cells, directly perform tumor killing by secreting effector molecules such as perforin and granzyme B. NK cells also upregulate cell-intrinsic ligands such as FasL, or the TNF-related apoptosis-inducing ligand (TRAIL), which engage receptors on tumor cells and induce tumor cell apoptosis. On activation, NK cells also secrete cytokines such as interferon (IFN)-γ and TNFα that exert antitumor affects.[21] NKT cells and $\gamma\delta$ T cells can also induce antitumor activity by the recognition of specific ligands on tumor cells and subsequently trigger tumor cell killing.

Dying tumor cells also release damage-associated molecular patterns (DAMPs), which serve as "danger signals" and further activate innate immune responses. Recognition of DAMPs results in macrophage, dendritic cell, and NK-cell activation, release of antitumor cytokines by these cells, and efficient maturation and antigen presentation by DCs.[22] DCs engulf dying cells, participate in antigen processing, and cross present these

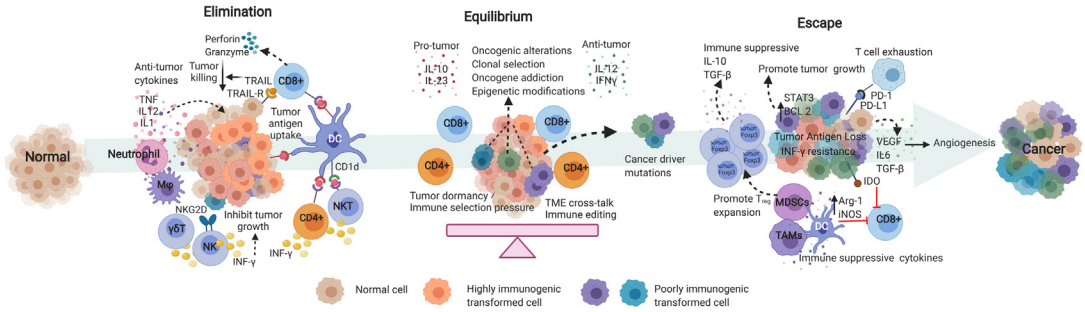

Fig. 1. The 3 E's of immunoediting. Cancer immunoediting comprises 3 processes called the 3 E's: elimination, equilibrium, and escape. The elimination phase results in the activation of the innate and adaptive immune response. Macrophages and dendritic cells orchestrate the innate immune response resulting in antitumor activity and release of cytokines such as TNF and ILs. NK and γδ T cells bind to the cancer cell via NKG2D ligands and release IFN-γ that inhibits tumor growth and causes tumor apoptosis. Tumor antigens released from dying cancer cells are recognized by dendritic cells that cross present these antigens to T cells, resulting in CD4+ T-cell activation, release of IFN- γ and CD8+ T-cell activation. Dendritic cells can also activate NK cells via the engagement of CD1d receptors. Activated CD8+ T cells inhibit tumor growth by releasing antitumor cytokines and direct cell killing by effector molecules such as perforin and granzyme B. CD8+ T cell also induce cytolytic activity by activating TRAIL-dependent apoptotic pathways. Specific cancer cells that escape elimination enter the equilibrium phase, the most prolonged phase of immunoediting. During the equilibrium phase, the adaptive immune cells are maintained in a state of dormancy. Both antitumor and pro-tumor cytokines compete in the tissue environment resulting in selection pressure on tumor cells. During this phase, tumor cells undergo oncogenic alterations, genomic modifications, and clonal selection. Specific cancer driver mutations enable cancer cells to escape immune evasion. In the escape phase, tumor cells become insensitive to proinflammatory cytokines such as IFN-γ. TAMs that are incapable of phagocytosis secrete immune-suppressive cytokines that allow the expansion of MDSCs and T_{regs}. These cells further amplify the immune-suppressive milieu that inhibits CD8+ T-cell function. Tumor cells express ligands that cause T-cell exhaustion, inhibit CD8+ T-cell function, and upregulate pathways that allow tumor growth. These processes allow angiogenesis that facilitates the dissemination of cancer cells, leading to primary and metastatic tumors. (*Data from* Refs.[12,14–17])

antigens to T cells, thus enabling adaptive immune activation. DCs can acquire tumor antigens by direct opsonization (tagging) of tumor cells and can migrate away from the tissue site to draining lymph nodes where they present these antigens to CD4+ and CD8+ T cells. DCs activate CD4+ T-helper cells, which produce IFN-γ, which then stimulate tumor-specific CD8+ T cells.[23–25]

Activated CD4+ and CD8+ T cells swarm the tumor site and dispose of the remaining antigen-bearing tumor cells.[26] Tumor-specific antigens presented by antigen-presenting cells (APCs) engage T-cell receptors (TCRs) on CD8+ T cells allowing their rapid proliferation and induction of cytolytic activity through cytotoxic granules like granzyme B. CD8+ T-cell activation is often considered a 3-signal process in which the first signal is generated by interactions between major histocompatibility complex class I (MHC-I) on APCs and TCR on T cells, and the second signal is provided by costimulatory molecules such as CD28 on T cells, which engage receptors like CD80 on the surface of APCs. Finally, cytokines such as IL12 and IFNα/β may serve as a third signal for maximum proliferation and stronger effector responses.

Cytokines and chemokines secreted by immune cells also function as chemoattractants to recruit additional immune cells at the tumor site. Infiltrating lymphocytes such as NKT, NK, or T cells recognize transformed cells and stimulate the production of IFN-γ[27–29] and antiproliferative,[30] apoptotic mechanisms[31] contributing to tumor death. Type I IFNs secreted via innate immune cells, as well as type II interferons released by T cells, are key cytokines in mediating antitumor effects. Where type I IFNs secreted by innate immune cells promote T-cell survival, DC activation, and chemokine secretion, type II IFNs such as IFN-γ play a critical role in cytolytic T-cell function, upregulation of MHC molecules, antigen recognition, and T-cell differentiation. IFN-γ receptor, JAK kinases, and IRF1 and STAT1 are key transcription factors indispensable for type II IFN signaling in T-cell function. In line with this process, deletion of key signaling molecules of interferon pathways such as STATs or IRFs results in enhanced disease burden in tumor mouse models. Although these mechanisms are in place to evade tumors, successful elimination is dependent on tumor type, tumor origin, and the nature of the tissue involved.

Certain tumor cells escape immune attack and enter the equilibrium phase, existing in tandem with the host immune system. In this phase, the tissue environment contains both pro-tumor IL-10, IL-23, and antitumor cytokines such as IL-12, TNF-α, and IFN-γ. Equilibrium is predominantly governed by CD8+ T cells, NK cells, and $\gamma\delta$ T cells, which maintain tumor dormancy. Although lymphocytes and IFN-γ exercise sufficient pressure to contain tumor cells, they are not able to fully remove lingering tumor cells and therefore cause tumor cells to become unstable and undergo rapid mutations. Through natural selection, many remaining variants are destroyed while new variants simultaneously develop, expressing novel mutations, allowing them to evade immune attack. Tumor cells during the equilibrium phase undergo genomic instability, epigenetic modifications, exhibit a defective DNA damage repair response, and evolve tumor clones that succeed in immune evasion. Mutating tumor cells during this phase are often referred to as tumor-sculpting immune "editors." Equilibrium is believed to be the longest of the 3 processes, perhaps occurring over many years.

The final stage, or escape, is characterized by loss of dormancy and an uncontrolled expansion of surviving tumor variants that have remained immunologically insensitive, thereby surviving earlier stages and ultimately leading to clinically significant malignant disease. This phase is characterized by the presence of immune-suppressive cells such as myeloid-derived suppressor cells (MDSCs), regulatory T cells (T_{regs}), M2s, or anti-inflammatory macrophages. MDSCs are undifferentiated, heterogeneous cells that proliferate quickly and become potent immune suppressors. They suppress T-cell activation by production of arginase, reactive oxygen species, or nitric oxide synthase. Besides immunologic functions, MDSCs promote tumor growth by contributing to cancer cell survival, metastasis, and angiogenesis. T_{regs} originate from CD4+ T cells that constitutively express CD25 and Foxp3 and inhibit T-cell function by expressing inhibitory molecules such as CTLA-4, PD-1, and TIM-3. These cells promote an immune-suppressive cytokine milieu comprising transforming growth factor (TGF)-β, vascular endothelial growth factor (VEGF), and IL-10 and allow the establishment of metastatic tumors.

An important component of immune evasion and tumor metastasis is the regulation and expression of cell adhesion molecules (CAMs) and their ligands.[32] Cell adhesion molecules mediate interactions with other cells and with the extracellular matrix (ECM) in the environment.[33,34] Malignant transformation disrupts cell adhesion expression and cell-surface glycosylation.[35]

A large contributing factor in determining whether cells will help promote or halt malignant growth is their metabolic needs and properties.[36] The diverse makeup of the tumor microenvironment (TME) can make it difficult for cancer cells to thrive, creating competition for access to nutrients and forcing cancer cells to undergo physical pressure, oxidative stress, hypoxia, and immune surveillance.[36] For example, when cancer-associated fibroblasts are activated and proliferate, they release growth factors and cytokines, as well as depositing ECM proteins.[37–39] Cytokines induce immune cells to release ECM remodeling factors, altering the architecture of the organ and hindering vascular activity, thus affecting the success of cancer cells.[40–42] Yet, various expression factors within the ECM contribute to tumor promotion. Overexpression of cytokines such as TNFα, a cytokine implicated in inflammation and various signaling pathways, contributes to tumor development.[43]

The theory of immunoediting represents an expansive concept of immunosurveillance that emphasizes the immune system's dual roles in both tumor-eliminating and tumor-promoting. Thorough analysis of immunoediting and of the tumor microenvironment can lead to better understanding of host-tumor interactions and to the development of more effective immune-related therapies to address these complex interactions.

PROSTATE CANCER DIAGNOSIS AND TREATMENT

Prostate cancer is the second most common malignancy in men worldwide and the fifth leading cause of death worldwide, with 1,276,106 new cases and 358,989 deaths in 2018 alone.[44] In the United States, estimated figures for 2020 suggest there will be 191,930 new cases of prostate cancer and 33,300 deaths from the disease.[45] Older age is associated with higher rates of incidence and mortality from prostate cancer, and African American men have higher incidence rates than Caucasian men, which is believed to result from a combination of socioeconomic, environmental, and genetic factors.[46–50] Modern diagnostic techniques for prostate cancer include the evaluation of prostate specific antigen (PSA) levels and multiparametric MRI with subsequent systematic and possible targeted prostate biopsy using transrectal ultrasound to obtain tissue samples that are then assigned a Gleason score, based on histologic patterns.

Combining the Gleason score, PSA level, and the clinical stage, clinicians stratify risk as low,

intermediate, or high. Depending on the patient, additional genetic testing assays on prostate biopsy tissue further help with risk stratification and treatment decisions by predicting oncological outcomes, including clinical progression, adverse pathology at prostatectomy, biochemical recurrence, and metastases.[51–53] Genetic assays such as the Decipher test, which assesses RNA expression of 22 known high-risk PCA biomarkers, can provide additional information on tumor indolence or aggression over and above the Gleason score. MRIs serve to add prognostic value through reporting and scoring of Prostate Imaging Reporting and Data System lesions and by clinical staging, as well as offering guidance for patients who may be in active surveillance programs and are not receiving radical treatment.[54]

The options for treatment differ based on risk stratification. When managing localized cancer, primary treatment options include active surveillance, surgery, or radiation. Active surveillance is an option for low-risk disease and involves monitoring the cancer for progression while avoiding the complications of definitive therapy. The patient undergoes PSA testing every 3 months, along with physical examinations, annual MRIs, and prostate biopsies every 2 to 3 years to ensure that the cancer has not progressed. If at any time patients develop more significant disease, they are offered definitive treatment.

Patients with intermediate and high-risk disease may be offered surgery or radiotherapy. Robotic radical prostatectomy has largely replaced open radical prostatectomy due to its minimally invasive approach with lower transfusion rates, shorter recovery time, and improved sexual and functional outcomes. Recent advances have led to the replacement of 3-dimensional conformal radiation therapy with intensity-modulated radiation therapy, which allows the radiation dose to be delivered more accurately to the prostate without affecting the surrounding normal tissues. Brachytherapy using implantation of radiation seeds, is a radiation treatment modality used alone or in combination with external beam radiotherapy as a “boost” for higher risk disease.

Focal therapy using high-intensity focused ultrasound (HIFU) or cryotherapy, are minimally invasive procedures that allow for precise targeting of the cancer as seen on MRI, sparing normal prostate tissue and reducing the complications of either surgery or radiotherapy. Focal therapies have been thoroughly investigated in clinical trials and treatment efficacy has been demonstrated.[55,56]

Prostate cancer cells have been shown to be androgen dependent, and chemical castration with androgen deprivation therapy (ADT) is indicated for localized disease in combination with radiotherapy. The treatment is administered for 6 months to 2 years along with, and after, radiotherapy, the duration depends on risk stratification at the time of diagnosis.[57,58]

For men with metastatic prostate cancer, ADT is the recommended first-line treatment, sometimes given orally, but more commonly by depot injection over 1 to 3 months. ADT is not without toxicity and established adverse effects include decreased bone mineral density, metabolic changes, sexual dysfunction, cardiac morbidity, and cognitive dysfunction.[59,60] To mitigate these effects, intermittent ADT has been investigated and may avoid some of the known adverse effects, including the restoration of sexual function.[61] Some patients may not respond to ADT initially or become unresponsive, leading to metastatic castration-resistant prostate cancer (mCRPC). When a patient becomes castration resistant, a number of therapies, including chemotherapies, novel cancer vaccines, or drugs targeting different aspects of the androgen pathway (abiraterone, enzalutamide, docetaxel[62–65]) are used as alternatives to slow disease progression, restore quality of life, and increase life expectancy.[66]

Sipuleucel-T is the first vaccine to be approved for prostate cancer in patients who are asymptomatic or minimally symptomatic with mCRPC, with clinical trials showing increased median survival by 4.1 months.[67,68] Another strategy for targeting prostate cancer unresponsive to ADT has focused on bone health, which is the site of most metastatic disease. Denosumab blocks receptors on the nuclear factor k-B ligand, preventing bone degradation, and has been shown to delay bone involvement in men with mCRPC by 3.6 months.[65] Radium works by specifically binding to bone-related metastasis and was shown to extend average survival by 3.6 months, in addition to delaying skeletal-related events by 5.8 months.[69]

ADT significantly abolishes immune tolerance to the prostate gland, suggesting that joint treatment of ADT and immunotherapy could be beneficial.[70] Clinical trials combining the CPI pembrolizumab (anti-PD-1) and ADT showed reduced PSA levels and a radiographic response.[71] Tumors often rely on the programmed death-1 (PD-1) pathway to ensure immune escape. Typically, PD-1 serves to prevent cytotoxic T cells from acting on normal cells in the body by binding to its ligand PD-L1. Tumor cells are able to exploit this process by expressing PD-L1 and binding to the PD-1–bearing T cells, thereby rendering these T cells ineffective at removing tumor cells.

To enable this type of immune resistance and ensure that T cells carry out their effector function on tumor cells, the PD-1/PD-L1 interaction needs to be blocked.[72] Given the presence of PD-L1 in prostate cancer, checkpoint blockade may be an effective treatment option, although response has been weak compared with other tumor types.[73] Moderate success with this approach led to further investigation of combination therapies testing anti-PD-L1 with various established therapeutic options.

Cytotoxic T-lymphocyte associated protein 4 (CTLA-4) is another prevalent immune checkpoint molecule restricted to CD4+ T cells[74] with immunosuppressive capabilities.[75] Anti-CTLA-4 antibodies can prevent blocking to coreceptors allowing for an immune-driven response against the growing tumor.[76] Monotherapy clinical trials with mCPRC cohorts demonstrated that anti-CTLA-4 exhibits insignificant effects on curbing prostate cancer.[77,78] However, in animal models treated with combination CTLA-4 with anti-PD-1, the observed response was sufficient to warrant anti-PD-1/CTLA-4 trials in humans.[79,80]

As research advances, increasing emphasis is being placed on precision or personalized medicine approaches. As some patients may have higher levels of immune-related genes, DNA repair genes, and androgen receptor (AR)-related genes, it will be critical to understand specific pathways and gene aberrations contributing to cancer, which will help clinicians select combinations of therapeutic strategies, including immunotherapies, that will most effectively match the individual's needs.[81,82]

HOT VERSUS COLD TUMORS

The immunologic classification of tumors as "hot" or "cold" was initially based on the presence of T cells within the tumor's center and in the tumor margins. Over the years, this system of classification has expanded to incorporate other parameters, such as the presence and activation of different tumor-infiltrating immune cells, expression of new tumor antigens, and mutation burden within cancer cells (**Fig. 2**).[83–85] Currently, hot tumors are classified as those with a high immunologic score based on increased infiltration of T cells, the increased activation of dendritic cells and macrophages, increased MHC-I expression, and the presence of neo-tumor antigens.[83] An efficient antitumor response triggered by the immune system is reliant on the combined expression of these factors.

In hot tumors, on immunologic cell death, highly mutating cancer cells express new antigens that are recognized by APCs such as dendritic cells. APCs cross present these antigens to T cells within the tumor site, as well as to T cells present in the lymph nodes. APC-dependent T-cell activation allows T-cell migration and infiltration to the tumor site where these cells identify specific antigen-bearing cancer cells and induce cell killing that feeds forward into the release of both neoantigens and ligands that further activate an innate and adaptive immune response.[84,86]

In contrast, cold tumors have low T-cell infiltration, impaired dendritic cell activation, presence of immune-suppressive cell types such as MDSCs, T_{regs}, tumor-associated macrophages (TAMs), and the immune-suppressive cytokines produced by these cells, such as VEGF, TGF-β, IL-10, and others.[86] Tumor-associated macrophages are alternatively activated by M2 macrophages that produce low proinflammatory cytokines, exhibit poor phagocytic activity, and low antigen processing. MDSCs and T_{regs} inhibit T-cell activation by sequestering amino acids such as cysteine and cytokines IL-2, IL-15, and IL-7 that are necessary for optimal T-cell function. In addition, tumor cells within a cold tumor also express reduced MHC-I and tumor antigen expression that increases poor immune recognition of these tumors.[87]

Prostate cancer is a slow-growing cancer as compared with other types of malignancies, and immunotherapy in this setting has shown only modest results. Although the precise reason remains unclear, a number of hypotheses have been proposed. The prostate tumor microenvironment is incompatible with tumor-infiltrating cells meant to fight against growing tumors, which makes immunotherapies much less effective.[88] Prostate cancer is also known to have a low tumor mutation burden and lack of T-cell presence that contributes to the immunosuppressive nature of the prostate TME, accounting for a lack of response to immune CPIs.[89]

In addition to the lack of T cells, a number of other issues contribute to the inability to mount an adaptive immune response in prostate cancer. Lack of tumor antigens makes it challenging to identify specific targets to bind and remove. Reduced tumor antigens also cause failure of T-cell priming and activation by dendritic cells. Low production of DAMPs contributes to reduced dendritic cell activation and production of immunosuppressive chemokines and cytokines like TGF-β that contribute to absence of T cells around the tumor.[84]

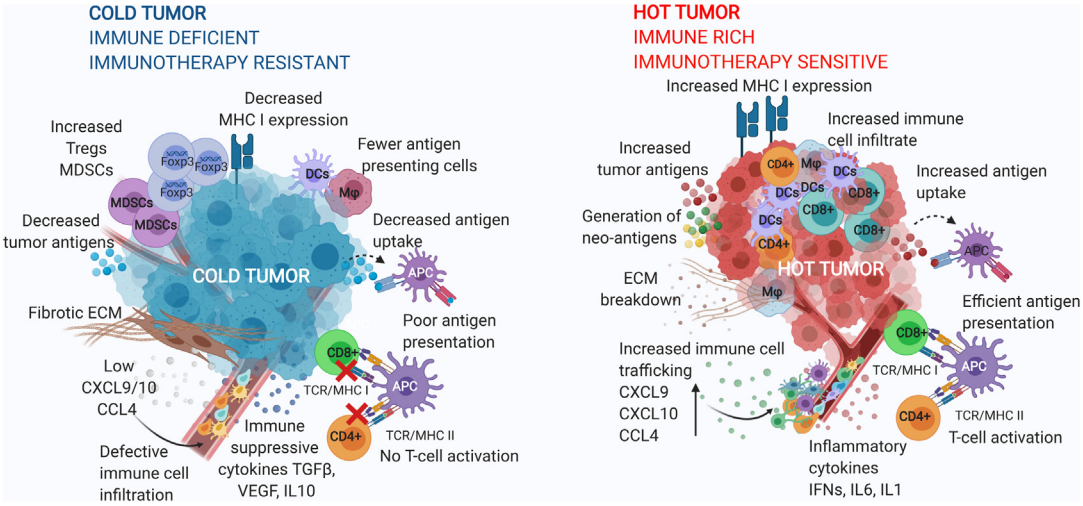

Fig. 2. Hot and cold tumors. Hot tumors are rich in infiltrating immune cells and sensitive to immunotherapy treatments. Tumor cells in a hot tumor present with increased MHC-I expression, increased tumor antigen, and neoantigens' release on cell death. APCs such as dendritic cells present tumor antigens to CD4+ T cells via MCH II/TCR and CD8+ T cells via MCH I/TCR interactions that result in the release of inflammatory cytokines such as IFN-γ and tumor cell death. The breakdown of the ECM surrounding the tumor releases ligands that trigger innate and adaptive immune responses. Activated immune cells secrete proinflammatory cytokines such as IFNs, IL-6, and IL-1 and chemokines such as CXCL9/10 and CCL4 that allow increased immune-cell homing and infiltration of the tumor bed. Cold tumors have low or no immunogenicity and decreased expression of MHC-I, resulting in poor antigen presentation by APCs, ultimately leading to impaired T-cell activation and defective immune-cell infiltration. Cold tumors are enriched with immune-suppressive cells such as regulatory T cells (T_{regs}), and MDSCs that enhance immune suppression by releasing cytokines, such as TGF-β, IL-10, and VEGF. The ECM within these tumor sites is fibrotic, contributing to immune-cell deficiency and resistance to immunotherapy treatments in these tumor types. (*Data from* Refs.[81,82,84])

Various strategies are used to address these challenges to transform cold tumors into hot ones.[83,90] Cancer genomics can assist in the isolation of specific mutations, particularly in heterogeneous tumors. Identification of cancer stem cells in combination with cell-type–specific immunotherapy can be beneficial in heterogeneous tumors with a focus on cell populations not typically looked at in prostate cancer.[7]

PROSTATE CANCER AND THE TUMOR MICROENVIRONMENT

Stromal-Epithelial and Growth Factor Interactions During Cancer Progression

The tumor microenvironment is composed of malignant cells, fibroblasts, inflammatory cells, and a noncellular matrix composed of collagen, elastin, and glycoproteins[80,91] (**Fig. 3**A). The nonepithelial components of tissue or stroma are more responsive to anticancer therapies due to their more genetically stable makeup.[80] Within the prostate, the stroma is made up of smooth muscle cells that engage the epithelium to modulate proliferation, differentiation, and migration of epithelial cells, maintaining homeostasis.[92,93] Smooth muscle-epithelial interactions maintain normal prostate structure and function.

Genetic damage to the epithelium disrupts signaling in the epithelium, which is passed on to the smooth muscle. Aberrations within various signaling pathways contribute to carcinogenesis, as the equilibrium between the epithelium and smooth muscle has been disrupted leading to a loss of differentiation and uncontrolled progression of tumor cells.[92,94] Fibroblast growth factors (FGF) coordinate communication between the epithelium and stroma in the prostate.[95] FGF-related stromal-epithelial receptors are potentially involved in prostate cancer establishment and progression due to their altered expression leading to proliferation, blocked apoptosis, and angiogenesis.[96] Targeting FGF receptors and signaling would not only aim at removing tumor cells directly but also be beneficial in preventing angiogenesis via cytokines such as TGF-β, an antiproliferative and pro-apoptotic cytokine.

Typically, TGF-β inhibits proliferation, prompts apoptosis, and assists in prostatic epithelial cell differentiation.[97] Despite the high levels of TGF-β found in the tumor microenvironment[98] due to a

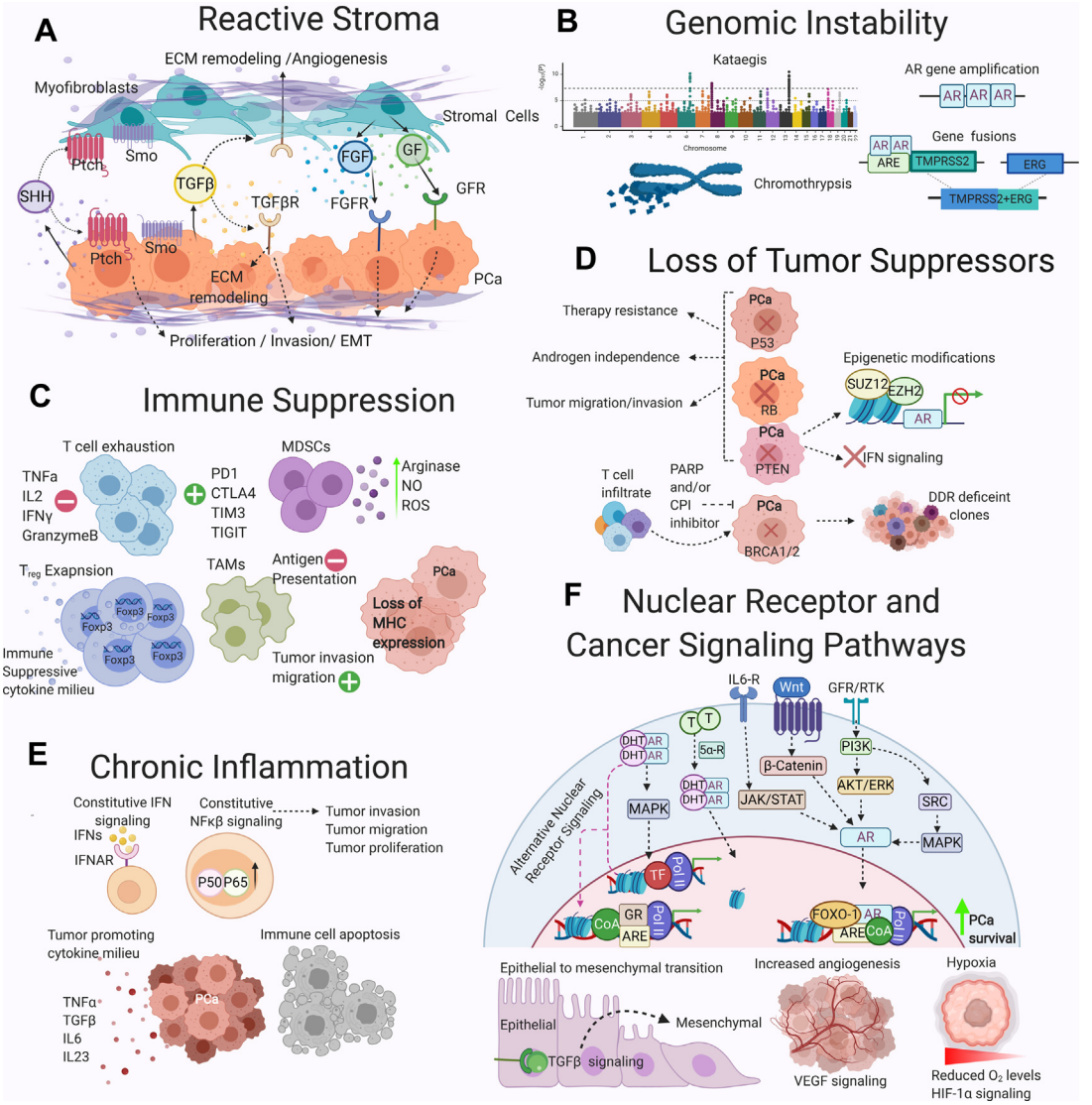

Fig. 3. Hallmarks of prostate cancer and tumor microenvironment. (*A*) Reactive stroma: Cancer-associated stromal cells secrete several growth factors like FGFs that bind to their cognate receptor on prostate cancer cells. Altered FGFs allow myofibroblast expansion that leads to extracellular remodeling and angiogenesis. Specific cytokines like TGFβ and SHH secreted from cancer cells trigger TGF-β and sonic hedgehog signaling via Ptch and Smo proteins to impact both stromal and cancer cells. TGF-β signaling contributes to extracellular remodeling, angiogenesis, and other pathways such as GFR, FGFR and SHH, cause cancer cell proliferation, invasion, and EMT. (*B*) Genomic instability: Complex structural genomic rearrangements resulting in gene fusions, chromothripsis, kataegis, and AR amplification are often observed in prostate cancer. (*C*) Immune suppression: The prostate tumor microenvironment comprises immune-suppressive cells such as MDSCs, T_{regs}, that secrete immune-suppressive cytokines and molecules such as NO, ROS, and arginase. The TME also contains exhausted T cells that release reduced proinflammatory cytokines and upregulate cell-surface proteins such as PD-1, CTLA-4, TIGIT, TIM-3. Dysfunctional macrophages present in the tumor TME are poor antigen presenters that do not activate adaptive immune responses effectively. Prostate cancer cells also express low tumor antigens and reduced MHC expression causing the suboptimal induction of immune responses. Taken together, these events allow immune suppression, which causes increased tumor invasion and migration. (*D*) Loss of tumor suppressors: PTEN, P53 or RB, gene deletions in prostate cancer cells results in aggressive phenotype, androgen independence, and therapeutic resistance. Loss of tumor suppressors drives lineage plasticity and results in metastatic clones that are resistant to anti-androgens. Epigenetic regulators like EZH2 and SUZ12 that mediate repressor histone modification of chromatin play a key role in this process. DDR deficiencies including BRCA1/2 are prevalent in prostate cancer and contribute to cancers with invasive phenotype. Tumors with DDR deficiency respond to PARP or CPIs by increasing

combination of decreased TGF-β receptor expression, along with other influences,[99–105] the inhibitory effects of TGF-β are reduced. Only when interacting with stromal cells does TGF-β adopt a tumor promoting role.[101,106] TGF-β plays an important role in epithelial differentiation in cancer-associated stroma,[107] contributing to the modeling and maintenance of the tumor stroma.[108] As a tumor promoter, TGF-β induces angiogenesis and suppresses immune surveillance mechanisms.[91] Androgen resistance may be associated with TGF-β[109] and serves as a mediator of bone metastasis.[110] Due to its significant role in tumor progression at various stages, targeting TGF-β is largely dependent on the cancer stage.[111,112]

A hallmark feature of malignant epithelial neoplasms, a precursor to invasive carcinomas, is a reaction in which cancer cells invade the stroma transforming the microenvironment into one that will assist and enhance tumor development.[80] Tumor cells within the stroma activate fibroblasts, recruit inflammatory cells, transform the ECM, enhance angiogenesis, and release ECM-bound growth factors.[113] As the stromal reaction progresses and the number of myofibroblasts increases, the Gleason score rises.[114] Stromal reaction is an indicator of adverse clinicopathologic parameters and recurrence.[115,116] A recent study further demonstrates how a stromogenic environment drives prostate cancer progression.[117]

Normal-behaving cells of the stroma are reprogrammed to aid cancer cells, which now rely on each other for survival.[118] This dependent loop depends on both cancer cells and the microenvironment to adapt to evade immune-infiltrating cells.[119] Strategies for evasion include tumor cell alteration of the expression and presentation of tumor-associated antigens by MHC-I proteins and transformation of macrophages to immune-suppressive phenotypes.[120–125] Decreased expression of MHC-I expression correlates with more aggressive tumor phenotypes.[126–128] This downregulation is significant because it leads to further impairment of T-cell capability in identifying and removing tumor cells, leading to the tolerance of cancerous cells, further contributing to successful immune evasion.

Androgen Receptor Signaling in the Tumor Microenvironment

AR, a nuclear receptor transcription factor, mediates cellular signaling events critical for prostate development during embryonic and fetal stages and is required for optimal function and the physiologic homeostasis of the adult prostate. The binding of the ligand dihydrotestosterone (DHT) triggers AR signaling, which results in dimerization and nuclear localization of AR, where it binds specific DNA sequences within the genome (**Fig. 3**F). In conjunction with nuclear receptor co-activators, chromatin and histone remodelers, RNA polymerase II, and mediator proteins, AR controls the expression of target genes vital for prostate physiology. Because of its vital role, the aberrant deregulation of AR signaling results in oncogenic events.

Interestingly, AR signaling, which is predominantly mediated by ligand binding, also can occur in a ligand-independent manner, a strategy co-opted by cancer cells to override the need for androgens during ADT. Ligand-independent AR signaling, also called nongenomic AR signaling,

T-cell immune infiltration. (*E*) Chronic inflammation: Chronic inflammation is characterized by immune-cell apoptosis, release of tumor-promoting cytokines such as IL-23, TGF-β, IL-6 secreted by prostate cancer cells and constitutive activation of immune pathways, such as IFN and NFκB signaling pathways, that add to immune-cell exhaustion and allow for tumor promotion. (*F*) Nuclear receptor and cancer signaling pathways: The AR signaling pathway plays a crucial role in prostate cancer cells. Ligand (hormone)-dependent canonical AR signaling plays a central role in prostate physiology. Circulating testosterone (T) is converted to DHT by 5AR. DHT binds AR and results in dimerization and nuclear translocation of AR. In the nucleus, AR binds to the ARE element, followed by the recruitment of co-regulators like FOXO-1 and RNA Polymerase II to the genome's AR binding region and transcription of AR-regulated genes. Several other cellular pathways can augment AR signaling in a ligand-dependent or independent manner; for example, IL-6 receptor signaling via JAK-STAT, Wnt- β Catenin signaling, growth factor receptor signaling through PI3K, AKT, and ERK, as well as PI3K signaling cascade via SRC and MAPK, all lead to translocation of AR from cytoplasm into the nucleus. Other nuclear receptor transcription factors like GR can bypass AR signaling and activate genes involved in cancer progression. EMT regulated by TGFβ, increased angiogenesis via VEGF signaling, and hypoxia regulated by HIF-1a are hallmarks of aggressive tumor cells that render the tumor microenvironment more conducive to cancer invasion and migration. (*Data from* [A] Karlou M, Tzelepi V, Efstathiou E. Therapeutic targeting of the prostate cancer microenvironment. Nat Rev Urol. Sep 2010;7(9):494-509. https://doi.org/10.1038/nrurol.2010.134 And [F] Lonergan PE, Tindall DJ. Androgen receptor signaling in prostate cancer development and progression. J Carcinog. 2011;10:20. https://doi.org/10.4103/1477-3163.83937.)

is mediated by crosstalk with cytoplasmic kinases, including MAPK, PI3-K, AKT, and SRC.[129–131] This crosstalk often results in posttranslational modification of AR followed by cooperative interactions with nuclear transcription factors and co-activators for successful transcription of AR targets. Ligand-independent AR signaling is often seen in advanced metastatic castrate-resistant prostate cancer (mCRPC) and is associated with the emergence of AR variants, including AR-V7, which lacks the ligand-binding domain.[80,132–135] Multiple ligands and signaling pathways, including (TGF β1), Nuclear Factor Kappa B (NFκB), WNT-β-catenin, insulin-like growth factor, epidermal growth factor, and interleukins can cooperate with AR signaling.

This crosstalk results in the right balance of AR signaling in the normal prostate. However, during oncogenesis, one or more of these pathways are constitutively activated and hijacked by cancer cells that facilitate sustained growth and cancer cell maintenance.[129,132,136,137] Emerging studies indicate that prostate cancer cells override the need for androgen and AR signaling by using other nuclear receptor transcription factors like the glucocorticoid receptor (GR). Several lines of evidence demonstrate common transcriptional targets for AR and GR, and the upregulation of GR signaling has been associated with aggressive prostate cancer.[138–140]

Androgen signaling is crucial to epithelial crosstalk during both benign prostate growth and throughout the initiation and development of malignancies. Whereas epithelial growth in normal prostate relies on androgen signaling, tumor development and CRPC are not disrupted by lack of stromal AR.[141] Although stromal androgen signaling promotes tumor growth, patients with prostate cancer tend to have lower levels of AR present in the stroma. Nevertheless, androgen signaling is still implicated in metastases and is believed to be involved in the bone microenvironment during metastasis.[142]

Many therapies targeting AR focus on pre-receptor and post-receptor AR regulation in an attempt to thwart resistance. Hedgehog signaling (Hh), a major network that coordinates cell-cell communication in developing organisms and, specifically within the prostate, mediates differentiation and homeostasis within stromal-epithelial interactions. The high correlation between Hh activity and tumor aggressiveness provides a useful biomarker to distinguish between patients requiring immediate intervention versus those who can rely on monitoring. Therapies focusing on Hedgehog signaling aim to inhibit Hh in an effort to slow down or stop tumor progression.

Integrins and Kinases

Src, a proto-oncogene involved in the androgen-prompted propagation of tumor cells, likely aids in cell sustainability without relying on androgen.[95] In tumor cells, Src works with proangiogenic factors (VEGF and IL-8) to increase vascular permeability and neovascularization in the tumor mass.[143] Src family kinase signaling pathways are involved in guiding primary prostate epithelial cell migration and might be involved in bone metastasis.[144] Src inhibitors are particularly attractive therapeutic targets as they affect both tumor and host cells. Blocking Src from functioning in the host cells can prevent expression of pro-angiogenic factors that aid developing tumors, eliminating a main source of vasculature necessary for the tumor to thrive.

Integrins, a family of transmembrane receptors, facilitate the attachment of epithelial cells to the basement membrane.[145] Prostate glands express integrins only on the basal epithelial cells, except in prostatic carcinoma where these cells disappear, allowing integrin expression to adapt with disease progression. The presence of integrins is accompanied by angiogenesis and overall tumor growth both locally and at distant sites.[145] Disease progression impacts the adoption of immune evasion through multilayered cellular alterations reaching a point where cancer cells are able to neutralize immune and stromal components, forming an immunosuppressive tumor microenvironment.[146] To circumvent this phenotypic heterogeneity, which develops rapidly as the disease progresses, therapeutic intervention would likely be most beneficial in the early stages.

Chronic Inflammation, Loss of Tumor Suppressors, and Genomic Alterations in Prostate Cancer

Pro-tumorigenic immunoediting begins at the onset of immune infiltration to primary prostate tumors.[147,148] Chronic inflammation is often detected, along with cancerous prostate tissue, creating implications for immunotherapy strategies (**Fig. 3**E). The prostate tumor microenvironment is a prime locus for inflammation given its immune inflammatory cell population, including TAMs, tumor-infiltrating T cells, mesenchymal stem cells (MSCs), MDSCs, and cancer-associated fibroblasts, among others.[149] Molecules such as TNF, IL-6, IL-7, IL-2, IL-15, and IL-17 also inhabit the TME, contributing to inflammation. Prostate cancer cells themselves act as mediators of inflammation through their release of various stimulating factors such as colony stimulating factor 1 (CSF-1), granulocyte macrophage

colony stimulating factor receptor (GM-CSFR), and CCL2, which recruit immune cells.

Chemokines are crucial for promoting and sustaining inflammation by coordinating the movement of immune cells and thus can either help to defend against the tumor and inflammation or can promote inflammation, contributing to an immunosuppressive environment.

Chemokines are released by innate immune cells, such as dendritic cells, mast cells, and macrophages, and serve to recruit neutrophils and monocytes (effector innate cells) to the site of inflammation, which in turn release their own chemokines.

TAMs are a predominant population within the TME and are often recruited by chemokines, including CCL2, CCL3, CCL4, CCL5, and many others149 (**Fig. 3**C). Depending on the accompanying mediators, TAMs can either aid in tumor growth or be associated with good prognosis. TAMs, for example, have been found to activate CCL2, an inflammation-promoting chemokine that induces STAT3-mediated epithelial-to-mesenchymal transition (EMT). Activation of the EMT has been thought to play a key role in affecting the function of immune cells in the tumor microenvironment, contributing to immunosuppression and immunoresistance.[150] TAMs have confirmed pro-tumorigenic effects that can be used as a predictive marker of clinical outcome in patients with prostate cancer.[151–153] Although they have not been equated with clinical prognosis, immunosuppressive immune cells, such as T_{regs}[154,155] and MDSCs[156–158] in prostate cancer tissue, indicate advanced disease. B cells, although not as commonly looked at in prostate cancer, are also important to progression, specifically in the castration-resistant setting.[159–161]

MSCs are characterized by their ability to differentiate into cells of varying lineage, including osteoblasts, chondrocytes, adipocytes, fibroblasts, and others. These cells are typically found at the sites of inflammation, often corresponding with tumor regions. CXCR4 and its corresponding ligand CXCL12 are often found in conjunction with MSCs and are responsible for activating major cellular signaling pathways like RAS-MAPK, PI3K-AKT-mTOP, JAK-STAT, and PLC.[162] The CXCR4 axis has antiapoptotic effects and promotes immunosuppression via recruitment of dendritic cells with defective tumor-associated antigen presentation capabilities.

The IL family of cytokines, along with TNF, TGFβ, and interferons, is a key driver of chronic inflammation in the TME.[163] The sustained activation of cytokines such as IL-6 and TNF results in reactive oxygen species (ROS) production[164,165] which further contributes to immune suppression and tissue damage. In prostate cancer cells, ROS production results in DNA damage and genomic rearrangements causing the recurrent fusion between TMPRSS2-ERG genes.[165] Besides ROS production, IL-6 also contributes to cancer migration, invasion, and the development of castration-resistant prostate cancer.[166]

Complex and catastrophic structural DNA rearrangements resulting in deletions of tumor suppressors, amplification, copy number alterations, translocation, and gene fusions are often observed in prostate cancer and result in genomic instability[167–170] (**Fig. 3**B). Development of gene fusions like TMPRSS2-ERG contribute to the development and progression of prostate cancer. Gene fusions are typically categorized as either within the ETS gene family (accounting for 50% of fusions associated with prostate cancer) or as non-ETS fusions. Although rare, non-ETS genes can be crucial drivers of tumorigenesis. Structural rearrangements can be traced to genomic instability resulting from transcription, specifically androgen-stimulated transcription, which is associated with ETS fusion and predisposition for TMPRSS2-ERG fusion.[167] TMPRSS2-ERG fusion tends to be associated with poorer outcomes, although further research is necessary to elucidate the biology behind the detrimental effects of structural rearrangements as they correlate with clinical outcomes.

In prostate cancer, genomic rearrangements also result in amplification of oncogenes like MYC and AR, and deletion of tumor suppressors like PTEN, RB1, TP53, and NKNX3.1.[167] Alterations in these genes are also associated with the adverse biology of both castration-sensitive and castration-resistant prostate cancer. The cumulative loss of PTEN, RB1, and TP53 results in genomic instability and differentiation of CRPC to a lethal variant with neuroendocrine phenotype CRPC-NE (**Fig. 3**D). Cooperative alterations in PTEN, RB1, and TP53 result in lineage plasticity or transdifferentiation, which may drive therapeutic resistance and prostate cancer metastasis. Studies using novel genetically engineered mouse models demonstrate that functional PTEN, TP53, and RB1 status are critical genetic determinants of therapeutic resistance and metastasis (see **Fig. 3**D). Co-inactivation of PTEN/TP53 or PTEN/RB1 or PTEN/RB1/TP53 results in prostate tumors with molecular features that promote aggressive phenotypes such as human mCRPC. These studies also highlight the critical roles of histone-modifying enzymes EZH2, DOT1L, DNMT1, and DNMT3A in the epigenetic regulation of target genes and

SOX2, a driver of lineage plasticity and stemness.[171–176]

Although prostate cancer has low mutation burden, it is prone to genomic instabilities such as chromothripsis and kataegis (see Fig. 3B). Chromothripsis, defined as abnormal breakages and rearrangements of chromosomal fragments within cancer genomes, is often accompanied by kataegis in which several mutations occur in clusters within a smaller region of the genome. These mutations are mostly dinucleotide substitutions (C > T and C > G) catalyzed by the APOBEC3A/B family of enzymes. Several cellular alterations contribute to chromothripsis and kataegis, including loss of P53, aberrant cell-cycle progression, the failure of DNA repair programs, and the shortening and fusion of telomeres.[177,178] Nearly 30% to 45% of genomes of patients with prostate cancer exhibit massive chromothripsis and kataegis.[179,180] Chromothryptic events are also enriched in a subgroup of metastatic castration-resistant prostate cancers.[181]

Certain prostate cancer types such as TMPRSS2 gene (ETS+) tumors present with "chromoplexy," which is thought to be a chained event of intrachromosomal gene rearrangements spanning over several chromosomes, as opposed to chromothripsis, in which genomic rearrangements occur only in 1 or 2 chromosomes.[182] Bioinformatic analysis reveals that chromoplexy transcriptionally dysregulates several key genes simultaneously impacting DNA damage responses and cell-cycle regulation and generating tumor-promoting fusion events.[183] Chromothripsis and chromoplexy in prostate cancer cells are hypothesized to be a single clonal event, playing a role during tumor initiation rather than tumor promotion.[184]

Kataegis, on the other hand, is thought to be the diversification of a clone into clonal subsets harboring regions of hypermutations. These mutations may allow a specific clone to expand during later stages of cancer progression and increase the risk of relapse in high-risk patients presenting with polyclonal tumors.[185] Chromothripsis and kataegis are important hallmarks of prostate cancer cells that may aid in patient risk stratification and biomarker discovery, revealing important therapeutic targets. However, further genomic and molecular studies are required to explore these possibilities.

DNA Repair Mechanisms

Germline and somatic alterations in DNA damage and repair (DDR) genes are associated with prostate cancer and contribute to genomic instability.[186–188] Aberrations in DDR genes, including the Breast Cancer 1 and 2 (BRCA1 and BRCA2) genes, are prevalent in both localized and castration-resistant prostate cancer[189,190](see Fig. 3D). These tumor suppressor genes are involved in DNA repair processes, such as homologous recombination[191,192] and nonhomologous end-joining pathways. Mutations in the mismatch repair genes MutS homolog 2 and 6 (MSH2 and MSH6) are linked to prostate cancer.[193,194] Aberrations in DDR pathway results in a DNA repair deficient phenotype with an increased likelihood of developing aggressive disease. Although DDR defects are observed in localized primary prostate cancer, the alteration frequencies of DDR genes are higher mCPRC.[189,190,195] Prostate cancer with DDR defects and lethal phenotype can be targeted with Poly (ADP-ribose) polymerase (PARP) inhibitors.[196–198] Defects in the DDR pathway lead to an accumulation of neoantigens, which can, in turn, lead to a more potent response to CPIs.[199] Thus, a combination of PARP inhibitors with immune CPIs has the potential for even greater therapeutic response.[199] Additionally, preclinical models indicate that copious amounts of DNA damage contribute to activating stimulator of interferon genes, which release type I interferons, initiating a T-cell response against immunogenic tumors.[200] Isolating tumor types with DDR deficiencies that present with a high tumor mutation burden and neoantigens will allow for targeted selection of inhibitors that may be more effective.

Targeting DDR genes in primary prostate cancer tumors is challenging due to their limited detection rate, as well as the complex molecular mechanisms underlying their biology. The difficulty in targeting DDR pathways includes their extensive variation influenced by AR signaling, epigenetic changes, and transcriptional and post-translational regulation. Lack of significant biomarkers and panels to classify the molecular subtypes of the cancer in early stages, along with their high prevalence in castrate-resistant prostate cancer, has focused treatment during metastatic settings, with little improvement in targeting DDR deficiencies in primary tumors.

Recent sequencing tools, panels, and biomarkers that use biopsy tissue[201] and circulating cell-free DNA[202] make capturing the subset of patients in earlier stages more plausible. In the primary setting, the tumor is still local while also being at the height of increased mutational burden, allowing for a greater number of treatment options. The tumor may be more receptive to various combinations of approaches, especially CPIs, as it has yet to establish immune tolerance. Rethinking DDR targeted strategies in

combination with other treatments may suggest new possibilities for helping patients with therapies that not only improve their quality of life but prolong it.

BLADDER CANCER DIAGNOSIS AND TREATMENT

In contract to the poor response of prostate cancer to immunotherapy, bladder cancer has shown more success, making it a useful model for advancing the field of genitourinary cancers. Bladder cancer is the ninth most common cancer and ranks 13th in cancer-related deaths.[203] Men are at higher risk than women, and disease incidence increases with age, with half of all the cases attributed to smoking. Tobacco is the number 1 risk factor for bladder cancer.[204]

Bladder cancer typically develops within the epithelium (urothelium) with urothelial carcinomas representing most bladder cancer diagnoses.[205] These are typically classified as either non–muscle-invasive (NMIBC) or muscle-invasive bladder cancer (MIBC). NMIBC is restricted to the inner layer of cells (the transitional epithelium). Conversely, invasion of the detrusor muscle characterizes MIBC, which differs in tumor biology, has a worse prognosis and, as a result, is managed differently.[206] MIBC typically presents as either a luminal or basal tumor. Luminal tumors are characterized by their papillary histology and expression of differentiation markers like E-cadherin, FGFR3, and other early cell-cycle genes. Basal tumors contain markers of the basal layer of the urothelium and show squamous cell differentiation.[207]

The diagnosis of bladder cancer commonly results from the observation of hematuria or blood in the urine.[208] A cystoscopy and biopsy or transurethral resection of bladder tumor is diagnostic[209] and pathologic examination of tissue that includes detrusor muscle confirms whether the disease is muscle or non–muscle invasive. Urine cytology is also used during diagnosis, although its sensitivity is most effective for those patients with high-grade disease. Imaging of the upper urinary tract plays a minor role in actual diagnosis but is important in evaluating the upper tracts for the purpose of hematuria screening.[210,211]

The management of bladder cancer is dependent on its histopathology (NMIBC or MIBC)[210,211] and risk stratification. For NMIBC, risk stratification helps determine whether the treatment should be cystoscopic surveillance, intravesical chemotherapy or potentially early cystectomy, whereas MIBCs definitely require surgical removal of the bladder, potentially with neoadjuvant or adjuvant chemotherapy, depending on disease staging.[212,213] For NMIBC patients in whom there is no muscle tissue in the pathologic specimen at initial biopsy or trans urethral resection of bladder tumor (TURBT), a redo TURBT is done at 4 to 6 weeks to prevent more invasive disease being missed.

Patients with intermediate and high-risk disease NMIBC undergo a course of intravesical Bacillus Calmette-Guerin (BCG), which has shown success in preventing NMIBC tumor recurrence or progression to MIBC.[214,215] For patients who fail BCG therapy, options include radical cystectomy or novel clinical trials.[216] Radical cystectomy is the gold standard treatment for MIBC, and as mentioned previously may also be performed on NMIBC patients in whom clinical assessment suggest progression to MIBC is inevitable. As a part of radical cystectomy, pelvic lymphadenectomy is performed, as there is evidence that this strategy improves long-term survival. Chemotherapy before surgery (neoadjuvant therapy) is an option for selected patients with locally advanced disease who are able to withstand the toxic side effects[217,218] and it is also the only standard treatment for those with metastatic bladder cancer.[219] Checkpoint-based immunotherapies have also shown promising results in this setting.[220]

THE BLADDER TUMOR MICROENVIRONMENT

The bladder tumor microenvironment comprises immune cells, mesenchymal cells, endothelial cells, ECM molecules, and inflammatory mediators. The interaction between these various components and tumor cells contributes to the regulation of bladder cancer progression, as well as to treatment response. Immune gene expression patterns are enriched in bladder cancer,[221–223] with tumor-infiltrating lymphocytes (TILs) serving as useful predictors of a patient's response to immuno-oncological treatments.[224]

Within NMIBC, the tumor microenvironment is characterized by immune cells (macrophages, dendritic cells, mast cells, neutrophils, and lymphocytes), cytokines such as TNF and interleukins that serve to support the stroma and tumor areas. TAMs function in aiding tumor development and angiogenesis, guiding cell invasion and muting an adaptive immune response.[223,225] Within NMIBC, TAMs tend to localize in the stroma tumor margin with increased infiltration in more aggressive tumors, correlate with cancer recurrence and are thought to reduce patient response to BCG.[226–229]

T_{regs} localize in the stroma irrespective of tumor aggressiveness[226] and serve as an indicative

marker for relapse post-BCG therapy.[226,227] The absence of NK cells renders BCG ineffective, suggesting that NK cells are key for successful BCG therapy.[230] Various mechanisms of the bladder microbiome modulate the bladder microenvironment.[231] Bacterial strains reduce inflammation by blocking key pathways, potentially affecting immunotherapies that depend on inflammatory factors.[232] Oncolytic viruses, which target tumor cell lysis and trigger an immune response, are commonly used to treat NMIBC and have shown success in altering the TME immune landscape to eradicate growing tumors through activation of both the innate and adaptive immune systems.[233]

As in prostate cancer, combination strategies are necessary when addressing these complex microenvironments. Combining CPIs, such as anti-PD-1 therapies, with BCG has shown success as result of the infiltration of CD8+ T cells and suppression of MDSCs.[234] The IL-15 super agonist is currently being tested in clinical trials and appears to functionally activate and multiply NK and T cells.[235] Used in combination with BCG, BCG-induced immunity will likely exhibit better response.[236]

Certain chemotherapy agents have broader effects in diminishing T_{regs},[237] leading to an upregulation of MHC-I expression, stimulating T-cell function, and simultaneously depleting MDSCs, recruiting type I interferons, and inducing immunogenic cell death.[238] A combination of chemotherapy with BCG was more successful than treatment with a single agent.[239] BCGs clear out TAMs and T_{regs} while engaging NK cells, which provide an unobstructed path for chemotherapeutic agents to increase the presence of antitumoral cells and, in turn, strengthen the potency of BCG.

BLADDER CANCER AS A MODEL FOR SUCCESSFUL IMMUNOTHERAPY

Immunotherapy has demonstrated remarkable success in bladder cancer at various stages of the disease. BCG has become the standard of care for higher risk NMIBC with demonstrated efficacy in large cohorts of patients. Because bladder tumors exhibit an upregulation of CPIs, PD-L1/PD-1 and CTLA-4 inhibitors have also proven effective.[240] The many Food and Drug Administration (FDA)-approved PD-L1 checkpoint blockade immuno-therapeutics include Durvalumab, Nivoluma, and Avelumab. Ipilimumab is an anti-CTLA-4 antibody being tested in clinical trials both as a single treatment and in combination with other therapies.[3]

Autologous cell-based therapies are also showing promising results in clinical trials for bladder cancer.[1] In trials currently under way (https://clinicaltrials.gov/ct2/show/NCT04184232; https://clinicaltrials.gov/ct2/show/NCT02886897), growth factors/cytokines and/or antigens are applied to a patient's own immune cells ex vivo to induce differentiation into dendritic cells. These activated dendritic cells are then infused back into patients to achieve an antitumor response. T-cell subsets, such as central memory T cells (Tcm), have also been activated ex vivo and infused back into patients to achieve antitumor response. Autologous Tcm therapy is being tested in combination with standard first-line gemcitabine plus cisplatin chemotherapy to treat metastatic bladder urothelial carcinoma (https://clinicaltrials.gov/ct2/show/NCT03389438).

Another preclinical study reported efficacy for autologous T cells isolated from draining metinel nodes in patients with metastatic urinary bladder cancer. An increased CD4+/CD8+ ratio was maintained ex vivo by providing autologous tumor antigens and cytokines. These T cells showed in vitro tumor killing and were safely infused back into patients. Although results from a larger cohort and demonstration of long-term effects are required, the study shows success of autologous T-cell therapy in a metastatic setting.[241]

T cells also can be modified using cell transduction strategies to generate chimeric or transgenic cells that exhibit strong tumor-killing activities. The main strategies to modify T cells include genetically engineering T-cell receptors (transgenic TCRs) and creation of a chimeric antigen receptor (CAR). Transgenic TCRs are engineered to identify tumor antigens and target both extracellular and intracellular antigens that are processed via MHC. Several clinical trials (https://clinicaltrials.gov/ct2/show/NCT02869217; https://clinicaltrials.gov/ct2/show/NCT03132922) are ongoing for bladder cancer. These trials are using transgenic TCRs focused on common tumor antigens such as cancer-testis antigen MAGE-A4 (melanoma-associated antigen) and NY-ESO-1 (New York esophageal squamous cell carcinoma 1). Although broadly reactive tumor antigens are currently being used, engineering novel antigens specific to tumor type will allow enhanced specificity and reduced off-target toxicities.[242]

CARs are transmembrane proteins with an intracellular antigen-binding domain that can lead to antigen binding and T-cell activation.[243] Engineering of CAR-T cells is continually evolving to enable better engraftment, proliferation, and activation of antitumor response in patients. CAR-T cells are

engineered, as well, to enhance proinflammatory cytokines in the tumor microenvironment, recognize a broad variety of tumor antigens, and reduce off-target effects.[244] A clinical trial testing the safety and efficacy of CAR-T strategies in bladder cancer is currently recruiting patients (https://clinicaltrials.gov/ct2/show/NCT03185468).

Analogous to T cells, NK cells are also being engineered to deliver antitumor responses. CAR-NKs are thought to induce fewer off-target effects and to activate an increased immune response as compared with CAR-T cells. Most clinical trials using CAR-NKs are currently being tested in hematological tumors; however, in vitro studies using CAR-NK cells show efficient killing in bladder cancer lines.[245]

Overall, immunotherapies that have not been effective in other solid tumors have shown success in bladder cancer. Bladder cancer has a high mutation rate[246] and various staged cancers have been sequenced to assess the tumor mutation load[247] to develop neoantigen strategies that can enhance immune checkpoint blockade response. The anatomic location of the tissue contributes to well-controlled local therapy administration and the ability to sample urine to assess the systemic effects of therapies allows for better response monitoring. These characteristics make bladder cancer a useful model for the development and testing of novel treatments that can then be applied to other cancers. The clear mechanistic understanding and significant clinical response of bladder immunotherapy serve as a resource for the eventual broadening of such therapies to other cancer types.[2]

VACCINES: AN EMERGING STRATEGY TO INDUCE IMMUNE RESPONSE

An emerging strategy to address anticancer immunity is vaccination: injection of a foreign pathogen induces an antigen-specific immune response and immunologic memory. As with other immunotherapies, the use of vaccines in prostate cancer requires detailed understanding of the complex tumor environment, mutational load, and expression of tumor antigens.[248] In addition, prostate cancer immune escape mechanisms prevent the immune system from having an effective impact on tumor development, limiting the effect vaccines may have. Vaccine design for prostate cancer must consider various pathways and targets capable of maintaining ongoing tumor cell death and preventing immune escape. The vaccine should transform the tumor environment from anti-inflammatory to proinflammatory and initiate an influx of T-cell clones that recognize antigens building up an expansive T-cell repertoire.[249,250]

Effective vaccination design must incorporate 3 key steps. First, a response must be elicited via a conventional vaccination method.[248] Second, the response must be "shaped." For the vaccine to be efficacious, cytotoxic T lymphocytes (CTLs) must be primed with "help" signals. A lack of proper help signals contributes to immune exclusion phenotypes[251] and exhausted or dysfunctional T cells.[252–254] Finally, infiltration and removal of tumor cells must be properly executed via primed and activated T cells. Through these steps, T cells are adequately equipped to tailor their response to the evolving disease, leading to successful eradication of the tumor and establishment of immunologic memory to ensure protection against a future malignant invasion.

A number of cancer vaccine platforms with varying ability to create or shape a desired outcome have been developed. Prostate cancer vaccines belong to 1 of 4 categories, described briefly here.

Dendritic Cell Vaccines

Dendritic cells (DCs) provide antigen-presenting function, triggering a T-cell memory response and facilitating a naïve T-cell response to tumor-associated antigens (TAAs). DCs are highly immunogenic and control the presentation of TAAs but are expensive to manufacture and require leukapheresis, which is a complex process. Sipuleucel-T (FDA approved for mCRPC without visceral metastasis) and DCVAC/prostate cancer Sipuleucel-T administration involve the collection of peripheral DCs by leukapheresis which are then incubated with GM-CSF and PAP-fusion proteins before reintroduction into the patient to activate PAP-specific CTLs.[255] DCVAC/prostate cancer consists of autologous poly-dendritic cells activated by polyinosinic: polycytidylic protein then pulsed with a LNCaP, prostate cancer cell line to stimulate an active immune response to prostate cancer tumor antigens. Thus far, despite a documented immune response, survival outcomes for metastatic prostate cancer patients have been mixed. The results of the VIABLE trial in mCRPC assessing its combination with docetaxel and prednisolone are being awaited (https://clinicaltrials.gov/ct2/show/NCT02111577).

Peptide/Protein-Based Vaccines

A multitude of immunomodulatory changes in prostate cancer cells result in the dampening of antitumor immunity. Defects in T-cell signaling and MHC-I expression; an immunosuppressive microenvironment; impairment of NK-cell

signaling; and expansion of T_{reg} within tumors are significant tumor-driven immune tolerance mechanisms in prostate cancer. Interestingly, prostate cancer cells express neoantigens, along with frequently presented antigens, albeit at lower levels. Further, the expression and modification of neoantigens are progressively modulated, resulting in immune escape and tolerance of tumor antigens. Neoantigens are an invaluable and promising source for peptide-based cancer immunotherapy and, therefore, strategies to identify these neoantigens have garnered significant interest.

Cancer neoantigens arise from somatic mutations in the genome that affect the coding region and, consequently, alter the protein phenotype. Neoantigens are sought-after targets for peptide-based vaccines and adoptive T-cell engineering. Neoantigens are not attenuated by central and peripheral tolerance mechanisms and are better vaccine candidates when compared to TAAs or germline antigens. Sequencing-based analysis of patient mutanomes (neoantigens that result from somatic mutations in the genome) has enabled rapid elucidation of the unique features of a patient's cancer and vaccines targeting these neoantigens can elicit a stronger antitumor immune response.[256]

Generally, peptide vaccines are easy to manufacture and have low toxicity, but have shown, at best, moderate immunogenicity and are restricted to HLA subtypes.[255] In bladder cancer, a recent Phase 1 study assessed the combination of M-phase (MPHOSPH1) and DEP domain containing 1 (DEPDC1) peptide vaccines with intravesical BCG for the management of high-risk NMIBC. Results showed good immunogenicity with minimal toxicity, and oncological outcomes suggested that peptide vaccines may contribute to further reducing recurrence rates for NMIBC.[257] Peptide-based vaccines are also being explored for prostate cancer and are in various stages of development.[256]

Viral Vector Vaccines

Several viruses have been used as vectors for vaccines with the advantage that human immune systems are activated by viral stimuli with both effective and prolonged adaptive and innate responses. Viruses are specifically recognized by pattern recognition receptors that facilitate the actions of APCs. Their disadvantages include potential toxicity, infection, and reactivation of the immune response against the viral vector on re-dosing.[258,259] To counteract these effects, vaccination strategies have been developed in which different viral vectors or vector types linked to the same tumor antigen are used on second and subsequent injections. For example, PROSTVAC-VF/Tricom, based on the PSA antigen, starts with a vaccinia viral vector for priming, followed by boosters with a fowlpox viral vector.[259] An initial Phase 2 trial using this strategy in 125 men with mCRPC showed prolonged overall survival, but could not be reproduced in a Phase 3 trial[259,260] most likely due to the lack of PSA antigen-dependent T-cell response. Nevertheless, the vaccine holds promise, and to improve its efficacy, as with other cancer vaccines, combination therapies are under investigation. A recent study showed that PROSTVAC administered in patients with prostate cancer in a neoadjuvant setting before surgery induces T-cell infiltration into the prostate tumor microenvironment and generates a peripheral immune response post-vaccination.[261]

Gene-Based Vaccines

Gene-based vaccines use either RNA or DNA platforms, with the advantage that they are not HLA-type restricted, are inexpensive to produce, can be dosed repeatedly, can be used to present a diverse range of TAAs, and are known to induce both humoral and cellular immunity. Several delivery methods have been developed to improve their efficacy, including gene guns, micro-injection, and electroporation.[262] A number of phase 1 and 2 trials have investigated their clinical effectiveness. For example, pTVG-HP, plasmid DNA-encoding human PAP vaccine, demonstrated a PAP-specific T-cell response with prolonged PSA doubling time in patients who presented with localized prostate cancer and biochemical recurrence after surgery or radiotherapy.[263] More recently in a mouse model, vaccine pTVG-AR, a plasmid DNA vaccine encoding the androgen binding domain of the AR, demonstrated cellular-specific antigen response and improved survival in mice with induced prostate cancer.[264]

In Situ Vaccine

In situ vaccine (tumor site vaccine) is an alternative to conventional approaches (away from the tumor site) for vaccine delivery and has shown success in some cancers.[265–270] A broad variety of immune modulators are injected directly at the site of tumors; however, monitoring patient response can be challenging in this setting[271] and lack of sufficient signals to complement antigen release may prevent a full response from occurring.

A number of studies have explored strategies to improve clinical response to the various types of vaccines, including combination therapies. These approaches combine treatments with synergistic actions, for example, the use of CPIs in

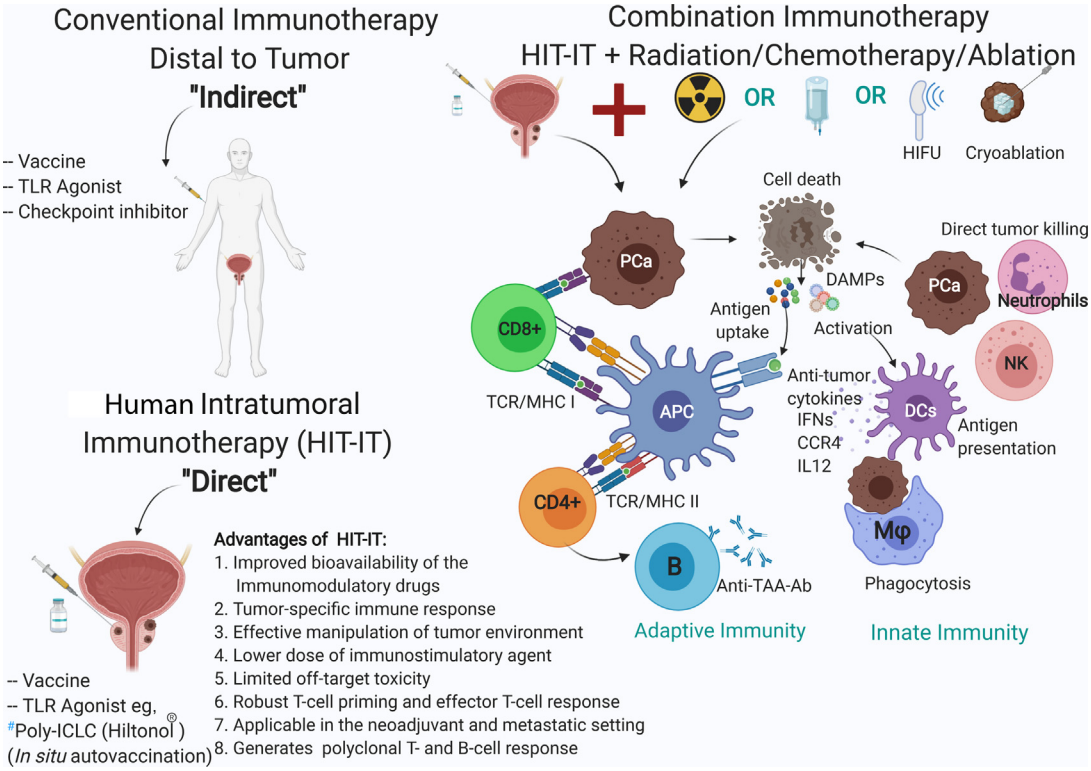

Fig. 4. Overview of conventional and HIT-IT immunotherapy focuses on the systemic delivery of immunomodulatory agents distal from the tumor site. As compared with conventional immunotherapy, HIT-IT is the direct delivery of immunomodulatory agents directly to the tumor site. The direct delivery activates innate and adaptive immune responses in the tumor microenvironment. A combination of HIT-IT with other treatment modalities, such as radiation, chemotherapy, HIFU, or cryoablation, amplifies immune responses. HIT-IT combined with other treatment strategies has the potential to increase therapeutic benefit. Combination immunotherapy enhances the function of APCs by increasing antigen uptake and efficient antigen presentation to T cells. It also induces immunogenic tumor cell death by enhancing tumor-killing properties of NK cells and neutrophils. Dying tumor cells release DAMPs that further activate innate immune responses by releasing antitumor cytokines and enhanced phagocytosis by macrophages and DCs. # Poly-ICLC (Hiltonol ®), Oncovir Inc, is being investigated in a Phase-1 dose-escalation study for prostate cancer in a neoadjuvant setting at the Icahn School of Medicine at The Mount Sinai Hospital (NCT03262103).

combination with vaccination to improve the effects of tumor antigen-specific T-cell responses induced by therapeutic vaccination.[259]

NEOADJUVANT AND HUMAN-INTRATUMORAL IMMUNOTHERAPY

Neoadjuvant immunotherapy refers to an adjunctive therapy to induce antitumor priming before surgery or radiation (Fig. 4). The neoadjuvant approach (before surgery) has been found to have improved efficacy over adjuvant immunotherapy (following surgery), providing an earlier and more effective treatment for "micrometastases," which are known to later cause recurrent disease.[272] Neoadjuvant immunotherapy may take the form of a vaccine, systemic immunotherapy, or human-intratumoral immunotherapy (HIT-IT), and has a number of potential therapeutic advantages.[273,274] Benefits may include local and systemic efficacy, reversal of immune escape resistance mechanisms, treatment of micrometastases and formal metastases, reduction of primary tumor size, improvement of symptoms, the use of pathologic response data as biomarkers for oncological outcomes, and, specifically for HIT-IT, drug escalation at the site of injection.[272,275]

Recent preclinical and clinical studies have demonstrated that neoadjuvant immunotherapy is more efficacious than adjuvant therapy. Higher tumor load before surgery or radiotherapy results in a more effective T-cell response.[272,276] One study using 2 mouse models of triple negative metastatic breast cancer, found neoadjuvant

immunotherapy (anti-PD-1 with or without anti-CD137 or antiCD25) was more effective than adjuvant therapy at reducing micro-metastasis and resulted in cure for a significant number of treated mice.[272] Similarly, a clinical study assessing neoadjuvant versus adjuvant immune CPIs (nivolumab and ipilimumab) for advanced but resectable melanoma, found that 80% and 90% of patients were free from long-term term disease progression and had improved overall survival, respectively, compared with 60% and 67%, respectively, in the adjuvant group.[261]

Applying the neoadjuvant approach in prostate cancer, the use of vaccines has been explored with a recent Phase 2 trial using PSA-targeted vaccination in 27 patients with localized prostate cancer (Gleason scores 6–9) before radical prostatectomy (RP).[277] The vaccine was well tolerated with no serious adverse effects or significant toxicities resulting from the vaccine itself. Comparing RP with preoperative biopsies, CD4+ T cell infiltrates were significantly increased in the surgical specimens, with an increase in CD4+ and CD8+ T cells at the surgical specimen tumor margin and core, respectively. Using 3 TAAs as markers, peripheral immune responses were noted in more than half the patients. A clinical assessment of oncological outcomes was limited by term follow-up (12–15 months), but at the time of publication only 4 patients demonstrated biochemical recurrence (defined as a PSA rise after definitive treatment).[277] A more detailed assessment of biochemical recurrence and longer follow-up may yet reveal more insight into the vaccine's effect on long-term disease progression and overall survival.

Neoadjuvant immunotherapy may also be given by direct human-intratumoral injection (HIT-IT), potentially gaining the benefits of a focused local and secondary peripheral polyclonal immune response, without systemic adverse effects and toxicities. HIT-IT remains in its infancy with only ~20 trials investigating HIT-IT of the more than 130 assessing neoadjuvant immunotherapies.[278] HIT-IT modalities include ex situ dendritic cell vaccines, which have shown promising results in high-grade sarcoma with more than half of patients demonstrating a T-cell response,[279] and immune-stimulating agents such as intratumoral gene therapy, immunostimulatory antibodies, and small molecule immune modulators.[278] Ex situ dendritic cell vaccines are, however, expensive and time consuming to produce. Immune-stimulating agents have the potential advantage of promoting a stronger local and systemic immune response. Examples of immune-stimulating agents include intratumoral injections of Trimix mRNA (mRNA coding for CD70, CD40, and Toll-like receptor 4) as gene therapy, intratumoral injections of antibodies to PD-1 and CTLA-4, and intratumoral injection of toll-like receptor (TLR) agonist compounds. Some of these have shown promising results in early trials but all require further investigation.[278]

A number of questions surrounding HIT-IT in prostate cancer persist, including pharmacokinetic and pharmacodynamic assessment of these therapies as they may translate to improved clinical outcomes[273]; whether to use multiple injection sites, including primary and metastatic site or the combined injections of compounds with different modalities of action; whether repeated injections are beneficial; and if concomitant surgical lymphadenectomy is required.[275]

Our group has initiated a novel intratumoral in situ strategy which uses the patient's tumor as the antigen source. We expect this approach to convert the prostate tumor into a favorable immune ecosystem and improve the antitumor immune response. We are currently testing this strategy in a Phase 1 dose-escalation study designed to define a safe dose and schedule of preoperative intratumoral (IT) plus intramuscular (IM) Polyinosinic-polycytidylic acid stabilized with polylysine and carboxymethylcellulose (poly-ICLC, Hiltonol) before radical prostatectomy for patients with intermediate and high-risk prostate cancer undergoing RP (https://clinicaltrials.gov/ct2/show/NCT03262103). Poly-ICLC is a synthetic agonist that activates TLR3, resulting in upregulation of innate immune genes that also activate adaptive immune responses. Poly-ICLC injections generate a broad immune response in humans that will aid generation of tumor suppressive immune milieu.[280]

Other future directions for neoadjuvant therapy in prostate cancer include combination strategies such as HIT-IT with CPIs to provide synergistic efficacy, or intratumoral TLR agonists combined with PD-1.[281,282] In immunologic "cold" tumors like prostate cancer, injection of TLR agonists facilitates the immune-cell response and may increase the effects of CPIs. A recent study examined the role of intratumoral injection of TLR 7/8 agonist MD19197 in human cell lines and primary cells, as well as in several syngeneic mouse tumor models, and found that the TLR agonist enhanced immune-cell responses. The antitumor immune-cell activity was dependent on the T-helper cell pathway, and also increased the action of CPIs.[281] Examples of neoadjuvant therapies for bladder and prostate cancer that are currently being investigated are presented in **Tables 1** and **2**, respectively.

Table 1
Trials in neoadjuvant immunotherapies for bladder cancer

Clinical trials.gov Identifier	Treatment	Combination	Official Title of Clinical Trial	Phase	Status (Updated as of Nov, 23, 2020)
https://clinicaltrials.gov/show/NCT03529890 [283]	Nivolumab (PD-1 checkpoint inhibitor)	Radiation therapy to the pelvis	A Prospective, Single Arm, Multicenter, Phase II-Trial to Assess Safety and Efficacy of Preoperative RAdiation Therapy Before Radical CystEctomy Combined with ImmunoTherapy in Locally Advanced Urothelial Carcinoma of the Bladder	2	Recruiting Estimated Completion Date August 2022
https://clinicaltrials.gov/show/NCT03520491[284]	Nivolumab (PD-1 checkpoint inhibitor)	With or without Ipilimumab (CTLA-4 checkpoint inhibitor)	A Pilot Study to Evaluate the Safety of Neoadjuvant Nivolumab Alone or in Combination with Ipilimumab for Cisplatin-Ineligible Patients With Muscle Invasive Bladder Cancer (CA209-9DJ)	Pilot	Recruiting Estimated Completion Date January2021
https://clinicaltrials.gov/show/NCT03472274[285]	Durvalumab and Tremelimumab	Standard of care (cisplatin-based chemotherapy) with or without combined PD-1 and CTLA4 checkpoint inhibitors	The DUTRENEO Trial: A Prospective Study to Individualize the Approach with Durvalumab (MEDI4736) and Tremelimumab in neoadjuvant bladder Cancer Patients	2	Recruiting Estimated Completion Date December2022

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Table 1
(*continued*)

Clinical trials.gov Identifier	Treatment	Combination	Official Title of Clinical Trial	Phase	Status (Updated as of Nov, 23, 2020)
https://clinicaltrials.gov/show/NCT04209114[286]	Nivolumab	With or without NKTR-214 (IL-2 pathway agonist)	A Phase 3, Randomized, Study of Neoadjuvant and Adjuvant Nivolumab Plus NKTR-214, vs Nivolumab Alone vs Standard of Care in Participants with Muscle-Invasive Bladder Cancer (MIBC) Who Are Cisplatin Ineligible	3	Recruiting Estimated Completion Date September 2024
https://clinicaltrials.gov/show/NCT03732677[287]	Durvalumab	With or without Gemcitabine/Cisplatin (GC) chemotherapy	A Phase III, Randomized, Open-Label, Multicenter, Global Study to Determine the Efficacy and Safety of Durvalumab in Combination with Gemcitabine + Cisplatin for Neoadjuvant Treatment Followed by Durvalumab Alone for Adjuvant Treatment in Patients With Muscle-Invasive Bladder Cancer	3	Recruiting Estimated Completion Date December 2025
https://clinicaltrials.gov/show/NCT04099589[288]	Toripalimab (PD-1 Inhibitor)	With and without Gemcitabine/Cisplatin (GC) Chemotherapy	Multicenter Phase II Study of Gemcitabine/Cisplatin (GC) Chemotherapy Combined With PD-1 Inhibitor (Toripalimab) in the Neoadjuvant Treatment of Upper Urinary and Muscular Invasive Bladder Urothelial Carcinoma	2	Recruiting Estimated Completion Date October 2022

https://clinicaltrials.gov/show/NCT04383743[289]	Pembrolizumab (PD-1 Inhibitor)	With and without aMVAC chemotherapy (methotrexate, vinblastine, doxorubicin and cisplatin)	Pembrolizumab and aMVAC Chemotherapy as Neoadjuvant Therapy in Non-Urothelial Histology Muscle-Invasive Bladder Cancer r: A Pilot Trial	Pilot	Recruiting Estimated Completion Date February 2023
https://clinicaltrials.gov/show/NCT03912818[290]	Durvalumab (PD-1 Inhibitor)	With 3 different chemotherapy regimens (Gemcitabine/Cisplatin or Gemcitabine/Carboplatin or Methotrexate/Vinblastine/Doxorubicin/Cisplatin)	Phase 2 Open-Label Study of Durvalumab With Neoadjuvant Chemotherapy in Variant Histology Bladder Cancer	2	Recruiting Estimated Completion Date August 2022
https://clinicaltrials.gov/show/NCT03387761[291]	Nivolumab and Ipilimumab	Different combined neoadjuvant dosing schedules	Phase 1B Study to Assess Safety and Efficacy of Neo-Adjuvant Bladder Urothelial Carcinoma COmbination-immunotherapy (NABUCCO)	IB	Recruiting Estimated Completion Date June 2021

Data from Refs.[283–291]. Information as of Nov 23, 2020.

Table 2
Trials in neoadjuvant immunotherapies for prostate cancer

Clinical trials.gov Identifier	Treatment	Combination	Official Title of Clinical Trial	Phase	Status (Updated as of Nov, 23, 2020)
https://clinicaltrials.gov/show/NCT00715104[292]	Sipuleucel-T	With and without booster	An Open-Label, Phase 2 Trial of Immunotherapy with Sipuleucel-T (Provenge®) as Neoadjuvant Treatment in Men With Localized Prostate Cancer	2	Completed in December 2013 with results available
https://clinicaltrials.gov/show/NCT01696877[293]	Degalerix	GVAX Low-dose Cyclophosphamide	A Neoadjuvant Immunologic Study of Androgen Deprivation Therapy Combined With a Granulocyte macrophage-colony Stimulating Factor F-secreting Allogeneic Prostate Cancer Vaccine and Low-dose Cyclophosphamide in Men With High-risk Localized Prostate Cancer Undergoing Radical Prostatectomy	1/2	Completed in December 2018 with results available
https://clinicaltrials.gov/show/NCT04009967[294]	Pembrolizumab	None	Phase II Clinical and Translational Study of Neoadjuvant Pembrolizumab Before Radical Prostatectomy in Non-metastatic Gleason $\geq$8 Prostate Cancer Patients Positive by 18FDG-PET Scanning (PICT-01)	2	Recruiting Estimated Completion Date November 2022

https://clinicaltrials.gov/show/NCT04301414[295]	Non-fucosylated Anti-CTLA-4 (BMS-986218)	Degarelix	A Pilot Study of Neoadjuvant Non-fucosylated Anti-CTLA-4 (BMS-986218) + Degarelix Acetate vs Degarelix Acetate Alone in Men with High-risk Localized Prostate Cancer	Pilot	Not Yet Recruiting Estimated Completion Date May 2023
https://clinicaltrials.gov/show/NCT04020094[296]	Atezolizumab	MVA-BN-Brachyury PROSTVAC	Perioperative Atezolizumab with MVA-BN-Brachyury and PROSTVAC For Intermediate-Risk And High-Risk Localized Prostate Cancer	2	Recruiting Estimated Completion Date March 2024
https://clinicaltrials.gov/show/NCT03262103[297]	Poly-ICLC, Hiltonol®	None	Phase I Study of In situ autologous vaccination against Prostate Cancer with intratumoral and systemic Hiltonol® (Poly-ICLC) prior to radical prostatectomy	1	Recruiting Estimated Completion Date May 2022
https://clinicaltrials.gov/show/NCT03821246[298]	Atezolizumab	Tocilizumab	An Open-Label Multicenter Phase II Study of Neoadjuvant Atezolizumab-Based Combination Therapy in Men with Localized Prostate Cancer Prior to Radical Prostatectomy	2	Recruiting Estimated Completion Date August 2022
https://clinicaltrials.gov/show/NCT02933255[299]	PROSTVAC	Nivolumab	Phase I/II Study of PROSTVAC in combination with Nivolumab in men with Prostate Cancer	1/2	Recruiting Estimated Completion Date August 2022

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Table 2
(*continued*)

Clinical trials.gov Identifier	Treatment	Combination	Official Title of Clinical Trial	Phase	Status (Updated as of Nov, 23, 2020)
https://clinicaltrials.gov/show/NCT02153918[261]	PROSTVAC-V/TRICOM	PROSTVAC-F/TRICOM boost	A Phase II Study of Neoadjuvant rFowlpox-PSA (L155)-TRICOM (Prostvac-F/TRICOM) in Combination With rVaccinia-PSA (L155)-TRICOM (Prostvac-V/TRICOM) in Men with Prostate Cancer Undergoing Treatment With Radical Prostatectomy	2	Recruiting Completed in January 2018 with results available

Data from Refs.[261,292–299]. Information as of Nov 23, 2020.

COMBINATION STRATEGIES TO IMPROVE THE EFFICACY OF HUMAN-INTRATUMORAL IMMUNOTHERAPY

A putative synergistic effect has been suggested for the combination of radiation and immunotherapeutic treatment with the potential for involvement of tumor cells, the tumor microenvironment and improved innate and adaptive immune response (see **Fig. 4**). The effect of radiation on cancer cells is an increase in tumor antigen exposure preventing immune escape mechanisms, modifying the TME to reduce its immunosuppressive function, and facilitating activation of immune response pathways by the release of DAMPs.[300,301] DAMPs activate several innate immune pathways, including inflammasomes. The inflammasome complex activates capsase-1, leading to the secretion of proinflammatory cytokines such as IL-8 and IL-1.[302,303] NLRP3, a well-known inflammasome[302] when removed in mice led to a noticeably reduced tumor burden, induced NK-cell infiltration, and increased CCL5 and CXCL9 chemokine production.[304]

Radiation also results in upregulation of membrane calreticulin, which promotes phagocytosis and T-cell priming,[305] downregulation of CD47, and upregulation of tumor MHC-I expression, enhancing the presentation of TAAs and the activation of effector cells.[305–307] By inducing cytokines in the tumor microenvironment, radiation has the ability to change TME cellular organization from immunosuppressive "cold" to immunostimulatory "hot," the latter with high T-cell infiltration and inflammatory phenotypes, which would be especially important in prostate cancer, given its nature as a cold tumor.[282]

Early clinical trials have demonstrated promising feasibility and efficacy in some cancers. For example, combining dendritic cell HIT-IT with localized fractionated radiotherapy for high-risk sarcoma[279] has proven efficacious. Similarly, a recent retrospective analysis studied more than 750 patients who received combined CPI and radiotherapy (RT) for metastatic solid tumors. Overall survival was increased in patients who received concurrent CPI and RT.[282] Because the effect of radiation occurs primarily during treatment, the most effective timing for combined RT/CPI regimes will be in the neoadjuvant (before surgery) and concurrent setting. Nevertheless, further studies are required to strengthen these results and address several questions regarding dose and timing of such combinatorial treatments. For instance, whether conventional RT dosing or hypofractionation that is, giving higher radiation doses per treatment but over a shorter time period is more effective. Similarly, whether regional nodal irradiation by influencing the immune specific local T-cell response has any effect if RT is combined with immunotherapy[275]. It is possible that different immunotherapies require specific RT regimes to harness the most effective therapeutic response[308]. The option of tailoring immunotherapies other than checkpoint blockade with RT has been suggested and some studies[308] have discussed the rationale for combining RT with CAR-T-cell therapies. These strategies are based on the ability of RT to increase MHC-I expression and TAA exposure since CAR-T cells are genetically engineered to recognize specific TAAs. In this context, the interaction between a given immunotherapy and RT is therefore highly individualized, and further studies may reveal pathway-specific synergies that may lead to precision combination therapies.

Similar to radiation, therapies like HIFU that can convert tumors from "immune deserts" to "T-cell inflamed" are also highly sought after as a basis for rational combination approaches.[309–311] Thermal energy generated during HIFU destroys the tissue at the site of delivery. Mechanical tissue damage results in the release of danger signals like DAMPs and activation of heat shock proteins that induce an immune response. Heat shock proteins like HSP60 function as molecular chaperones for exogenous antigens released during mechanical lysis. HSP60 chaperoned antigens can stimulate antigen presentation on dendritic cells, cytotoxic T-cell infiltration and tumor killing.[312] HIFU can induce immune changes to reset the tumor microenvironment and has the potential to stimulate a local and systemic immune response to eliminate tumor cells.[313–316] For these reasons, future explorations combining HIFU with immunotherapy hold significant promise with, further investigation needed.

SUMMARY

Bladder and prostate cancer differ significantly in their response to immunotherapy. Bladder cancer has shown genuine efficacy in response to various immunotherapies and has contributed to our understanding of the complex interaction of the immune system with cancer. Conversely, prostate cancer is a "cold" tumor and has yet to demonstrate significant response to immunotherapeutic approaches. The immunoediting hypothesis elucidates the host-tumor interaction and the delicate balance between tumor degradation and promotion by the critical processes of elimination, equilibrium, and escape.

It is clear that with the onset of cancer, different cell types take on new roles linked to gene regulation, resulting in either the prevention or facilitation of tumor growth. Cellular mutations alter signal transduction, the epigenome, and gene expression resulting in expansion of cancer cells. In addition, oncogenic alterations within cancer cells and in the tumor microenvironment (TME) evade immune responses and dictate the efficiency of immunotherapies.

Developing effective immunotherapies for prostate cancer will depend on greater understanding of the underlying mechanisms of immune-related pathways in tumors with the goal of developing combination therapies that target novel components within the tumor microenvironment.[274,317,318] Systematic analysis of the TME will lead to greater success using systemic oncolytic virotherapy,[319] improvement in tumor permeability to T-cell infiltration,[320] and improved preclinical animal models that accurately model human immune mechanisms.[321]

Current results from trials on potential immunotherapeutic targets suggest that immunotherapy for cancers shows promise for the future. Nevertheless, despite the large number of immunotherapies that have so far been tested either as single therapies or in combination with other treatments, success has yet to be achieved in prostate cancer. The number of trials for both neoadjuvant prostate and bladder cancer immunotherapies is increasing and as the scientific community gains experience, it is hoped that neoadjuvant options will contribute to a multimodal approach to cancer treatment and that the combinations of immunotherapies, surgery, and radiotherapy in both neoadjuvant and adjuvant settings will significantly prolong life for patients living with cancer. The current interest in, and determination to, find effective immunotherapy strategies for prostate cancer indicate that the field will continue to evolve, and that greater success will be achieved.

CLINICS CARE POINTS

- Improvements in our understanding of the differences in the TME for prostate and bladder cancer will hopefully catalyze the development of novel immunotherapeutics for urogenital cancers.
- Identifying tumor neoantigens and defining the role of genes related to DNA repair functions, reactive stroma, tumor immunology, and cancer-specific signaling pathways will contribute to the provision of “Precision Medicine,” which aims to provide precise, tailored treatment based on an individual’s unique tumor genetics.
- Bladder cancers of different stages have been sequenced to assess tumor mutation load in order to develop neoantigen strategies that can enhance immune checkpoint blockade response. Furthermore, administering intravesical therapy and sample urine to assess systemic effects allows for better response monitoring. These characteristics make bladder cancer a useful model for developing and testing novel treatments that can then be applied to other cancers.
- Exploring the use of vaccines and intratumoral therapy potentially combined with more traditional treatments such as radiotherapy, chemotherapy, or HIFU in both neoadjuvant and adjuvant settings may unlock novel therapeutic strategies that improve oncological outcomes and patient survival.

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AUTHOR CONTRIBUTIONS

S.S. Nair and A.K. Tewari conceived the idea, developed the conceptual framework, and supervised the project. R. Weil and Z. Dovey co-wrote the original first draft. S.S. Nair, R. Weil, Z. Dovey, and A.K. Tewari co-wrote, edited, and reviewed the article. S.S. Nair and A. Davis prepared figures and Z. Dovey prepared tables. All authors contributed to reviewing literature and provided critical feedback. All authors reviewed and approved the article. This article was screened using iThenticate software before submission.

COMPETING INTERESTS

The authors declare no competing interests.

DISCLOSURE

Dr A.K. Tewari has served as a site-PI on pharma/industry-sponsored clinical trials from Kite Pharma, Lumicell Inc, Dendreon, and Oncovir Inc. He has received research funding (grants) to his institution from DOD, NIH, Axogen, Intuitive Surgical, AMBFF, and other philanthropy. Dr A.K. Tewari has served as an unpaid consultant to

Roivant Biosciences and advisor to Promaxo. He owns equity in Promaxo. S.S. Nair, R. Weil, Z. Dovey, and A. Davis declare no conflicts.

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