Molecular Therapy
Oncolytics
Review

New insights and options into the mechanisms and effects of combined targeted therapy and immunotherapy in prostate cancer

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Chronic inflammation is believed to drive prostate carcinogenesis by producing reactive oxygen species or reactive nitrogen species to induce DNA damage. This effect might subsequently cause epigenetic and genomic alterations, leading to malignant transformation. Although established therapeutic advances have extended overall survival, tumors in patients with advanced prostate cancer are prone to metastasis, transformation into metastatic castration-resistant prostate cancer, and therapeutic resistance. The tumor microenvironment (TME) of prostate cancer is involved in carcinogenesis, invasion and drug resistance. A plethora of preclinical studies have focused on immune-based therapies. Understanding the intricate TME system in prostate cancer may hold much promise for developing novel therapies, designing combinational therapeutic strategies, and further overcoming resistance to established treatments to improve the lives of prostate cancer patients. In this review, we discuss nonimmune components and various immune cells within the TME and their putative roles during prostate cancer initiation, progression, and metastasis. We also outline the updated fundamental research focusing on therapeutic advances of targeted therapy as well as combinational options for prostate cancer.

INTRODUCTION

Prostate cancer is a leading cause of cancer morbidity and mortality in men worldwide, with approximately 1.3 million new cases annually.[1] Approximately 10 million men are currently diagnosed with prostate cancer, and approximately 7% of them have the metastatic subtype. Moreover, metastatic prostate cancer contributes to over 400,000 deaths every year.[2] The main risk factors for prostate cancer include age, ethnicity, genomic changes, infection, obesity, and diet.[3] The oncogenesis of prostate cancer is related to the complex interplay between these intrinsic and extrinsic factors. Chronic inflammation, frequently detected in preneoplastic prostates, has been implied to drive prostate carcinogenesis and progression.[4–8] The inflammatory effects may recruit diverse immune cells within the tumor microenvironment (TME) and in turn function in the inflammatory microenvironment.[9]

Cellular entities within the TME may have intriguing roles in prostate cancer development and progression. On the one hand, the TME contributes to immune remodeling and immune surveillance. On the other hand, the TME may induce tumor growth, metastasis, and invasion of immune surveillance.[10] Understanding the landscape of the TME and its biological underpinnings in both primary and metastatic prostate cancer is crucial to designing promising molecule-targeting methods and overcoming therapeutic resistance.

In this review, we first briefly describe possible causes of the initiation and development of prostate cancer, highlighting the potentially procarcinogenic role of both endogenous and exogenous factors during the development of chronic inflammation. We also describe how carcinogenesis-associated inflammation is related to the accumulation of multiple immune cells within the TME. Then, we describe the TME to provide a deep understanding of the immunobiology of prostate cancer. Subsequently, we thoroughly outline novel therapeutic approaches in prostate cancer, with an emphasis on the immunotherapies and clinical insights provided by associated research. However, the therapeutic efficiency of immunotherapy, especially immune checkpoint blockade (ICB) therapies, in prostate cancer patients remains limited and unsatisfactory. Resistance to ICB treatment is inevitable, and combination strategies to convert tumor cells from a "cold" immune state into a "hot" immune state are urgently needed. Finally, we summarize many agents from recent advances that can potentially be harnessed in combinational therapies for the treatment of prostate cancer.

INITIATION AND DEVELOPMENT OF PROSTATE CANCER

The tumorigenesis of prostate cancer is related to diverse interactions between inherent germline susceptibility loci, acquired somatic gene alterations, and microenvironmental and macroenvironmental factors.[11] Chronic inflammation is thought to promote the development of several types of solid cancers, with well-described examples including colon, stomach, and liver cancer. Although the clarified underlying mechanisms between inflammation and prostate cancer have yet to be defined, substantial data from genetic epidemiological and histopathological studies have suggested that chronic

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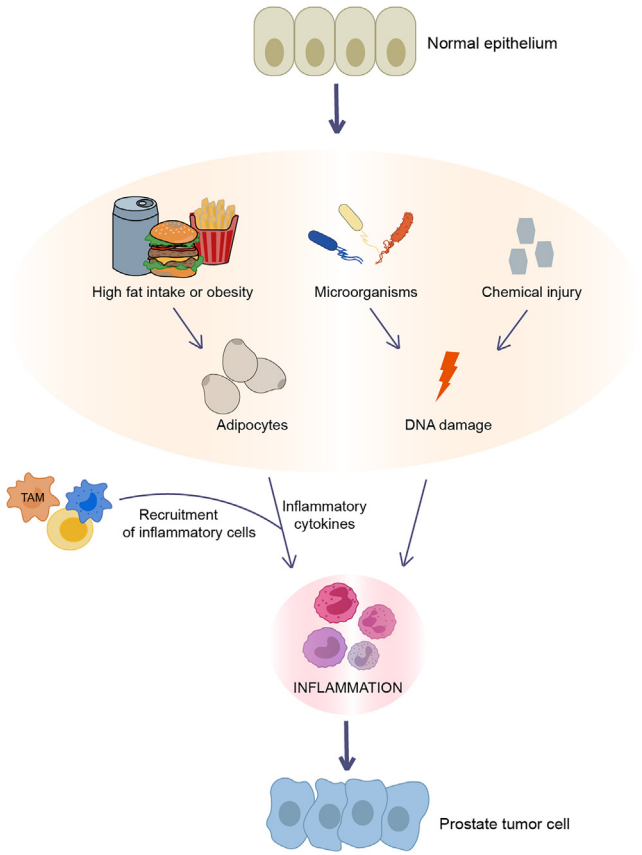

Figure 1. The normal prostatic epithelium is exposed to various putative factors that may cause inflammation in the microenvironment and thus promote prostate carcinogenesis and progression

Chemical injury caused by uric acid and microorganisms lead to epithelial injury and subsequent DNA damage by oxidative stress, leading to chronic inflammation and compensatory proliferation, indicating the formation of prostatic intraepithelial neoplasia. Dietary fat intake and obesity result to accumulation of adipocytes. Adipocytes secrete various proinflammatory cytokines, enhancing the recruitment and infiltration of inflammatory cells, such as TAMs and MDSCs within the TME that fuel the emergence and progression of prostate adenocarcinoma.

inflammation might initially contribute to prostate carcinogenesis (Figure 1).[12] A recent study of the molecular mechanisms described inflammation as a driver of somatic genome and epigenome alterations via oxidative stress induced by inflammatory cytokines.[9] Moreover, inflammation might induce the prostate carcinogenic process on the basis of its role in stimulating and transforming pre-malignant cells into cancer cells.[13] Putative factors of chronic inflammation implicated in the development of prostate cancer include microbial stressors, high fat intake or obesity, chemical injury, and physical trauma.[3] Notably, commensal microbiota have been proven to colonize the gland and are regarded as a pivotal component of the TME.[14] Recent evidence has implicated that the microbiome contributes to prostate carcinogenesis as well as tumor progression, and it may also influence the efficacy of antitumor immunotherapies.[15–17] Microorganisms in the prostate may possibly originate from the urinary tract and cause prostatic infection. Then, microbial infection results in prostatic injury, leading to damage to epithelial defenses and eventually resulting in chronic, persistent inflammation.[12] Another microbiome source may be the gastrointestinal (GI) microbiome. Emerging evidence suggests that metabolites and androgens produced by the GI) microbiome may enhance the development of prostate cancer.[18–20] Complete characterization of the relationship between potential microbiota-associated chronic inflammation might be critical for prostate cancer prevention.

Another major putative factor of chronic inflammation in the development of prostate cancer is high dietary fat intake and obesity. A myriad of mechanisms proposed that adipocytes surrounding the prostate gland can release chemokines or inflammatory cytokines to promote prostate cancer progression and migration,[21–23] which may explain the higher risk between obesity and prostate cancer and provide therapeutic targets. A preclinical study identified a key oncogenic *SREBP* in the lipogenic program. Aberrant sterol-regulatory element binding proteins (SREBPs) caused by a Western high-fat diet (HFD) was sufficient to promote prostate cancer growth and metastasis through lipid biosynthesis.[24] Another study demonstrated that HFD enhanced the oncogenic *MYC* transcriptional signature through histone methylation at the promotor regions of MYC-targeted genes, leading to tumor burden in a murine prostate cancer model.[25]

TME IN PROSTATE CANCER

Prostate cancer cells are located in a microenvironment that consists of endothelial cells, fibroblasts, mesenchymal stem cells, and a plethora of immune cells.[26] We discuss TME-associated nonimmune parts as well as immune parts including innate immune cells (macrophages, natural killer cells, and others), adaptive immune cells (T cells), and immune-suppressive cells (T_{reg} cells).

The immune landscape of the TME may differ among prostate cancer patients because of clinical heterogeneity. Recently, Chen et al. utilized single-cell analysis to identify an endothelial subset (activated endothelial cells) enriched in castration-resistant prostate cancer (CRPC) and associated with cancer cell invasion.[27] Basic and novel knowledge of the characteristics of the TME prompted us to assess potential treatment options. Below, we describe the major cellular components facilitating the emergence and progression of prostate cancer (Figure 2 and Table 1).

NONIMMUNE COMPONENTS

Cancer-associated fibroblasts

Fibroblasts, myofibroblasts and smooth muscle cells mainly contribute to secreting extracellular matrix (ECM) proteins such as collagen, fibronectin, and laminin, defining the biophysical properties of tissues and maintaining homeostasis between the ECM and cells.[26] Emerging evidence has shown that alteration of this homeostasis caused by tensile and compressive forces of proliferating cancer cells may enhance tumor growth.[28] Furthermore, this kind of force can activate fibroblasts and myofibroblasts to secrete specific enzymes

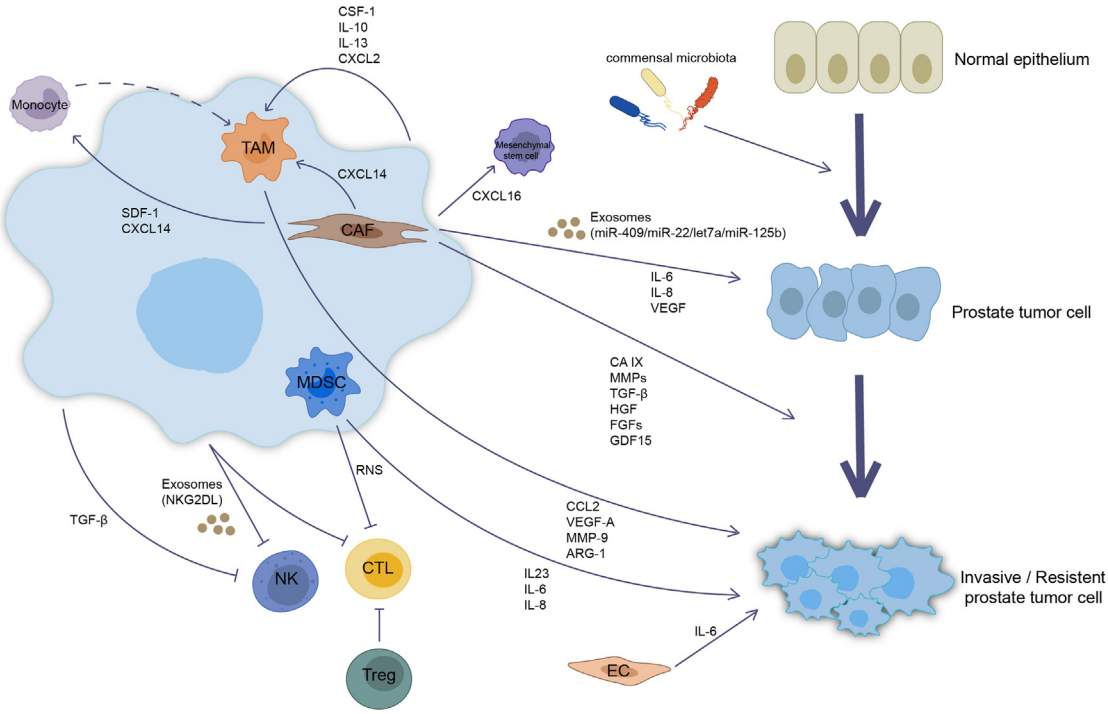

Figure 2. The crosstalk between the TME and prostate cancer cells during carcinogenesis, metastasis, and therapeutic resistance

Complicated interactions affect tumor growth and lead to resistance of the antitumor immune responses through fostering and modulating immune cells with suppressive properties. Commensal microbiota lead to an inflammatory environment via causing DNA damage to promote tumorigenesis. Exosomes, cytokines, and growth factors delivered by CAFs promote tumorigenesis, progression, and metastasis. Increased secreting of CXCL14 and SDF-1 by CAFs facilitates the infiltration of M2 macrophages as well as the transformation of monocytes toward M2-like macrophages. T_{reg} cells suppress CTLs to enhance immune tolerance. Additionally, the release of CSF-1, IL-10, IL-13, and CXCL2 by prostate cancer cells in the TME result in M2 macrophage polarization phenotype, accelerating tumor progression. Macrophages lead to drug resistance and tumor invasion by delivering cytokines, such as VEGF and ARG-1. Similarly, MDSCs and ECs also deliver cytokines like IL-6 to stimulate oncogenic pathways in prostate cancer cells and induce proliferation, metastasis, and treatment resistance. High levels of TGF-β secreted by cancer cells mediate the suppressive effects on NK cells. Moreover, exosomes delivered by cancer cells downregulate the NKG2D on NK cells and CTLs, leading to their impaired cytotoxic function and evasion of tumor surveillance. Simultaneously, MDSCs and T_{reg} cells within the TME also negatively regulate CTLs, leading to their anergy and exhaustion.

within the tumor microenvironment, resulting in ECM remodeling, which is closely related to tumor malignancy.[29–31] Apart from the secretion of ECM proteins, cancer-associated fibroblasts (CAFs) secrete growth factors, such as fibroblast growth factors (FGFs) or growth differentiation factor 15 (GDF15), immunomodulating chemokines and cytokines, as well as diverse metalloproteinase (MMP) enzymes and exosomes.[32,33] An earlier study found that a novel autocrine factor CXC motif chemokine ligand 14 (CXCL14) secreted by CAFs stimulates monocyte migration and increases macrophage infiltration, resulting in enhanced proliferation and migration of prostate cancer cells.[34] Interestingly, an increasing number of autocrine factors have been described with different functions. CAFs actively secrete stromal-derived factor-1 (SDF-1), also known as CXC motif chemokine ligand 12 (CXCL12), to recruit monocytes toward tumor sites and promote their transdifferentiation toward the M2 macrophage polarization phenotype, mainly by SDF-1 delivery, promoting their transdifferentiation toward the M2 macrophage polarization phenotype.[35] Jung and colleagues indicated that CAFs can also recruit mesenchymal stem cells (MSCs) into tumor cells, mainly by CXC motif chemokine ligand 16 (CXCL16) delivery, stimulating the conversion of MSCs into CAFs and secreting SDF-1 to induce metastasis of prostate tumors.[36] Furthermore, it was shown that CAFs deliver soluble factors carbonic anhydrase IX (CA IX) to cause extracellular acidification upon their activation by prostate cancer cells, helping cancer cells to achieve epithelial-mesenchymal transition.[37] In addition, CAFs promote tumorigenesis by delivering cytokines, such as interleukin-6 (IL-6), interleukin-8 (IL-8), and vascular endothelial growth factor (VEGF), to facilitate tumor angiogenesis.[38,39] Finally, CAFs release exosomes containing noncoding RNAs such as miR-409 to enhance EMT in prostate cancer cells.[40] In addition, several microRNAs (miRNAs) contained in CAF-derived exosomes were found to have other functions. A review by Zhao et al.. described the ability of CAF-derived exosomes containing miR-22, let7a, and miR-125b to reprogram metabolic pathways in prostate cancer cells.[41]

Endothelial cells

Endothelial cells (ECs) participate in ECM reshaping and the formation of immature vessels. Previous evidence suggested that ECs may play an integral part during the development of prostate cancer.[42,43]

Table 1. TME components and their major effects on prostate cancer development

Component	Secreted factors	Effect	Publication
CAFs	SDF-1	promoting transdifferentiation of monocyte toward the M2 macrophage polarization phenotype	Comito et al.[35]
	CXCL14	stimulation of monocyte migration and macrophage infiltration, promoting tumor angiogenesis	Augsten et al.[34]
	CXCL16	recruitment of mesenchymal stem cells into prostate tumors to promote prostate cancer metastasis	Jung et al.[36]
	IL-6, IL-8, VEGF	stimulating tumor angiogenesis and supporting tumor growth	Ishii et al. and Bonollo et al.[38,39]
	TGF-β, HGF, FGFs, GDF15	Enhancing prostate cancer cell migration, invasion, and tumor growth	Levesque and Nelson, Bruzzese et al.[32,33]
	exosomes (miR-409)	promotion of EMT transition and prostate tumorigenesis	Josson et al.[40]
	Exosomes (miR-22, let7a and miR-125b)	downregulation of mitochondrial oxidative phosphorylation and reprogram metabolic pathways in prostate cancer cells	Zhao et al.[41]
ECs	IL-6	blockade of androgen receptor (AR) signaling and activation of TGF-β/matrix metalloproteinase-9 (MMP-9) signaling, leading to tumor invasion	Wang et al.[44]
Prostate cancer cells	Exosomes (ligands for NKG2D)	downregulation of the NKG2D on CD8+ T cells and NK cells, leading to impaired cytotoxic function and tumor escape	Lundholm et al.[61]
	CSF-1, IL-10, IL-13, CXCL2	recruitment and polarization of TAMs toward an M2 phenotype, leading to tumor progression	Escamilla et al.[85]
	TGF-β	mediating the immunosuppressive effects on NK cells	Pasero et al.[69]
	Exosomes (PD-L1)	suppression of T cell activation in the draining lymph node, resulting to evasion of immune surveillance and resistance to anti-PD-L1 antibody blockade	Poggio et al.[173]
MDSCs	IL-23	driver of castration-resistant prostate cancer	Calcinotto et al.[75]
	IL-6, IL-8	Activation of multiple oncogenic pathways in prostate cancer cells, induce proliferation, metastases and chemoresistance	Nguyen et al. and Araki et al.[78,79]
	RNS	Induction of tyrosine nitration to LCK, leading to T cell anergy	Li et al.[74]
Macrophages	CCL2	enhancing metastasis of prostate cancer cells	Maolake et al.[83]
	VEGF-A, MMP-9, ARG-1	causing treatment failure and tumor progression	Escamilla et al.[85]

A study demonstrated that CD31-positive and CD34-positive ECs were increased in prostate cancer and associated with the castration-resistant stage. The mechanism indicated that increased IL-6 secreted from ECs blocks androgen receptor (AR) signaling and induces transforming growth factor (TGF)-β/matrix metalloproteinase-9 (MMP-9) signaling, leading to enhanced invasion of prostate cancer cells.[44] The review by Georgiou et al. pointed out that bone-marrow-derived circulating endothelial progenitors and circulating ECs contribute to neovascularization and are associated with prostate progression and the therapeutic response in different contexts, suggesting that they may be biomarkers of prostate cancer.[45]

Commensal microbiota

Emerging evidence has suggested that commensal microbiota are a crucial component of the TME. Hieken et al. utilized 16S rDNA hypervariable tag sequencing to generate microbial signatures in human breast tissues, confirming the differences of breast microbiome between benign and malignant breast disease.[46] Their work identified that the relative abundance of genera, including *Fusobacterium*, *Atopobium*, *Gluconacterobacter*, *Hydrogenophaga,* and *Lactobacillus,* increased from the invasive breast tissue. Recently, Nejman and colleagues noted that intratumor bacteria vary across tumor types and may influence the response to immunotherapy.[14] Regulatory T cells

(T_{regs}) promote human symbiote enterotoxigenic *Bacteroides fragilis*-triggered neoplasia through limiting the availability of IL-2 in the TME.[47] Others pointed out that metabolites from commensal microbiota may mediate the crosstalk between microbiota and the host immune system.[48,49] A recent study reported that gut microbial metabolites, especially butyrate, could boost the antitumor cytotoxic $CD8^+$ T cell responses in the TME in an ID2-dependent manner.[48] Another study also reported that the microbial metabolite trimethylamine N-oxide improved the efficacy of immunotherapy in triple-negative breast cancer (TNBC).[49] Earlier studies detected and identified microorganisms in prostate cancer tissues, and microbial DNA was likely to be present in focal regions associated with acute or chronic inflammation.[50–52] Due to the complexity of the potential crosstalk between the commensal microbiome and TME in prostate cancer, it is pivotal to further explore the mechanisms that are involved.

IMMUNE COMPONENTS OF THE TME

T lymphocytes

Diverse subsets of T cells, including cytotoxic $CD8^+$ T lymphocytes (CTLs) and regulatory T cells (T_{regs}), have been described to infiltrate the prostate TME and might be responsible for tumor progression. CTLs are capable of recognizing tumors and exhibiting antitumor properties, while T_{reg} cells suppress antitumor immune responses by transmitting inhibitory signaling to cytotoxic T cells.[53,54] The protein cell death ligand (PD-L1) is present on the surface of diverse cells in the TME, including prostate cancer cells, T_{reg} cells, tumor-associated macrophages (TAMs), and CAFs. The protein cell death protein 1 (PD-1)/PD-L1 pathway transfers inhibitory signaling to T cells, leading to impaired T cell activation and proliferation. A previous study indicated that prostate-infiltrating $CD8^+$ T lymphocytes were prevalent in prostate cancer patients, but they were unable to exhibit immune responses due to high expression of PD-1,[55] suggesting that PD-1 blockade might present therapeutic relevance. For patients who have high microsatellite instability (MSI-High) tumors or high tumor mutational burdens, it is possible that PD-1 inhibition alone may have significant antitumor effects.[56] However, the strategy of PD-1 inhibition was thought to be controversial on account of low mutational spectrum in prostate cancer and MSI occurs only in 3%–12% of patients with metastatic castration-resistant prostate cancer (mCRPC).[57,58] An early trial reported on 17 men with mCRPC treated with nivolumab, and no patients showed a significant response to the therapy.[59] The failure was attribute to "non-inflamed" phenotype of prostate cancer.[60] Thus, future study should focus on how to transform prostate cancer from "cold" to "hot" status. Alteration of activated receptors caused by cancer cells can explain T cell dysfunction, resulting in tumor immune evasion. Lundholm et al. found that treatment with prostate tumor-derived exosomes containing ligands for NKG2D could downregulate NKG2D on $CD8^+$ T cells and natural killer (NK) cells, leading to their impaired cytotoxic function *in vitro.* Accordingly, *in vivo*, compared with healthy donors, patients with CRPC presented a marked decrease in surface NKG2D expression on $CD8^+$ T cells.[61] T_{reg} cells, which are distinguished by high expression levels of *CD25* and *FOXP3*, were found to be enriched in the tumor tissue of early-stage prostate cancer patients.[62] Furthermore, a clinical analysis based on a tissue microarray demonstrated that a higher number of intratumoral T_{reg} cells was associated with a more advanced tumor stage in prostate cancer.[63] The results from multiregion sequencing indicated that activation of Wnt/β-catenin mutations was associated with a lower $CD8^+$/$FOXP3^+$ ratio in high-risk prostate cancer tissues, demonstrating an overall immunosuppressive environment.[64]

NK cells

As a critical part of the innate immune system, NK cells exhibit immune surveillance with cytotoxic activity and cytokine production against infections and tumors.[65] Evidence has suggested that highly effective NK cells are related to a longer castration response and better prognosis in patients with metastatic prostate cancer.[66] In a murine prostate cancer model, CD1d-restricted invariant natural killer (iNKT) cells directly contacted macrophages in the TME and sustained their proinflammatory M1-like phenotype, delaying tumor progression. In addition, aggressive human prostate cancer is closely associated with reduced intratumoral iNKT cells, increased M2-like macrophages, and increased expression of proangiogenic $TIE2^+$, shedding light on the clinical significance of the crosstalk.[67] In prostate cancer, the TME has been described as immunosuppressive partially due to immature NK cells with poor cytolytic activity.[68] This phenomenon may partially contribute to increased TGF-β secretion by prostate tumors, mediating immunotolerance to NK cells and potentially advancing disease progression.[69] Several mechanisms may also explain how tumor cells evade attack from NK cells. NKG2D, a lectin-like type-2 transmembrane stimulatory immunoreceptor, is expressed preferentially by NK cells. The engagement of NKG2D and its ligands is a sufficient stimulus to activate NK cells. Moreover, it elicits a costimulatory signal to activate the functions of CD8+ T cells.[70] Downregulation of NKG2D ligands by tumor cells may result in tumor immune evasion. Interestingly, a previous study described that prostate cancer cells shed NKG2D ligands to avoid immune surveillance by NK cells and T cells.[71] Xu et al. discovered that IL-6 secreted by prostate cancer cells conversely modulates PD-L1/NKG2D ligand levels through the JAK/STAT3 signaling pathway, effectively decreasing the susceptibility of tumor cells to NK-cell cytotoxicity.[72]

A published study by Saga et al. proposed that prostate cancer cells upregulate NANOG, a pluripotent-related transcription factor, mediating suppression of adhesion molecules to escape cytotoxic attack from NK cells during tumorigenesis.[73]

MDSCs

Myeloid-derived suppressor cells (MDSCs), known as tumor motivators with immunosuppressive effects, play critical roles in prostate cancer development, metastasis and therapeutic resistance.[74] Calcinotto et al. identified that IL-23 secreted by MDSCs induced the AR pathway and promoted cell survival and proliferation in prostate tumor cells. Clinically, the IL-23 concentration and MDSC infiltration in the TME are upregulated in tissue samples from patients with CRPC.[75] Moreover, other clinically relevant evidence indicates that MDSCs are associated with the development of metastatic prostate cancer, with higher levels indicating higher tumor stage and worse overall survival.[76,77]

MDSCs undoubtedly mediate immunosuppression through indirect contact, including paracrine and endocrine signaling, or direct contact with other immune cells within the TME. For example, circulating MDSCs were indicated to be closely related to higher levels of IL-6 and IL-8 in prostate cancer patients.[76] Notably, both IL-6 and IL-8, as critical proinflammatory cytokines, are capable of triggering diverse oncogenic pathways to enhance proliferation, invasion, and chemoresistance in prostate cancer cells.[78,79] Indeed, it was reported that MDSCs secrete reactive nitrogen species to induce tyrosine nitration of lymphocyte-specific protein tyrosine kinase (LCK), leading to T cell anergy with reduced IL-2 production and proliferation.[80] However, the precise molecular mechanisms of MDSC recruitment and expansion during prostate carcinogenesis remain elusive and need to be further explored.

Macrophages

In prostate cancer, TAMs have been described as linkers connecting inflammation and immunosuppression in the context of prostate cancer progression.[35] A spectrum of TAM functional phenotypes has been described, ranging from M1-like (classically activated) with proinflammatory and antitumor properties to M2-like macrophages (alternatively activated) with anti-inflammatory and protumor M2 characteristics.[81] M2-like macrophages correlate with the aggressiveness of prostate cancer and castration resistance.[35,82] Functionally, TAMs promote tumor progression through a myriad of mechanisms by supporting tumor proliferation, angiogenesis, migration, and escape immune surveillance. A study demonstrated that TAMs enhance prostate cancer metastasis by delivering C-C motif chemokine ligand 2 (CCL2) to upregulate C-C motif chemokine ligand 22 (CCL22) and C-C motif chemokine receptor 4 (CCR4) in tumor cells.[83] The inflammatory TME within prostate cancer cells may upregulate cytokines and chemokines derived from tumor cells and stromal cells, impacting TAM recruitment and the M2-like phenotype.[35] Di Mitri et al. found that CXC motif chemokine ligand 2 (CXCL2) secreted by Pten-null prostate cancer cells strongly polarized infiltrating TAMs toward an anti-inflammatory phenotype, inducing tumor growth.[84] Although the existence of macrophages can exacerbate tumorigenesis, the interaction is not a simple one-way street, implying that future studies may be required to elucidate how macrophages affect tumor cells and vice versa to exploit novel therapeutic strategies. Escamilla et al. found that androgen blockade therapy (ABT), either by MDV3100 or castration treatment, induced colony-stimulating factor 1 (CSF1) and other cytokine secretion from tumor cells, which, in turn, promoted a pro-tumorigenic M2 phenotype in TAMs. Then, M2 TAM-delivered VEGF-A, MMP-9, and ARG-1 further cause treatment failure and cancer progression. Subsequent blockade of the CSF1-CSF1R axis could improve the efficacy and sustain a durable response to ABT for prostate cancer.[85]

Overall, these findings indicate that the tumorigenesis and progression of human prostate cancer is mediated by a complicated and interconnected interaction of diverse nonimmune and immune cell types in the TME.

The interplay leads to the dysregulated secretion of immunomodulatory and proangiogenic factors and secretion of exosomes or cytokines by CAFs, facilitating tumor growth. Moreover, the reshaping of the TME in prostate cancer is characterized by a suppressive immune system, whereby the function of CTLs and NK cells is inhibited by exosomes delivered by tumor cells. This interaction brings about the exhausted phenotypes of CTLs and NK cells and increases the infiltration of T_{reg} cells and tumor-promoting M2-like macrophages. Hence, these changes in the TME play a critical role in prostate tumor emergence, progression, and response to treatments and could be therapeutically targeted to improve the clinical outcome of prostate patients.

TREATMENT OF PROSTATE CANCER

Discovering therapeutic methods to suppress progression of prostate cancer is urgently needed. Conventional treatments include radical prostatectomy, radiotherapy, endocrine therapy, and chemotherapy.[86–88] Given that the TME plays a critical role during progression and drug resistance in prostate cancer, immunotherapies are beneficial approaches. Below, we summarize treatments at the development stage for prostate cancer patients as well as immunotherapies and potential combination regimens.

THERAPEUTICS AT THE DEVELOPMENT STAGE

RNAi therapy

RNA interference (RNAi), a strategy in which RNA molecules inhibit gene expression, has recently attracted research attention and showed promise in treatment of prostate cancer. By identifying therapeutic gene targets, RNAi therapies may augment the effect of current therapeutic regimens.[89] Vainio and colleagues combined microarray and RNAi techniques to identify biomarkers and therapeutic targets, providing potential personalized approaches for prostate cancer management.[90] A study reported that effective promoter-targeting small interfering RNA (siRNA) mediating *MYC* silencing could reduce prostate cancer stem-like cell, diminishing tumor-initiating and metastatic capability. Notably, delivery of the siRNA in the xenograft model significantly suppressed prostate tumor development.[91] However, the potential off-target of RNAi therapy may reduce its efficacy. Among various carriers designed to deliver RNAi molecules, nanoparticles stand out as efficient carriers with more precise targeting ability. Additionally, nanoparticles enable the co-delivery of RNAi molecules with antitumor drugs, paving the way into drug delivery to prostate cancer.[92] Binzel et al. used RNA nanotechnology for efficient and specific miRNA delivery. RNA nanoparticles were constructed with anti-prostate-specific membrane antigen (PSMA) RNA aptamer as a targeting ligand and anti-miR17 or anti-miR21 as therapeutic modules, strongly inhibiting tumor growth in murine prostate cancer models.[93]

Aptamer-drug conjugates strategy

Aptamer-drug conjugates is a novel strategy for drug delivery to reduce side effects of drugs. Theoretically, aptamer-drug conjugates can specifically recognize prostate cancer cells, inducing tumor cell death without assault to normal tissues.[94] To date, at least nine

compounds have been conducted as aptamer-drug conjugates and their anti-prostate cancer effects are evaluated. Among them, doxorubicin (Dox) is the most studied with various known sides effects including heart injuries, allergic reactions, bone marrow suppression, and vomiting, which largely limit their clinical application.[95] Aptamer-Dox conjugate therapies have been developed into liposomes, nanoparticles, micelles, myristilated chitosan nanogels, etc. Nanoparticles, with low molecular weight and easy absorption, are regarded as main preparation for aptamer-Dox delivery systems. In a study, an aptamer called A10 and Dox were constructed to form a polylactide nanoconjugate named A10 Dox-PLA NCs. By controlling drug release kinetics and targeting tumor-associated ECs, A10 Dox-PLA NCs showed well tolerance in murine models as supported by the absence of histologic organ toxicity.[96] Taghdisi et al. developed a three-way junction pocket DNA nanostructure for efficient Dox delivery. Results from cellular uptake studies and cell viability assays demonstrated that the Dox-loaded three-way junction pocket DNA nanostructure specifically internalized into target cancer cells and was preferably against cancer cells, largely reducing cytotoxic effects on nontarget cells.[97] However, the transformation from cell or animal experiments to clinical applications still remains as a challenge.

PROTAC strategy

Proteolysis-targeting chimera (PROTAC), one of the strategies of targeted protein degradation, is attracting interest on account of its therapeutic potential to modulate proteins that are arduous to target with conventional small molecules. PROTAC consists of two ligands mediated by a linker. One ligand recruits and binds a target protein of interest (POI). Simultaneously, the other ligand recruits and binds an E3 ligase, such as Von Hippel-Lindau (VHL) and Cereblon (CRBN), forming a stable POI–PROTAC–E3 ternary complex.[98] Finally, the ternary complex induces polyubiquitylation and subsequent proteasomal degradation of the POI, after which the PROTAC can be recycled to target another POI.[99] AR, a well-known driver of prostate cancer, is an outstanding example of a PROTAC target owing to its potential insensitivity or resistance to anti-androgenic therapies.[100] Preclinical findings demonstrated that two VHL-recruiting PROTAC degraders, ARD-69 and ARD-61, achieved potent degradation of AR in prostate cancer and exhibited efficacious tumor growth-inhibitory effects in xenograft models.[101,102] Most excitingly, the CRBN-recruiting PROTAC degrader ARV-110 has made great progress and has shown encouraging preliminary clinical results in enzalutamide-resistant prostate cancer. A phase I/II clinical trial (NCT03888612) demonstrated that ARV-110 exhibited antitumor activity with an acceptable safety profile in patients with mCRPC after enzalutamide and/or abiraterone treatments.[99] This clinical advancement represents an essential milestone for the PROTAC therapeutic field.

IMMUNOTHERAPY

In recent years, immunotherapies have become options for the treatment of various solid tumors, such as non-small cell lung cancer and melanoma.[103,104] The focus of cancer immunotherapy is to promote an immune response against tumor cells. In prostate cancer, therapeutic activity has been observed for immunotherapy strategies based on vaccination, immune cell-targeted modifications, and those based on ICB.

Dendritic cell vaccines

Dendritic cells (DCs) form a bridge between the innate and adaptive immune response by presenting tumor-associated antigens, boosting potent antigen-specific T cell responses against tumor cells. To leverage these features, DC-based vaccines were developed. The common practice of preparation of DC vaccines is to isolate monocytes from patients and culture these monocytes with stimulatory cytokines, such as granulocyte macrophage-CSF and IL-4. Next, DCs are loaded with diverse tumor antigens, such as tumor peptides, proteins, mRNAs, cell lysates, and apoptotic tumor cells, and finally injected back into the patient.[105] Sipuleucel-T, an autologous cellular immunotherapy, was the first US Food and Drug Administration (FDA)-approved DC-based vaccine in 2010 for the treatment of patients with asymptomatic or minimally symptomatic mCRPC. This vaccine targets prostate acid phosphatase to stimulate an immune response. Sipuleucel-T represented a reduction of 22% in the risk of death and prolonged survival up to 25.8 months among metastatic castration-resistant prostate patients compared with 21.7 months in the placebo group.[106] However, concerns and skepticism about the trial results and high cost of this cellular immunotherapy have limited its universal uptake in the clinic.[107] Unfortunately, PROSTVAC, a dendritic cell-based vaccine similar to Sipuleucel-T, showed no overall survival advantage in a phase III study.[108] Our understanding of DC vaccines has expanded over the past decade, yet no further DC therapy has been established to date, suggesting a potential gap between fundamental research and clinical application to be investigated. Thus, recent studies of Sipuleucel-T and PROSTVAC have focused on combination therapies. A phase II study of Sipuleucel-T combined with radium-223 for patients with mCRPC were conducted and presented a synergistic effect of the combination therapy.[109] In details, combination of Sipuleucel-T and radium-223 exhibited significant prostate-specific antigen (PSA) decline, longer progression-free survival (PFS) (39 vs. 12 weeks; hazard ratio [HR], 0.32; 95% confidence interval [CI], 0.14–0.76) and improved overall survival (OS) (not reached vs. 2.6 years; HR, 0.32; 95% CI, 0.08–1.23). Unfortunately, another phase II trial reported that combination of Sipuleucel-T and stereotactic ablative radiotherapy for patients with mCRPC did not increase time to progression or OS compared with the original IMPACT clinical trial.[110] PROSTVAC was also applied in combination with androgen deprivation therapy (ADT) for patients with mCRPC.[111]

γδ T cells

Another novel choice of immunotherapy for prostate cancer patients relies on gamma-delta T cells (γδ T cells). γδ T cells have universally protective effects in cancer, largely due to their ability to produce interferon-γ and their enduring cytotoxicity,[112] providing a strategy for developing clinical approaches on the basis of their innate antitumor properties. In a transgenic mouse model of prostate cancer (TRAMP), studies were performed to first propose that γδ T cells

can definitely activate immunosurveillance against developing mouse prostate cancer.[113] In a phase I clinical trial, patients treated with a γδ T cell agonist (zoledronate) combined with low-dose IL-2 presented declining PSA levels and positive clinical outcomes with manageable adverse events, suggesting a novel and feasible therapeutic method to be considered.[114]

CAR T cell therapies

Chimeric antigen receptor (CAR) T cell therapies are a promising treatment option involving gene-transfer techniques that confer a defined specificity onto T cells to augment T cell function and mediate enhanced elimination of tumor cells.[115] Each CAR T cell can extensively proliferate and elicit a long-lasting immune response against tumor cells. Simultaneously, CAR T cells may facilitate immune surveillance to prevent tumor relapse by their own persistence.[116,117] Several CAR T products, which target diverse antigens, including prostate stem cell antigen (PSCA), PSMA, epithelial cell adhesion molecule (EpCAM), and NKG2DL, are under preclinical investigation or clinical trials for prostate cancer.[118–121] A phase I/II study showed that the combination of anti-PSCA CAR T cells with the rimiducid-inducible MyD88/CD40 coactivation switch could enhance T cell proliferation and persistence in patients with prostate cancer.[118] Unfortunately, the Bellicum PSCA CAR T program has been terminated due to immune adverse events. An early phase I trial of anti-PSMA CAR T cell therapy in prostate cancer reported partial responses in 2 of 5 patients with PSA declines of 50% and 70% and PSA delays of 78 and 150 days.[119] A preclinical study showed that intravenous application of EpCAM-targeting CAR T cells significantly reduced tumor growth in a xenograft model.[120] Recent research reported that natural killer group 2D ligand (NKG2DL), an alternative tumor marker, provides a possible target for conventional CAR T cell therapy in prostate cancer. NKG2DL-targeting CAR T cells exhibited markedly increased cytotoxicity against prostate cancer *in vitro* and *in vivo*. Furthermore, the application of NKG2DL-targeting CAR T cells with *IL-7* gene modification exhibited enhanced antitumor efficacy and resulted in prolonged OS in xenograft models.[121] The limitations of utilizing CAR T therapies include CAR T cell trafficking, low T cell infiltration, weak CAR T cell persistence, and an immunosuppressive TME. Thus, the improved and optimal CAR T format combined with other therapies may be proposed for individual application.

T_{reg} therapies

T_{reg} cells, as immunosuppressive components in the TME, have emerged as promising targets for harnessing the immune system to destroy cancer cells.

Targeting T_{reg} cells has achieved impressive results in fundamental research. Kobayashi and colleagues found that depletion of T_{reg} cells in the tumor environment with near-infrared photoimmunotherapy (NIR-PIT) could unleash anticancer immune responses in mouse models of prostate cancer.[122] In fact, studies that focus on depleting T_{reg} cells in patients are impeded by the fact that T_{reg} cells do not express a unique surface molecule that can be exclusively targeted. Current methods for T_{reg} cell depletion are largely based on agents that target upregulated surface molecules such as IL-2R, CCR4, and cytotoxic T lymphocyte-associated protein 4 (CTLA-4). Denileukin diftitox (DAB_{389}IL-2), a fusion protein of IL-2 and diphtheria toxin, exhibited a strategy of targeting T_{reg} cells in combination with a poxviral vaccine to promote antigen-specific immune responses.[123] Other than humans, canines are the only large mammals that develop spontaneous prostate cancer. Moreover, canine prostate cancer shares a plethora of similarities with its human counterpart.[124] Updated research from Japan reported that mogamulizumab, a monoclonal antibody targeting CCR4, remarkably depleted circulating T_{reg} cells and improved survival rates with a low abundance of adverse events in a canine model of advanced prostate cancer. Moreover, immunohistochemistry confirmed that tumor-infiltrating T_{regs} expressed CCR4 in human patients with prostate cancer.[125] Although preliminary, these results demonstrated the possible utilization of mogamulizumab in humans. Notably, mogamulizumab has been approved by the FDA as a treatment for patients with T cell lymphoma and other cancers,[126] paving the way for mogamulizumab as a clinical treatment in human prostate cancer. CTLA-4, which is expressed by effector T cells and T_{reg} cells, transmits inhibitory signals and restrains antitumor activity. The application of CTLA4-specific antibodies, a strategy partially relying on T_{reg} depletion, can enhance effector T cell activity and simultaneously inhibit T_{reg} cell-dependent immune suppression.[127] Although anti-CTLA-4 therapy could recruit T cells, anti-CTLA-4 (ipilimumab) monotherapy failed to be of substantial clinical benefit in patients with prostate cancer, implying the existence of other compensatory inhibitory pathways of tumor-infiltrating T cells.[128] A recently published study described a novel mechanism by which the B7-family immune checkpoint B7x is expressed in various cancer cells and promotes the conversion of $CD4^+$ T cells into T_{reg} cells, leading to resistance to anti-CTLA-4 therapy. Thus, anti-B7x in combination with anti-CTLA-4 is an encouraging strategy of synergistic therapeutic efficacy.[129] We will further discuss CTLA-4 and other ICB methods in the next part of this review. Another potential strategy to modulate T_{reg} cells is focusing on molecules that are involved in T_{reg} cell migration. Evidence has shown that functional T_{reg} cells are increased in the bone marrow microenvironment and that CXC chemokine receptor 4 (CXCR4) and CXC motif chemokine ligand 12 (CXCL12) contribute to T_{reg} cell migration in patients with bone metastasis-associated prostate cancer.[130] Thus, blockade of the CXCL12/CXCR4 axis should be considered.

Immune checkpoint blockade

Evasion of immune surveillance is an essential characteristic of tumorigenesis.[131] To evade antitumor immune responses, upregulation of inhibitory receptors (IRs), such as CTLA-4 and PD-1, is frequently observed in the TME. Basic research conducted by James Allison and Tasuku Honjo characterized these IRs as efficacious anticancer immunotherapeutic targets for reinvigorating antitumor responses via ICB.[132] Immune checkpoint inhibitors targeting CTLA4, the PD-1 protein, or their ligands have become novel therapeutic options for various cancers. The application of pembrolizumab and ipilimumab, two monoclonal antibodies against PD-1 and

CTLA4, respectively, has been evaluated in patients.[59] Nevertheless, the distinct role of ICB for patients with prostate cancer has yet to be clarified. Early evidence demonstrated that pembrolizumab might present clinical benefits in patients who had potential progression on enzalutamide.[133] The results from a phase Ib KEYNOTE-028 trial of pembrolizumab represented an overall response rate of approximately 17% (95% CI, 5.0%–38.8%) in patients with advanced prostate adenocarcinoma. Median PFS and OS of patients were 3.5 and 7.9 months, respectively.[134] The phase II KEYNOTE-199 study demonstrated that pembrolizumab monotherapy exhibited durable responses and encouraging OS improvements in a subset of patients with mCRPC who were previously treated with docetaxel and endocrine therapy. Objective response rate of patients was 5% (95% CI, 2%–11%) in cohort 1 and 3% in cohort 2 (95% CI, <1%–11%) and median OS was 9.5 months in cohort 1, 7.9 months in cohort 2, and 14.1 months in cohort 3.[135] Another phase II trial evaluated the efficacy of enzalutamide plus pembrolizumab in 28 patients with mCRPC. Results showed that 18% of patients had declined PSA and 25% of patients achieved an objective response. For all patients, median OS was 21.9 months (95% CI, 14.7–28.4 months) compared with 41.7 months (95% CI, 22.16 to not reached [NR]) in the responders.[136] A phase III KEYNOTE-641 study (ClinicalTrials.gov: NCT03834493) is being conducted, and its final results may help us to evaluate the efficacy of pembrolizumab plus enzalutamide as a treatment for mCRPC.[137] Apart from pembrolizumab, ipilimumab was simultaneously assessed in prostate cancer. In two phase III trials including patients with mCRPC who were pretreated with docetaxel or chemotherapy-naive, the median OS in the ipilimumab group did not differ significantly from that in the placebo group.[138,139] Interestingly, long-term responders with survival benefits were observed in an additional follow-up analysis.[140] In a preliminary analysis of patients from the CheckMate 650 trial (NCT02985957), Sharma and colleagues reported that combination of ipilimumab (anti-CTLA-4) plus nivolumab (anti-PD-1) can generate responses in a subset of patients with mCRPC.[141] In detail, patients from the pre-chemotherapy cohort exhibited 25% overall response rate and 19.0 months median OS and in patients from the post-chemotherapy cohort, the overall response rate was 10% and median OS was 15.2 months. These early results support the rationale for further evaluation of immune checkpoint blockade-based combinational therapies in patients with prostate cancer, but problems remain related to dosing regimens (optimal dose/schedule).

The probable reasons for limited therapeutic activity to immunotherapy in prostate cancer include (1) existence of an alternative mechanism of immune checkpoint pathways that are not completely understood and targeted; (2) low mutational burden of tumor cells; and (3) a scarcity of antitumor infiltrating immune cells including T cells in prostate cancer ("cold" signature) compared with immunological cancers ("hot" signature). These effects can partially explain the limited benefits of ICB therapies in prostate cancer. Thus, fundamental mechanism-associated studies and preclinical research on the basis of combination ICB therapies are needed to transform prostate cancer from "cold" to "hot" status.

COMBINATION-IMMUNOTHERAPY STRATEGIES

Over 90% of prostate cancers are identified as immune-cold and do not display a T-cell-inflamed property. Therefore, therapies that can promote T cell infiltration may create a breakthrough for efficient ICB in patients with prostate cancer. It was noted in our current study that cryoablation increases postablative immunogenicity.[142] Thus, an innovative modified strategy was developed combining cryoablation with the blockade of CTLA-4. A preclinical model of prostate cancer showed that cryoablation and CTLA-4 blockade combination therapy synergized to mediate rejection of a second tumor challenge. The results also showed that tumors at challenge sites exhibited greater infiltration of $CD8^+$ T cells and had a higher ratio of effector T cells to T_{reg} cells in the combination therapy group.[143] Another study also showed that synergism of cryoablation and CTLA-4 blockade delayed the growth of distant tumors and decreased the mortality rate.[144] mCRPC exhibits overwhelming resistance to ICB. A study indicated that MDSC-targeted therapy in combination with anti-CTLA-4 and anti-PD-1 antibodies synergized to increase the tumor regression rate, stemming from the increase in IL-1 receptor antagonists and blockade of MDSC-activating cytokines delivered by tumor cells.[145] Interferon-γ, a cytokine with few benefits in clinical trials, was identified to induce major histocompatibility class-I (MHC-I) and PD-L1 gene expression in prostate cancer cells *in vitro*, suggesting a potential combination regimen to overcome ICB resistance for mCRPC.[146] Peng et al. proposed a new therapeutic combination strategy targeting EP4, which was identified in various immune cells by single-cell analysis. Blocking EP4 with a novel antagonist, known as YY001, efficiently reversed the immunosuppressive phenotype of MDSCs while enhancing the infiltration and antitumor cytotoxicity of CD8+ T cells. In particular, combining YY001 with anti-PD-1 antibodies strongly inhibited tumor progression and resulted in long-term survival and durable immunologic memory, implying a transformation from a cold tumor state into a hot tumor state.[147] Assessment of clinical samples showed that upregulated expression of IL-8 from prostate epithelial cells after castration might contribute to ICB resistance by recruiting tumor-promoting polymorphonuclear myeloid-derived suppressor cells (PMN-MDSCs). Blocking IL-8 signaling in combination with ICB could remarkably increase the number of $CD8^+$ T cells within the TME.[148] *Pten*-null prostate cancers are usually resistant to anti-PD-1 immunotherapy. A recent study indicated that intermittent but not daily usage of the PI3Kα/β/δ inhibitor BAY1082439 could overcome ICB resistance by promoting $CD8^+$ T cell infiltration and enhancing antitumor immunity, turning cold tumors into T-cell-inflamed tumors.[149] Endogenous tumor-specific tissue-resident memory T (T_{RM}) cells have attracted our attention during cancer immunotherapy. In a murine model of prostate carcinoma, dual therapy combining irreversible electroporation and anti-CTLA-4 antibodies confirmed T_{RM} cell establishment and was related to the protection of subsequent tumor challenge. Furthermore, a triple-therapy strategy adding anti-PD-1 antibodies demonstrated positive efficiency in nonresponsive cases.[150]

Microbiota-based strategies

Typical treatments in prostate cancer, such as ADT and radiotherapy, can potentially reprogram the microbiota, resulting in finite immune

responses. Preclinical studies indicated that the gut microbiota can be influenced by castration, and another clinical study revealed the same alteration for pelvic radiotherapy by massive pyrosequencing.[151,152] Moreover, evidence has shown that conventional therapies might cause resistance to ICB. Thus, microbiota-based mechanisms and strategies involving the gut microbiome, commensal microbiota, and microbial metabolites were considered in several preclinical models.

Gut microbiome

Multiple studies have focused on the potential effects of varied compositions of gut microbiota on antitumor immune responses.[153–155] Cimadamore et al. demonstrated that Ruminococcaceae species and *Akkermansia muciniphila* were associated with a better response to anti-PD-1 antibodies. Administration of Ruminococcaceae species antibiotic was correlated with worse progressive disease.[156] In addition, Sfanos et al. found that a higher abundance of *Akkermansia muciniphila* and Ruminococcaceae species, previously described as positive markers for favorable efficiency in patients receiving anti-PD-1 immunotherapy,[156] was detected in prostate cancer patients with androgen axis-targeted therapies, implying the potential effects of gastrointestinal microbiota in modulating the response to antitumor immunotherapies.[17] A newly published study by Pernigoni et al. found that during ADT, a specific intestinal microbial community was enriched in patients with the emergence of CRPC. Administration of *Prevotella stercorea* efficiently overcame endocrine resistance, providing a possible clue for combination treatment in the future.[157]

Commensal microbiota

Recently, many efforts have been made to confirm the role of commensal microbiota in refashioning the host immune system. Some have reported that lipopolysaccharide can induce an immune response via the TLR4 (toll-like receptor 4) signaling pathway.[158,159] An earlier study demonstrated that commensal *Bifidobacterium* was associated with improved antitumor effects in murine melanoma models. Strikingly, oral administration of *Bifidobacterium* alone could suppress tumor growth to the same degree as administration of anti-PD-1 antibodies. Combination therapy with *Bifidobacterium* and anti-PD-1 antibodies strongly abolished tumor growth by enhancing $CD8^+$ T cell accumulation and priming.[160] Anker et al. found that CP1, a patient-derived prostate-colonized microbe, in combination with anti-PD-1 antibodies could decrease tumor burden by enhancing the tumor infiltration of $CD8^+$ T cells, M1 macrophages, and NK cells while decreasing the abundance of intratumoral T_{reg} cells in oncogene-driven prostate cancer models. This article pointed out that tissue-specific microbes can sensitize resistant cancer types to immunotherapy.[161]

Microbial metabolites

He et al. previously found that the microbial metabolite butyrate can directly modulate the antitumor response of $CD8^+$ T cells and improve the efficacy of anti-PD-L1 antibodies.[48] A recently published study by Wang et al. discovered that the microbial metabolite trimethylamine N-oxide (TMAO), derived from *Clostridiales*, could strikingly improve the therapeutic efficacy of anti-PD-1 antibodies in patients with TNBC by enhancing $CD8^+$ T-cell-mediated antitumor immunity, providing a clue that a choline-rich diet, which is the primary source of TMAO, could be a clinical treatment in patients.[49] Another study described that higher dietary fiber, which yields higher levels of fiber-fermenting short-chain fatty acids derived from gut microbiota, could enhance the immune response to ICB in preclinical melanoma models by increasing T cell infiltration and reactivating cytotoxicity toward tumors.[162]

To date, few studies on the mechanisms connecting the gut microbiome, commensal microbiota, related metabolites, and ICB in patients or models of prostate cancer have been published. However, similar mechanisms identified in other cancer types could provide clues for treatments in prostate cancer, but further study is needed.

Autophagy inhibition

Accumulated evidence implies that cancer cells may use autophagy to develop resistance to antitumor therapies, rendering the autophagy process an ideal target for combinatorial regimens.[163] Vacuolar sorting protein 34 (VPS34) plays a central role in the control of autophagic activity through the formation of Beclin 1-VPS34 protein complexes.[164] A study reported that genetic or pharmacological inhibition of VPS34 with SB02024 or SAR405 (VPS34i) could inhibit tumor growth by establishing a T cell-inflamed TME, improving the efficacy of anti-PD-L1/PD-1 blockade therapies.[165] Natural compounds have been applied for cancer treatment based on their tumor roles in inhibiting autophagy. A study showed that rhizochalinin (Rhiz), a novel sphingolipid-like marine compound, exhibited antitumor properties through the inhibition of prosurvival autophagy in human prostate cancer cells. Thus, Rhiz resensitized tumor cells to conventional therapies.[166] A recent article demonstrated that ESK981, a phase I-cleared orally bioavailable multityrosine kinase inhibitor (MTKI), presented autophagy inhibitory characteristics that efficiently promoted functional T cell infiltration, leading to tumor regression in prostate cancer. ESK981 targets the lipid kinase PIKfyve and converts prostate tumor cells from cold to hot phenotypes via inhibition of autophagy, enhancing the therapeutic response to ICB.[167]

Epigenomic therapy

Emerging evidence has suggested that epigenomic reprogramming results in reduced T cell infiltration in tumor cells, causing immune evasion.

Diverse epigenetic modulators have been identified as potential targets for improving the efficiency of ICB. Epigenetic therapy combining DNA methyltransferase inhibitors with histone deacetylase inhibitors was described to reverse tumor immune evasion and transfer the T cell exhaustion phenotype toward memory and effector T cell states in murine non-small cell lung cancer models.[168] Another article showed that ablation of the histone demethylase lysine demethylase 1 (LSD1) correlated with increased $CD8^+$ T cell infiltration. Importantly, LSD1 blockade in combination with anti-PD-1 therapy elicited strong antitumor responses of T cells and restrained tumor

growth in poorly immunogenic tumors.[169] Given the overexpression of LSD1 protein in prostate cancer, novel therapeutic therapy targeting LSD1 could be considered and evaluated.[170] Liu et al. discovered that the m^6A demethylase FTO increased tumor glycolysis and simultaneously restricted the activation of $CD8^+$ T cells. Administration of the FTO inhibitor Dac51 synergized with anti-PD-L1 blockade and efficiently increased $CD8^+$ T cell infiltration and antitumor responses in murine melanoma models.[171] Given the paucity of $CD8^+$ T cell infiltration in the TME of prostate cancer cells, the mechanisms and therapeutic strategies associated with epigenetic factors should be considered and further investigated to convert the cold tumor state into the hot state.

Finally, prostate cancer cells might employ alternative mechanisms of immune checkpoint pathways that are not entirely investigated and targeted to escape ICB therapies. For instance, Wang et al. reported that fibrinogen-like protein 1 (FGL1), a protein secreted by liver or cancer cells, is a major ligand for the inhibitory receptor lymphocyte-activation gene 3 and that blockade of FGL1 could remarkably enhance the efficacy of immunotherapies in lung and liver cancer models.[172] Notably, supplementary data from this article showed that *FGL1* mRNA expression in human prostate cancer tissues is higher than that in corresponding normal tissues from The Cancer Genome Atlas database, implying a potential evasion mechanism in prostate cancer cells. Additional results and targeted combination ICB therapies are keenly awaited.

DISCUSSION

Overall, both intrinsic and external factors contribute to prostate cancer. In this review, we focused on the potential pro-carcinogenic role of the inflammatory microenvironment and described the interaction between diverse cellular entities within the TME in prostate cancer development. The consequence of the interplay is the formation of an immunosuppressive TME, establishing a more invasive and resistant status of prostate cancer. Although conventional therapeutic methods, such as endocrine therapy, represent improved OS in clinical trials, they have limited efficacy and may lead to the development of tumor resistance. Given the pivotal role of the TME during prostate cancer initiation, progression, and metastasis, a plethora of immunotherapeutic strategies targeting immunosuppressive cells within the TME have been considered and evaluated. Moreover, ICB regimens involving pembrolizumab and ipilimumab manifest in preclinical models. Nevertheless, ICB strategies did not yield adequate clinical benefits, largely due to the complexity of the TME in prostate cancer, which is delineated as a cold signature. Therefore, combinatorial checkpoint immunotherapies will be an outstanding choice for prostate cancer. Combinatorial therapies, including those targeting immunosuppressive cells within the TME, microbiota, autophagy, and epigenetic factors, have made great progress in overcoming ICB resistance in animal models. In light of findings from other cancer models, more therapeutic agents and combinational targets will be prospectively evaluated for the treatment of prostate cancer, with the goal of achieving sustained clinical benefits for patients.

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AUTHOR CONTRIBUTIONS

M.L. played major roles in reading literature, collecting information, and writing the manuscript; X.S. helped to proofread the manuscript; L.L. supervised the writing.

DECLARATION OF INTERESTS

The authors declare no competing interests.

REFERENCES

1. Ferlay, J., Soerjomataram, I., Dikshit, R., Eser, S., Mathers, C., Rebelo, M., Parkin, D.M., Forman, D., and Bray, F. (2015). Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. Int. J. Cancer *136*, E359–E386.
2. Foreman, K.J., Marquez, N., Dolgert, A., Fukutaki, K., Fullman, N., McGaughey, M., Pletcher, M.A., Smith, A.E., Tang, K., Yuan, C.W., et al. (2018). Forecasting life expectancy, years of life lost, and all-cause and cause-specific mortality for 250 causes of death: reference and alternative scenarios for 2016-40 for 195 countries and territories. Lancet *392*, 2052–2090.
3. de Bono, J.S., Guo, C., Gurel, B., De Marzo, A.M., Sfanos, K.S., Mani, R.S., Gil, J., Drake, C.G., and Alimonti, A. (2020). Prostate carcinogenesis: inflammatory storms. Nat. Rev. Cancer *20*, 455–469.
4. De Marzo, A.M., Marchi, V.L., Epstein, J.I., and Nelson, W.G. (1999). Proliferative inflammatory atrophy of the prostate: implications for prostatic carcinogenesis. Am. J. Pathol. *155*, 1985–1992.
5. Sfanos, K.S., and De Marzo, A.M. (2012). Prostate cancer and inflammation: the evidence. Histopathology *60*, 199–215.
6. Gurel, B., Lucia, M.S., Thompson, I.M., Jr., Goodman, P.J., Tangen, C.M., Kristal, A.R., Parnes, H.L., Hoque, A., Lippman, S.M., Sutcliffe, S., et al. (2014). Chronic inflammation in benign prostate tissue is associated with high-grade prostate cancer in the placebo arm of the prostate cancer prevention trial. Cancer Epidemiol. Biomarkers Prev. *23*, 847–856.
7. Mani, R.S., Amin, M.A., Li, X., Kalyana-Sundaram, S., Veeneman, B.A., Wang, L., Ghosh, A., Aslam, A., Ramanand, S.G., Rabquer, B.J., et al. (2016). Inflammation-induced oxidative stress mediates gene fusion formation in prostate cancer. Cell Rep. *17*, 2620–2631.
8. Kwon, O.J., Zhang, L., Ittmann, M.M., and Xin, L. (2014). Prostatic inflammation enhances basal-to-luminal differentiation and accelerates initiation of prostate cancer with a basal cell origin. Proc. Natl. Acad. Sci. USA. *111*, E592–E600.
9. Sfanos, K.S., Yegnasubramanian, S., Nelson, W.G., and De Marzo, A.M. (2018). The inflammatory microenvironment and microbiome in prostate cancer development. Nat. Rev. Urol. *15*, 11–24.
10. Neophytou, C.M., Panagi, M., Stylianopoulos, T., and Papageorgis, P. (2021). The role of tumor microenvironment in cancer metastasis: molecular mechanisms and therapeutic opportunities. Cancers (Basel) *13*, 2053.
11. Sandhu, S., Moore, C.M., Chiong, E., Beltran, H., Bristow, R.G., and Williams, S.G. (2021). Prostate cancer. Lancet *398*, 1075–1090.
12. De Marzo, A.M., Platz, E.A., Sutcliffe, S., Xu, J., Grönberg, H., Drake, C.G., Nakai, Y., Isaacs, W.B., and Nelson, W.G. (2007). Inflammation in prostate carcinogenesis. Nat. Rev. Cancer *7*, 256–269.
13. van Leenders, G.J.L.H., Gage, W.R., Hicks, J.L., van Balken, B., Aalders, T.W., Schalken, J.A., and De Marzo, A.M. (2003). Intermediate cells in human prostate epithelium are enriched in proliferative inflammatory atrophy. Am. J. Pathol. *162*, 1529–1537.
14. Nejman, D., Livyatan, I., Fuks, G., Gavert, N., Zwang, Y., Geller, L.T., Rotter-Maskowitz, A., Weiser, R., Mallel, G., Gigi, E., et al. (2020). The human tumor

microbiome is composed of tumor type-specific intracellular bacteria. Science *368*, 973–980.

15. Liss, M.A., White, J.R., Goros, M., Gelfond, J., Leach, R., Johnson-Pais, T., Lai, Z., Rourke, E., Basler, J., Ankerst, D., and Shah, D.P. (2018). Metabolic biosynthesis pathways identified from fecal microbiome associated with prostate cancer. Eur. Urol. *74*, 575–582.

16. Shrestha, E., White, J.R., Yu, S.H., Kulac, I., Ertunc, O., De Marzo, A.M., Yegnasubramanian, S., Mangold, L.A., Partin, A.W., and Sfanos, K.S. (2018). Profiling the urinary microbiome in men with positive versus negative biopsies for prostate cancer. J. Urol. *199*, 161–171.

17. Sfanos, K.S., Markowski, M.C., Peiffer, L.B., Ernst, S.E., White, J.R., Pienta, K.J., Antonarakis, E.S., and Ross, A.E. (2018). Compositional differences in gastrointestinal microbiota in prostate cancer patients treated with androgen axis-targeted therapies. Prostate Cancer Prostatic Dis. *21*, 539–548.

18. Ridlon, J.M., Ikegawa, S., Alves, J.M.P., Zhou, B., Kobayashi, A., Iida, T., Mitamura, K., Tanabe, G., Serrano, M., De Guzman, A., et al. (2013). Clostridium scindens: a human gut microbe with a high potential to convert glucocorticoids into androgens. J. Lipid Res. *54*, 2437–2449.

19. Golombos, D.M., Ayangbesan, A., O'Malley, P., Lewicki, P., Barlow, L., Barbieri, C.E., Chan, C., DuLong, C., Abu-Ali, G., Huttenhower, C., and Scherr, D.S. (2018). The role of gut microbiome in the pathogenesis of prostate cancer: a prospective, pilot study. Urology *111*, 122–128.

20. Poutahidis, T., Cappelle, K., Levkovich, T., Lee, C.W., Doulberis, M., Ge, Z., Fox, J.G., Horwitz, B.H., and Erdman, S.E. (2013). Pathogenic intestinal bacteria enhance prostate cancer development via systemic activation of immune cells in mice. PLoS One *8*, e73933.

21. Hayashi, T., Fujita, K., Nojima, S., Hayashi, Y., Nakano, K., Ishizuya, Y., Wang, C., Yamamoto, Y., Kinouchi, T., Matsuzaki, K., et al. (2018). High-fat diet-induced inflammation accelerates prostate cancer growth via IL6 signaling. Clin. Cancer Res. *24*, 4309–4318.

22. Laurent, V., Guérard, A., Mazerolles, C., Le Gonidec, S., Toulet, A., Nieto, L., Zaidi, F., Majed, B., Garandeau, D., Socrier, Y., et al. (2016). Periprostatic adipocytes act as a driving force for prostate cancer progression in obesity. Nat. Commun. *7*, 10230.

23. Allott, E.H., Masko, E.M., and Freedland, S.J. (2013). Obesity and prostate cancer: weighing the evidence. Eur. Urol. *63*, 800–809.

24. Chen, M., Zhang, J., Sampieri, K., Clohessy, J.G., Mendez, L., Gonzalez-Billalabeitia, E., Liu, X.S., Lee, Y.R., Fung, J., Katon, J.M., et al. (2018). An aberrant SREBP-dependent lipogenic program promotes metastatic prostate cancer. Nat. Genet. *50*, 206–218.

25. Labbé, D.P., Zadra, G., Yang, M., Reyes, J.M., Lin, C.Y., Cacciatore, S., Ebot, E.M., Creech, A.L., Giunchi, F., Fiorentino, M., et al. (2019). High-fat diet fuels prostate cancer progression by rewiring the metabolome and amplifying the MYC program. Nat. Commun. *10*, 4358.

26. Chiarugi, P., Paoli, P., and Cirri, P. (2014). Tumor microenvironment and metabolism in prostate cancer. Semin. Oncol. *41*, 267–280.

27. Chen, S., Zhu, G., Yang, Y., Wang, F., Xiao, Y.T., Zhang, N., Bian, X., Zhu, Y., Yu, Y., Liu, F., et al. (2021). Single-cell analysis reveals transcriptomic remodellings in distinct cell types that contribute to human prostate cancer progression. Nat. Cell Biol. *23*, 87–98.

28. Butcher, D.T., Alliston, T., and Weaver, V.M. (2009). A tense situation: forcing tumour progression. Nat. Rev. Cancer *9*, 108–122.

29. Tomasek, J.J., Gabbiani, G., Hinz, B., Chaponnier, C., and Brown, R.A. (2002). Myofibroblasts and mechano-regulation of connective tissue remodelling. Nat. Rev. Mol. Cell Biol. *3*, 349–363.

30. Tuxhorn, J.A., Ayala, G.E., Smith, M.J., Smith, V.C., Dang, T.D., and Rowley, D.R. (2002). Reactive stroma in human prostate cancer: induction of myofibroblast phenotype and extracellular matrix remodeling. Clin. Cancer Res. *8*, 2912–2923.

31. Zhang, Y., Nojima, S., Nakayama, H., Jin, Y., and Enza, H. (2003). Characteristics of normal stromal components and their correlation with cancer occurrence in human prostate. Oncol. Rep. *10*, 207–211.

32. Levesque, C., and Nelson, P.S. (2018). Cellular constituents of the prostate stroma: key contributors to prostate cancer progression and therapy resistance. Cold Spring Harb. Perspect. Med. *8*, a030510.

33. Bruzzese, F., Hägglöf, C., Leone, A., Sjöberg, E., Roca, M.S., Kiflemariam, S., Sjöblom, T., Hammarsten, P., Egevad, L., Bergh, A., et al. (2014). Local and systemic protumorigenic effects of cancer-associated fibroblast-derived GDF15. Cancer Res. *74*, 3408–3417.

34. Augsten, M., Hägglöf, C., Olsson, E., Stolz, C., Tsagozis, P., Levchenko, T., Frederick, M.J., Borg, A., Micke, P., Egevad, L., and Ostman, A. (2009). CXCL14 is an autocrine growth factor for fibroblasts and acts as a multi-modal stimulator of prostate tumor growth. Proc. Natl. Acad. Sci. USA. *106*, 3414–3419.

35. Comito, G., Giannoni, E., Segura, C.P., Barcellos-de-Souza, P., Raspollini, M.R., Baroni, G., Lanciotti, M., Serni, S., and Chiarugi, P. (2014). Cancer-associated fibroblasts and M2-polarized macrophages synergize during prostate carcinoma progression. Oncogene *33*, 2423–2431.

36. Jung, Y., Kim, J.K., Shiozawa, Y., Wang, J., Mishra, A., Joseph, J., Berry, J.E., McGee, S., Lee, E., Sun, H., et al. (2013). Recruitment of mesenchymal stem cells into prostate tumours promotes metastasis. Nat. Commun. *4*, 1795.

37. Fiaschi, T., Giannoni, E., Taddei, M.L., Cirri, P., Marini, A., Pintus, G., Nativi, C., Richichi, B., Scozzafava, A., Carta, F., et al. (2013). Carbonic anhydrase IX from cancer-associated fibroblasts drives epithelial-mesenchymal transition in prostate carcinoma cells. Cell Cycle *12*, 1791–1801.

38. Ishii, K., Sasaki, T., Iguchi, K., Kajiwara, S., Kato, M., Kanda, H., Hirokawa, Y., Arima, K., Mizokami, A., and Sugimura, Y. (2018). Interleukin-6 induces VEGF secretion from prostate cancer cells in a manner independent of androgen receptor activation. Prostate *78*, 849–856.

39. Bonollo, F., Thalmann, G.N., Kruithof-de Julio, M., and Karkampouna, S. (2020). The role of cancer-associated fibroblasts in prostate cancer tumorigenesis. Cancers (Basel) *12*, 1887.

40. Josson, S., Gururajan, M., Sung, S.Y., Hu, P., Shao, C., Zhau, H.E., Liu, C., Lichterman, J., Duan, P., Li, Q., et al. (2015). Stromal fibroblast-derived miR-409 promotes epithelial-to-mesenchymal transition and prostate tumorigenesis. Oncogene *34*, 2690–2699.

41. Zhao, H., Yang, L., Baddour, J., Achreja, A., Bernard, V., Moss, T., Marini, J.C., Tudawe, T., Seviour, E.G., San Lucas, F.A., et al. (2016). Tumor microenvironment derived exosomes pleiotropically modulate cancer cell metabolism. Elife *5*, e10250.

42. Godoy, A., Montecinos, V.P., Gray, D.R., Sotomayor, P., Yau, J.M., Vethanayagam, R.R., Singh, S., Mohler, J.L., and Smith, G.J. (2011). Androgen deprivation induces rapid involution and recovery of human prostate vasculature. Am. J. Physiol. Endocrinol. Metab. *300*, E263–E275.

43. Godoy, A., Watts, A., Sotomayor, P., Montecinos, V.P., Huss, W.J., Onate, S.A., and Smith, G.J. (2008). Androgen receptor is causally involved in the homeostasis of the human prostate endothelial cell. Endocrinology *149*, 2959–2969.

44. Wang, X., Lee, S.O., Xia, S., Jiang, Q., Luo, J., Li, L., Yeh, S., and Chang, C. (2013). Endothelial cells enhance prostate cancer metastasis via IL-6–>androgen receptor–>TGF-beta–>MMP-9 signals. Mol. Cancer Ther. *12*, 1026–1037.

45. Georgiou, H.D., Namdarian, B., Corcoran, N.M., Costello, A.J., and Hovens, C.M. (2008). Circulating endothelial cells as biomarkers of prostate cancer. Nat. Clin. Pract. Urol. *5*, 445–454.

46. Hieken, T.J., Chen, J., Hoskin, T.L., Walther-Antonio, M., Johnson, S., Ramaker, S., Xiao, J., Radisky, D.C., Knutson, K.L., Kalari, K.R., et al. (2016). The microbiome of aseptically collected human breast tissue in benign and malignant disease. Sci. Rep. *6*, 30751.

47. Geis, A.L., Fan, H., Wu, X., Wu, S., Huso, D.L., Wolfe, J.L., Sears, C.L., Pardoll, D.M., and Housseau, F. (2015). Regulatory T-cell response to enterotoxigenic Bacteroides fragilis colonization triggers IL17-dependent colon carcinogenesis. Cancer Discov. *5*, 1098–1109.

48. He, Y., Fu, L., Li, Y., Wang, W., Gong, M., Zhang, J., Dong, X., Huang, J., Wang, Q., Mackay, C.R., et al. (2021). Gut microbial metabolites facilitate anticancer therapy efficacy by modulating cytotoxic CD8(+) T cell immunity. Cell Metab. *33*, 988–1000.e7.

49. Wang, H., Rong, X., Zhao, G., Zhou, Y., Xiao, Y., Ma, D., Jin, X., Wu, Y., Yan, Y., Yang, H., et al. (2022). The microbial metabolite trimethylamine N-oxide promotes antitumor immunity in triple-negative breast cancer. Cell Metab. *34*, 581–594.e8.

50. Sfanos, K.S., Sauvageot, J., Fedor, H.L., Dick, J.D., De Marzo, A.M., and Isaacs, W.B. (2008). A molecular analysis of prokaryotic and viral DNA sequences in prostate tissue from patients with prostate cancer indicates the presence of multiple and diverse microorganisms. Prostate *68*, 306–320.

51. Keay, S., Zhang, C.O., Baldwin, B.R., and Alexander, R.B. (1999). Polymerase chain reaction amplification of bacterial 16s rRNA genes in prostate biopsies from men without chronic prostatitis. Urology *53*, 487–491.

52. Krieger, J.N., Riley, D.E., Vesella, R.L., Miner, D.C., Ross, S.O., and Lange, P.H. (2000). Bacterial dna sequences in prostate tissue from patients with prostate cancer and chronic prostatitis. J. Urol. *164*, 1221–1228.

53. Davidsson, S., Ohlson, A.L., Andersson, S.O., Fall, K., Meisner, A., Fiorentino, M., Andrén, O., and Rider, J.R. (2013). CD4 helper T cells, CD8 cytotoxic T cells, and FOXP3(+) regulatory T cells with respect to lethal prostate cancer. Mod. Pathol. *26*, 448–455.

54. Kiniwa, Y., Miyahara, Y., Wang, H.Y., Peng, W., Peng, G., Wheeler, T.M., Thompson, T.C., Old, L.J., and Wang, R.F. (2007). CD8+ Foxp3+ regulatory T cells mediate immunosuppression in prostate cancer. Clin. Cancer Res. *13*, 6947–6958.

55. Sfanos, K.S., Bruno, T.C., Meeker, A.K., De Marzo, A.M., Isaacs, W.B., and Drake, C.G. (2009). Human prostate-infiltrating CD8+ T lymphocytes are oligoclonal and PD-1+. Prostate *69*, 1694–1703.

56. Le, D.T., Uram, J.N., Wang, H., Bartlett, B.R., Kemberling, H., Eyring, A.D., Skora, A.D., Luber, B.S., Azad, N.S., Laheru, D., et al. (2015). PD-1 blockade in tumors with mismatch-repair deficiency. N. Engl. J. Med. *372*, 2509–2520.

57. Robinson, D., Van Allen, E.M., Wu, Y.M., Schultz, N., Lonigro, R.J., Mosquera, J.M., Montgomery, B., Taplin, M.E., Pritchard, C.C., Attard, G., et al. (2015). Integrative clinical genomics of advanced prostate cancer. Cell *161*, 1215–1228.

58. Pritchard, C.C., Morrissey, C., Kumar, A., Zhang, X., Smith, C., Coleman, I., Salipante, S.J., Milbank, J., Yu, M., Grady, W.M., et al. (2014). Complex MSH2 and MSH6 mutations in hypermutated microsatellite unstable advanced prostate cancer. Nat. Commun. *5*, 4988.

59. Topalian, S.L., Hodi, F.S., Brahmer, J.R., Gettinger, S.N., Smith, D.C., McDermott, D.F., Powderly, J.D., Carvajal, R.D., Sosman, J.A., Atkins, M.B., et al. (2012). Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. N. Engl. J. Med. *366*, 2443–2454.

60. Martin, A.M., Nirschl, T.R., Nirschl, C.J., Francica, B.J., Kochel, C.M., van Bokhoven, A., Meeker, A.K., Lucia, M.S., Anders, R.A., DeMarzo, A.M., and Drake, C.G. (2015). Paucity of PD-L1 expression in prostate cancer: innate and adaptive immune resistance. Prostate Cancer Prostatic Dis. *18*, 325–332.

61. Lundholm, M., Schröder, M., Nagaeva, O., Baranov, V., Widmark, A., Mincheva-Nilsson, L., and Wikström, P. (2014). Prostate tumor-derived exosomes down-regulate NKG2D expression on natural killer cells and CD8+ T cells: mechanism of immune evasion. PLoS One *9*, e108925.

62. Miller, A.M., Lundberg, K., Ozenci, V., Banham, A.H., Hellström, M., Egevad, L., and Pisa, P. (2006). CD4+CD25high T cells are enriched in the tumor and peripheral blood of prostate cancer patients. J. Immunol. *177*, 7398–7405.

63. Flammiger, A., Weisbach, L., Huland, H., Tennstedt, P., Simon, R., Minner, S., Bokemeyer, C., Sauter, G., Schlomm, T., and Trepel, M. (2013). High tissue density of FOXP3+ T cells is associated with clinical outcome in prostate cancer. Eur. J. Cancer *49*, 1273–1279.

64. Linch, M., Goh, G., Hiley, C., Shanmugabavan, Y., McGranahan, N., Rowan, A., Wong, Y.N.S., King, H., Furness, A., Freeman, A., et al. (2017). Intratumoural evolutionary landscape of high-risk prostate cancer: the PROGENY study of genomic and immune parameters. Ann. Oncol. *28*, 2472–2480.

65. Vivier, E., Tomasello, E., Baratin, M., Walzer, T., and Ugolini, S. (2008). Functions of natural killer cells. Nat. Immunol. *9*, 503–510.

66. Pasero, C., Gravis, G., Granjeaud, S., Guerin, M., Thomassin-Piana, J., Rocchi, P., Salem, N., Walz, J., Moretta, A., and Olive, D. (2015). Highly effective NK cells are associated with good prognosis in patients with metastatic prostate cancer. Oncotarget *6*, 14360–14373.

67. Cortesi, F., Delfanti, G., Grilli, A., Calcinotto, A., Gorini, F., Pucci, F., Lucianò, R., Grioni, M., Recchia, A., Benigni, F., et al. (2018). Bimodal CD40/fas-dependent crosstalk between iNKT cells and tumor-associated macrophages impairs prostate cancer progression. Cell Rep. *22*, 3006–3020.

68. Ge, R., Wang, Z., and Cheng, L. (2022). Tumor microenvironment heterogeneity an important mediator of prostate cancer progression and therapeutic resistance. NPJ Precis. Oncol. *6*, 31.

69. Pasero, C., Gravis, G., Guerin, M., Granjeaud, S., Thomassin-Piana, J., Rocchi, P., Paciencia-Gros, M., Poizat, F., Bentobji, M., Azario-Cheillan, F., et al. (2016). Inherent and tumor-driven immune tolerance in the prostate microenvironment impairs natural killer cell antitumor activity. Cancer Res. *76*, 2153–2165.

70. Raulet, D.H. (2003). Roles of the NKG2D immunoreceptor and its ligands. Nat. Rev. Immunol. *3*, 781–790.

71. Wu, J.D., Higgins, L.M., Steinle, A., Cosman, D., Haugk, K., and Plymate, S.R. (2004). Prevalent expression of the immunostimulatory MHC class I chain-related molecule is counteracted by shedding in prostate cancer. J. Clin. Invest. *114*, 560–568.

72. Xu, L., Chen, X., Shen, M., Yang, D.R., Fang, L., Weng, G., Tsai, Y., Keng, P.C., Chen, Y., and Lee, S.O. (2018). Inhibition of IL-6-JAK/Stat3 signaling in castration-resistant prostate cancer cells enhances the NK cell-mediated cytotoxicity via alteration of PD-L1/NKG2D ligand levels. Mol. Oncol. *12*, 269–286.

73. Saga, K., Park, J., Nimura, K., Kawamura, N., Ishibashi, A., Nonomura, N., and Kaneda, Y. (2019). NANOG helps cancer cells escape NK cell attack by downregulating ICAM1 during tumorigenesis. J. Exp. Clin. Cancer Res. *38*, 416.

74. Li, K., Shi, H., Zhang, B., Ou, X., Ma, Q., Chen, Y., Shu, P., Li, D., and Wang, Y. (2021). Myeloid-derived suppressor cells as immunosuppressive regulators and therapeutic targets in cancer. Signal Transduct. Target. Ther. *6*, 362.

75. Calcinotto, A., Spataro, C., Zagato, E., Di Mitri, D., Gil, V., Crespo, M., De Bernardis, G., Losa, M., Mirenda, M., Pasquini, E., et al. (2018). IL-23 secreted by myeloid cells drives castration-resistant prostate cancer. Nature *559*, 363–369.

76. Chi, N., Tan, Z., Ma, K., Bao, L., and Yun, Z. (2014). Increased circulating myeloid-derived suppressor cells correlate with cancer stages, interleukin-8 and -6 in prostate cancer. Int. J. Clin. Exp. Med. *7*, 3181–3192.

77. Idorn, M., Køllgaard, T., Kongsted, P., Sengeløv, L., and Thor Straten, P. (2014). Correlation between frequencies of blood monocytic myeloid-derived suppressor cells, regulatory T cells and negative prognostic markers in patients with castration-resistant metastatic prostate cancer. Cancer Immunol. Immunother. *63*, 1177–1187.

78. Nguyen, D.P., Li, J., and Tewari, A.K. (2014). Inflammation and prostate cancer: the role of interleukin 6 (IL-6). BJU Int. *113*, 986–992.

79. Araki, S., Omori, Y., Lyn, D., Singh, R.K., Meinbach, D.M., Sandman, Y., Lokeshwar, V.B., and Lokeshwar, B.L. (2007). Interleukin-8 is a molecular determinant of androgen independence and progression in prostate cancer. Cancer Res. *67*, 6854–6862.

80. Feng, S., Cheng, X., Zhang, L., Lu, X., Chaudhary, S., Teng, R., Frederickson, C., Champion, M.M., Zhao, R., Cheng, L., et al. (2018). Myeloid-derived suppressor cells inhibit T cell activation through nitrating LCK in mouse cancers. Proc. Natl. Acad. Sci. USA. *115*, 10094–10099.

81. Martinez, F.O., and Gordon, S. (2014). The M1 and M2 paradigm of macrophage activation: time for reassessment. F1000prime Rep. *6*, 13.

82. Nonomura, N., Takayama, H., Nakayama, M., Nakai, Y., Kawashima, A., Mukai, M., Nagahara, A., Aozasa, K., and Tsujimura, A. (2011). Infiltration of tumour-associated macrophages in prostate biopsy specimens is predictive of disease progression after hormonal therapy for prostate cancer. BJU Int. *107*, 1918–1922.

83. Maolake, A., Izumi, K., Shigehara, K., Natsagdorj, A., Iwamoto, H., Kadomoto, S., Takezawa, Y., Machioka, K., Narimoto, K., Namiki, M., et al. (2017). Tumor-associated macrophages promote prostate cancer migration through activation of the CCL22-CCR4 axis. Oncotarget *8*, 9739–9751.

84. Di Mitri, D., Mirenda, M., Vasilevska, J., Calcinotto, A., Delaleu, N., Revandkar, A., Gil, V., Boysen, G., Losa, M., Mosole, S., et al. (2019). Re-Education of tumor-associated macrophages by CXCR2 blockade drives senescence and tumor inhibition in advanced prostate cancer. Cell Rep. *28*, 2156–2168.e5.

85. Escamilla, J., Schokrpur, S., Liu, C., Priceman, S.J., Moughon, D., Jiang, Z., Pouliot, F., Magyar, C., Sung, J.L., Xu, J., et al. (2015). CSF1 receptor targeting in prostate cancer reverses macrophage-mediated resistance to androgen blockade therapy. Cancer Res. *75*, 950–962.

86. Hamdy, F.C., Donovan, J.L., and Neal, D.E. (2017). 10-Year outcomes in localized prostate cancer. N. Engl. J. Med. *376*, 180.

87. de Bono, J.S., Logothetis, C.J., Molina, A., Fizazi, K., North, S., Chu, L., Chi, K.N., Jones, R.J., Goodman, O.B., Jr., Saad, F., et al. (2011). Abiraterone and increased survival in metastatic prostate cancer. N. Engl. J. Med. *364*, 1995–2005.

88. Nader, R., El Amm, J., and Aragon-Ching, J.B. (2018). Role of chemotherapy in prostate cancer. Asian J. Androl. *20*, 221–229.

89. Fitzgerald, K.A., Evans, J.C., McCarthy, J., Guo, J., Prencipe, M., Kearney, M., Watson, W.R., and O'Driscoll, C.M. (2014). The role of transcription factors in prostate cancer and potential for future RNA interference therapy. Expert Opin. Ther. Targets *18*, 633–649.

90. Vainio, P., Mpindi, J.P., Kohonen, P., Fey, V., Mirtti, T., Alanen, K.A., Perälä, M., Kallioniemi, O., and Iljin, K. (2012). High-throughput transcriptomic and RNAi analysis identifies AIM1, ERGIC1, TMED3 and TPX2 as potential drug targets in prostate cancer. PLoS One *7*, e39801.

91. Civenni, G., Malek, A., Albino, D., Garcia-Escudero, R., Napoli, S., Di Marco, S., Pinton, S., Sarti, M., Carbone, G.M., and Catapano, C.V. (2013). RNAi-mediated silencing of Myc transcription inhibits stem-like cell maintenance and tumorigenicity in prostate cancer. Cancer Res. *73*, 6816–6827.

92. Ashrafizadeh, M., Hushmandi, K., Rahmani Moghadam, E., Zarrin, V., Hosseinzadeh Kashani, S., Bokaie, S., Najafi, M., Tavakol, S., Mohammadinejad, R., Nabavi, N., et al. (2020). Progress in delivery of siRNA-based therapeutics employing nano-vehicles for treatment of prostate cancer. Bioengineering (Basel) *7*, 91.

93. Binzel, D.W., Shu, Y., Li, H., Sun, M., Zhang, Q., Shu, D., Guo, B., and Guo, P. (2016). Specific delivery of MiRNA for high efficient inhibition of prostate cancer by RNA nanotechnology. Mol. Ther. *24*, 1267–1277.

94. Wang, X., Zhou, Q., Li, X., Gan, X., Liu, P., Feng, X., Fang, G., and Liu, Y. (2022). Insights into aptamer-drug delivery systems against prostate cancer. Molecules *27*, 3446.

95. Sheibani, M., Azizi, Y., Shayan, M., Nezamoleslami, S., Eslami, F., Farjoo, M.H., and Dehpour, A.R. (2022). Doxorubicin-induced cardiotoxicity: an overview on preclinical therapeutic approaches. Cardiovasc. Toxicol. *22*, 292–310.

96. Tang, L., Tong, R., Coyle, V.J., Yin, Q., Pondenis, H., Borst, L.B., Cheng, J., and Fan, T.M. (2015). Targeting tumor vasculature with aptamer-functionalized doxorubicin-polylactide nanoconjugates for enhanced cancer therapy. ACS Nano *9*, 5072–5081.

97. Taghdisi, S.M., Danesh, N.M., Ramezani, M., Yazdian-Robati, R., and Abnous, K. (2018). A novel AS1411 aptamer-based three-way junction pocket DNA nanostructure loaded with doxorubicin for targeting cancer cells in vitro and in vivo. Mol. Pharm. *15*, 1972–1978.

98. Donovan, K.A., Ferguson, F.M., Bushman, J.W., Eleuteri, N.A., Bhunia, D., Ryu, S., Tan, L., Shi, K., Yue, H., Liu, X., et al. (2020). Mapping the degradable kinome provides a resource for expedited degrader development. Cell *183*, 1714–1731.e10.

99. Dale, B., Cheng, M., Park, K.S., Kaniskan, H.Ü., Xiong, Y., and Jin, J. (2021). Advancing targeted protein degradation for cancer therapy. Nat. Rev. Cancer *21*, 638–654.

100. Salami, J., Alabi, S., Willard, R.R., Vitale, N.J., Wang, J., Dong, H., Jin, M., McDonnell, D.P., Crew, A.P., Neklesa, T.K., and Crews, C.M. (2018). Androgen receptor degradation by the proteolysis-targeting chimera ARCC-4 outperforms enzalutamide in cellular models of prostate cancer drug resistance. Commun. Biol. *1*, 100.

101. Han, X., Wang, C., Qin, C., Xiang, W., Fernandez-Salas, E., Yang, C.Y., Wang, M., Zhao, L., Xu, T., Chinnaswamy, K., et al. (2019). Discovery of ARD-69 as a highly potent proteolysis targeting chimera (PROTAC) degrader of androgen receptor (AR) for the treatment of prostate cancer. J. Med. Chem. *62*, 941–964.

102. Kregel, S., Wang, C., Han, X., Xiao, L., Fernandez-Salas, E., Bawa, P., McCollum, B.L., Wilder-Romans, K., Apel, I.J., Cao, X., et al. (2020). Androgen receptor degraders overcome common resistance mechanisms developed during prostate cancer treatment. Neoplasia *22*, 111–119.

103. Brahmer, J., Reckamp, K.L., Baas, P., Crinò, L., Eberhardt, W.E.E., Poddubskaya, E., Antonia, S., Pluzanski, A., Vokes, E.E., Holgado, E., et al. (2015). Nivolumab versus docetaxel in advanced squamous-cell non-small-cell lung cancer. N. Engl. J. Med. *373*, 123–135.

104. Robert, C., Ribas, A., Schachter, J., Arance, A., Grob, J.J., Mortier, L., Daud, A., Carlino, M.S., McNeil, C.M., Lotem, M., et al. (2019). Pembrolizumab versus ipilimumab in advanced melanoma (KEYNOTE-006): post-hoc 5-year results from an open-label, multicentre, randomised, controlled, phase 3 study. Lancet Oncol. *20*, 1239–1251.

105. Sutherland, S.I.M., Ju, X., Horvath, L.G., and Clark, G.J. (2021). Moving on from sipuleucel-T: new dendritic cell vaccine strategies for prostate cancer. Front. Immunol. *12*, 641307.

106. Kantoff, P.W., Higano, C.S., Shore, N.D., Berger, E.R., Small, E.J., Penson, D.F., Redfern, C.H., Ferrari, A.C., Dreicer, R., Sims, R.B., et al. (2010). Sipuleucel-T immunotherapy for castration-resistant prostate cancer. N. Engl. J. Med. *363*, 411–422.

107. Huber, M.L., Haynes, L., Parker, C., and Iversen, P. (2012). Interdisciplinary critique of sipuleucel-T as immunotherapy in castration-resistant prostate cancer. J. Natl. Cancer Inst. *104*, 273–279.

108. Gulley, J.L., Borre, M., Vogelzang, N.J., Ng, S., Agarwal, N., Parker, C.C., Pook, D.W., Rathenborg, P., Flaig, T.W., Carles, J., et al. (2019). Phase III trial of PROSTVAC in asymptomatic or minimally symptomatic metastatic castration-resistant prostate cancer. J. Clin. Oncol. *37*, 1051–1061.

109. Marshall, C.H., Fu, W., Wang, H., Park, J.C., DeWeese, T.L., Tran, P.T., Song, D.Y., King, S., Afful, M., Hurrelbrink, J., et al. (2021). Randomized phase II trial of sipuleucel-T with or without radium-223 in men with bone-metastatic castration-resistant prostate cancer. Clin. Cancer Res. *27*, 1623–1630.

110. Hannan, R., Dohopolski, M.J., Pop, L.M., Mannala, S., Watumull, L., Mathews, D., Gao, A., Garant, A., Arriaga, Y.E., Bowman, I., et al. (2022). Phase II trial of sipuleucel-T and stereotactic ablative body radiation for patients with metastatic castrate-resistant prostate cancer. Biomedicines *10*, 1419.

111. Einstein, D.J., Arai, S., and Balk, S.P. (2019). Targeting the androgen receptor and overcoming resistance in prostate cancer. Curr. Opin. Oncol. *31*, 175–182.

112. Silva-Santos, B., Serre, K., and Norell, H. (2015). Gammadelta T cells in cancer. Nat. Rev. Immunol. *15*, 683–691.

113. Liu, Z., Eltoum, I.E.A., Guo, B., Beck, B.H., Cloud, G.A., and Lopez, R.D. (2008). Protective immunosurveillance and therapeutic antitumor activity of gammadelta T cells demonstrated in a mouse model of prostate cancer. J. Immunol. *180*, 6044–6053.

114. Dieli, F., Vermijlen, D., Fulfaro, F., Caccamo, N., Meraviglia, S., Cicero, G., Roberts, A., Buccheri, S., D'Asaro, M., Gebbia, N., et al. (2007). Targeting human {gamma}delta} T cells with zoledronate and interleukin-2 for immunotherapy of hormone-refractory prostate cancer. Cancer Res. *67*, 7450–7457.

115. Sadelain, M., Rivière, I., and Riddell, S. (2017). Therapeutic T cell engineering. Nature *545*, 423–431.

116. Davenport, A.J., Jenkins, M.R., Cross, R.S., Yong, C.S., Prince, H.M., Ritchie, D.S., Trapani, J.A., Kershaw, M.H., Darcy, P.K., and Neeson, P.J. (2015). CAR-T cells inflict sequential killing of multiple tumor target cells. Cancer Immunol. Res. *3*, 483–494.

117. Scholler, J., Brady, T.L., Binder-Scholl, G., Hwang, W.T., Plesa, G., Hege, K.M., Vogel, A.N., Kalos, M., Riley, J.L., Deeks, S.G., et al. (2012). Decade-long safety and function of retroviral-modified chimeric antigen receptor T cells. Sci. Transl. Med. *4*, 132ra53.

118. Wolf, P., Alzubi, J., Gratzke, C., and Cathomen, T. (2021). The potential of CAR T cell therapy for prostate cancer. Nat. Rev. Urol. *18*, 556–571.

119. Junghans, R.P., Ma, Q., Rathore, R., Gomes, E.M., Bais, A.J., Lo, A.S.Y., Abedi, M., Davies, R.A., Cabral, H.J., Al-Homsi, A.S., and Cohen, S.I. (2016). Phase I trial of anti-PSMA designer CAR-T cells in prostate cancer: possible role for interacting interleukin 2-T cell pharmacodynamics as a determinant of clinical response. Prostate *76*, 1257–1270.

120. Deng, Z., Wu, Y., Ma, W., Zhang, S., and Zhang, Y.Q. (2015). Adoptive T-cell therapy of prostate cancer targeting the cancer stem cell antigen EpCAM. BMC Immunol. *16*, 1.

121. He, C., Zhou, Y., Li, Z., Farooq, M.A., Ajmal, I., Zhang, H., Zhang, L., Tao, L., Yao, J., Du, B., et al. (2020). Co-expression of IL-7 improves NKG2D-based CAR T cell therapy on prostate cancer by enhancing the expansion and inhibiting the apoptosis and exhaustion. Cancers (Basel) *12*, 1969.

122. Sato, K., Sato, N., Xu, B., Nakamura, Y., Nagaya, T., Choyke, P.L., Hasegawa, Y., and Kobayashi, H. (2016). Spatially selective depletion of tumor-associated regulatory T cells with near-infrared photoimmunotherapy. Sci. Transl. Med. *8*, 352ra110.

123. Litzinger, M.T., Fernando, R., Curiel, T.J., Grosenbach, D.W., Schlom, J., and Palena, C. (2007). IL-2 immunotoxin denileukin diftitox reduces regulatory T cells and enhances vaccine-mediated T-cell immunity. Blood *110*, 3192–3201.

124. Keller, J.M., Schade, G.R., Ives, K., Cheng, X., Rosol, T.J., Piert, M., Siddiqui, J., Roberts, W.W., and Keller, E.T. (2013). A novel canine model for prostate cancer. Prostate *73*, 952–959.

125. Maeda, S., Motegi, T., Iio, A., Kaji, K., Goto-Koshino, Y., Eto, S., Ikeda, N., Nakagawa, T., Nishimura, R., Yonezawa, T., et al. (2022). Anti-CCR4 treatment depletes regulatory T cells and leads to clinical activity in a canine model of advanced prostate cancer. J Immunother Cancer *10*, e003731.

126. Kim, Y.H., Bagot, M., Pinter-Brown, L., Rook, A.H., Porcu, P., Horwitz, S.M., Whittaker, S., Tokura, Y., Vermeer, M., Zinzani, P.L., et al. (2018). Mogamulizumab versus vorinostat in previously treated cutaneous T-cell lymphoma (MAVORIC): an international, open-label, randomised, controlled phase 3 trial. Lancet Oncol. *19*, 1192–1204.

127. Peggs, K.S., Quezada, S.A., Chambers, C.A., Korman, A.J., and Allison, J.P. (2009). Blockade of CTLA-4 on both effector and regulatory T cell compartments contributes to the antitumor activity of anti-CTLA-4 antibodies. J. Exp. Med. *206*, 1717–1725.

128. Gao, J., Ward, J.F., Pettaway, C.A., Shi, L.Z., Subudhi, S.K., Vence, L.M., Zhao, H., Chen, J., Chen, H., Efstathiou, E., et al. (2017). VISTA is an inhibitory immune checkpoint that is increased after ipilimumab therapy in patients with prostate cancer. Nat. Med. *23*, 551–555.

129. John, P., Pulanco, M.C., Galbo, P.M., Jr., Wei, Y., Ohaegbulam, K.C., Zheng, D., and Zang, X. (2022). The immune checkpoint B7x expands tumor-infiltrating Tregs and promotes resistance to anti-CTLA-4 therapy. Nat. Commun. *13*, 2506.

130. Zhao, E., Wang, L., Dai, J., Kryczek, I., Wei, S., Vatan, L., Altuwaijri, S., Sparwasser, T., Wang, G., Keller, E.T., and Zou, W. (2012). Regulatory T cells in the bone marrow microenvironment in patients with prostate cancer. Oncoimmunology *1*, 152–161.

131. Chen, D.S., and Mellman, I. (2017). Elements of cancer immunity and the cancer-immune set point. Nature *541*, 321–330.

132. Ledford, H., Else, H., and Warren, M. (2018). Cancer immunologists scoop medicine Nobel prize. Nature *562*, 20–21.

133. Graff, J.N., Alumkal, J.J., Drake, C.G., Thomas, G.V., Redmond, W.L., Farhad, M., Cetnar, J.P., Ey, F.S., Bergan, R.C., Slottke, R., and Beer, T.M. (2016). Early evidence of anti-PD-1 activity in enzalutamide-resistant prostate cancer. Oncotarget *7*, 52810–52817.

134. Hansen, A.R., Massard, C., Ott, P.A., Haas, N.B., Lopez, J.S., Ejadi, S., Wallmark, J.M., Keam, B., Delord, J.P., Aggarwal, R., et al. (2018). Pembrolizumab for advanced prostate adenocarcinoma: findings of the KEYNOTE-028 study. Ann. Oncol. *29*, 1807–1813.

135. Antonarakis, E.S., Piulats, J.M., Gross-Goupil, M., Goh, J., Ojamaa, K., Hoimes, C.J., Vaishampayan, U., Berger, R., Sezer, A., and Alanko, T. (2020). Pembrolizumab for treatment-refractory metastatic castration-resistant prostate cancer: multicohort, open-label phase II KEYNOTE-199 study. J. Clin. Oncol. *38*, 395–405.

136. Graff, J.N., Beer, T.M., Alumkal, J.J., Slottke, R.E., Redmond, W.L., Thomas, G.V., Thompson, R.F., Wood, M.A., Koguchi, Y., Chen, Y., et al. (2020). A phase II single-arm study of pembrolizumab with enzalutamide in men with metastatic castration-resistant prostate cancer progressing on enzalutamide alone. J. Immunother. Cancer *8*, e000642.

137. Graff, J.N., Liang, L.W., Kim, J., and Stenzl, A. (2021). KEYNOTE-641: a Phase III study of pembrolizumab plus enzalutamide for metastatic castration-resistant prostate cancer. Future Oncol. *17*, 3017–3026.

138. Kwon, E.D., Drake, C.G., Scher, H.I., Fizazi, K., Bossi, A., van den Eertwegh, A.J.M., Krainer, M., Houede, N., Santos, R., Mahammedi, H., et al. (2014). Ipilimumab versus placebo after radiotherapy in patients with metastatic castration-resistant prostate cancer that had progressed after docetaxel chemotherapy (CA184-043): a multicentre, randomised, double-blind, phase 3 trial. Lancet Oncol. *15*, 700–712.

139. Beer, T.M., Kwon, E.D., Drake, C.G., Fizazi, K., Logothetis, C., Gravis, G., Ganju, V., Polikoff, J., Saad, F., Humanski, P., et al. (2017). Randomized, double-blind, phase III trial of ipilimumab versus placebo in asymptomatic or minimally symptomatic patients with metastatic chemotherapy-naive castration-resistant prostate cancer. J. Clin. Oncol. *35*, 40–47.

140. Fizazi, K., Drake, C.G., Beer, T.M., Kwon, E.D., Scher, H.I., Gerritsen, W.R., Bossi, A., den Eertwegh, A.J.M.v., Krainer, M., Houede, N., et al. (2020). Final analysis of the ipilimumab versus placebo following radiotherapy phase III trial in postdocetaxel metastatic castration-resistant prostate cancer identifies an excess of long-term survivors. Eur. Urol. *78*, 822–830.

141. Sharma, P., Pachynski, R.K., Narayan, V., Fléchon, A., Gravis, G., Galsky, M.D., Mahammedi, H., Patnaik, A., Subudhi, S.K., Ciprotti, M., et al. (2020). Nivolumab plus ipilimumab for metastatic castration-resistant prostate cancer: preliminary analysis of patients in the CheckMate 650 trial. Cancer Cell *38*, 489–499.e3.

142. Jansen, M.C., van Hillegersberg, R., Schoots, I.G., Levi, M., Beek, J.F., Crezee, H., and van Gulik, T.M. (2010). Cryoablation induces greater inflammatory and coagulative responses than radiofrequency ablation or laser induced thermotherapy in a rat liver model. Surgery *147*, 686–695.

143. Waitz, R., Solomon, S.B., Petre, E.N., Trumble, A.E., Fassò, M., Norton, L., and Allison, J.P. (2012). Potent induction of tumor immunity by combining tumor cryoablation with anti-CTLA-4 therapy. Cancer Res. *72*, 430–439.

144. Benzon, B., Glavaris, S.A., Simons, B.W., Hughes, R.M., Ghabili, K., Mullane, P., Miller, R., Nugent, K., Shinder, B., Tosoian, J., et al. (2018). Combining immune check-point blockade and cryoablation in an immunocompetent hormone sensitive murine model of prostate cancer. Prostate Cancer Prostatic Dis. *21*, 126–136.

145. Lu, X., Horner, J.W., Paul, E., Shang, X., Troncoso, P., Deng, P., Jiang, S., Chang, Q., Spring, D.J., Sharma, P., et al. (2017). Effective combinatorial immunotherapy for castration-resistant prostate cancer. Nature *543*, 728–732.

146. Korentzelos, D., Wells, A., and Clark, A.M. (2022). Interferon-gamma increases sensitivity to chemotherapy and provides immunotherapy targets in models of metastatic castration-resistant prostate cancer. Sci. Rep. *12*, 6657.

147. Peng, S., Hu, P., Xiao, Y.T., Lu, W., Guo, D., Hu, S., Xie, J., Wang, M., Yu, W., Yang, J., et al. (2022). Single-cell analysis reveals EP4 as a target for restoring T-cell infiltration and sensitizing prostate cancer to immunotherapy. Clin. Cancer Res. *28*, 552–567.

148. Lopez-Bujanda, Z.A., Haffner, M.C., Chaimowitz, M.G., Chowdhury, N., Venturini, N.J., Patel, R.A., Obradovic, A., Hansen, C.S., Jacków, J., Maynard, J.P., et al. (2021). Castration-mediated IL-8 promotes myeloid infiltration and prostate cancer progression. Nat. Cancer *2*, 803–818.

149. Qi, Z., Xu, Z., Zhang, L., Zou, Y., Li, J., Yan, W., Li, C., Liu, N., and Wu, H. (2022). Overcoming resistance to immune checkpoint therapy in PTEN-null prostate cancer by intermittent anti-PI3Kalpha/beta/delta treatment. Nat. Commun. *13*, 182.

150. Burbach, B.J., O'Flanagan, S.D., Shao, Q., Young, K.M., Slaughter, J.R., Rollins, M.R., Street, T.J.L., Granger, V.E., Beura, L.K., Azarin, S.M., et al. (2021). Irreversible electroporation augments checkpoint immunotherapy in prostate cancer and promotes tumor antigen-specific tissue-resident memory CD8+ T cells. Nat. Commun. *12*, 3862.

151. Harada, N., Hanaoka, R., Horiuchi, H., Kitakaze, T., Mitani, T., Inui, H., and Yamaji, R. (2016). Castration influences intestinal microflora and induces abdominal obesity in high-fat diet-fed mice. Sci. Rep. *6*, 23001.

152. Nam, Y.D., Kim, H.J., Seo, J.G., Kang, S.W., and Bae, J.W. (2013). Impact of pelvic radiotherapy on gut microbiota of gynecological cancer patients revealed by massive pyrosequencing. PLoS One *8*, e82659.

153. Viaud, S., Saccheri, F., Mignot, G., Yamazaki, T., Daillère, R., Hannani, D., Enot, D.P., Pfirschke, C., Engblom, C., Pittet, M.J., et al. (2013). The intestinal microbiota modulates the anticancer immune effects of cyclophosphamide. Science *342*, 971–976.

154. Vétizou, M., Pitt, J.M., Daillère, R., Lepage, P., Waldschmitt, N., Flament, C., Rusakiewicz, S., Routy, B., Roberti, M.P., Duong, C.P.M., et al. (2015). Anticancer

immunotherapy by CTLA-4 blockade relies on the gut microbiota. Science *350*, 1079–1084.

155. Gopalakrishnan, V., Spencer, C.N., Nezi, L., Reuben, A., Andrews, M.C., Karpinets, T.V., Prieto, P.A., Vicente, D., Hoffman, K., Wei, S.C., et al. (2018). Gut microbiome modulates response to anti-PD-1 immunotherapy in melanoma patients. Science *359*, 97–103.

156. Cimadamore, A., Santoni, M., Massari, F., Gasparrini, S., Cheng, L., Lopez-Beltran, A., Montironi, R., and Scarpelli, M. (2019). Microbiome and cancers, with focus on genitourinary tumors. Front. Oncol. *9*, 178.

157. Pernigoni, N., Zagato, E., Calcinotto, A., Troiani, M., Mestre, R.P., Calì, B., Attanasio, G., Troisi, J., Minini, M., Mosole, S., et al. (2021). Commensal bacteria promote endocrine resistance in prostate cancer through androgen biosynthesis. Science *374*, 216–224.

158. Rathinam, V.A.K., Zhao, Y., and Shao, F. (2019). Innate immunity to intracellular LPS. Nat. Immunol. *20*, 527–533.

159. Santos, J.C., Boucher, D., Schneider, L.K., Demarco, B., Dilucca, M., Shkarina, K., Heilig, R., Chen, K.W., Lim, R.Y.H., and Broz, P. (2020). Human GBP1 binds LPS to initiate assembly of a caspase-4 activating platform on cytosolic bacteria. Nat. Commun. *11*, 3276.

160. Sivan, A., Corrales, L., Hubert, N., Williams, J.B., Aquino-Michaels, K., Earley, Z.M., Benyamin, F.W., Lei, Y.M., Jabri, B., Alegre, M.L., et al. (2015). Commensal Bifidobacterium promotes antitumor immunity and facilitates anti-PD-L1 efficacy. Science *350*, 1084–1089.

161. Anker, J.F., Naseem, A.F., Mok, H., Schaeffer, A.J., Abdulkadir, S.A., and Thumbikat, P. (2018). Multi-faceted immunomodulatory and tissue-tropic clinical bacterial isolate potentiates prostate cancer immunotherapy. Nat. Commun. *9*, 1591.

162. Spencer, C.N., McQuade, J.L., Gopalakrishnan, V., McCulloch, J.A., Vetizou, M., Cogdill, A.P., Khan, M.A.W., Zhang, X., White, M.G., Peterson, C.B., et al. (2021). Dietary fiber and probiotics influence the gut microbiome and melanoma immunotherapy response. Science *374*, 1632–1640.

163. Levy, J.M.M., Towers, C.G., and Thorburn, A. (2017). Targeting autophagy in cancer. Nat. Rev. Cancer *17*, 528–542.

164. Funderburk, S.F., Wang, Q.J., and Yue, Z. (2010). The Beclin 1-VPS34 complex–at the crossroads of autophagy and beyond. Trends Cell Biol. *20*, 355–362.

165. Noman, M.Z., Parpal, S., Van Moer, K., Xiao, M., Yu, Y., Viklund, J., De Milito, A., Hasmim, M., Andersson, M., Amaravadi, R.K., et al. (2020). Inhibition of Vps34 reprograms cold into hot inflamed tumors and improves anti-PD-1/PD-L1 immunotherapy. Sci. Adv. *6*, eaax7881.

166. Dyshlovoy, S.A., Otte, K., Alsdorf, W.H., Hauschild, J., Lange, T., Venz, S., Bauer, C.K., Bähring, R., Amann, K., Mandanchi, R., et al. (2016). Marine compound rhizochalinin shows high in vitro and in vivo efficacy in castration resistant prostate cancer. Oncotarget *7*, 69703–69717.

167. Qiao, Y., Choi, J.E., Tien, J.C., Simko, S.A., Rajendiran, T., Vo, J.N., Delekta, A.D., Wang, L., Xiao, L., Hodge, N.B., et al. (2021). Autophagy inhibition by targeting PIKfyve potentiates response to immune checkpoint blockade in prostate cancer. Nat. Cancer *2*, 978–993.

168. Topper, M.J., Vaz, M., Chiappinelli, K.B., DeStefano Shields, C.E., Niknafs, N., Yen, R.W.C., Wenzel, A., Hicks, J., Ballew, M., Stone, M., et al. (2017). Epigenetic therapy ties MYC depletion to reversing immune evasion and treating lung cancer. Cell *171*, 1284–1300.e21.

169. Sheng, W., LaFleur, M.W., Nguyen, T.H., Chen, S., Chakravarthy, A., Conway, J.R., Li, Y., Chen, H., Yang, H., Hsu, P.H., et al. (2018). LSD1 ablation stimulates anti-tumor immunity and enables checkpoint blockade. Cell *174*, 549–563.e19.

170. Loo Yau, H., Ettayebi, I., and De Carvalho, D.D. (2019). The cancer epigenome: exploiting its vulnerabilities for immunotherapy. Trends Cell Biol. *29*, 31–43.

171. Liu, Y., Liang, G., Xu, H., Dong, W., Dong, Z., Qiu, Z., Zhang, Z., Li, F., Huang, Y., Li, Y., et al. (2021). Tumors exploit FTO-mediated regulation of glycolytic metabolism to evade immune surveillance. Cell Metab. *33*, 1221–1233.e11.

172. Wang, J., Sanmamed, M.F., Datar, I., Su, T.T., Ji, L., Sun, J., Chen, L., Chen, Y., Zhu, G., Yin, W., et al. (2019). Fibrinogen-like protein 1 is a major immune inhibitory ligand of LAG-3. Cell *176*, 334–347.e12.

173. Poggio, M., Hu, T., Pai, C.C., Chu, B., Belair, C.D., Chang, A., Montabana, E., Lang, U.E., Fu, Q., Fong, L., and Blelloch, R. (2019). Suppression of exosomal PD-L1 induces systemic anti-tumor immunity and memory. Cell *177*, 414–427.e13.