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Review Article

Tumor–Immune Interactions in Prostate Cancer: Insights from Single-Cell and Spatial Genomics

Rita Onyebuchi Ogboh[1], Ejembi Bright O.[2], Adebola Ayisat Effa[3], Blessing Chinonye Okoro[4], Anthony Kosisochukwu Anyabuoke[5]

[1]*Department of Pharmacology and Toxicology, Niger Delta University, Yenagoa, Nigeria*
[2]*Department of Medicine and Surgery, University of Jos, Jos, Nigeria*
[3]*Department of Product Development and Quality Assurance, Nigeria Natural Medicine Development Agency, Lagos, Nigeria*
[4]*Department of Medical Laboratory Science, Imo State University, Owerri, Imo State, Nigeria*
[5]*Department of Biology, Columbus State University, Columbus, GA, USA*

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Corresponding author's email: ritzypearl367@gmail.com

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ABSTRACT

Prostate cancer still remains one of the most common cancers in men worldwide, and it is a great therapeutic challenge, especially in the field of immunotherapeutics. The tumour microenvironment (TME) is immunologically "cold" in prostate cancer, and influenced by intrinsic molecular characteristics of the disease such as androgen receptor (AR) signalling, PTEN loss, and lineage plasticity towards neuroendocrine prostate cancer (NEPC). Together, these aspects inhibit antigen presentation, block the entry of cytotoxic T cells and help to establish spatially organised immunosuppressive niches, providing a rational explanation for the clinical variability and partial efficacy of immune-based therapies.

Traditional bulk genomic approaches have provided important insights into tumour biology but are unable to capture the cellular and spatial complexity of tumour–immune interactions. These developments have been spurred by recent advancements in single-cell RNA sequencing (scRNA-seq) and spatial transcriptomics, which allow to detect individual cell subpopulations within intact tumour tissues, such as exhausted T cells co-expressing PD-1, TIM-3, LAG-3 and TIGIT, immunosuppressive SPP1+ macrophages and various cancer-associated fibroblast subpopulations. These technologies have identified specific immune exclusion sites, stromal–epithelial immune silencing barriers, and therapeutic resistance and immune evasion regulatory programs in the context of prostate cancer specifically.

However, there are still many technical challenges that need to be overcome, such as the lack of patient samples and their demographic diversity, data integration, lack of spatial characterisation of bone metastases and difficulties in clinical translation. Comprehensive multi-omics atlases, AI-driven spatial pattern recognition, functional validation of potential targets and prospective clinical trials based on biomarkers are all important areas for future research. They show significant potential for the creation of better, personalized immunotherapeutic treatment for prostate cancer.

Keywords: Prostate Cancer; Tumour Microenvironment; Tumour–Immune Interactions; Single-Cell RNA Sequencing; Spatial Transcriptomics; Androgen Receptor Signalling; PTEN Loss; T-Cell Exhaustion; Immunotherapy Resistance; Precision Oncology

Introduction

Clinical and Biological Context

Prostate cancer is a highly prevalent form of cancer among men and is a major cause of cancer-related death (1). It is an increasing problem worldwide due partly to an aging population, better screening and higher life expectancy. While the application of earlier detection and targeted treatment has led to better patient outcomes, there is a significant proportion of patients who develop advanced- or metastatic-stage disease and who have limited treatment choices with poor prognosis. Prostate cancer has a relatively weak and unpredictable response to immunotherapy compared to other cancers, which are more immunogenic and driven by mutations.Prostate cancer is less immunogenic than other cancers and the response to immunotherapy is less predictable and poor, compared to other cancers that are driven by mutations. Immune checkpoint inhibitors have provided durable clinical benefit in a small proportion of patients (those who are mismatch repair deficient or high microsatellite instability, < 3-5% of metastatic cases). Single agent checkpoint blockade has not been shown to be effective in prostate cancer, with clinical trials of pembrolizumab, nivolumab and ipilimumab in unselected prostate cancer populations reporting objective response rates of <10% in most cases (11). The mechanisms by which prostate cancer immunobiology differs from more immunogenic tumours is not just the lack of immune infiltrates, but the active co-option of specific molecular pathways: AR signalling, PTEN loss, Wnt/β-catenin activation, and TGF-β secretion, which are all known to promote suppression of antigen presentation, exclusion of T cells and the establishment of spatially organised immunosuppressive niches. They stand out by the presence of low T-cell infiltration, less neoantigen load, and the prevalence of immunosuppressive myeloid populations, the latter of which is referred to as the immunologically “cold” TME. It is essential to have knowledge of these mechanisms at cellular and tissue-level resolution to design rationally tailored immunotherapies (9).

Limitations of Conventional Approaches

Conventional approaches suffer from a number of limitations, such as: Traditional genomics and transcriptomics approaches have yielded important information about prostate cancer such as recurrent genetic changes and deregulated signalling pathways. These methods are, however, mainly bulk sequencing methods, which combine signals from a heterogenous cell population. Consequently, they mask the activity of rare or distinct subsets of cells within the tumor that may fulfill important roles in tumor progression and immune regulation (3). Bulk methods are also unable to uncover the cellular sources of molecular signals in the TME. They are unable to accurately separate or identify signals emanating from cancer cells from signals from infiltrating immune and stromal cells, or to describe the physical organisation of cells. However these techniques offer little insight into the spatial distribution of cells within tumour tissue, into the distribution of immune cells relative to the malignant glands or whether immune exclusion is a result of physical barriers in the stromal matrix, of repulsive chemokine gradients, or of other stromal barriers — some of the most crucial and poorly understood features of the prostate cancer microenvironment (4).

Emergence of Next-Generation Genomic Technologies

How can we realize the next generation of genomic technologies?How to achieve next-generation genomic technologies? Single-cell RNA sequencing has become a paradigm-shifting approach for studying the transcriptome transcriptome-wide at single-cell resolution (5), thus allowing for a dissection of the cellular heterogeneity of complex tissues. This allows to identify distinct subpopulations, rare cell states, and dynamic programs of cell function that may go undetected in bulk measurements. scRNA-seq applications have been used in prostate cancer to classify a variety of T-cell states, from naïve to progenitor exhausted and terminally exhausted, to catalogue the transcriptional diversity of tumour-associated macrophages, and to define tumour epithelial subpopulations with features of lineage plasticity associated with treatment resistance. To complement scRNA-seq, there are technologies that enable mapping of gene expression to defined coordinates within intact tissue sections, called spatial transcriptomics platforms, including 10x Visium, Slide-seq, MERFISH, Visium HD and CosMx SMI (6). Platforms have different attributes depending on the resolution, number of genes they can detect, and how they fit with clinical samples (Table 3). They preserve spatial context to allow identification of immune-excluded and immune-infiltrated regions, spatially constrained immunosuppressive zones and the architecture of tumour–stroma boundaries that influence immune cell access. These two modalities have now been recognized to be important for a systems level understanding of prostate cancer TME in conjunction with epigenomic and proteomic and clinical data (7).

Scope and Objectives of the Review

This review summarises the knowledge on tumour–immune interactions in prostate cancer, focusing on new knowledge gained from single-cell

and spatial genomic technologies. It explores the ways these approaches have contributed to the understanding of cellular composition, functional states and spatial organisation of the TME and critically reviews the key studies that have driven the field. Another goal is to shift from a descriptive to a mechanistic and critical analysis of how single-cell and spatial techniques can complement each other to better understand the mechanisms behind immune evasion in prostate cancer. As disease-specific features of immune suppression, we explore AR signalling, loss of PTEN, castration resistance, neuroendocrine differentiation, bone metastatic biology, and how these features might be translated to the development of immunotherapies and patient stratification based on biomarkers.

Literature Search Strategy

The material for this narrative review was identified by systematic literature search in PubMed, Scopus, Web of Science and Google Scholar of articles published till mid of 2026. Combinations of the following terms were used in searching: "prostate cancer" and "tumour microenvironment", "single-cell RNA sequencing" and "spatial transcriptomics", "immune checkpoint" and "T-cell exhaustion", "tumour-associated macrophage" and "myeloid-derived suppressor cells", "androgen receptor signalling" and "PTEN loss", "caspitation-resistant prostate cancer" and "neuroendocrine prostate cancer", "bone metastasis" and "lineage plasticity", "multi-omics integration" and "immunotherapy". Identified articles were manually screened for additional relevant articles according to reference lists. Special focus was given to landmark prostate cancer publications, high impact single-cell and spatial genomics publications and recent advances in computational integration. This is a narrative review, therefore, a systematic search protocol and a PRISMA diagram were not applied and no synthesis of the number of studies was conducted. Wherever possible, relevant studies from other solid tumour types are cited, but prostate cancer evidence is used more heavily throughout.

Disease-Specific Drivers of Immune Suppression

Androgen Receptor Signalling and Immune Regulation

The androgen receptor is the key driver of prostate cancer biology, and has broad and diverse effects on the prostate tumour immune microenvironment. There are at least 3 major ways that AR signalling blunts anti-tumour immunity. First, in tumour epithelial cells, AR directly down-modulates the machinery of antigen presentation, such as peptide loading complex and proteasomal processing pathways, that are required for the recognition of CTLs. Second, AR decreases the expression of CCL5 and CXCL9/10, chemokines that are essential for the recruitment of cytotoxic T cells and promote a chemokine profile of immunosuppression. Third, androgens have direct immunomodulatory effects on T cells and myeloid populations; androgens are inhibitory to the proliferation and effector function of CD8+ T cells.

In mice and in humans, androgen deprivation therapy (ADT) can promote infiltration of T cells and partially restore immune function, supporting the concept of using this with immune checkpoint inhibition. These combinations are being studied in phase II trials in metastatic CRPC, including one trial called KEYLINK-010 and another trial named KEYNOTE-641, with inconsistent results. Importantly, scRNA-seq analysis of on-treatment biopsies is starting to show that the immune effects of AR pathway inhibition are context specific and can be bidirectional, with early activation of immune markers in infiltrating T-cells followed by compensatory upregulation of other immune checkpoints and myeloid-driven suppression. It is a major open question if these changes reflect permanent changes to immunity or temporary fluctuations that are repelled by resistance systems, and single-cell profiling over time appears to be the only way to answer this question.

The loss of PTEN and immune exclusion

Loss of PTEN (which occurs in about 20–40% of localized prostate cancers and more frequently in CRPC) leads to activation of PI3K/AKT/mTOR signalling pathway, and has been linked to impacts on the immune system beyond effects on tumor growth intrinsic to the cells. PI3K/AKT activation in tumour cells has several effects on antigen presentation, such as the downregulation of MHC class I expression and interference with interferon γ signalling. In the absence of PI3K signalling, activation of β-catenin downstream of PI3K signalling alone is sufficient to suppress CCL4 secretion, which limits dendritic cell recruitment, and affects T-cell priming.

The link between PTEN loss and immune exclusion is strongly suggested by the TCGA correlative data, as PTEN null prostate tumours have significantly lower immune cell scores and lower levels of T-cell gene expression signatures, but there are limited definitive mechanistic studies in prostate-specific models. To date, there are no reports of single-cell analyses stratified by PTEN status to characterise immune microenvironment remodelling that is specific to PTEN loss, which is highly relevant. In terms of therapeutic evaluation, PI3K/AKT inhibition, in this case by using AKT inhibitors like ipatasertib, is being

assessed in CRPC and may be used as an immune sensitisation approach, especially in combination with checkpoint inhibitors targeting the PTEN-loss immune suppression axis (8, 12).

Cancer is a condition that can be triggered by a variety of causes.There are various factors that can cause cancer, including castration-resistant prostate cancer and immune remodelling.

Significant immune microenvironment remodelling is associated with the transition to CRPC. In the CRPC, there are increased frequencies of immunosuppressive myeloid populations, deeper T-cell exhaustion programs and increased stromal density. Single-cell studies, such as the recent landmark atlas by Hirz et al. (2023) that profiled 12 CRPC specimens, have revealed a macrophage-rich immune microenvironment, in which M2-polarized TAMs with pro-angiogenic and ECM-remodelling transcriptional programs dominate. Both T-cell frequency and function in CRPC is poor – single cell analyses show that CRPC is enriched for terminally exhausted T-cell phenotypes with high expression of TOX, NR4A1, HAVCR2 (TIM-3), and LAG3, which are largely resistant to checkpoint blockade, explaining the poor efficacy of PD-1/PD-L1 inhibitors in unselected CRPC populations.

One of the drawbacks of the current CRPC single-cell literature is that it is relatively small. Although the study by Hirz et al. was the most extensive so far, it was limited to 12 patients, which was not sufficient for the detection of rare cell states or patient-specific immune patterns. Although clinically most relevant, metastatic and CRPC specimens are technically difficult to obtain and process, as well as likely under-representative of the entire biological diversity of advanced prostate cancer. There is therefore a priority need to expand the single-cell and spatial datasets in CRPC.

Lineage Plasticity: NEP and ARCaP.Lineage Plasticity: NEP and ARCaP.

Neuroendocrine prostate cancer is a rapidly emerging treatment-emergent form that occurs by lineage plasticity (LP) — a transcriptional reprogramming event in which AR-driven luminal prostate cancer cells gain the characteristics of neuroendocrine cells in response to strong suppression of the AR pathway. NEPCs share expression defects of AR and PSA with high expression of NE markers such as CHGA, SYP and NCAM1, activation of ASCL1 or FOXA2 transcriptional programs, and frequent alterations of TP53 and RB1. They're very aggressive and don't respond to AR targeted therapy.

NEPC is fundamentally different in its immune biology than conventional CRPC and more immunosuppressive. Here, single-cell and bulk transcriptomic analyses indicate that NEPC tumours are characterized by extremely low levels of immune infiltration, low expression of PD-L1 and a low expression of MHC class I. Single-cell data revealed luminal and NE cells with intermediate cell states during transdifferentiation, which may possess a different antigen presentation capacity or differential susceptibility to immune cells killing. The immunophenotypes of these transitional states are not well known, and are an area of significant biological interest. Further studies are warranted to explore the immunological implications of epigenetic therapies targeting NEPC, such as EZH2 and BET bromodomain inhibition.

Prostate cancer is the most common site of metastasis, with more than 80% of patients having metastatic CRPC in bone. The bone microenvironment has immune cells, adipocytes, bone marrow stromal cells, osteoblasts and osteoclasts which form an immune environment that is very different from the primary prostate site. The bone metastases associated with prostate cancer are mostly osteoblastic, and reactive bone formation as a result of these lesions will result in high densities of sclerotic bone microenvironments that will not allow immune cell trafficking.

The immune microenvironment of prostate cancer bone metastasis (PCBM) is highly immunosuppressive, characterized in single-cell analysis by a macrophage-rich tumor-infiltrating immune infiltrate and a highly exhausted T cell type. Spatial transcriptomic characterisation of bone metastasis is limited, as it is difficult to decalcify and process bone samples in FFPE. Spatial transcriptomics of decalcified bone tissues is an emerging area of spatial biology that is particularly impactful when applied to the spatial transcriptomics of prostate cancer bone metastases. In addition, tumour-associated MDSCs are enriched in the bone marrow of prostate cancer patients with metastasis and can systemically influence T-cell responses which is an under-explored aspect of disease biology that should be explored in an integrated spatial and systemic immune profile of prostate cancer.

The Prostate Tumour Microenvironment

Cellular Composition

The prostate tumour microenvironment is a complex and dynamic environment which is comprised of a variety of immune cell subsets, cancer-associated fibroblasts, endothelial cells and other stromal cell populations that all play a role in the development of

prostate cancer and in response to therapy (8). Single-cell transcriptomics studies have revealed subpopulations of cells within tumour epithelial cells with luminal, basal and intermediate states. Myeloid cells, specifically tumour-associated macrophages (TAMs), make up the majority of the immune compartment and include far more cells in most prostate tumour specimens than do T lymphocytes. T cells are present in the tumour core, but are generally scarce and are characterised by a higher abundance of exhausted or dysfunctional cell types. Infiltrating NK cells are usually low and myeloid-derived suppressor cells (MDSCs) and regulatory T cells (Tregs) are immune-suppressive cells with different actions. Cancer-associated fibroblasts (CAFs) are the predominant stromal cell type in prostate cancer and are not uniform. scRNA-seq analysis revealed at least two types of CAF: myofibroblastic (myCAFs) and inflammatory (iCAFs), which displayed distinct cytokine secretion profiles and differential effects on recruitment of immune cells. Signaling of T-cell exclusion by CAF-derived TGF-β and skewing of myeloid cells by CAF-derived CXCL12 and IL-6 have been linked. Figure 1 offers a conceptual view of these interactions.

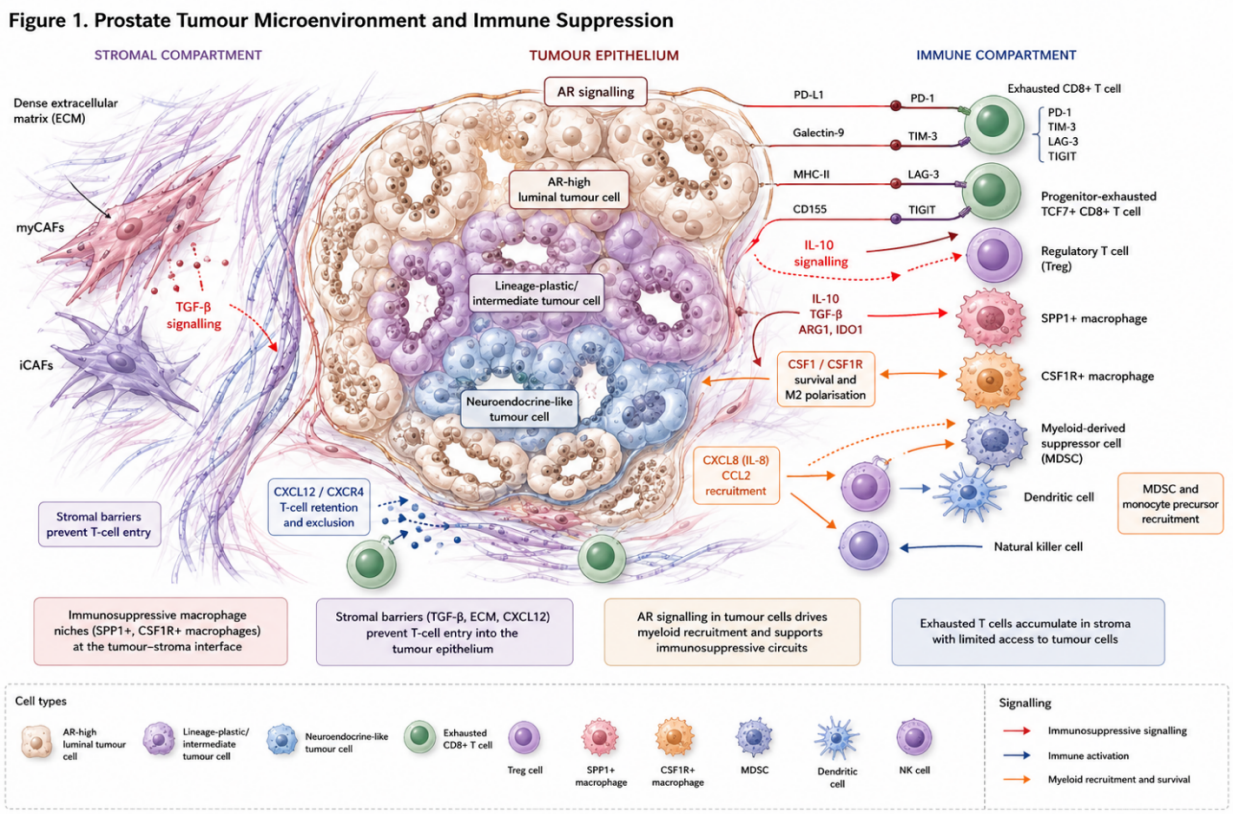

Figure 1. Prostate Tumour Microenvironment Schematic.

Illustration of the prostate TME showing malignant epithelial cells (with luminal and plastic/neuroendocrine subpopulations), major immune populations (CD8+ T cells in exhausted and progenitor-exhausted states, CD4+ Tregs, TAMs with SPP1+ and CSF1R+ subsets, MDSCs, NK cells, and dendritic cells), and cancer-associated fibroblasts (myCAF and iCAF). Key molecular interactions depicted include AR/androgen signalling, PD-1/PD-L1, TIM-3/Galectin-9, LAG-3/MHC-II, and TIGIT/CD155 checkpoint axes, TGF-β and IL-10 immunosuppressive cytokine signalling, CXCL12/CXCR4-mediated T-cell stromal trapping, and CSF1/CSF1R tumour–macrophage crosstalk. Spatial features of immune exclusion at the tumour–stroma boundary and immunosuppressive niches within the tumour core are highlighted.

Immunobiology of Prostate Cancer The difficulty of generating an effective immune response against prostate cancer is not a single cause but is due to convergent biology of the disease. The Neoantigen load of prostate cancer is one of the lowest of all common solid cancers, with a median TMB of ~2–3 mutations/Mb in localised disease. The low neoantigen density limits the antigen repertoire for presentation by MHC class I, and limits the repertoire of antigen-specific T-cell primings that are productive. In addition, up to 60% of prostate cancers show downregulation of MHC class I expression, which further restricts the ability of CTLs to recognize prostate cancer. The TME

also inhibits anti-tumour immunity by various mechanisms such as: T-cell suppression via TGF-β, secretion of IL-10 and PGE2 by tumour-associated macrophages and CAFs, tryptophan depletion via IDO1 and adenosine generation via CD39/CD73 ectonucleotidase activity (9). The prostate cancer is also a cold tumour, and the molecular and architectural coldness is a key difference from more immunogenic tumour types. In tumors where CD8+ T-cell infiltrates are visible, spatial analyses of the type of tumor and its location repeatedly show that the T cells are almost exclusively located within the stromal compartment, but rarely in direct contact with the tumor glands themselves, with the exception of shrub-like glands with abundant tumor-reactive T cells in their interiors, where they are surrounded by a dense fibrotic ECM and repulsive chemokine gradients. This architectural exclusion differs from the mere absence of T cells, as the absence of T cells could also be due to their inability to carry out their anti-tumour activity (both physically and molecularly).

Current Immunotherapeutic Landscape

Prostate cancer has seen a great deal of improvement in the field of immunotherapy, but results have only been seen in a very small minority of patients who have been molecularly selected. The FDA approved pembrolizumab for dMMR/TMB-high solid tumours, irrespective of histology, a small proportion of prostate cancer patients. The prostate cancer group had an objective response rate of ~5% in unselected patients in KEYNOTE-158. The CHECKMATE 650 trial with nivolumab plus ipilimumab revealed an objective response rate of ~25% in a carefully selected and heavily pretreated patient population that was enriched for CDK12 alterations, a population now known to have higher neoantigen burden as a result of tandem gene duplications. The only FDA-approved overall survival benefit prostate cancer immunotherapy has shown to date is for sipuleucel-T, which targets prostatic acid phosphatase (11). These findings are all together indicative that immune checkpoint therapy is not enough to overcome the multi-faceted immune resistance of prostate cancer. The field has thus shifted towards combination approaches such as checkpoint inhibition and AR targeting, PARP inhibitors and therapeutic cancer vaccines. The molecular understanding of the TME at single cell and spatial levels is crucial to rational design of these combinations and for elucidation of the molecular basis of response heterogeneity (12).

Single-Cell Genomics Insights

Mapping Cellular Heterogeneity

Single cell sequencing technologies have greatly improved our understanding of heterogeneity in the prostate TME. Table 1 below summarizes the most significant single-cell sequencing studies of prostate cancer.

Table 1. Major Single-Cell Sequencing Studies in Prostate Cancer

Study (Year)	Cohort/Stage	Cells (~)	Key Findings
Karthaus et al. (2020)	Human n=6; Localized	~13,000	Luminal progenitor cells as cells of origin; luminal plasticity under androgen deprivation; AR-dependent differentiation hierarchy
Chen et al. (2021)	Human n=14; Localized + CRPC	~45,000	T-cell exhaustion landscape; MDSC subpopulations; AR-dependent immune suppression; PD-1/TIM-3 co-expression on CD8+ T cells
Hirz et al. (2023)	Human n=12; CRPC (bone/liver mets)	~166,000	Comprehensive CRPC TME atlas; macrophage dominance; terminally exhausted T cells (TOX+/TIM-3+); spatial macrophage exclusion of T cells
Brady et al. (2021)	Human n=13; Localized	~35,000	Epithelial heterogeneity; ERG-fusion subpopulations; myCAF vs iCAF subtypes; stromal–epithelial transcriptional gradients
Stultz et al. (2022)	Human n=8; Localized + metastatic	~22,000	Myeloid diversity; SPP1+ macrophage enrichment in aggressive disease; pro-tumorigenic macrophage programs
Song et al. (2022)	Human + mouse; Localized + CRPC	~85,000	AR pathway modulation of immune composition; ADT increases T-cell infiltration; rationale for ADT + ICI combination
Jing et al. (2022)	Human n=18; Localized	~54,000	TCR clonality analysis; TCF7+ progenitor-exhausted T cells as candidate ICI response predictors

Study (Year)	Cohort/Stage	Cells (~)	Key Findings
Liang et al. (2023)	Human n=24; Localized + metastatic	~76,000	Integrated scRNA-seq + spatial; spatially restricted immunosuppressive niches; SPP1+ macrophage co-localization with exhausted T cells
Bian et al. (2024)	Human; Localized + CRPC	~90,000	Multi-omics single-cell integration; prostate cancer heterogeneity; AR-driven immune modulation; treatment resistance subpopulations

While there are several common themes throughout these studies, there are also significant differences and shortcomings that need to be critically examined. While the definition of exhausted T-cell states is consistent across datasets, the exact boundaries between the progenitor-exhausted (TCF1+/PD-1+) and the terminally exhausted (TOX+/TIM-3+/PD-1+) states are somewhat different across studies, depending on the computational method employed for clustering and annotation. In prostate cancer, the clinical significance of progenitor-exhausted T cells, which are the most likely to show a response to checkpoint inhibition, has been established in small, retrospective series only.In prostate cancer the prognostic or predictive value of progenitor-exhausted T cells has been demonstrated in small, retrospective series only. One of the drawbacks is the lack of diversity in the datasets published with regards to the treatment stage or lack of treatment. Technically difficult to collect and process, the technically more relevant specimens to develop immunotherapies for prostate cancer, such as CRPC and metastatic prostate cancer, have fewer and potentially less representative scRNA-seq datasets.

T-Cell Dysfunction: Exhaustion Programs and Checkpoint Expression

The highest level of systematic characterisation of the immune populations profiled in prostate cancer by scRNA-seq is CD8+ T cells. Prostate cancer T cells have a distinct profile of inhibitory receptors (PD-1 [PDCD1], TIM-3 [HAVCR2], LAG-3, and TIGIT) and transcription factors (TOX and NR4A family) that are master regulators of the cancer-exhaustion program (16, 20). There is no uniform co-expression of these inhibitory receptors, but their co-expression depends on the depth of exhaustion and the length of antigen exposure. The exhaustion state is not entirely characterized by PD-1 expression. TIM-3 and LAG-3 co-expression with PD-1 is more specifically associated with terminally exhausted states that are devoid of effector capacity. CD155 (PVR) is targeted by TIGIT on tumour cells and myeloid cells and TIGIT+ T cells are found in abundance in prostate cancer specimens including CRPC. Combined blocking of TIGIT and PD-1 seems to work together to enforce exhaustion, thus morphological and biological basis for combined blocking therapies. In the context of prostate cancer, the Tpex population appears to be smaller and less functional in this disease compared to more immunogenic tumours, possibly contributing to the poor performance of anti-PD-1 immunotherapy as single agent and a potential important mechanism that could be explored in the future. Myeloid Heterogeneity and Immunosuppressive Macrophage Programs

Androgen Receptor–Mediated Remodeling of the Myeloid Tumor Microenvironment

Contrary to the myeloid compartment in prostate cancer being a dichotomy of two states — M1 and M2 — scRNA-seq analysis of macrophages has essentially replaced this concept with the idea of continuous states of macrophages. There are multiple macrophage subpopulations that are recurrently identified by single-cell analyses and the one that expresses SPP1 has become a clinically important one. SPP1+ macrophages also have elevated expression of pro-tumorigenic genes such as APOE, CD44 and cathepsins and are enriched in more aggressive prostate cancer samples, which are also associated with more transcriptional immune activity. Their spatial association with exhausted T cells indicates that they may directly affect the function of T cells via the interaction with galectin-9 and TIM-3, and via their ability to release IL-10 or to physically block the entry of T cells into tumour epithelium (17). Tumour cells that express AR can produce CXCL8 (IL-8) and CCL2, which are potent chemoattractants for MDSCs and monocyte precursors, and the binding of CSF1 (macrophage colony-stimulating factor) to its receptor, CSF1R (macrophage colony-stimulating factor receptor), between tumour cells and macrophages can promote macrophage survival and M2-polarisation. These interaction axes, identified by the analysis of scRNA-seq data for ligands and receptors, indicate that the tumour epithelial AR signalling indirectly influences the myeloid microenvironment, thus offering a rationale for combining the use of AR pathway inhibitors with anti-CSF1R drugs and suggesting that the AR pathway may be partially disrupted by these combinations. A unique mechanism of how the primary oncogenic driver is linked to the composition of the immune microenvironment is a prostate cancer-specific mechanism with potential therapeutic implications.

Molecular Regulation of the Tumour Microenvironment

Single-cell genomic studies not only enable identification of cellular subsets, they also enable reconstruction of cellular regulatory networks that control cellular behaviour (18). The application of TCF applied to prostate cancer scRNA-seq datasets has revealed TOX, NR4A1, and NR4A2 as the master regulators of T-cell exhaustion and IRF2, MAFB, and C/EBPβ as regulators of immunosuppressive macrophage states. Combining scRNA-seq with scATAC-seq offers complementary chromatin accessibility landscapes that allow identification of regulatory elements that are active in different cell states. The study of prostate cancer by scATAC-seq is still limited (a problem considering the role of epigenomic profiling in prostate cancer mechanism) and will greatly improve the understanding of how role of AR signalling, PTEN loss and lineage plasticity is encoded at the single-cell level in prostate cancer.

Strengths and Limitations of Single-Cell Genomics

Although single-cell genomics represents an irreplaceable approach to address cellular heterogeneity, there are some limitations which can clearly be stated. Enzymatic dissociation techniques have their limitations: they favor the dissociation of easily dissociated cells, the loss of tissue-resident macrophages and stromal cells, and the dissociation induced stress response may change gene expression profiles — which is particularly important in prostate cancer because of the dense fibrous stroma (23). Most published studies have limited cohorts to less than 20 patients, because of cost and computational requirements, which reduces the power of the study to detect heterogeneity in patients and/or to correlate cellular profiles with clinical outcome. Interpretation of cell–cell communication analyses must be done with care: the tools used to make inferences about cell–cell communication assume that a cell expressing a particular ligand also expresses its corresponding receptor and that the presence of a receptor in a cell implies the presence of the corresponding ligand, but do not actually capture whether the cell is undergoing a signalling event; and even when the receptor is identified in a cell, it is not always functionally validated using orthogonal methods, as is often done in published analyses.

Spatial Genomic Organisation of the Tumour

The Importance of Spatial Context

However, the cellular composition is a limited description of the TME, and spatial transcriptomics has become an indispensable resource for studying the architecture of prostate cancer and its role in immune regulation. Cells' functional behaviour dictates their response to the paracrine signals, contact mediated interactions and gradients of nutrients and oxygen to which they are exposed, which cannot be deduced from dissociated single-cell data. Spatial context is especially important in prostate cancer as malignant glands are partially compartmentalised from adjacent stromal components and immune cells that enter the prostate are concentrated in the stromal compartment between glands, rather than invading the glandular epithelium (24, 25), which can be molecularly defined using spatial transcriptomics.

Spatial Transcriptomics Technologies

To date, there are a number of spatial transcriptomics platforms that have been used in prostate cancer, and each has unique technical characteristics described in Table 3. The 10x Visium platform is the most commonly used because it is commercially available, can be used on FFPE tissue (which is the most common type of prostate tissue used for archival research), and has paired analytical pipelines. Its main drawback is that it has a limited resolution with only 5-20 cells per capture spot (55μm), which means it needs computational deconvolution. The newer Visium HD platform shrinks the spot size to 2 μm, closer to single-cell resolution and with full-transcriptome coverage, but will probably be the standard for future studies as the cost comes down. MERFISH and CosMx SMI are able to achieve single-cell and sub-cellular resolution with high multiplexing capability, but require pre-selected gene panels for unbiased discovery (26, 28).

Table 3. Comparison of Spatial Genomics Technologies

Technology	Resolution	Genes Detected	Key Strengths	Key Limitations
10x Visium	55 μm spots (~5–20 cells)	~3,000–5,000	Widely adopted; FFPE compatible; full transcriptome; paired scRNA-seq deconvolution pipelines	Multi-cell spots require deconvolution; uncertainty propagates to downstream analyses

Technology	Resolution	Genes Detected	Key Strengths	Key Limitations
10x Visium HD	2 μm bins (near single-cell)	~3,000–5,000	Near single-cell resolution; full transcriptome; FFPE compatible; evolving to standard	Higher cost; computationally demanding; still maturing
Slide-seq v2	10 μm beads	~3,000	High spatial resolution; compatible with frozen tissue	Lower sensitivity than Visium; technically demanding; limited commercial support
MERFISH / seqFISH+	Sub-cellular	100–10,000 (selected panel)	Single-cell and sub-cellular resolution; high multiplex imaging	Pre-selected gene panel limits unbiased discovery; lower throughput
CosMx SMI (Nanostring)	Sub-cellular	~6,000–18,000	High multiplex; near-transcriptome-wide options; FFPE compatible	High cost; complex analysis pipelines; panel-based selection required
Xenium (10x)	Sub-cellular	100–5,000 (panel)	High sensitivity; FFPE compatible; rapid workflow	Panel-based; gene number limited by panel design
Imaging Mass Cytometry (IMC)	Single-cell (~1 μm)	~40 proteins	Single-cell resolution; protein-level; multiplexed	Protein-only (no transcriptomics); destructive; panel-based
CODEX / PhenoCycler	Single-cell	~50+ proteins	Single-cell spatial proteomics; high-plex IHC; FFPE compatible	Protein-only; panel-based; no transcriptome coverage

Spatial Architecture of the Prostate Cancer Immune Microenvironment

Early spatial transcriptomic studies in prostate cancer have confirmed and extended findings from conventional immunohistochemistry. Table 2 summarizes major spatial transcriptomics studies in prostate cancer.

Table 2. Major Spatial Transcriptomics Studies in Prostate Cancer

Study (Year)	Platform	Disease Stage	Key Spatial Findings
Berglund et al. (2018)	Spatial Tx (Visium precursor)	Localized	Demonstrated feasibility; immune cell spatial heterogeneity across tumor regions; proof-of-concept for prostate cancer TME mapping
Brady et al. (2021)	10x Visium	Localized	Stromal–epithelial transcriptional gradients; immune exclusion patterns at glandular boundaries; CAF spatial distribution
Espiritu et al. (2021)	10x Visium + scRNA-seq	Localized	CAF spatial distribution relative to immune exclusion; TGF-β signaling highest at stromal–epithelial boundaries
Liang et al. (2023)	10x Visium + scRNA-seq	Localized + metastatic	Spatially restricted immunosuppressive niches; SPP1+ macrophage–exhausted T-cell co-localization; spatial determinants of immune exclusion
Chen et al. (2023)	CosMx SMI	CRPC	High-resolution mapping of exhausted T cells and myeloid cells; sub-cellular checkpoint ligand localization; spatial macrophage barrier structure
Dong et al. (2023)	10x Visium	Localized + CRPC	Tumor epithelial–macrophage crosstalk via CSF1/CSF1R; AR-associated spatial immune patterns; spatial validation of scRNA-seq interaction predictions

Regardless of the spatial study, immune-excluded zones and immune-infiltrated zones within tumours are consistently identified. In immune-excluded areas, CD8+ T cells are located at the stromal–epithelial border or perivascular regions, and do not enter the malignant glandular epithelium. Spatial analyses have also identified that SPP1+ macrophages are localized at sites of active immune exclusion, where they form a physical and molecular barrier to T-cell entry, and that this macrophage localization is associated with regions of high TGF-β signalling and dense collagen deposition, indicating that macrophage positioning is a mechanistically coupled aspect of immune exclusion driven by the remodelling of the

extracellular matrix by myeloid cells (27). A novel idea is that within prostate tumours, there are organised ectopic lymphoid aggregates, also known as tertiary lymphoid structures, that are associated with better anti-tumour immunity and responses to checkpoint inhibitors in other tumour types. TLS have been detected in prostate cancer, but to a lesser degree the prevalence, spatial distribution, and role of TLS in prostate cancer remains less defined than in breast cancer or melanoma. Spatial transcriptomics is ideally suited for the characterisation of TLS architecture and to determine the relationship between TLS and the enrichment of progenitor exhausted T-cell subsets and potential therapeutic benefit for immunotherapy. The spatial patterns are shown in Figure 2.

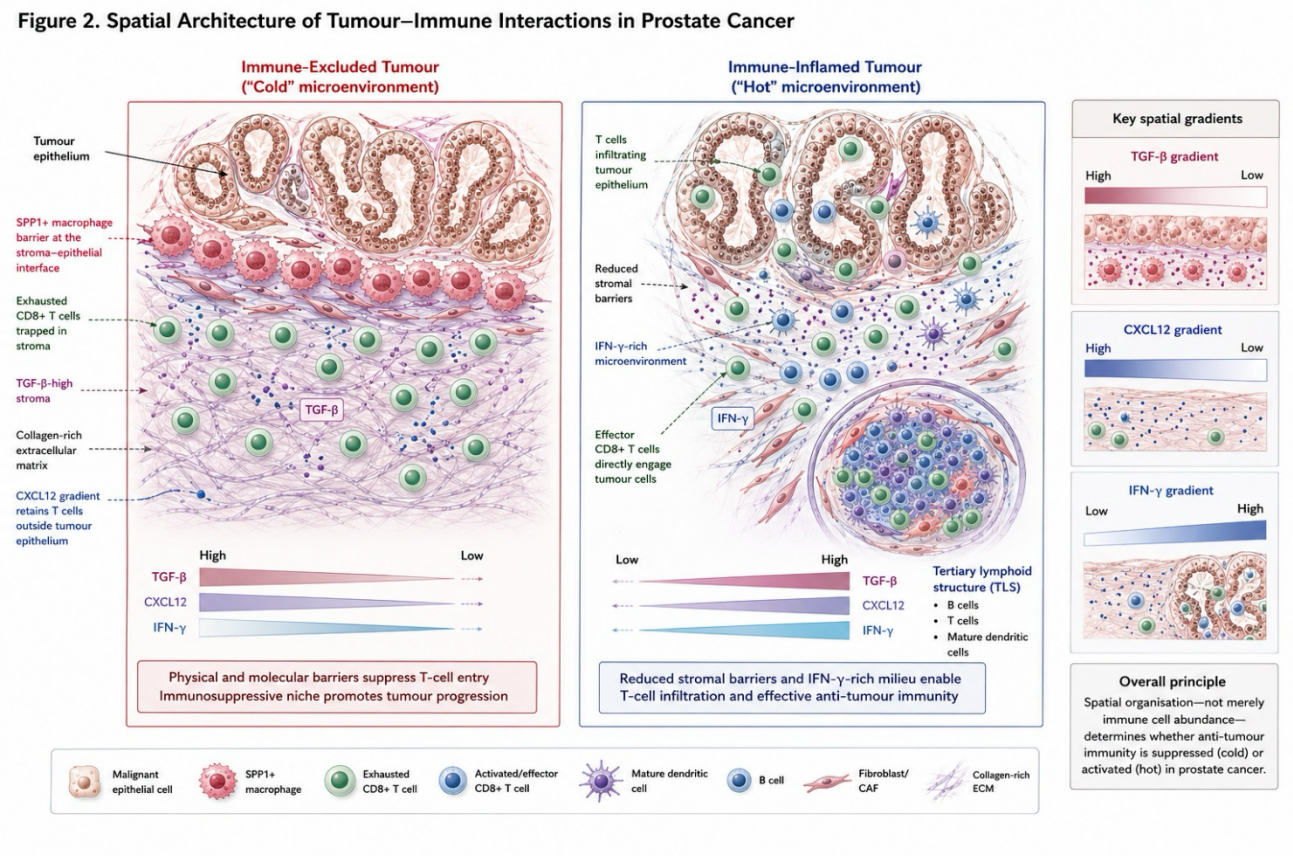

Figure 2. Spatial Organisation of Tumour–Immune Interactions.

Schematic representation of spatial immune patterns within the prostate TME. Left panel depicts an immune-excluded tumour region, where CD8+ T cells are confined to the stromal–epithelial boundary and SPP1+ macrophages form a cellular barrier between stroma and malignant glands, co-localising with TGF-β-rich, collagen-dense ECM. Right panel depicts an immune-infiltrated region with homogeneous T-cell distribution and proximity to tumour epithelium. A tertiary lymphoid structure (TLS) containing B cells, T cells, and mature DCs is shown at the tumour periphery. Spatial distribution of key signalling gradients (TGF-β, CXCL12, IFN-γ) is indicated. The figure highlights how spatial architecture — rather than immune cell presence alone — determines functional anti-tumour immunity.

Integrative Single-Cell and Spatial Multi-Omics

Rationale for Integration

Single-cell and spatial genomic technologies give partial and complementary views of the TME. scRNA-seq provides a high level of molecular detail about cells at the single-cell level, but cannot capture spatial information. Spatial transcriptomics preserves tissue architecture, but with lower molecular resolution. A crucial challenge they can tackle — one that neither technology could address separately — is whether T-cell exhaustion depth varies in stromal vs. intraepithelial locations. Are there more immunosuppressive transcriptomes in SPP1+ macrophages physically associated with exhausted T cells? Are progenitor-exhausted T cells, also, found in proximity to TLS? Integrated analysis (28, 29) is needed to answer these spatially contextualised molecular questions.

Table 1 in the original manuscript compared genomic technologies; Table 4 below extends this with a comprehensive comparison of computational integration methods.

Computational Integration Strategies

The integration of scRNA-seq and spatial transcriptomic datasets has been an area of rapid methodological development. Table 4 summarises the major computational methods available.

Table 4. Computational Methods for Single-Cell and Spatial Data Integration

Method	Primary Function	Key Strengths	Limitations
RCTD	Cell type deconvolution of spatial spots	Statistically rigorous; FFPE compatible; well-validated	Requires complete scRNA-seq reference; no continuous state modelling
cell2location	Spatially resolved cell type mapping	Probabilistic Bayesian framework; models multi-cell spots; accounts for technical variation	Computationally intensive; requires high-quality reference dataset
SPOTlight	Cell type deconvolution	Fast and interpretable; accessible for non-specialists	Less accurate for rare or transitional populations
Seurat (anchor-based)	Cross-platform data integration	Widely used; established and documented workflows	Assumes linear relationships; sensitive to batch effects
Harmony	Batch correction for single-cell data	Fast and scalable; minimal parameter tuning	Linear correction only; may remove genuine biological variation
LIGER	Multi-modal data integration	Joint matrix factorization; robust to batch effects; integrates epigenomic data	Computationally demanding; interpretability challenges
CellChat	Cell–cell communication inference	Biologically curated ligand–receptor database; signalling pathway context	Predicts potential interactions only; requires functional validation
NicheNet	Ligand–receptor activity prediction	Prioritizes functionally relevant interactions using regulatory prior knowledge	Requires comprehensive prior knowledge networks; context-dependent accuracy
SCENIC / pySCENIC	Transcription factor network inference	Identifies co-expressed gene regulatory modules; applicable to any species	Computationally heavy; quality depends on reference gene regulatory network
BANKSY	Spatial domain identification	Integrates cellular identity with neighbourhood context; robust to noise	Relatively new; validation in prostate cancer ongoing
Squidpy	Spatial analysis framework	Comprehensive; integrates morphology and expression; community-maintained	Requires high-quality preprocessing; steep learning curve
scVI / totalVI	Probabilistic deep learning integration	Scalable; handles technical variation; multi-modal (RNA + protein)	Black-box; interpretability limited; requires large datasets

Decovolution of spatial transcriptomics spots to infer cell type composition is a basic component of many spatial analyses, and is a meaningful source of uncertainty that is often not fully reported in published analyses. The accuracy of deconvolution will strongly depend on the quality and completeness of the scRNA-seq reference data set, and rare or transitional cell states that are poorly represented in reference data sets will be systematically underestimated in spatial analyses. This is especially applicable to the intermediate lineage

plasticity states and transitional macrophage phenotypes in prostate cancer (29, 30). A major focus of scRNA-seq analysis has been the analysis of cell–cell communication, which has sparked great interest as a framework for the identification of therapeutic targets at the level of ligand–receptor interactions.

CellChat and NicheNet identify co-expression of ligands and receptors required but not sufficient for functional signalling). Functional relevance must be confirmed by in vitro co-culture studies, spatial co-localisation and/or in vivo perturbation, and is often lacking in prostate cancer publications. Mechanical view is enriched by Multi-modal integration which goes beyond epigenomic data (scATAC-seq) and proteomic data (CITE-seq, CODEX) and beyond to metabolomic data. CITE-seq is able to profile the surface protein expression and transcriptome of the same single cells, and has specific benefits for profiling and characterizing the phenotypic expression of immune cells in which surface protein and mRNA expression do not always agree. The integrated analytical workflow is shown in figure 3.

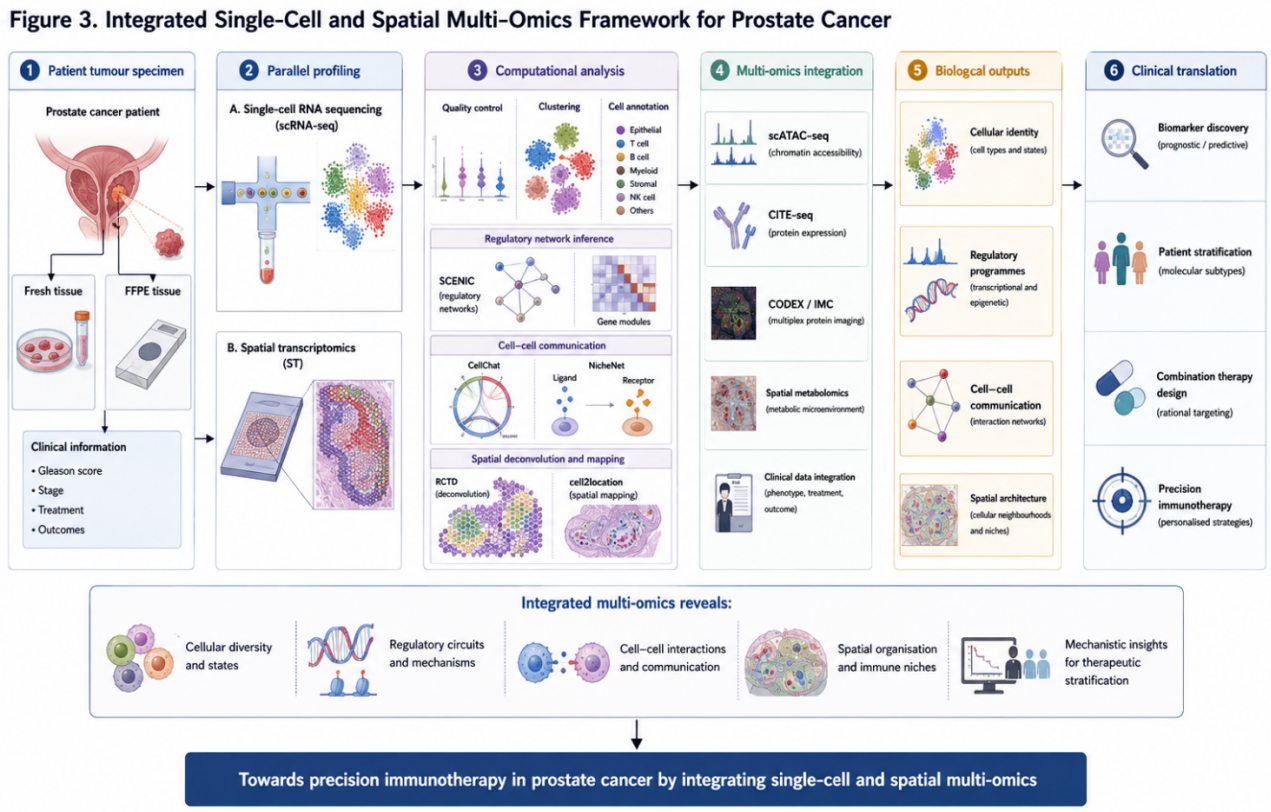

Figure 3. An integrated single-cell and spatial multi-omics workflow.

The integrated analytical workflow used for prostate cancer TME characterisation. Steps include: (1) tissue acquisition and processing (fresh-frozen and FFPE); (2) parallel scRNA-seq (dissociation-based) and spatial transcriptomics (intact tissue sections); (3) data preprocessing, quality control, dimensionality reduction and unsupervised clustering; (4) cell type annotation using canonical markers and SCENIC transcription factor inference; (5) analysis of ligand–receptor interactions (CellChat/NicheNet); (6) spatial deconvolution (cell2location/RCTD) mapping single-cell identities onto spatial coordinates; (7) identification of spatially restricted immunosuppressive niches and immune-excluded zones; (8) integration with epigenomic (scATAC-seq), proteomic (CITE-seq, CODEX), and clinical data for biomarker discovery in the context of translation. Key output layers are identified: cellular identity, regulatory programmes, spatial architecture and clinical correlates.

Integrative Models of Tumour–Immune Interactions

The most advanced existing methods integrate cellular identity (derived from scRNA-seq) with spatial transcriptomic coordinates with epigenomic or proteomic information in a single analytical framework. An emerging idea is the notion of the "immunosuppressive spatial niche" which is a spatially defined area where tumour cells, SPP1+ macrophages, CAFs and exhausted T cells are co-localised, and TGF-β, SPP1 and VEGF signalling is co-expressed. This finding by Liang et al. on how functional units of immune exclusion are established in prostate cancer spatial datasets, rather than by the mere presence of immunosuppressive cells, has therapeutic implications.

If these niches can be structurally disrupted, such as through anti-TGF-β therapy or anti-CSF1R therapy or VEGF pathway inhibition, it may be possible to restore immune access and sensitise tumours to checkpoint inhibition (30, 31).

Tumour–Immune Interaction Networks

Cell–Cell Communication Landscapes Several signalling axes emerge consistently from scRNA-seq-based ligand–receptor analyses in prostate cancer. The TGF-β axis functions primarily to regulate T-cell dysfunction, by repressing effector function via SMAD2/3-dependent transcriptional reprogramming and promoting exclusion of T cells by driving ECM deposition, mainly from CAFs and macrophages to T cells. Spatial analyses validate that the axis of interaction between stromal and epithelial cells identified via scRNA-seq is consistent with the highest activity of the TGF-β signalling pathway at stromal–epithelial boundaries, where immune exclusion is most intense.

Tumour epithelial cells express CSF1/CSF1R, which is involved in macrophage recruitment, survival and M2-polarization. A vicious circle is generated whereby the hormone dependency of the tumour influences the myeloid immune environment via AR-induced CSF1 secretion from tumour cells. The single-cell analyses show that CSF1R expression overlaps with SPP1 and GPNMB, suggesting that the most immunosuppressive macrophage subsets are maintained via CSF1R. The CXCL12/CXCR4 axis not only controls immune cell positioning, trapping of T cells in stromal CXCL12-rich areas away from tumour epithelium, but also tumour cell homing to bone at metastatic sites. In addition to immune exclusion, CXCR4 is also a factor in prostate cancer metastatic tropism, making it an appealing combined therapeutic target; however, its immunological implications in prostate cancer clinical studies have not been systematically explored in combination therapies (25).

In this section, learners will explore how prostate cancer cells evade the immune system.In this section students will examine prostate cancer specific mechanisms to evade the immune system. Prostate cancer uses multiple mechanisms to escape the immune system. The number of tumour-specific T-cell responses is restricted due to low levels of neoantigens. Restriction of MHC class I expression confines CTL recognition. T-cell exclusion by stromal barriers, TGF-β signalling and CXCL12 trapping are barriers to immune access of malignant epithelium. Exhaustion represents a progressive process as a result of chronic exposure to the same antigen in the presence of high levels of checkpoint ligands expressed on tumour cells and macrophages.Exhaustion is a progressive process due to chronic exposure to the same antigen in the presence of high levels of checkpoint ligands expressed on tumour cells and macrophages.

It is important to note that there are differences in the relative importance of these evasion mechanisms between prostate and more immunogenic tumours. In melanoma, neoantigen burden is high, while MHC class I downregulation is less prevalent, and the immune evasion relies more on the upregulation of checkpoint pathways. High neoantigen burden and immune infiltration are enough to induce strong checkpoint inhibitor responses in colorectal cancer (CRC) with MSI-H. Checkpoint inhibition is not enough for prostate cancer because it is able to resist at the antigen generation and presentation part of the immune cycle. Certain strategies that enhance immunogenicity, such as cancer vaccines, epigenetic modification of antigen presentation and mutational loading with PARP inhibitors, may be prerequisites for successful checkpoint blockade in this disease.

Key Signalling Pathways and Therapeutic Intersections

Specific pathways have been consistently associated with either immune suppression or activation in prostate cancer and have been identified through integrative multi-omics analysis. TGF-β, CSF1/CSF1R and CXCL12/CXCR4 signalling converge in spatially localized immunosuppressive niches, suggesting that these pathways are interdependent and coordinate each other to create an immunosuppressive network. Attacks on one node in this network might not be enough to stop the niche architecture if it is maintained by compensatory activity in other pathways. It has stimulated increasing interest in multi-target combination strategies, and in the creation of spatial biomarkers that could be used to inform the selection of a specific therapeutic axis for each patient's tumor, thereby selecting the appropriate treatment.

Translational and Clinical Implications

Emerging Immunotherapeutic Targets

In the field of prostate cancer, the toolkit of potential immune therapeutics has been expanded from the PD-1/PD-L1 pathway to include other targets, as identified by integrative single-cell and spatial analyses. Summarized in Table 5 are emerging biomarkers and immunotherapeutic targets and their current development status.

Table 5. Emerging Biomarkers and Immunotherapeutic Targets in Prostate Cancer

Target / Biomarker	Biological Context	Development Stage	Evidence Source
PD-1 / PD-L1	T-cell exhaustion checkpoint; PD-L1 on macrophages and tumour cells	FDA-approved (dMMR/MSI-H); clinical trials in selected PCa	Clinical trials; scRNA-seq
CTLA-4 (Tregs)	Treg-mediated immune suppression; ipilimumab-sensitive	Phase II/III trials (combination)	Clinical trials; scRNA-seq
TIGIT	Co-expressed with PD-1 on exhausted T cells; engaged by CD155 on macrophages/tumour	Phase I/II trials	scRNA-seq; spatial genomics
TIM-3 (HAVCR2)	Terminal exhaustion marker; Galectin-9 expressed on SPP1+ macrophages	Phase I trials	scRNA-seq
LAG-3	Inhibitory receptor; MHC-II engagement on APCs; exhaustion co-regulator	Phase I/II trials	scRNA-seq
SPP1 (Osteopontin)	Immunosuppressive macrophage state marker; spatial barrier to T cells	Preclinical	scRNA-seq; spatial genomics
CSF1R	Macrophage survival and M2-polarisation; AR-driven CSF1 secretion from tumour cells	Phase I/II trials (+ checkpoint)	scRNA-seq; functional studies
TGF-β pathway	T-cell exclusion master regulator; stromal remodelling; ECM deposition	Phase I/II (bintrafusp alfa + checkpoint)	Spatial genomics; functional
CXCR4 / CXCL12	T-cell trapping in stroma; bone metastatic homing of tumour and myeloid cells	Phase I/II	Spatial genomics; functional
PSMA	High tumour epithelial expression; CAR-T, BiTE and radioligand therapy target	FDA-approved (177Lu-PSMA-617); Phase I/II CAR-T/BiTE	Clinical trials
CCR8 (Tregs)	Selective intratumoural Treg depletion without peripheral autoimmunity	Preclinical / Phase I	scRNA-seq
TCF7+ Tpex cells	Progenitor-exhausted T cells with proliferative capacity; candidate ICI predictor	Investigational biomarker	scRNA-seq
TLS density / location	Organised anti-tumour immunity; correlates with ICI response in other tumour types	Investigational biomarker	Spatial genomics; IHC
Spatial SPP1+ macrophage density	Immunosuppressive niche structural marker; T-cell barrier quantification	Investigational biomarker	Spatial genomics

PSMA targeted therapies represent a significant translational breakthrough. Lutetium-177–PSMA-617 (Pluvicto) is FDA-approved for PSMA-positive mCRPC, based on the VISION trial. In addition to radioligand therapy, PSMA's high tumour expression makes it a good target for CAR-T cell therapy and bispecific T-cell engager (BiTE) strategies. Sustained PSA responses have been seen with early phase trials of PSMA-directed CAR-T cells, which have proven to be feasible, likely reflecting rapid CAR-T exhaustion in the immunosuppressive TME and persistence of suppressive myeloid populations. BiTE molecules including pasotuxizumab (AMG 160) and acapatamab (CC-1) have shown clinical activity in early trials. scRNA-seq analysis of tumour biopsies from patients treated with PSMA-directed BiTE therapy could provide mechanistic insight into response and resistance at resolution not previously available (11, 32).

Biomarker Discovery and Patient Stratification

Therefore, the clinical value of single-cell and spatial genomic profiling will rely on the discovery of biomarkers that can inform selection of patients for immunotherapy. Current candidates for analysis as biomarkers include: abundance of TCF1+ progenitor-exhausted T cells (Tpex), spatial density of TLS, depth of infiltration of T cells into the tumour core, and density of SPP1+ macrophages at the stromal–epithelial boundary (32, 36). These spatial biomarkers convey architectural determinants of immune function that can't be conveyed by single-cell composition measures alone. To translate these biomarkers into clinical assays, standardised image analysis protocols, validated computational pipelines, and reference ranges for large prospective cohorts are needed. Clinical biomarker development can be realised through multiplexed immunofluorescence or chromogenic IHC panels that are similar to the key spatial features extracted from the spatial transcriptomic analysis. Combined TCF1/PD-1 IHC could be a surrogate for scRNA-seq defined Tpex abundance and enable clinical translation for TCF1+ Tpex cells. These methods need to be validated in tissue from prospective immunotherapy trials including KEYNOTE-641. Incorporating tumour biopsy with multi-omics profiling in prostate cancer immunotherapy trials would provide invaluable mechanistic information as well as clinical outcomes, which would enable the identification of biomarkers alongside therapeutic development. This has been shown to be possible for other tumour types, and is now being applied in some prostate cancer trials. To make a more rapid progress in the field expanding this practice and sharing the biomarker data through open access platforms will be essential.

Clinical Applications of Single-Cell and Spatial Genomics

Table 6 summarises the current and emerging clinical applications of these technologies.

Table 6. Clinical Applications of Single-Cell and Spatial Genomics in Prostate Cancer

Application	Clinical Context	Current Status	Key Challenges
TME cellular characterisation	Patient stratification for ICI trials	Research; selected clinical trials	Cost; tissue quantity; standardisation of pipelines
Spatial immune biomarker discovery	Predicting ICI response; prognostic stratification	Investigational	Large validation cohorts; clinical-grade assay development
Resistance mechanism characterisation	Understanding CRPC evolution; AR-targeted therapy resistance	Research	Longitudinal tissue access; serial biopsy feasibility
CAR-T / BiTE response profiling	Mechanisms of CAR-T exhaustion in TME; informing next-generation designs	Research; early clinical	Biopsy feasibility; pairing infusion product with tumour profiling
Neoadjuvant treatment response assessment	Evaluating pre-operative immunotherapy microenvironment effects	Phase II trials (selected)	Serial biopsy requirements; tumour heterogeneity sampling
Metastatic biopsy profiling	Characterising bone/lymph node metastatic TME	Research	Bone decalcification technical challenges; tissue access
Lineage plasticity monitoring	Detecting NEPC emergence; guiding treatment adaptation	Research	NEPC rarity; biopsy accessibility at transition state
Multi-omics-guided combination therapy design	Identifying co-targets; overcoming multimodal immune resistance	Preclinical; early clinical	Functional validation; complex adaptive trial design

Embedding mandatory or optional tumour biopsy protocols with multi-omics profiling into immunotherapy trials in prostate cancer would generate invaluable mechanistic data alongside clinical outcomes, enabling identification of biomarkers in parallel with therapeutic development. The feasibility of this approach has been demonstrated in other tumour types and is now being implemented in selected prostate cancer trials. Expanding this practice and ensuring that biomarker data are shared through

open-access platforms will be essential for rapid progress in the field.

Combination Therapy Rationale

The complexity of immune resistance in prostate cancer argues strongly for combination therapeutic strategies that simultaneously address multiple evasion mechanisms. Single-cell and spatial data provide a rational framework for combination design by identifying which resistance mechanisms co-occur spatially and molecularly within the same tumour compartments (33).

The combination of AR pathway inhibition with checkpoint inhibition is supported by evidence that ADT/ARPI increases T-cell infiltration while checkpoint inhibition may activate those T cells. Several phase II trials are evaluating these combinations in mCRPC, with mixed results to date. Anti-TGF-β combined with anti-PD-1/PD-L1 addresses the immune exclusion mechanism most directly implicated by spatial analyses — bintrafusp alfa, a bifunctional fusion protein trapping TGF-β while blocking PD-L1, is under evaluation in multiple solid tumours including prostate cancer. Myeloid reprogramming through anti-CSF1R or anti-IL-8 combined with checkpoint inhibition addresses the dominant immunosuppressive myeloid landscape. A critical unmet need is biomarker-guided patient selection for these combinations, so that the right patients receive the right combination rather than applying all combinations broadly.

Challenges and Limitations

The multifaceted nature of immune resistance in prostate cancer is an excellent rationale for combination therapies targeting multiple mechanisms of immune evasion. Single-cell and spatial data can be used to provide a rational design of combinations; associations of resistance mechanisms that occur spatially and molecularly within the same tumour compartments (33). There is evidence that the AR pathway inhibition in combination with the checkpoint inhibition can enhance T-cell infiltration, which can be activated by checkpoint inhibition by the combination. These combinations are being tested in a number of phase II trials in mCRPC with variable results so far. Treatment with anti-TGF-β in combination with anti-PD-1/PD-L1 targets the immune exclusion mechanism most directly implicated by the spatial analyses: bifunctional fusion protein anti-TGF-β, anti-PD-L1, bintrafusp alfa is being investigated in various solid tumors, including prostate cancer. Combination of anti-CSF1R or anti-IL-8 with checkpoint inhibition targets the predominant suppressive myeloid cell compartment. One large unmet need in these combinations is the need for biomarker-driven patient selection to ensure that appropriate combinations of therapies are used for specific patients, and not all combinations given to all patients. 9. Challenges and Limitations

Technical Challenges

There are still a number of technical issues that need to be addressed. Different platform data can have different technical biases, sequencing depths and resolutions, making analysis more complex. Comparisons across studies are impeded by variations in sample processing and sequencing methods, as well as computational approaches to analysis. However, scRNA-seq involves dissociation of tissue which introduces cellular bias, especially for prostate cancer, where dense fibrostromal tissue is hard to dissociate. Although batch correction methods have improved, they can cause the loss of biological variations if not used carefully. Spatial resolution of widely used platforms requires deconvolution, adding uncertainty in the downstream analyses (34, 35).

Biological Challenges

Prostate cancer is a complex disease, adding further complications. There are many difficulties in identifying common patterns from individual biopsy samples, due to the heterogeneity of the tumour, both within each tumour (which tends to be multifocal), and between patients. The sampling problem is paramount for immunotherapeutic decisions: clonal population that is the most immunologically excluded can be responsible for immunotherapy failure even if regions of the tumour are perceived as being more immunogenic. Longitudinal studies with paired biopsies before, during and after therapy are required to understand the time course of changes in the immune microenvironment, which have not been widely used in prostate cancer (35). Current datasets are very limited in terms of patient diversity. Published scRNA-seq and spatial analyses have largely been performed on patients of European descent and systematically ignored the prostate cancer biological diversity across racial and ethnic groups, which is particularly relevant, as African American men have a significantly higher incidence of prostate cancer, are more likely to be diagnosed at later stages, and have poorer outcomes than European American men. These differences are in part due to differences in the immunobiology of their tumours.

Clinical Translation Barriers

Large-scale validation in various patient groups, uniformity in experimental and computational approaches, and demonstration of clinical utility in

prospective trials are needed to translate multi-omics findings into clinical practice. The number of candidate biomarkers identified from single cell studies in 10-20 patients is numerous, and must be confirmed in several hundreds or thousands of patients before they can be considered for clinical use. Multi-omics-based diagnostic assays are in their infancy in terms of regulatory and reimbursement pathways and development of more straightforward, clinically scalable surrogate assays will likely be required for their widespread adoption. The cost and analytical resources needed for scRNA-seq and spatial transcriptomics are still significant, with costs declining for every new generation of platform (36).

Future Directions

Multi-Omics Atlases

The goal of disease stage-specific, comprehensive, public multi-omics atlases of prostate cancer biology is a high priority. Ideally, specimens from localised disease in Gleason grade groups, biochemically recurrent disease, hormone sensitive metastatic disease, CRPC, and NEPC should be included, both primary and metastatic (including bone), from demographically diverse patients and should have comprehensive clinical annotation with treatment history and outcomes. Steps towards this vision are underway, such as contributions to the Human Tumour Atlas Network (HTAN), and will significantly enhance the capacity of the field to make reproducible, cross-study analyses.

Robotic Systems and Automation

The extent and complexity of multi-omics data require sophisticated computational methods. Cell type annotation, cross-dataset integration, trajectory inference, and gene regulatory network prediction are just a few examples of potential applications of foundation models in computational biology, the field of which is growing in promise as large neural networks are pre-trained on vast genomic data. There are also spatially-aware deep learning models, such as graph neural networks that encode the spatial context among cells, which have been used to analyze spatial transcriptomic data and could be beneficial to detect spatial patterns related to clinical outcome (38).Caution is warranted: overfitting to small datasets, lack of interpretability, and failure to generalise across patient populations or sequencing platforms are real risks. Rigorous external validation of all AI-derived predictive models is essential, and open sharing of code, trained models, and processed data must accompany publication.

Functional Validation and Mechanistic Studies

A critical gap in the field is functional validation of the cellular interactions and therapeutic targets predicted by single-cell and spatial analyses. Most published studies are observational and do not demonstrate causality. Advancing mechanistic understanding requires complementary functional approaches: organoid co-culture systems incorporating defined immune populations; syngeneic mouse models with genetic manipulations targeting specific ligand–receptor interactions; spatial transcriptomic characterisation of murine tumour models before and after genetic or pharmacological perturbations; and patient-derived xenograft models with humanised immune systems. Incorporating immune cells into patient-derived tumour organoid co-culture systems — particularly autologous peripheral blood or tumour-infiltrating immune cells — creates platforms for testing the functional consequences of predicted interactions in a patient-specific context.

Expanding Population Diversity and Equity

Addressing the demographic homogeneity of existing prostate cancer genomics research will require intentional strategies, including focussing on recruitment of diverse patient populations, engaging partnerships with institutions that service specific populations, establishing data sharing programs that allow the pooling of diverse datasets for analysis. The scientific return is significant: the molecular differences identified between prostate cancer in men of different ethnicities – such as differences in tumour molecular subtypes, features of the immune microenvironment, and biomarkers for immunotherapy – could provide important insights into the biology of prostate cancer that would not be apparent in racially homogenous data sets (39).

New directions in PSMA-directed radioligand therapy, antibody-drug conjugates (ADC), PARP inhibitors, and bispecific T-cell engagers (BiTE) will provide the potential for integration with single-cell and spatial tumour profiling. All of these classes alter the TME in an immunogenic manner: radioligand therapy results in immunogenic cell death; PARP inhibitors create genomic instability and may raise neoantigen burden and; BiTE therapy will activate T cells locally in the tumour. Single-cell and spatial profiling of pre- and post-treatment specimens collected from clinical trials will be crucial in understanding these immunological effects to enable rational combination therapy design, and to realise the potential of combination immunotherapy in prostate cancer.

Conclusion

The application of single-cell RNA sequencing and spatial transcriptomics has revolutionized the study of prostate cancer and the tumour–immune interactions and now allowed researchers to investigate the TME at the cellular, molecular and spatial levels, aspects that would not have been possible using bulk molecular analyses. Genetic characterization of exhausted T-cell states has been done with great accuracy, the immune repertoire and immunosuppressive programs of tumor-associated macrophages have been catalogued, and the immune environment in space has been described as immunosuppressive and disease-specific niche programs have started to be defined, all of which have a direct impact on therapeutic responsiveness.

These advances have led to a significantly more detailed understanding of why prostate cancer is resistant to immunotherapy – rational targets for therapeutic intervention are represented by T-cell exhaustion programs, SPP1+ macrophage barriers, exclusion by transforming growth factor beta, and antigen presentation suppression by AR. Translation of these insights to successful clinical strategies will need prospective clinical trials with integrated biomarker programmes, the development of clinically scalable surrogate assays, and combination therapeutic strategies aimed at conquering the multifactorial nature of prostate cancer immune resistance.

Critical gaps remain. Spatial genomic characterisation of bone metastases is at the early stages. Single cell and spatial data from patient populations of diverse demographics is very limited. Description of predicted cell–cell interactions significantly outstrips function validation. The temporal evolution of immune microenvironment in disease stages and upon treatment has also the need for more designs that are longitudinal, which are not widely used. The way forward is in a multi-modal, collaborative and mechanistically rigorous science with the ultimate goal of improving the outcome for prostate cancer patients, which single-cell and spatial genomics are now well placed to help.

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