Tumor-immune spatiotemporal co-evolution: A new paradigm for understanding and overcoming therapy resistance in metastatic castration-resistant prostate cancer (Review)

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Abstract. Metastatic castration-resistant prostate cancer (mCRPC) remains an incurable disease characterized by relentless progression and universal resistance to standard therapies. The limited efficacy of immunotherapies in this malignancy reflects an immunosuppressive tumor microenvironment orchestrated by regulatory T cells, myeloid-derived suppressor cells, tumor-associated macrophages and cancer-associated fibroblasts, along with androgen receptor signaling, metabolic reprogramming and spatially organized immune exclusion zones. The present review synthesized emerging evidence from single-cell and spatial transcriptomics to propose a spatiotemporal coevolution paradigm, wherein resistance emerges not through linear genetic selection alone but through dynamic, reciprocal interactions among tumor cells, stromal components and immune populations across both temporal and spatial dimensions. The framework integrates clonal evolution dynamics, preexisting castration-tolerant progenitors, fibroblast-dominated barriers and metabolic crosstalk that collectively enforce immune evasion. Key therapeutic vulnerabilities, including YAP1-TGF-β1 axis disruption, PKMYT1 inhibition, metabolic targeting and biomarker-guided patient stratification, are critically evaluated within this ecological context. Ultimately, the present review aimed to establish spatiotemporal co-evolution as a unifying framework that transforms mCRPC from a uniformly fatal disease into a chronically manageable condition through precisely timed, spatially informed and mechanistically rational interventions.

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Abbreviations: ADT, androgen deprivation therapy; AR, androgen receptor; AR-V7, androgen receptor splice variant 7; ARSIs, androgen receptor signaling inhibitors; ASCL1, achaete-scute family bHLH transcription factor 1; ATGL, adipose triglyceride lipase; BiTE, bispecific T-cell engager; CAFs, cancer-associated fibroblasts; CAR, chimeric antigen receptor; CRPC, castration-resistant prostate cancer; CTLA-4, cytotoxic T-lymphocyte-associated protein 4; CXCL, C-X-C motif chemokine ligand; CXCR, C-X-C motif chemokine receptor; DHODH, dihydroorotate dehydrogenase; FOXA1, Forkhead box protein A1; FOXA2, Forkhead box protein A2; ICIs, immune checkpoint inhibitors; MDSCs, myeloid-derived suppressor cells; mCRPC, metastatic castration-resistant prostate cancer; NEPC, neuroendocrine prostate cancer; NF-κB, nuclear factor-kappa B; PARPi, PARP inhibitor; PCa, prostate cancer; PD-L1, programmed death-ligand 1; PKM2, pyruvate kinase M2; PNI, perineural invasion; PRRX2, paired related homeobox 2; PSMA, prostate-specific membrane antigen; RLT, radioligand therapy; SPOP, speckle-type POZ protein; SREBP, sterol regulatory element-binding protein; STING, stimulator of interferon genes; TAMs, tumor-associated macrophages; TGF-β, transforming growth factor-beta; TIGIT, T-cell immunoglobulin and ITIM domain; TIM3, T-cell immunoglobulin and mucin domain 3; TMB, tumor mutational burden; TME, tumor microenvironment; Treg, regulatory T cell; YAP1, Yes-associated protein 1

Key words: spatiotemporal co-evolution, metastatic castration-resistant prostate cancer, tumor microenvironment, immune evasion, cancer-associated fibroblasts, metabolic reprogramming, immune exclusion zones, spatial transcriptomics

Contents

1. Introduction

Metastatic castration-resistant prostate cancer (mCRPC) remains a lethal disease state characterized by relentless progression despite the availability of multiple life-prolonging therapies (1,2). The median overall survival for patients with mCRPC is 2-3 years, and <30% of patients respond to any

given second-line therapy, underscoring the substantial clinical challenge posed by therapeutic resistance (2,3). While androgen deprivation therapy (ADT) and androgen receptor (AR) signaling inhibitors constitute the backbone of treatment, nearly all patients eventually develop resistance, driven by both tumor-intrinsic adaptations and dynamic remodeling of the tumor microenvironment (TME) (4,5). The clinical management of mCRPC is further complicated by the modest and unpredictable efficacy of immunotherapies, which have transformed outcomes in other solid malignancies but have yet to deliver comparable benefits in this disease context (3,6). Recent platform trials have demonstrated that no single immune checkpoint inhibitor combination provides uniform benefit across unselected mCRPC populations, underscoring the urgent need for biomarker-driven and combination strategies (7,8).

The immunological characterization of prostate cancer (PCa) as a 'cold tumor' has traditionally been attributed to its low mutational burden, sparse neoantigen generation and an immunosuppressive TME replete with regulatory T cells, myeloid-derived suppressor cells and tumor-associated macrophages (9,10). However, existing reviews have largely focused on isolated mechanisms such as *AR* signaling, immune checkpoint pathways, or metabolic reprogramming, without integrating these factors into a unified framework that accounts for the dynamic interplay between tumor cells and their microenvironment across both time and space (3,6,9). However, this classification overlooks a more nuanced reality: Even within the same tumor, spatially distinct immune-excluded and immune-infiltrated niches can coexist, and temporal shifts in immune architecture accompany disease progression from hormone-sensitive to castration-resistant states (11,12). For instance, a regulatory T-cell-specific signature has been shown to correlate with poor prognosis and therapeutic resistance, while tumor-associated macrophages expressing PD-L1 may actively suppress cytotoxic T-cell function, demonstrating that immune exclusion is not merely a passive consequence of low antigenicity but an actively enforced process (10). Moreover, chemotherapy such as docetaxel can paradoxically remodel the immune microenvironment, depleting myeloid-derived suppressor cells while enhancing $CD8^+$ T-cell infiltration, indicating that standard-of-care agents may themselves modulate immunogenicity in ways that could be harnessed therapeutically (11).

Traditional models of therapy resistance have largely focused on linear, cell-autonomous mechanisms such as AR amplification, AR-V7 splice variant expression, and alterations in tumor suppressor genes (4,13). While these factors are undoubtedly important, they do not fully account for the complex ecological dynamics that govern treatment response and resistance in mCRPC. An accumulating body of evidence indicates that resistance emerges not as a simple consequence of genetic selection, but through a process of spatiotemporal co-evolution, defined as the reciprocal, adaptive interactions among tumor cells, stromal fibroblasts, immune populations and the metabolic microenvironment that unfold across both temporal (disease progression, treatment sequencing) and spatial (intratumoral heterogeneity, immune exclusion zones) dimensions, collectively driving therapeutic escape (12,14,15). For example, yes-associated protein 1 (YAP1) inhibition has been shown to induce phenotype switching of cancer-associated fibroblasts from tumor-promoting to tumor-suppressive, thereby enhancing $CD8^+$ T-cell infiltration and improving responses to immune checkpoint blockade, while targeting PKMYT1 can enhance antitumor immune responses to PD-L1 blockade in castration-resistant models (12,14). Similarly, epigenetic profiling has identified markers of endocrine resistance that also modulate immune-related pathways, linking chromatin remodeling to immune evasion (13). These findings collectively argue that resistance is an emergent property of the entire tumor ecosystem rather than a cell-intrinsic trait, challenging the static reductionist models that have historically guided therapeutic development.

The present review introduced a spatiotemporal co-evolution paradigm as a unifying framework for understanding and overcoming therapy resistance in mCRPC. Rather than viewing resistance as a fixed endpoint, this paradigm conceptualized it as a dynamic, multi-dimensional process that unfolds across both temporal (disease progression, treatment sequencing) and spatial (intratumoral heterogeneity, immune exclusion zones) axes (15,16). This work addresses four key gaps in the current literature: The lack of an integrated framework that synthesizes cellular, metabolic and spatial drivers of resistance; insufficient characterization of the spatiotemporal dynamics of immune exclusion zones and fibroblast-dominated barriers; limited understanding of how therapy-induced remodeling of the TME can be therapeutically exploited; and the absence of a cohesive strategy for biomarker-guided, temporally sequenced combination interventions (3,12,14,16). Recent integrative analyses have revealed that immune-derived long non-coding RNA signatures can predict clinical outcomes and immunotherapeutic response, while targeting monoamine oxidase A has been shown to reprogram the immunologically cold landscape of PCa, providing proof-of-concept that the immune ecosystem is amenable to pharmacological remodeling (15,16). Furthermore, neoantigen vaccines are emerging as a strategy to reverse the 'cold' microenvironment, suggesting that active immunization may complement checkpoint blockade (17). Critically, this ecological perspective reveals novel therapeutic vulnerabilities that may be exploited through rationally designed, temporally sequenced combination strategies, moving beyond the one-size-fits-all approach that has dominated previous clinical trials. The scope of the present review encompassed the cellular and molecular drivers of immunosuppression in the mCRPC TME, the metabolic reprogramming that fuels resistance, and the spatial architecture that enforces immune exclusion.

2. The immunosuppressive landscape of the mCRPC TME

The mCRPC TME is characterized by a complex network of cellular and molecular immunosuppressive mechanisms that collectively limit anti-tumor immunity and promote therapeutic resistance. The following subsections dissect the key cellular orchestrators, the central role of AR signaling, the cytokine/chemokine networks, alternative immune checkpoints beyond PD-1/programmed death-ligand 1 (PD-L1), and the effect of low mutational burden, all of which are summarized in Fig. 1. Understanding these interconnected drivers is essential for developing strategies to re-engineer the 'cold' mCRPC ecosystem into a therapeutically responsive state.

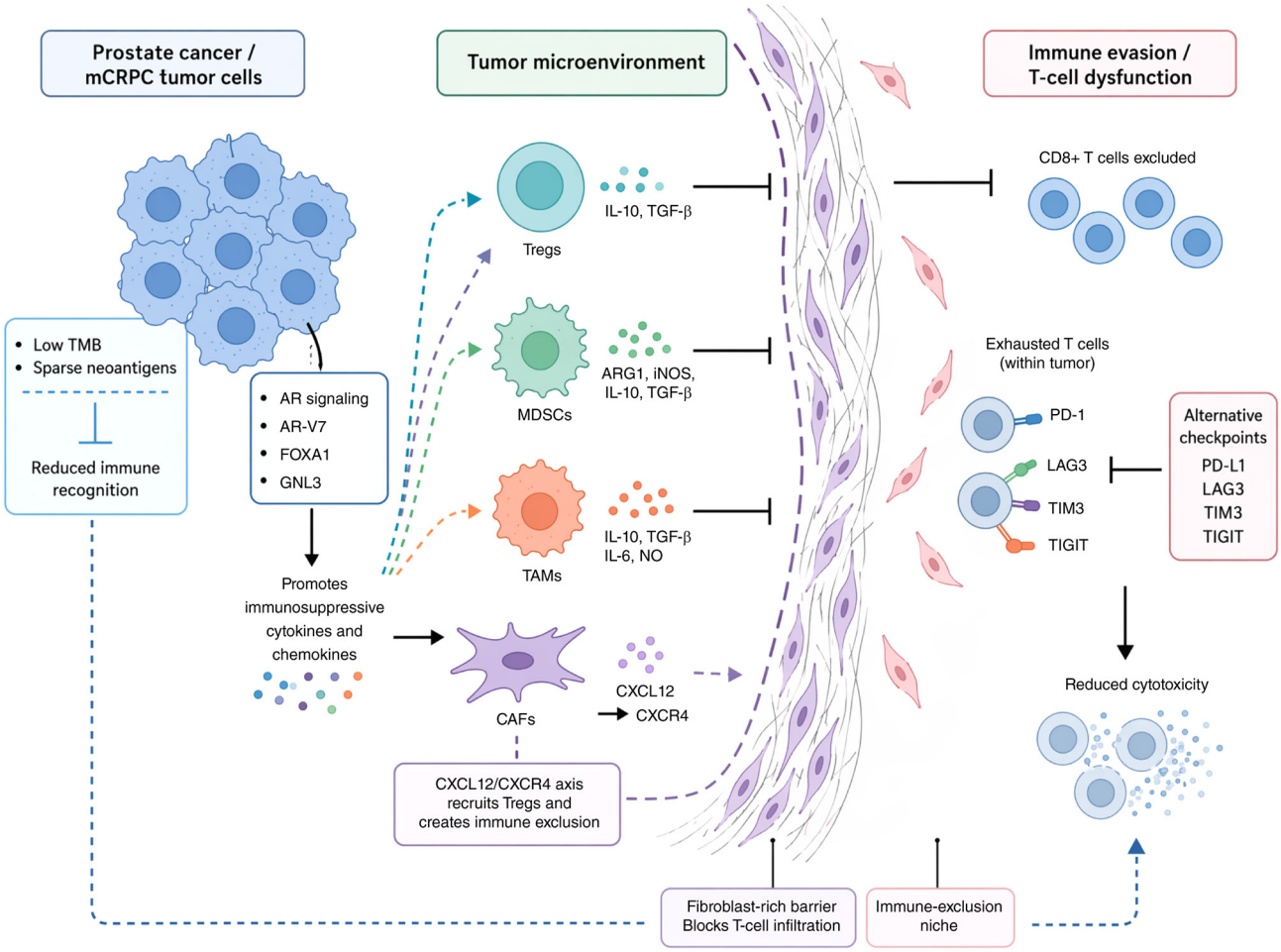

Figure 1. Immunosuppressive landscape of the mCRPC tumor microenvironment. Schematic illustrating major cellular and molecular components, directional intercellular relationships, and spatial niche organization driving immune evasion in mCRPC. Major components include mCRPC tumor cells, multiple immunosuppressive cellular populations (Tregs, MDSCs, TAMs and CAFs), soluble mediators, and spatial immune-exclusion niches. Established empirical findings (supported by cited literature) include: i) Low TMB and sparse neoantigen production intrinsically reduce tumor-specific immune recognition; ii) AR-related signaling axes (AR-V7, FOXA1, GNL3) within tumor cells drive the secretion of immunosuppressive cytokines and chemokines; iii) Tregs, MDSCs and TAMs secrete inhibitory mediators including IL-10 and TGF-β to directly suppress $CD8^+$ T-cell cytotoxic function and promote T-cell exhaustion characterized by upregulation of multiple alternative immune-checkpoint molecules (PD-L1, LAG3, TIM3, TIGIT); iv) CAFs release CXCL12 which engages the CXCL12/CXCR4 chemokine axis to recruit Tregs, while constructing a physical fibroblast-rich tissue barrier that blocks $CD8^+$ effector T-cell infiltration into tumor parenchyma, forming spatially restricted immune-exclusion niches. Paradigm-specific conceptual elements derived from the tumor-immune spatiotemporal co-evolution framework are highlighted: These immunosuppressive cellular networks are not static snapshots; the spatial arrangement of barrier-forming stromal and myeloid populations evolves progressively under sustained therapeutic selective pressure over time, dynamically reinforcing immune escape rather than functioning as fixed intrinsic tumor features. This schematic summarizes the key cellular and molecular drivers of immune evasion in mCRPC. Established mechanisms include immunosuppressive cell populations (Tregs, MDSCs, TAMs, CAFs), AR signaling, cytokine/chemokine networks, alternative immune checkpoints, and low mutational burden. Paradigm-specific elements integrate these drivers within the spatiotemporal framework, emphasizing that spatial organization of immunosuppressive populations creates physical barriers that evolve under therapeutic selection pressure across time. mCRPC, metastatic castration-resistant prostate cancer; Treg, regulatory T cell; MDSCs, myeloid-derived suppressor cells; TAMs, tumor-associated macrophages; CAFs, cancer-associated fibroblasts; TMB, tumor mutational burden; AR, androgen receptor; AR-V7, androgen receptor splice variant 7; FOXA1, Forkhead box protein A1; GNL3, G protein nucleolar 3, IL, interleukin; TGF-β, transforming growth factor-beta; PD-L1, programmed death-ligand 1; LAG3, lymphocyte activation gene 3; TIM3, T-cell immunoglobulin and mucin domain 3; TIGIT, T-cell immunoglobulin and ITIM domain; CXCL, C-X-C motif chemokine ligand; CXCR, C-X-C motif chemokine receptor.

Cellular orchestrators of immune evasion: regulatory T cells (Tregs), myeloid-derived suppressor cells (MDSCs), tumor-associated macrophages (TAMs) and cancer-associated fibroblasts (CAFs). Accumulation of immunosuppressive cell populations is a hallmark of the mCRPC TME. Using single-cell and spatial transcriptomics across healthy prostate, adjacent normal tissue and localized tumors, Li *et al* (18) demonstrated that macrophages regulate C-X-C motif chemokine ligand 12 (CXCL12)/C-X-C motif chemokine receptor 4 (CXCR4)-mediated recruitment of both $CD8^+$ effector T cells and Tregs, with Tregs competitively depleting IL-2 to drive $CD8^+$ T-cell exhaustion; combined CXCR4 inhibitor and IL-2 treatment reversed immune dysfunction and exerted superior anti-tumor effects. In a comprehensive multi-omics integration of bulk and single-cell RNA-seq from CRPC cohorts, Xiong *et al* (19) found that higher EZH2 expression correlates with adverse outcomes and increased enrichment of TAMs-related immunosuppressive programs; EZH2 inhibition upregulated immune-related genes such as TIMP3 and SOCS3, suggesting an epigenetic control of the suppressive milieu. Furthermore, using patient tissue analysis and a PCa bone metastasis mouse model, Dattilo *et al* (20) showed that

high MD2 expression is associated with increased infiltration of Tregs and MDSCs; pharmacological MD2 inhibition reduced tumor growth, establishing MD2 as a driver of immunosuppression in bone metastases. Collectively, these findings illustrate how Tregs, MDSCs, TAMs and CAFs form an integrated cellular network that actively silences anti-tumor immunity.

Molecular mediators: AR signaling as a master immunomodulator. AR signaling not only drives PCa growth but also functions as a central immunomodulator within the TME. In a prostate-specific forkhead box protein A1 (FOXA1) deletion model, Brea *et al* (21) demonstrated that FOXA1 loss drives tumor progression by reprogramming luminal cells toward a basal/squamous-like state and promotes an immunosuppressive TME characterized by accumulation of immunosuppressive myeloid cells and dysfunctional T cells; FOXA1 directly represses inflammatory genes, linking a key AR pioneer factor to immune evasion. Using proteomic profiling of CRPC vs. primary PCa cells, Zhang *et al* (22) identified GNL3 as a dual-function AR coregulator that physically interacts with AR, enhances its chromatin occupancy and coactivates proliferative programs (NEK2 and CDC20) while concurrently repressing immune-responsive genes (such as CXCL10 and TAP1) via class I histone deacetylases, thereby facilitating $CD8^+$ T-cell elimination; GNL3 expression progressively increases from normal prostate to CRPC. In a mechanistic study combining patient samples and mouse models, Wang *et al* (23) showed that AR^+TREM2^+ macrophages induce pathogenic immunosuppression to promote PCa progression; AR signaling within macrophages directly supports a myeloid circuit that drives immune exclusion during AR pathway inhibition. Furthermore, Zhang *et al* (24) demonstrated that androgen-activated AR suppresses antitumor immunity by inhibiting nuclear factor-kappa B (NF-κB) activation in T cells, providing a direct mechanistic link between AR activity and T-cell dysfunction. Collectively, these studies establish that AR signaling orchestrates immune suppression through both tumor-intrinsic (GNL3 and FOXA1) and myeloid-compartment (AR^+ $TREM2^+$ macrophages) mechanisms.

Cytokine and chemokine networks shaping the immunosuppressive milieu. Cytokine and chemokine networks profoundly influence the polarization and function of immune cells in the mCRPC TME. By systematically integrating patient cohort data, an analysis revealed that intra-tumoral IL-33 expression is markedly reduced in PCa tissues and correlates with aggressive disease, whereas IL-38 is markedly elevated, correlates with tumor severity and poorer overall survival, and mechanistically inhibits $CD8^+$ cytotoxic T-cell infiltration while upregulating Tregs (25). Using a time-dependent model of castration in Pten knockout mice, Sha *et al* (26) showed that androgen deprivation triggers a TNF-CCL2 cytokine switch: elevated TNF levels associate with expansion of basal-like stem cells during recurrence, and CCL2 secretion upon enzalutamide treatment drives T-cell depletion and TAMs recruitment, confirming that recurrent PCa harbors an immunologically 'cold' TME. In a multi-omics analysis integrating spatial transcriptomics and bulk data, Krossa *et al* (27) identified aggressive PCa signatures highlighting pro-inflammatory chemokine activity in the TME, with specific chemokine-receptor interactions (such as CXCL/CXCR2) organizing immunosuppressive niches. Collectively, these findings demonstrate that an imbalance between protective (IL-33) and pro-tumor (IL-38 and CCL2) cytokines, together with chemokine-driven niche remodeling, sustains immune evasion.

Checkpoint pathways beyond PD-1/PD-L1 in PCa. Beyond the canonical PD-1/PD-L1 axis, alternative immune checkpoint receptors such as lymphocyte activation gene 3, T-cell immunoglobulin and mucin domain 3 (TIM3) and T-cell immunoglobulin and ITIM domain TIGIT have been implicated in T-cell exhaustion and immunotherapy resistance in PCa, although direct mechanistic studies in mCRPC remain limited. In a functional exosome study using PCa cell lines, Liu *et al* (28) demonstrated that tumor-derived exosomes upregulate both PD-1 and TIM-3 on $CD8^+$ T cells, induce secretion of exhaustion-related cytokines and markedly decrease the cytotoxic activity of $CD8^+$ T cells; treatment with the exosome inhibitor GW4869 reversed these effects and rejuvenated T-cell function, providing evidence that TIM-3 can be co-induced with PD-1 in the PCa microenvironment. In the bone metastasis niche, Brauneck *et al* (29) characterized the expression of the TIGIT axis and the CD39/CD73 purinergic pathway in immune cells isolated from bone metastases, showing that TIGIT is co-expressed with exhaustion markers on T cells, suggesting that this alternative checkpoint may contribute to immunosuppression in metastatic sites. Additionally, Palicelli *et al* (30,31) systematically reviewed PD-L1 expression in PCa and noted that intracellular signaling pathways intersecting with alternative checkpoints [such as cytotoxic T-lymphocyte-associated protein 4 (CTLA-4)] are frequently dysregulated, though primary data on LAG3 and TIM3 in PCa tissues remain sparse. These observations indicate that alternative ICRs are indeed engaged in the mCRPC TME, but further original research is required to clarify their relative contributions and therapeutic potential.

Low mutational burden, neoantigen paucity and the 'cold tumor' revisited. The classification of PCa as an immunologically 'cold' tumor has traditionally been attributed to its low tumor mutational burden (TMB) and sparse neoantigen generation. However, emerging evidence indicates that additional tumor-intrinsic and spatial mechanisms actively enforce immune exclusion, and that molecularly defined subsets may display heightened immunogenicity.

Using integrated genomic and transcriptomic analyses, Liao *et al* (32) systematically evaluated TMB and immune infiltration patterns in PCa, revealing that low TMB is associated with reduced cytolytic activity and diminished antigen-presentation signatures, yet TMB alone does not stratify patients by immunotherapy response; instead, a combination of TMB with immune-related gene expression provided improved discrimination. In a multi-dataset analysis, Wang *et al* (33) confirmed that higher TMB correlates with aggressive clinicopathological features such as high Gleason score and prior hormone therapy, but lacks independent predictive value for immune checkpoint inhibitors (ICIs) outcomes, underscoring the need for complementary biomarkers.

Beyond mutational count, Liu *et al* (34) identified PGAP3 as a tumor-intrinsic factor that drives $CD8^+$ T-cell exclusion and promotes a 'cold' phenotype; PGAP3 upregulation was mechanistically linked to reduced chemokine expression and impaired T-cell infiltration, providing a functional explanation for immune desertification independent of mutational load. Using spatial whole-transcriptome profiling of primary tumors from patients with metastatic PCa, Salachan *et al* (35) demonstrated that neoantigen heterogeneity and defects in antigen-presentation machinery are spatially organized within the tumor, creating cold niches even in the presence of moderate TMB, and that these spatial patterns predict resistance to ICIs. Importantly, specific molecular subsets can overcome the cold state. Elliott *et al* (36) showed that biallelic loss-of-function of CDK12, which occurs in 3-5% of PCas, generates increased neoantigen load due to fusion-induced frameshift mutations, and tumors with CDK12 deficiency exhibit higher immune infiltration and may respond to ICIs therapy. Similarly, Calagua *et al* (37) found that a subset of localized prostate cancers with losses of key tumor suppressor genes (such as *PTEN, RB1 and TP53*) displays an immunogenic phenotype characterized by increased $CD8^+$ T-cell infiltration and upregulation of checkpoint molecules, suggesting that genomic alterations can partially reverse the cold microenvironment. Collectively, these findings reframe the 'cold tumor' paradigm: low mutational burden is a baseline feature, but active exclusion mechanisms (PGAP3, spatial neoantigen heterogeneity) and rare immunogenic exceptions (CDK12 deficiency, specific suppressor gene losses) determine the actual immune landscape of mCRPC.

3. Key cellular drivers of therapy resistance in the TME

The progression of mCRPC is driven by a complex interplay of stromal, immune and tumor-intrinsic factors that collectively enable therapeutic escape. This section dissects the major cellular drivers within the TME, focusing on CAFs, TAMs and neuroendocrine differentiation. As summarized in Fig. 2, these cellular players operate through interconnected mechanisms: CAFs display marked functional heterogeneity and engage in metabolic and exosomal crosstalk with tumor cells via the YAP1-TGF-β1 axis; $TREM2^+$ TAMs orchestrate immunosuppression and exhibit remarkable plasticity, including transition to a myofibroblast-like phenotype; and neuroendocrine cells arise from lineage plasticity programs driven by key transcription factors such as achaete-scute family bHLH transcription factor 1 (ASCL1) and forkhead box protein A2 (FOXA2). Understanding the functional heterogeneity, metabolic crosstalk and lineage plasticity of these components is essential for identifying actionable vulnerabilities.

Cancer-associated fibroblast heterogeneity, spatial organization and YAP1-TGF-β1 axis. CAFs are not a uniform population but comprise functionally distinct subtypes that shape the TME and influence therapy response. Using single-cell RNA sequencing of PCa specimens, Liu *et al* (38) identified multiple CAF subtypes with distinct gene expression profiles and demonstrated that their abundance correlates with biochemical recurrence, establishing CAFs as prognostic biomarkers. In a mechanistic study employing patient-derived xenografts and genetic/pharmacological targeting, Brunner *et al* (39) showed that *AR* loss in myofibroblastic CAFs is driven by an NFκB-TGF-β1-YAP1 axis; combined targeting of this axis synergistically repressed myofibroblastic hallmarks and impaired autophagic flux, effects potentiated by enzalutamide leading to enhanced myofibroblastic cancer-associated fibroblast cell death. Furthermore, Song *et al* (12) demonstrated that YAP1 inhibition induces phenotype switching of CAFs from tumor-promoting to tumor-suppressive, offering a potential stromal-reprogramming strategy. Shen *et al* (40) also reported that YAP1-TEAD1 mediates perineural invasion of PCa cells induced by CAFs, linking stromal YAP1 activity to neural invasion. Collectively, these studies highlight that CAF heterogeneity, spatial organization and the YAP1-TGF-β1 axis are critical determinants of therapy resistance in mCRPC.

Metabolic and exosomal crosstalk between CAFs and tumor cells. CAFs actively reprogram the TME through secreted metabolites and exosomes, driving resistance in adjacent tumor cells. Zhao *et al* (41) used single-cell transcriptomic profiling of PCa tissues before and after ADT alongside *in vivo* functional validation to identify $APCDD1^+$ CAFs as a distinct stromal population that secretes lactate in response to ADT. Mechanistically, lactate uptake by PCa cells induces lactylation of the spliceosome component SNRPA at Lys123, promoting alternative splicing that generates androgen receptor splice variant 7 (AR-V7) and confers castration resistance. Targeting lactate transport with monocarboxylate transporter inhibitors restored ADT sensitivity. Ippolito *et al* (42) further demonstrated that lactate rewires lipid metabolism and sustains a metabolic-epigenetic axis that supports metastatic behavior. Beyond metabolites, Wang *et al* (43) performed single-cell transcriptomic profiling of hormone-sensitive and CRPC tissues and identified $STEAP4^+$ myofibroblastic CAFs as a therapy-resistant subset with intrinsic enzalutamide resistance. These myoCAFs drive resistance via TFE3-mediated autophagy activation and PCYT1A-led overproduction of phosphatidylcholine, activating tumor HSP90/HIF1A signaling. Additionally, Zhao *et al* (44) showed that CAF-secreted exosomal *miR-432-5p* targets CHAC1 to inhibit ferroptosis and promote acquired chemoresistance. Thus, CAF-derived lactate and exosomes constitute two interconnected arms of a metabolic-exosomal axis that fuels CRPC progression.

$TREM2^+$ tumor-associated macrophages: metabolic symbiosis and immunosuppression. $TREM2^+$ TAMs have emerged as key drivers of immunosuppression and CRPC progression. Wang *et al* (23) combined patient tissue analysis and mouse models to demonstrate that macrophages co-expressing AR and TREM2 (AR^+TREM2^+ macrophages) induce pathogenic immunosuppression through *AR*-driven transcriptional activation of IL-10, TGF-β1, IL-23A, and CCL2. This myeloid circuit actively promotes immune exclusion during *AR* pathway inhibition. In a comprehensive immune landscape mapping study, Pervizou *et al* (45) used single-cell and spatial transcriptomics of healthy mouse prostate and PCa progression models to show that $Trem2^+$ TAMs differ from resident macrophages and exhibit a strong metabolic and immunosuppressive signature driven by the MIF/HIF1A axis. Similarly, Mei *et al* (46) performed single-cell and spatial transcriptomics on primary

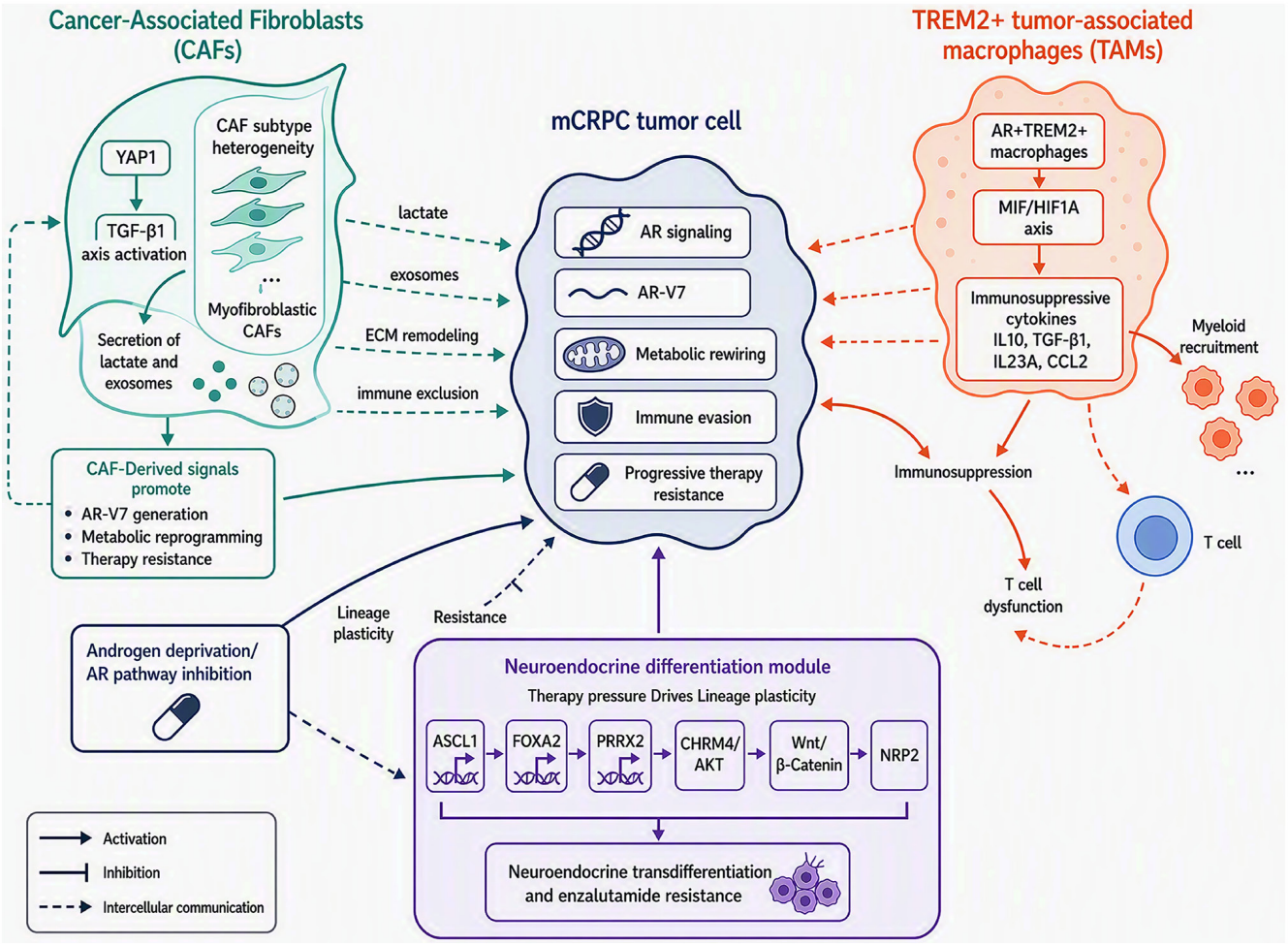

Figure 2. Key cellular drivers of therapy resistance in the mCRPC tumor microenvironment. Conceptual schematic depicting major cell subsets, directional signaling crosstalk, metabolic exchanges and lineage-plasticity programs mediating therapeutic resistance in mCRPC. Three core cellular modules are visualized: CAFs, TREM2⁺ and TAMs and the neuroendocrine differentiation module within tumor cells. Established empirical findings supported by existing literature: i) Heterogeneous CAF subsets, especially myofibroblastic CAFs, exhibit activated YAP1-TGF-β1 axis signaling; CAFs secrete lactate and exosomes to remodel the extracellular matrix, trigger immune exclusion, and induce AR-V7-mediated castration resistance in adjacent tumor cells via metabolic and exosomal crosstalk; ii) AR⁺TREM2⁺ TAMs activate the MIF/HIF1A signaling axis and secrete immunosuppressive mediators (IL10, TGF-β1, IL23A, CCL2), driving T-cell dysfunction and myeloid-cell recruitment; iii) therapeutic pressure from androgen-deprivation or AR-pathway-targeted therapy triggers tumor-cell lineage plasticity, where transcription factors including ASCL1, FOXA2, PRRX2, together with signaling cascades CHRM4/AKT and Wnt/β-catenin, drive neuroendocrine transdifferentiation and enzalutamide resistance. Paradigm-specific conceptual elements from spatiotemporal co-evolution theory: these resistance-related cellular modules do not function independently; reciprocal bidirectional crosstalk among CAFs, TAMs and tumor cells occur across both spatial niches and prolonged therapeutic exposure. Lineage-plasticity events represent temporal adaptive responses of the whole tumor ecosystem rather than purely tumor-cell-autonomous mutations. This schematic highlights CAFs, TAMs and neuroendocrine cells and their interconnected mechanisms. Established mechanisms include CAF heterogeneity and YAP1-TGF-β1 signaling, metabolic/exosomal crosstalk, TREM2⁺ TAM-mediated immunosuppression, macrophage plasticity and neuroendocrine lineage plasticity. Paradigm-specific elements frame these as an integrated ecosystem with reciprocal interactions across temporal and spatial dimensions. mCRPC, metastatic castration-resistant prostate cancer; CAFs, cancer-associated fibroblasts; TREM2, triggering receptor expressed on myeloid cells 2; TAMs, tumor-associated macrophages; YAP1, Yes-associated protein 1; TGF-β1, transforming growth factor-beta 1; AR-V7, androgen receptor splice variant 7; MIF, macrophage migration inhibitory factor; HIF1A, hypoxia-inducible factor 1-alpha; IL, interleukin; CXCL, C-X-C motif chemokine ligand; ASCL1, achaete-scute family bHLH transcription factor 1; FOXA2, Forkhead box protein A2; PRRX2, paired related homeobox 2; CHRM4, cholinergic receptor muscarinic 4.

and metastatic PCa samples and identified a TAMs subpopulation expressing TREM2 and SPP1 that mediates metastasis and progression. These data establish TREM2⁺ TAMs as central orchestrators of metabolic symbiosis and immune evasion in mCRPC, suggesting that targeting TREM2 or its downstream effectors may reverse immunosuppression.

Macrophage polarization, plasticity and transition to myofibroblasts. TAMs display remarkable phenotypic plasticity under therapeutic pressure, shifting between pro-inflammatory and immunosuppressive states. Boibessot *et al* (47) demonstrated that infiltrating prostate macrophages are subverted to a mixed immunosuppressive TAMs phenotype upon exposure to therapeutic drugs, characterized by upregulation of M2-associated markers and downregulation of antigen presentation. In a mechanistic study, Chaudagar *et al* (48) showed that reversal of lactate and PD-1-mediated macrophage immunosuppression controls growth of *PTEN*/*p53*-deficient aggressive PCa by restoring macrophage phagocytosis. Furthermore, Cui *et al* (49) identified that spindle pole body component 25 and PDGF mediate crosstalk between TAMs and PCa cells, promoting M2 polarization and tumor progression. Emerging

evidence supports the existence of macrophage-myofibroblast transition, whereby TAMs acquire myofibroblastic features and contribute directly to stromal remodeling. Wang *et al* (50) showed that CAF-secreted exosomal *miR-1290* promotes PCa cell growth and metastasis, in part by modulating TAMs polarization. Together, these findings highlight that TAMs plasticity, including macrophage-myofibroblast transition (MMT), represents a dynamic resistance mechanism that can be therapeutically targeted by reprogramming macrophages toward a pro-inflammatory state.

Neuroendocrine differentiation as a TME-driven lineage plasticity program. Neuroendocrine prostate cancer (NEPC) is an aggressive, therapy-resistant subtype arising from lineage plasticity. Within the spatiotemporal co-evolution framework, neuroendocrine transdifferentiation represents a canonical example of temporal lineage adaptation under therapeutic selection pressure, wherein AR-targeted therapies drive the emergence of *AR*-independent clones through transcriptional reprogramming that unfolds over months to years of treatment exposure. Using single-cell and bulk transcriptomics, Romero *et al* (51) demonstrated that the neuroendocrine transition in PCa is dynamic and dependent on ASCL1, with a three-phase model comprising de-differentiation, dormancy and re-differentiation. Han *et al* (52) identified that FOXA2 drives lineage plasticity and KIT pathway activation in NEPC, and that targeting FOXA2 suppresses neuroendocrine transformation. In a mechanistic study using patient samples and cell lines, Wen *et al* (53) showed that CHRM4/AKT/MYCN upregulates interferon alpha-17 in the TME to promote neuroendocrine differentiation, linking cytokine signaling to lineage plasticity. Furthermore, Wang *et al* (54) demonstrated that neuropilin-2 promotes lineage plasticity and progression to NEPC, providing a potential therapeutic target. Rodríguez *et al* (55) performed a genome-wide CRISPR activation screen and identified paired related homeobox 2 (PRRX2) as a regulator of enzalutamide resistance that also drives neuroendocrine features. Collectively, these studies establish that NEPC arises from a dynamic, TME-influenced lineage plasticity program involving key transcription factors (ASCL1, FOXA2, PRRX2) and signaling pathways (CHRM4/AKT, Wnt/β-catenin). Targeting these drivers may prevent or reverse neuroendocrine transdifferentiation in mCRPC.

Crucially, neuroendocrine transdifferentiation is not cell-autonomous but is spatially influenced by neighboring stromal and immune cells. Spatial transcriptomic profiling of primary tumors from metastatic patients has revealed active immune-stroma crosstalk within NEPC regions, with ligand-receptor interactions between CAFs and M2 macrophages (35). Integrated single-cell and spatial transcriptomic analyses across the prostate cancer progression continuum further demonstrate that specific CAF subtypes ($CXCL12^+$ inflammatory CAFs and $ACTA2^+$ myofibroblastic CAFs) co-localize with immunosuppressive myeloid populations that upregulate TGF-β signaling, creating spatially confined niches that may favor neuroendocrine lineage plasticity (45,56). Moreover, spatial analyses have identified that perineural niches, where neuroendocrine cells frequently reside, are associated with upregulation of monoamine oxidase A (MAOA) in both tumor and stromal compartments, and MAOA inhibition has been shown to reprogram the immunologically cold landscape of prostate cancer, suggesting that neural-associated microenvironments actively sustain neuroendocrine features and immune exclusion (16). Collectively, these spatial and mechanistic studies establish that NEPC arises from a dynamic, TME-influenced lineage plasticity program involving key transcription factors (ASCL1, FOXA2 and PRRX2) and signaling pathways (CHRM4/AKT and Wnt/β-catenin) and that this process is spatially organized within distinct stromal and immune niches. CAFs and TAMs, through paracrine signaling and spatial co-localization, create niche environments that promote neuroendocrine transdifferentiation while enforcing immune exclusion. Targeting these drivers may prevent or reverse neuroendocrine transdifferentiation in mCRPC, and spatially informed strategies that disrupt CAF-NEPC and TAM-NEPC crosstalk may offer novel therapeutic avenues.

The signaling pathways governing therapy resistance in mCRPC exhibit both convergent and divergent features across cellular compartments. The AR signaling axis operates as a central immunomodulator through tumor-intrinsic mechanisms (GNL3-mediated repression of immune-responsive genes, FOXA1-driven inflammatory gene suppression) and myeloid-compartment effects (AR^+TREM2^+ macrophages) (21-23). By contrast, the YAP1-TGF-β1 axis predominantly operates within CAFs, where NFκB-TGF-β1-YAP1 signaling drives AR loss and acquisition of the activated myofibroblastic state (39). WNT/β-catenin pathway activation, as implicated in neuroendocrine lineage plasticity, promotes enzalutamide resistance through transcriptional reprogramming (55). The JAK/STAT3 pathway, linked to cytokine-driven neuroendocrine differentiation, contributes to resistance in lineage-plastic variants (53). Notably, these pathways are not independent: AR signaling suppression can upregulate TGF-β1 expression in stromal cells (39), while YAP1 activation intersects with lineage-plasticity programs (12). This interconnectivity suggests that therapeutic targeting of individual pathways may be offset by compensatory activation of parallel signaling axes, underscoring the need for vertically integrated combination strategies.

4. Spatiotemporal dynamics of the prostate cancer immune ecosystem

The immune ecosystem of PCa is not static but evolves dynamically across disease stages and is spatially organized into discrete functional niches. This section synthesized evidence from clonal tracking, single-cell and spatial transcriptomics and emerging neural-immune-microbiome axes to illustrate how temporal selection pressures and spatial compartmentalization jointly shape therapy resistance. Fig. 3 provides a conceptual overview of this spatiotemporal transition, depicting the evolution from an immune-permissive state in hormone-sensitive PCa to an immune-excluded, therapy-resistant microenvironment in mCRPC, highlighting key temporal events (clonal selection, pre-existing castration-tolerant progenitors) and spatial features (fibroblast-dominated exclusion zones, CAFs-TAMs-T cells tripartite networks, and perineural invasion) as resolved by single-cell and spatial transcriptomics. A conceptual diagram summarizing these spatiotemporal

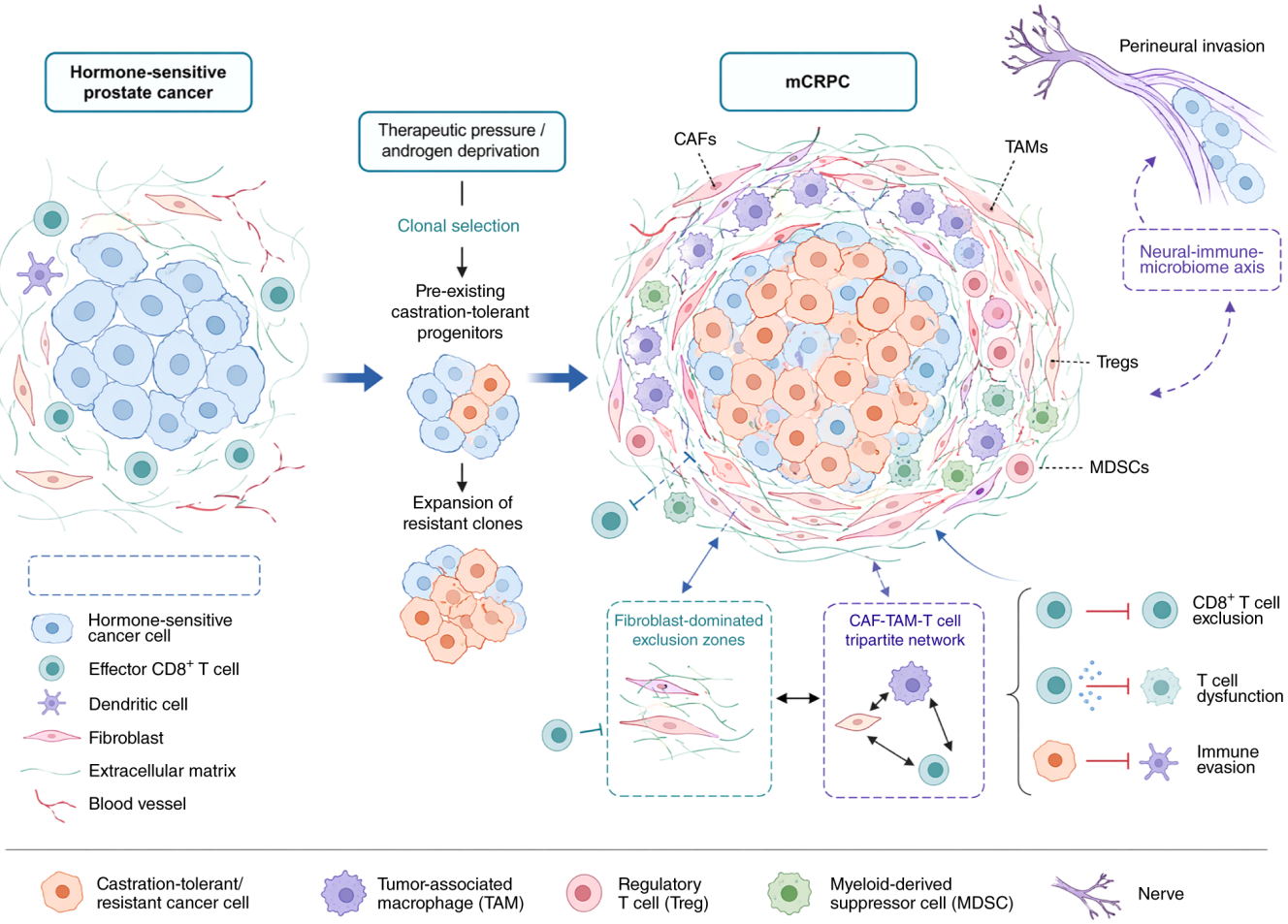

Figure 3. Spatiotemporal dynamics of the prostate cancer immune ecosystem during progression to mCRPC. Schematic illustrating sequential temporal disease transitions and corresponding spatial niche remodeling from hormone-sensitive prostate cancer towards therapy-resistant mCRPC. Temporal sequential events shown in the progression trajectory: starting from hormone-sensitive prostate cancer characterized by an immune-permissive state with abundant effector $CD8^+$ T-cell and dendritic-cell infiltration; therapeutic pressure induced by androgen-deprivation therapy drives clonal selection, expansion of pre-existing castration-tolerant progenitor tumor-cell populations, and outgrowth of therapy-resistant subclones. Major spatial architectural alterations observed upon transition to mCRPC include: formation of fibroblast-dominated physical immune-exclusion zones; establishment of the tripartite CAF-TAM-T-cell interaction network that mediates local immune-evasion; emergence of perineural-invasion niches; and functional coupling to the neural-immune-microbiome axis. Established empirical findings: immune-permissive tumor microenvironments in hormone-sensitive disease become progressively replaced by immune-suppressive niches enriched for Tregs and MDSCs, leading to $CD8^+$ T-cell exclusion and T-cell functional exhaustion. Paradigm-specific conceptual elements from the tumor-immune spatiotemporal co-evolution framework: temporal clonal evolution and spatial niche remodeling are tightly coupled reciprocal processes. Spatial stromal-immune structures shape which tumor subclones survive therapeutic stress; conversely, clonal-subclone expansion further reshapes local spatial micro-niches, collectively forming a self-reinforcing co-evolutionary cycle that establishes the therapy-resistant mCRPC phenotype. This schematic depicts evolution from an immune-permissive state to an immune-excluded, therapy-resistant microenvironment. Temporal events include clonal selection under therapeutic pressure, expansion of pre-existing castration-tolerant progenitors, and divergent clonal evolution. Spatial features include fibroblast-dominated immune exclusion zones, CAF-TAM-T cell tripartite networks, and perineural invasion. Established mechanisms are well-supported by evidence; paradigm-specific elements illustrate the interconnection of temporal and spatial phenomena forming a co-evolutionary cycle. mCRPC, metastatic castration-resistant prostate cancer; CAFs, cancer-associated fibroblasts; TAMs, tumor-associated macrophages; Treg, regulatory T cell; MDSCs, myeloid-derived suppressor cells.

transitions (from immune-permissive to immune-excluded states) is provided for integration here (Fig. 3).

Temporal evolution: clonal selection and immune pressure during progression to CRPC. Progression from hormone-sensitive to castration-resistant PCa is accompanied by profound remodeling of the immune landscape driven by clonal selection. Analyzing bulk and single-cell transcriptomes across primary and metastatic CRPC samples, Liu *et al* (57) identified extensive clonal heterogeneity, with distinct subclones emerging under therapeutic pressure that harbor immune-evasive gene expression programs. Using a genetically engineered mouse model and patient validation, Tshering *et al* (58) demonstrated that immune mechanisms actively shape the clonal landscape during early PCa progression and that an immune-specific gene signature of minor clones predicts biochemical recurrence, providing direct evidence that immune pressure acts as a selective force. In a subsequent longitudinal study integrating whole-exome and single-cell sequencing of metastatic CRPC, Hosseini *et al* (59) revealed that divergent clonal evolution and early metastatic dissemination generate substantial genetic heterogeneity among metastases, with clones displaying differential susceptibility to immune checkpoint blockade. More recently, Mikutenaite *et al* (60) performed integrative phylogenetic and spatial transcriptomic analyses on multi-regional prostate tumors, showing that clonal evolution and transcriptional plasticity define distinct

metastatic dissemination routes and that immune-related gene expression varies markedly among clones, highlighting the adaptive capacity of the tumor ecosystem. Collectively, these studies establish that the mCRPC immune ecosystem undergoes predictable temporal evolution under immune and therapeutic selection, moving from an immune-permissive state to progressively immune-suppressed niches.

Pre-existing castration-tolerant progenitors and adaptive resistance. Castration-tolerant progenitor cells that pre-exist in treatment-naïve tumors represent a reservoir for adaptive resistance. Using lineage tracing and single-cell RNA-seq in a Pten-deficient mouse model, Luo *et al* (61) identified a luminal intermediate cell state that maintains long-term prostate homeostasis and, upon androgen deprivation, expands and drives tumorigenesis, demonstrating that resistant progenitors are present before therapy. In human prostate tissues, Huang *et al* (62) characterized club-like cells, which express markers of progenitor potential and are enriched in regions of proliferative inflammatory atrophy; these cells show transcriptional signatures associated with castration resistance. Expanding on this, Kiviaho *et al* (63) combined single-cell and spatial transcriptomics across a large patient cohort, revealing that club-like cells interact physically with immunosuppressive myeloid cells in the TME and that their abundance predicts progression to CRPC. In a functional study using genetic ablation and pharmacological targeting in mouse models, Baurès *et al* (64) demonstrated that pre-existing club-like (LSCmed) progenitor cells acquire castration tolerance through Fosl1/AP-1-driven transcriptional reprogramming rather than intrinsic properties; dual targeting of Fosl1/AP-1 and PIM kinases suppressed progenitor expansion and restored ADT sensitivity, offering a therapeutic strategy to eliminate this resistance reservoir. Thus, pre-existing castration-tolerant progenitors, particularly club-like cells, are an early source of adaptive resistance.

Spatial architecture revealed by single-cell and spatial transcriptomics. High-resolution spatial technologies have unveiled the organized architecture of the PCa immune ecosystem. Hirz *et al* (65) performed integrated single-cell and spatial transcriptomic profiling of human prostate tumors and adjacent normal tissues, mapping the precise localization of immunosuppressive myeloid populations relative to tumor epithelium and demonstrating that TREM2$^+$ macrophages are enriched in peri-tumoral stromal niches. Using Visium spatial transcriptomics on radical prostatectomy specimens, Quan *et al* (66) identified intratumoral heterogeneity correlated with Gleason score progression, revealing that higher-grade regions are associated with specific fibroblast and immune cell spatial patterns. In an aggressive PCa signature study, Krossa *et al* (27) applied spatial multi-omics to characterize chemokine-enriched glands, showing that non-cancerous glands adjacent to aggressive tumors express high levels of pro-inflammatory chemokines and recruit club-like cells and immune infiltrates, creating a spatially defined premalignant niche. More recently, Apostolov *et al* (56) generated a comprehensive single-cell and spatial transcriptomic atlas of hormone-therapy-naïve localized PCa, defining epithelial functional states and fibroblast phenotypes, and identified a perineural fibroblast population with distinct spatial distribution. These spatial atlases provide a blueprint for understanding how tumor-stromal-immune interactions are physically organized to foster resistance.

Immune exclusion zones and fibroblast-dominated niches. Immune exclusion, where CD8$^+$ T cells are physically barred from tumor nests, is a hallmark of the 'cold' mCRPC TME and is often mediated by fibroblast-rich barriers. In a mechanistic study using patient-derived xenografts and genetic models, He *et al* (67) demonstrated that hormonal therapy drives a convergent evolution toward a specific ecological state (Ecotype 4) characterized by a transforming growth factor-beta (TGF-β)-driven rigid vascular-stromal barrier that enforces immune exclusion, accompanied by accumulation of *AR*-negative senescent fibroblasts; pharmacological blockade of the NF-κB2/p52 pathway reversed this barrier and restored antiandrogen sensitivity. Using single-cell and spatial transcriptomics, Pervizou *et al* (45) characterized the immune landscape in healthy mouse prostate and during PCa progression, showing that Trem2$^+$ macrophages and activated fibroblasts form spatially organized immunosuppressive niches that exclude effector T cells. Wu *et al* (68) performed multi-omics integration to uncover intercellular communication between FAP$^+$ fibroblasts and SPP1$^+$ macrophages, identifying CSF1/CSF1R and CXCL/ACKR1 as key signaling axes that drive the formation of fibroblast-dominant immune exclusion zones; these axes were further validated in human CRPC tissues. Finally, Li *et al* (18) demonstrated that the CXCL12/CXCR4 axis governs Treg spatial dominance over CD8$^+$ T cells via IL-2 sequestration, directly establishing a spatial proximity relationship that underlies immune suppression. Collectively, these findings indicate that physical exclusion by fibroblast-myeloid barriers is a major determinant of immunotherapy resistance.

Fibroblast-myeloid-lymphocyte tripartite interaction networks. The crosstalk among CAFs, TAMs and T lymphocytes forms a tripartite network that orchestrates immune suppression. Using multi-omics integration of single-cell and spatial transcriptomics, Wu *et al* (68) systematically mapped ligand-receptor interactions between FAP$^+$ fibroblasts and SPP1$^+$ macrophages, revealing that these two cell types co-localize and engage in reciprocal signaling through CSF1/CSF1R and CXCL/ACKR1, which in turn suppress T-cell activation and recruitment. Kiviaho *et al* (63) demonstrated that club-like epithelial cells, which are distinct from CAFs, interact with immunosuppressive myeloid cells, but more importantly, the spatial proximity of FAP$^+$ fibroblasts and SPP1$^+$ macrophages were validated in human CRPC samples and correlated with poor patient outcomes. Additionally, the CXCL12/CXCR4 axis, primarily emanating from CAFs and tumor cells, not only recruits Tregs but also directly impairs CD8$^+$ T-cell motility, as shown by Li *et al* (18). These tripartite interactions represent a functional unit where CAFs and TAMs synergistically create a physical and molecular barrier that renders T cells dysfunctional and excluded from the tumor parenchyma.

Emerging spatial regulators: perineural invasion and the neural-immune-microbiome axis. As well as classical

stromal-immune crosstalk, perineural invasion (PNI) and the neural-immune-microbiome axis are emerging as key spatial regulators. Several clinical studies have quantified the prognostic impact of PNI. Teramoto *et al* (69) demonstrated that the extent of perineural cancer invasion on needle core biopsy, particularly when quantified as the number of nerves involved, independently predicts biochemical recurrence and adverse pathology after radical prostatectomy. In a large cohort, Yang *et al* (70) reported that the presence of PNI after abiraterone resistance is associated with soft tissue progression and shorter survival in mCRPC patients, suggesting that neural invasion defines a more aggressive disease trajectory. Recent transcriptomic studies have begun to uncover molecular drivers: Aktan *et al* (71) identified a PNI-associated gene signature in PCa that correlates with poor prognosis and reduced immune infiltration, suggesting that neural invasion defines a disease trajectory associated with an immunologically cold microenvironment.

Concurrently, the gut microbiome has emerged as a systemic modulator of prostate cancer progression, with recent mechanistic studies delineating specific pathways relevant to spatiotemporal co-evolution. Gut microbiota-derived short-chain fatty acids promote tumor progression by inducing TLR3-triggered autophagy, activating NF-κB/MAPK signaling and upregulating CCL20 to recruit immunosuppressive M2 macrophages, thereby remodeling the TME. Additionally, certain gut bacteria can synthesize androgens, acting as an alternative testosterone source during ADT, while *Akkermansia muciniphila* metabolite inosine inhibits castration resistance by suppressing the LPS/NF-κB/AR axis. Using two-sample Mendelian randomization, Mingdong *et al* (72) found causal associations between specific gut microbial genera (such as Lachnospiraceae) and prostate cancer risk. Kure *et al* (73) demonstrated that androgen deprivation therapy markedly reduces gut microbial diversity and affects short-chain fatty acid production, which may influence systemic immune tone. Collectively, these findings indicate that gut microbiota influence mCRPC through short-chain fatty acid-driven immune reprogramming, microbial androgen synthesis, and metabolite-mediated barrier modulation pathways that operate across both temporal and spatial dimensions, embedding the microbiome as an active driver within the spatiotemporal co-evolution framework. Lachance *et al* (74) further showed that dietary polyunsaturated long-chain fatty acids modulate gut microbiome-prostate cancer crosstalk. While the precise molecular circuitry linking neural, microbial, and immune compartments remains to be fully elucidated, these insights establish the gut-prostate axis as a functionally significant regulator of mCRPC biology.

5. Metabolic reprogramming in the TME: Fueling resistance

The metabolic landscape of the mCRPC tumor microenvironment is profoundly rewired, shifting from oxidative phosphorylation towards aerobic glycolysis, lipid synthesis and glutamine dependence. Crucially, these metabolic alterations are not uniformly distributed but exhibit spatial heterogeneity, as revealed by recent spatial metabolomic and transcriptomic studies. These alterations are not merely cancer-cell autonomous but are orchestrated through reciprocal interactions with CAFs, TAMs, and other stromal cells. Table I summarized the major metabolic pathways implicated in therapy resistance, detailing their key mediators, downstream effects on immune cell function, corresponding model systems and therapeutic strategies currently under preclinical or early clinical investigation. The following subsections elaborate on these pathways, focusing on the Reverse Warburg effect and lactate shuttling, lactate-driven alternative splicing and AR-V7 generation, cholesterol-mediated immune suppression, and emerging targetable metabolic vulnerabilities including glutamine dependence, polyamine metabolism, and cuproptosis.

The reverse Warburg effect in PCa. Unlike classical Warburg metabolism, PCa displays a 'Reverse Warburg' phenotype wherein glycolytic CAFs produce lactate that is then utilized by oxidative tumor cells. Spatial metabolomic evidence using hyperpolarized [1-^{13}C] pyruvate magnetic resonance imaging has demonstrated that aggressive PCa lesions exhibit elevated lactate-to-pyruvate ratios, indicative of active lactate production and utilization and this metabolic phenotype correlates with cribriform histology and adverse clinical outcomes (75). Using a transgenic mouse model of PCa fed an obesogenic high-fat diet and MYC-overexpressing xenografts, Boufaied *et al* (76) performed metabolomic and transcriptomic profiling and demonstrated that a high-fat diet cooperates with MYC to drive lactate accumulation and extensive TME remodeling, including increased collagen deposition and infiltration of immunosuppressive myeloid cells; these changes were associated with accelerated tumor progression and poorer survival. In a separate study using hyperpolarized [1-^{13}C] pyruvate magnetic resonance imaging in patient-derived xenografts, Sushentsev *et al* (75) provided spatial metabolomic evidence that aggressive PCa lesions exhibit elevated lactate-to-pyruvate ratios, indicative of active lactate production and utilization, and this metabolic phenotype correlated with cribriform histology and adverse clinical outcomes. Complementing these findings, Awad *et al* (77) identified adipose triglyceride lipase (ATGL) as a critical regulator of metabolic plasticity in advanced PCa; genetic or pharmacological inhibition of ATGL reduced tumor growth in CRPC models by impairing lipid droplet mobilization and shifting metabolism toward oxidative stress, establishing a link between lipolysis and the Reverse Warburg-like metabolic adaptation. Together, these studies confirm that the Reverse Warburg effect is operational in aggressive PCa and that host-diet interactions together with lipolytic enzymes potentiate this metabolic rewiring.

Metabolic regulation of alternative splicing and AR-V7 generation. Emerging evidence indicates that metabolic reprogramming in the TME directly influences alternative splicing events that drive therapy resistance, particularly the generation of AR-V7. Spatial transcriptomic analyses by Zhao *et al* (41) revealed that lactate gradients are enriched in regions adjacent to CAF-rich stroma, where lactate uptake by PCa cells induces SNRPA lactylation at Lys123, promoting alternative splicing that generates AR-V7. Using metabolomic and transcriptomic analyses, Ippolito *et al* (42) demonstrated that lactate accumulation not only rewires lipid metabolism but also sustains a metabolic-epigenetic axis that alters splicing

Table I. Metabolic vulnerabilities in the metastatic castration-resistant prostate cancer tumor microenvironment: Pathways, mechanisms, immune consequences and therapeutic strategies.

Authors, year	Metabolic pathway/ vulnerability	Key mediators/mechanisms	Effect on TME/ immune function	Model system	Therapeutic strategy	(Refs.)
Boufaied *et al*, 2024	Reverse Warburg effect/ Lactate shuttling	High-fat diet + MYC cooperation; lactate accumulation	Promotes TME remodeling, collagen deposition, myeloid cell infiltration	Transgenic mouse model; MYC-overexpressing xenografts	Dietary intervention; lactate-targeting strategies	(76)
Sushentsev *et al*, 2024	Reverse Warburg effect	Elevated lactate-to-pyruvate ratio (hyperpolarized ^{13}C MRI)	Correlates with cribriform histology and adverse clinical outcomes	Patient-derived xenografts	Metabolic imaging for patient stratification	(75)
Awad *et al*, 2024	Reverse Warburg-like metabolism	Adipose triglyceride lipase (ATGL); lipid droplet mobilization	Supports metabolic plasticity and oxidative stress adaptation	CRPC cell lines and mouse models	ATGL inhibitors	(77)
Zhao *et al*, 2026	Lactate-driven alternative splicing	CAF-derived lactate; SNRPA lactylation (K123); AR-V7 splicing	Promotes castration resistance via AR-V7 generation	Single-cell trans-criptomics; *in vivo* PCa models	MCT inhibitors	(41)
Ippolito *et al*, 2024	Lactate-driven metabolic-epigenetic axis	Lactate rewires lipid metabolism; alters splicing factor activity	Supports metastatic behavior and ECM production	Prostate cancer cell lines	Metabolic-epigenetic inhibitors	(42)
Li *et al*, 2024	Metabolic regulation of splicing	hsa_circ_0085121; PI3K/ Akt/mTOR pathway; AR-V7 alternative splicing	Enhances AR-V7 generation and tumor progression	Prostate cancer cell lines	PI3K/Akt/mTOR pathway inhibitors	(78)
Yao *et al*, 2024	Glycolysis-associated splicing regulation	lncRNA SNHG3; miR-139-5p; PKM2 axis	Promotes CRPC development and enzalutamide resistance	CRPC cell lines and mouse models	PKM2-targeting strategies	(79)
Walker *et al*, 2024	Metabolic stress and splicing	Splicing factor requirements for AR variant synthesis	Modulates AR-V7 levels under metabolic stress	Advanced prostate cancer models	Splicing factor modulators	(80)
Xiong *et al*, 2024	Cholesterol biosynthesis	CXCL8; mTORC1/SREBP2 pathway; de novo cholesterol synthesis	Supports AR-negative cell survival; recruits myeloid cells; impairs $CD8^+$ T-cell activity	Human PCa specimens; RNA-seq; spatial transcriptomics	SREBP2 inhibitors	(81)
Sun *et al*, 2024	Cholesterol metabolism signature	Cholesterol-metabolism-related gene signature	Correlates with M2-polarised macrophages, exhausted T cells, and poor ICI response	Bulk and single-cell RNA-seq datasets	Cholesterol-targeting drugs; ICI combination	(82)
Dos Santos *et al*, 2024	Cholesterol biosynthesis	SREBP2 nuclear translo-cation; sterol-like drugs	Potentiates statin-triggered prostate cancer cell death	Prostate cancer cell lines	Sterol-like drugs + statins	(83)

Table I. Continued.

Authors, year	Metabolic pathway/ vulnerability	Key mediators/mechanisms	Effect on TME/ immune function	Model system	Therapeutic strategy	(Refs.)
Wang *et al*, 2024	Lipid metabolism	EXO1/p53/SREBP1 axis	Promotes CRPC progression via lipid synthesis	CRPC cell lines and mouse models	SREBP1-targeting agents	(84)
Liang *et al*, 2024	Cholesterol efflux/Lipid-immune crosstalk	Omega-3 fatty acids; GPR120; cholesterol efflux in TAMs	Reduces M2 polarization; improves T-cell infiltration	Prostate cancer mouse models	Dietary omega-3 supplementation	(85)
Guo *et al*, 2024	Pyrimidine synthesis (DHODH)	DHODH inhibition; fumarate accumulation; TCA cycle redirection	Impairs tumor proliferation; synergizes with glutamine blockade	Patient-derived organoids; high-throughput drug screen	DHODH inhibitors (e.g., brequinar)	(86)
Praharaj *et al*, 2024	Glutamine metabolism	Glutamine antagonist JHU083	Reprograms TAMs from M2 to M1; restores phagocytosis; enhances $CD8^+$ T-cell infiltration	Myeloid-rich prostate and bladder cancer models	Glutamine antagonists (JHU083)	(87)
Beier *et al*, 2024	Glutamine metabolism	Glutaminase (GLS); xCT transporter	Suppresses proliferation in mesenchymal docetaxel-resistant PCa cells	Docetaxel-resistant prostate cancer cell lines	GLS inhibitors; xCT inhibitors	(88)
Moon *et al*, 2024	Glutamine dependence	Glutamine antagonist DRP-104	Reduces proliferation and tumor growth in CRPC xenografts	CRPC xenograft models	Glutamine antagonists (DRP-104)	(89)
Zhang *et al*, 2025	Polyamine metabolism	Polyamine-based nanoparticle; oxidative/ carbonyl stress	Induces immunogenic cell death (ICD); converts 'cold' to 'hot' TME; durable memory responses	Established CRPC xenograft models	Polyamine-targeting nanoparticles	(90)
Affronti *et al*, 2025	Polyamine metabolism	Polyamine synthesis enzymes	Reduces tumor viability in *ex vivo* prostatectomy model	*Ex vivo* human prostatectomy specimens	Polyamine-targeting agents	(91)
Gao *et al*, 2024	Cuproptosis	Copper accumulation; mitochondrial respiration impairment	Enzalutamide sensitizes CRPC cells to copper-mediated cell death	CRPC cell lines and mouse models	Copper ionophores + enzalutamide	(92)

Table I. Continued.

Authors, year	Metabolic pathway/ vulnerability	Key mediators/mechanisms	Effect on TME/ immune function	Model system	Therapeutic strategy	(Refs.)
Yang *et al*, 2024	Cuproptosis	Cuproptosis-related gene signature	High expression correlates with improved prognosis and immune activation in PCa	Pan-cancer and PCa cohort analysis	Cuproptosis inducers	(93)
Li *et al*, 2026	Cuproptosis + glycolysis inhibition	Self-assembling copper-EGCG nanoreactor; TCA-dependent cuproptosis; glycolysis inhibition	Amplifies ICD; promotes DC maturation; M2-to-M1 macrophage repolarization; enhances $CD8^+$ T-cell infiltration	Prostate cancer mouse models	Copper-EGCG nanoreactors	(94)

Targeting metabolic vulnerabilities (lactate shuttling, glutamine dependence, polyamine metabolism, cholesterol synthesis, and cuproptosis) can simultaneously impair tumor growth and relieve immune suppression in metastatic castration-resistant prostate cancer, with combination strategies showing particular promise. AR-V7, androgen receptor splice variant 7; ATGL, adipose triglyceride lipase; CAF, cancer-associated fibroblast; CRPC, castration-resistant prostate cancer; DC, dendritic cell; DHODH, dihydroorotate dehydrogenase; ECM, extracellular matrix; EGCG, epigallocatechin gallate; GLS, glutaminase; ICD, immunogenic cell death; ICI, immune checkpoint inhibitor; MCT, monocarboxylate transporter; mTORC1, mechanistic target of rapamycin complex 1; PCa, prostate cancer; PKM2, pyruvate kinase M2; SREBP, sterol regulatory element-binding protein; TAM, tumor-associated macrophage; TCA, tricarboxylic acid; TME, tumor microenvironment.

factor activity, thereby promoting metastatic behavior in PCa cells. In a complementary study, Li *et al* (78) discovered that an androgen-targeted circular RNA, *hsa_circ_0085121*, encodes a novel protein that enhances AR-V7 alternative splicing via the PI3K/Akt/mTOR pathway, providing a direct link between metabolic signaling (PI3K/Akt) and splice variant generation. Furthermore, Yao *et al* (79) showed that the glycolysis-associated long non-coding RNA *SNHG3* promotes CRPC development and enzalutamide resistance through the *miR-139-5p*/pyruvate kinase M2 (PKM2) axis, indicating that the glycolytic enzyme PKM2 can be regulated by non-coding RNAs to influence splicing outcomes. Walker *et al* identified specific splicing factor requirements for *AR* variant synthesis, demonstrating that metabolic stress conditions alter the expression of these factors, thereby modulating AR-V7 levels (80). Collectively, these studies establish that metabolic cues, including lactate, glycolytic intermediates and lipid-derived signals, can orchestrate alternative splicing programs that generate AR-V7 and other resistance-associated splice variants, although the precise contribution of CAF-derived lactate vs. tumor-cell-autonomous metabolism remains to be fully dissected.

Lipid metabolism and immune evasion: from fatty acid synthesis to cholesterol signaling. Reprogramed lipid metabolism, particularly cholesterol biosynthesis and fatty acid oxidation, is intimately linked to immunosuppression in the mCRPC TME. Spatial transcriptomic analyses by Krossa *et al* (27) identified aggressive PCa signatures highlighting pro-inflammatory chemokine activity, with specific chemokine-receptor interactions organizing immunosuppressive niches. Moreover, Hirz *et al* (65) mapped the precise localization of $TREM2^+$ macrophages to peritumoral stromal niches, suggesting that immunosuppressive myeloid populations are spatially organized within lipid-rich microenvironments. Using a combination of RNA-seq, spatial transcriptomics and single-cell analyses of human PCa specimens, Xiong *et al* (81) demonstrated that androgen-ablative therapies induce the secretion of CXCL8 from tumor cells, which activates the mTORC1/sterol regulatory element-binding protein 2 (SREBP2) pathway and drives de novo cholesterol synthesis. This cholesterol-rich environment promoted the survival and proliferation of AR-negative PCa cells and was associated with increased infiltration of immunosuppressive myeloid cells and reduced $CD8^+$ T-cell activity. Sun *et al* (82) constructed a cholesterol-metabolism-related gene signature from bulk and single-cell data, revealing that high-risk patients exhibit a 'cold' immune phenotype with M2-polarised macrophages and exhausted T cells, and that this signature independently predicts poor response to immune checkpoint inhibitors. Moreover, Dos Santos *et al* (83) showed that sterol-like drugs potentiate statin-triggered PCa cell death by inhibiting SREBP2 nuclear translocation, offering a combinatorial approach to disrupt cholesterol-driven immune evasion. Wang *et al* (84) identified the EXO1/p53/SREBP1 axis as a key regulator of lipid metabolism that promotes CRPC progression, linking DNA damage signaling to sterol synthesis. Liang *et al* (85) further demonstrated that dietary omega-3 fatty acids enhance cholesterol efflux in tumor-associated macrophages via GPR120, reducing M2 polarization and

improving T-cell infiltration, highlighting that nutritional interventions can modulate the lipid-immune crosstalk. These results underscore that targeting cholesterol metabolism, either through SREBP2 blockade or dietary approaches, could simultaneously impair tumor growth and relieve immune suppression.

Targeting metabolic vulnerabilities in the TME. The metabolic dependencies of CRPC cells and the TME offer multiple actionable vulnerabilities. The recognition of spatial metabolic heterogeneity has direct therapeutic implications: Region-specific metabolic vulnerabilities suggest that combination metabolic targeting may be required to overcome spatially compartmentalized resistance (27,75). Guo *et al* (86) performed a high-throughput drug screen combined with metabolomic tracing in patient-derived organoids and identified dihydroorotate dehydrogenase (DHODH) as a critical metabolic vulnerability in both *AR*-positive and *AR*-negative CRPC cells. DHODH inhibition impaired pyrimidine synthesis, caused a >10-fold increase in fumarate, and redirected glucose carbons away from the tricarboxylic acid cycle, and combination with glutamine metabolism blockade was synergistic, suggesting a rational combinatorial strategy. In a separate approach, Praharaj *et al* (87) used a glutamine antagonist (JHU083) in myeloid-rich prostate and bladder cancer models and demonstrated that glutamine deprivation reprogramed tumor-associated macrophages from an immunosuppressive M2-like state to a pro-inflammatory M1 phenotype, restored phagocytosis and enhanced $CD8^+$ T-cell infiltration, leading to tumor control. Supporting this, Beier *et al* (88) showed that targeting glutamine metabolism suppressed cell proliferation in mesenchymal docetaxel-resistant PCa cells by inhibiting glutaminase and the xCT transporter, identifying a vulnerability in the resistant population. Moon *et al* (89) confirmed that pharmacological inhibition of glutamine uptake with DRP-104 (a glutamine antagonist) reduced proliferation and tumor growth in CRPC xenografts, validating glutamine dependence as a therapeutic target.

Polyamine metabolism has emerged as another actionable vulnerability. Zhang *et al* (90) engineered a polyamine-based nanoparticle that delivered a polyamine-targeting agent, inducing oxidative and carbonyl stress, triggering immunogenic cell death and converting 'cold' tumors into 'hot' ones, with complete regression of established CRPC xenografts and durable memory responses upon rechallenge. Affronti *et al* (91) further demonstrated that targeting polyamine metabolism in an *ex vivo* prostatectomy model reduced tumor viability, supporting clinical translation of this approach. Additionally, copper-induced cuproptosis represents a novel vulnerability. Gao *et al* (92) reported that enzalutamide sensitizes CRPC cells to copper-mediated cell death by promoting copper accumulation and impairing mitochondrial respiration and combination with copper ionophores enhanced anti-tumor efficacy. Yang *et al* (93) performed a pan-cancer analysis of cuproptosis-related genes and validated that high expression of cuproptosis regulators correlates with improved prognosis and immune activation in PCa, suggesting that cuproptosis inducers could be repurposed. Finally, Li *et al* (94) developed a self-assembling copper-EGCG nanoreactor that executed a dual-metabolic assault: Copper induced TCA-dependent cuproptosis while EGCG inhibited glycolysis. The nanoreactor depleted glutathione, downregulated ATP7B and amplified immunogenic cell death, converting the immunosuppressive TME into a highly inflamed state with robust dendritic cell maturation, M2-to-M1 macrophage repolarization, and $CD8^+$ T-cell infiltration. Collectively, these preclinical studies validate that targeting polyamine metabolism or cuproptosis can overcome metabolic resistance and re-awaken antitumor immunity.

6. Therapy-induced remodeling of the immune landscape

Standard-of-care therapies for mCRPC exert profound and often paradoxical effects on the tumor immune microenvironment. ADT, chemotherapy, PARP inhibitors and radiotherapy each induce distinct immunological changes that can either potentiate or undermine antitumor immunity. Table II summarized the key immunological effects of these modalities, detailing the model systems, underlying mechanisms and clinical implications, highlighting the dual nature of therapy-induced immune modulation and the importance of treatment sequencing. Understanding these therapy-induced dynamics is essential for rationally sequencing and combining treatments to optimize immune-mediated tumor control.

ADT and AR signaling inhibitors: The immunosuppressive paradox. ADT and AR signaling inhibitors (ARSIs) remain cornerstones of mCRPC management, yet their immunological consequences are complex and context-dependent. In a comprehensive single-cell and spatial transcriptomic analysis of prostate tumors from patients receiving neoadjuvant ADT, Obradovic *et al* (95) demonstrated that androgen deprivation enhances T-cell infiltration and induces adaptive Treg resistance, characterized by upregulation of PD-1 and CTLA-4 on tumor-infiltrating lymphocytes, suggesting that the timing of immune checkpoint blockade relative to ADT may critically influence efficacy. In a phase 2 study combining ADT with the PROSTVAC vaccine, Sater *et al* (96) showed that neoadjuvant ADT prior to radical prostatectomy markedly increased $CD8^+$ T-cell infiltration into the tumor microenvironment, providing evidence that ADT can prime the immune landscape for subsequent immunotherapies. Conversely, Xu *et al* (97) reported that enzalutamide-resistant PCa cells actively promote immunosuppressive alterations in the TME, including increased MDSCs infiltration and reduced $CD8^+$ T-cell activity, indicating that ARSIs resistance may establish a feed-forward loop of immune evasion. Qin *et al* (98) further synthesized evidence that inhibition of AR signaling ultimately leads to an immunosuppressive TME, an important consideration when combining ARSIs with immunotherapies. These contrasting findings highlight that the immunological effect of ADT/ARSIs depends on treatment duration, disease stage, and the presence of resistance mechanisms, underscoring the need for careful temporal coordination when combining hormonal therapies with immune checkpoint blockade.

Chemotherapy: immunogenic cell death vs. myeloid suppression. Taxane-based chemotherapy exerts dual and opposing effects on the immune landscape, inducing immunogenic cell death while simultaneously promoting immunosuppressive myeloid cell accumulation. Ma *et al* (11) demonstrated that

Table II. Therapy-induced remodeling of the immune landscape in metastatic castration-resistant prostate cancer.

Authors, year	Therapy/agent	Model/patient setting	Key immunological effects	Underlying mechanisms	Outcome/clinical implications	(Refs.)
Obradovic *et al*, 2020	ADT (neoadjuvant)	Localized prostate cancer patients	Enhanced T-cell infiltration; upregulated PD-1/CTLA-4 on TILs	Adaptive Treg resistance	Timing of ICI relative to ADT may be critical	(95)
Sater *et al*, 2020	ADT + PROSTVAC vaccine	Neoadjuvant setting prior to RP	Increased $CD8^+$ T-cell infiltration into TME	Vaccine-primed T-cell activation	ADT can prime immune landscape for subsequent immunotherapies	(96)
Xu *et al*, 2023	Enzalutamide (ARSI)	Enzalutamide-resistant PCa cells/mouse models	Increased MDSC infiltration; reduced $CD8^+$ T-cell activity	Feed-forward immunosuppressive loop	ARSI resistance establishes immune evasion	(97)
Qin *et al*, 2022	ARSIs (review)	Synthesis of evidence	Ultimately immunosuppressive TME	AR signaling inhibition promotes immunosuppression	Consider when combining ARSIs with ICIs	(98)
Ma *et al*, 2022	Docetaxel + anti-PD-1	Syngeneic mouse models	Depleted MDSCs; enhanced $CD8^+$ T-cell infiltration	Chemotherapy-induced ICD + MDSC depletion	Rationale for chemo-immunotherapy combinations	(11)
Laheurte *et al*, 2020	Metronomic cyclophosphamide	Biochemically recurrent PCa patients	Depleted Tregs; reactivated PSA-specific T cells	Dose-scheduling optimized for immunostimulation	Metronomic regimens favor immune activation	(99)
Chaudagar *et al*, 2023	Chemotherapy (lactate pathways)	PTEN/p53-deficient aggressive PCa models	Suppressed macrophage phagocytosis; immunosuppressive TME	Tumor cell lactate-generating pathways	Targeting lactate reverses chemotherapy-induced immunosuppression	(48,100)
Geng *et al*, 2023	PARPi (in SPOP-mutant models)	SPOP-mutant PCa models	Stimulated cGAS-STING; type I IFN response; $CD8^+$ T-cell infiltration	PARPi-induced growth suppression + STING activation	PARPi as immune-priming agents in SPOP-mutant tumors	(101)
Quinn *et al*, 2023	Niraparib + Radium-223	Phase 1 trial, mCRPC patients	Increased immune-related gene expression; enhanced T-cell activation	PARPi + RLT combination	Myeloid-suppressive pathways also upregulated; need combination strategies	(102)
Chen *et al*, 2025	ADT + PARPi (multi-omics)	Multi-omics integration	TAM plasticity continuum; SASP transition to MDSC-like phenotype	TREM2/SPP1 programs; senescence-associated secretory phenotype	Timing of combinations must account for myeloid-mediated suppression	(103)
Philippou *et al*, 2020	Anti-PD-L1 + Radiotherapy	Murine PCa model	Enhanced $CD8^+$ T-cell infiltration; delayed tumor growth	Abscopal effect	Compensatory immunosuppressive checkpoints also upregulated	(104)
Lin *et al*, 2021	High-dose per-fraction RT	Prostate tumor models	Increased $CD8^+$ T cells and MDSCs	Dual antitumor immunity and immunosuppression	Net effect determined by balance of opposing forces	(105)
Saylor *et al*, 2024	Radium-223 (RLT)	mCRPC patients; murine bone metastasis models	Immunomodulatory effects; facilitates anti-PD-1 therapy	RLT primes TME for checkpoint blockade	Clinical evidence for RLT-ICI combinations	(106)

Table II. Continued.

Authors, year	Therapy/agent	Model/patient setting	Key immunological effects	Underlying mechanisms	Outcome/clinical implications	(Refs.)
Czernin *et al*, 2021	^{225}Ac-PSMA-617 + ICI	Mouse model of PCa	Enhanced efficacy of RLT; prolonged survival	ICI enhances RLT efficacy	Combination markedly prolongs survival vs. monotherapy	(107)
Ferreira *et al*, 2025	Alpha vs. beta RLT	Murine PCa models	Alpha: DC activation, T-cell priming; Beta: depletion of myeloid populations	Distinct immunomodulatory profiles	Alpha and beta emitters have complementary immune effects	(108)

Each standard-of-care therapy exerts bidirectional immunomodulatory effects that evolve dynamically over time; successful combination strategies require careful temporal sequencing to first remodel the TME, then activate effector immunity, and finally sustain responses. ADT, androgen deprivation therapy; ARSI, androgen receptor signaling inhibitor; DC, dendritic cell; ICI, immune checkpoint inhibitor; ICD, immunogenic cell death; MDSC, myeloid-derived suppressor cell; PARPi, PARP inhibitor; PCa, prostate cancer; RLT, radioligand therapy; RP, radical prostatectomy; RT, radiotherapy; SASP, senescence-associated secretory phenotype; TAM, tumor-associated macrophage; TIL, tumor-infiltrating lymphocyte; TME, tumor microenvironment; Treg, regulatory T cell.

docetaxel remodels the PCa immune microenvironment by depleting MDSCs and enhancing CD8$^+$ T-cell infiltration; in syngeneic mouse models, docetaxel combined with anti-*PD-1* therapy exhibited superior antitumor efficacy compared with either agent alone, establishing a mechanistic rationale for chemotherapy-immunotherapy combinations. In a clinical study of biochemically recurrent PCa, Laheurte *et al* (99) showed that metronomic cyclophosphamide effectively depleted regulatory T cells and reactivated PSA-specific T cells, providing evidence that dose scheduling of chemotherapy can be optimized to favor immunostimulation over immunosuppression. However, Chaudagar *et al* (48,100) demonstrated that tumor cell lactate-generating signaling pathways, which are upregulated in chemotherapy-treated tumors, suppress macrophage phagocytosis and contribute to an immunosuppressive TME; targeting these pathways reversed the immunosuppressive effect and enhanced antitumor immunity. These observations indicate that the net immunological outcome of chemotherapy depends on dose, schedule and the specific TME context, with metronomic regimens and combination with agents that reverse myeloid suppression offering strategies to tilt the balance toward immune activation.

PARP inhibitors: Beyond DNA repair into TME modulation. *PARP* inhibitors (PARPi) extend beyond homologous recombination repair deficiency to exert direct and indirect effects on the immune TME. Geng *et al* (101) demonstrated that speckle-type POZ protein (SPOP) mutations in PCa target stimulator of interferon genes 1 (STING1) signaling, creating therapeutic vulnerabilities to PARPi-induced growth suppression; PARPi treatment stimulated the cyclic GMP-AMP synthase-STING pathway and induced a type I interferon response, promoting CD8$^+$ T-cell infiltration in *SPOP*-mutant models. In a phase 1 study, Quinn *et al* (102) evaluated niraparib in combination with radium-223 and reported that PARPi treatment was associated with increased expression of immune-related genes and enhanced T-cell activation, although myeloid-suppressive pathways were also upregulated, highlighting the need for combination strategies to overcome therapy-induced immunosuppression. Chen *et al* (103) integrated multi-omics analyses to demonstrate that TAM plasticity under ADT and PARP inhibition represents a continuum of states enriched for TREM2 and SPP1 programs, and that PARPi-induced senescence-associated secretory phenotypes ultimately transition to an MDSCs-like immunosuppressive phenotype. These findings suggest that PARPi can serve as immune-priming agents when combined with immune checkpoint inhibitors, but the timing of such combinations must account for the eventual emergence of myeloid-mediated suppression.

Radiotherapy and radioligand therapy: Abscopal effects and immune reprogramming. Radiotherapy and radioligand therapy (RLT) induce both local and systemic immune effects, offering opportunities for synergy with immunotherapy. In a murine PCa model, Philippou *et al* (104) demonstrated that combining anti-PD-L1 with radiotherapy markedly enhanced CD8$^+$ T-cell infiltration and delayed tumor growth compared with either treatment alone, but also noted upregulation of immunosuppressive checkpoints, indicating that the abscopal effect is potentiated yet constrained by compensatory immune

suppression. Lin *et al* (105) showed that high-dose per-fraction radiotherapy induces both antitumor immunity and immunosuppressive responses in prostate tumors, with increased infiltration of both $CD8^+$ T cells and MDSCs, suggesting that the net effect is determined by the balance between these opposing forces. Saylor *et al* (106) reported that the radiopharmaceutical radium-223 has immunomodulatory effects in patients and, in murine models, facilitates anti-PD-1 therapy, providing clinical evidence that RLT can prime the immune microenvironment for checkpoint blockade. Czernin *et al* (107) demonstrated that immune checkpoint blockade enhances the efficacy of ^{225}Ac-prostate-specific membrane antigen (PSMA)-617 in a mouse model of PCa, with combination treatment markedly prolonging survival compared with either monotherapy. Ferreira *et al* (108) compared α and β radiopharmaceutical therapy and found that both modalities induce distinct immunomodulatory profiles, with α emitters showing greater ability to activate dendritic cells and promote T-cell priming, while β emitters more effectively deplete immunosuppressive myeloid populations.

Collectively, the aforementioned evidence demonstrates that each standard-of-care modality exerts bidirectional immunomodulatory effects that are critically dependent on treatment timing, dosing schedule and disease stage. ADT and ARSIs enhance T-cell infiltration in the short term but ultimately promote adaptive Treg resistance and myeloid-mediated immunosuppression, particularly in the setting of acquired resistance (95-98). Chemotherapy, especially when administered metronomically, depletes MDSCs and Tregs while inducing immunogenic cell death, yet tumor cell lactate-generating pathways activated by chemotherapy can suppress macrophage phagocytosis and sustain an immunosuppressive TME (48,99,100). *PARP* inhibitors stimulate the cGAS-STING pathway and type I interferon responses, promoting $CD8^+$ T-cell infiltration, but prolonged treatment induces senescence-associated secretory phenotypes that transition to MDSC-like immunosuppression (101-103). Radiotherapy and RLT can convert 'cold' tumors into 'hot' ones through abscopal effects and dendritic cell activation, yet they also recruit MDSCs and upregulate compensatory immune checkpoints that constrain antitumor immunity (104-108). These observations underscore that the net immunological outcome of any given therapy is not fixed but evolves dynamically, necessitating careful temporal coordination when designing combination regimens. The 'prime, activate, sustain' framework, wherein therapies are sequenced to first remodel the TME, then activate effector immunity and finally sustain responses through maintenance strategies, offers a rational approach to harnessing these bidirectional effects (6).

7. Therapeutic strategies to overcome spatiotemporal resistance

The spatiotemporal co-evolution framework identifies multiple actionable vulnerabilities in the mCRPC ecosystem, ranging from immune checkpoint pathways to stromal and metabolic targets. This section reviewed emerging therapeutic strategies designed to overcome resistance, including immune checkpoint blockade, PKMYT1 inhibition, bispecific T-cell engagers, senolytic therapies, stroma-targeting approaches and biomarker-guided patient stratification. Table III summarized the key clinical and preclinical studies evaluating these approaches, outlining the therapeutic strategy, study type, major findings, clinical implications and current limitations for each modality, thereby providing a comprehensive evidence-based overview of the current landscape. These strategies and their mechanistic targets are summarized in Fig. 3.

Immune checkpoint blockade: current status and biomarker-driven approaches. ICIs have shown limited activity as monotherapy in unselected mCRPC patients, but biomarker-driven and combination approaches are refining their clinical utility. In the phase 2 NEPTUNES trial, Leone *et al* (109) evaluated nivolumab plus ipilimumab in mCRPC patients selected for an immunogenic signature ($CD8^+$ T-cell infiltration and PD-L1 expression); the combination achieved a 25% objective response rate in biomarker-positive patients, compared with 5% in unselected historical controls, demonstrating that patient selection can substantially improve ICIs efficacy. In the PORTER platform trial, Galsky *et al* (7) tested multiple ICIs combinations in mCRPC and identified that baseline $CD8^+$ T-cell density and interferon-γ gene expression signatures were associated with improved progression-free survival, though no single combination emerged as uniformly effective.

Negative trial results further underscore the limitations of unselected ICI approaches. The phase 1b/2 KEYNOTE-365 study evaluated pembrolizumab-containing combinations across multiple cohorts in mCRPC. In cohort C, which assessed pembrolizumab plus enzalutamide in abiraterone-pretreated patients, the combination showed limited antitumor activity, with a confirmed PSA response rate of only 24% and an objective response rate of 11% (95% CI 2.9-25%), accompanied by a 92% treatment-related adverse event rate (8). The phase III KEYNOTE-641 trial, which evaluated pembrolizumab plus enzalutamide vs. placebo plus enzalutamide in 1,244 mCRPC patients, subsequently failed to meet its dual primary endpoints of radiographic progression-free survival and overall survival, confirming the limited efficacy of this combination in an unselected population (7). These negative findings highlight that unselected ICIs combinations in mCRPC yield marginal efficacy at the cost of significant toxicity. The modest responses observed in a small subset of patients, particularly those with mismatch repair deficiency or high tumor mutational burden, suggest that the clinical benefit of ICIs in mCRPC is restricted to molecularly defined populations (2).

For patients with deleterious CDK12 alterations, Nguyen *et al* (110) conducted the phase 2 IMPACT trial and reported that *CDK12*-mutant mCRPCs exhibited enhanced neoantigen load and immune infiltration, and that anti-PD-1 therapy achieved a 38% PSA50 response rate in this molecularly defined subset, establishing CDK12 deficiency as a predictive biomarker for ICIs response. Markowski *et al* (111) investigated bipolar androgen therapy combined with nivolumab in the phase 2 COMBAT trial, showing that cyclic testosterone administration followed by ICIs induced tumor regression in 18% of patients and was associated with increased intratumoral $CD8^+$ T-cell infiltration, suggesting that androgen modulation can prime the immune microenvironment for

Table III. Emerging therapeutic strategies and biomarker approaches for overcoming spatiotemporal resistance in metastatic castration-resistant prostate cancer.

Authors, year	Therapeutic strategy/target	Study type/model	Key findings	Clinical/translational implications	Limitations/challenges	(Refs.)
Leone *et al*, 2025	Nivolumab + Ipilimumab (ICI combination)	Phase 2 trial (NEPTUNES)	25% ORR in biomarker-positive ($CD8^+$/PD-$L1^+$) mCRPC patients	Biomarker-driven patient selection substantially improves ICI efficacy	Requires validated immunogenic signature; limited to subset of patients	(109)
Galsky *et al*, 2025	Multiple ICI combinations (PORTER platform)	Phase 1 platform trial	Baseline $CD8^+$ T-cell density and IFN-γ signature associated with improved PFS	No single combination uniformly effective; platform trials enable parallel testing	Heterogeneous responses; biomarker refinement needed	(7)
Nguyen *et al*, 2024	Anti-PD-1 therapy in CDK12-altered mCRPC	Phase 2 trial (IMPACT)	38% PSA50 response rate in CDK12-mutant mCRPC	CDK12 deficiency as predictive biomarker for ICI response	CDK12 alterations occur in only 3-5% of prostate cancers	(110)
Markowski *et al*, 2024	Bipolar androgen therapy (BAT) + Nivolumab	Phase 2 trial (COMBAT)	18% tumor regression; increased intratumoral $CD8^+$ T-cell infiltration	Androgen modulation can prime TME for checkpoint blockade	Complex dosing schedule; requires further validation	(111)
Dorff *et al*, 2024	Acapatamab (PSMA-targeting BiTE)	Phase 1 trial	35% PSA decline; manageable at lower doses	Half-life extended BiTE shows clinical activity in heavily pretreated mCRPC	CRS and neurological toxicities dose-limiting	(112)
Hudson *et al*, 2026	JNJ-80038114 (PSMAxCD3 bispecific antibody)	Phase 1 trial	12% confirmed PSA response; neurologic AEs and CRS observed	Next-generation formats with attenuated CD3 affinity may be required	Limited efficacy; significant toxicity	(113)
Narayan *et al*, 2022	PSMA-targeting TGF-β-insensitive armored CAR-T cells	Phase 1 trial	40% PSA decline; 1 confirmed PR; enhanced CAR-T persistence	Armored design overcomes TGF-β-mediated immune suppression	CAR-T persistence still limited by hostile TME	(114)
Dorff *et al*, 2024	PSCA-CAR-T cells	Phase 1 trial	Feasible with objective responses in subset	PSCA as alternative target for CAR-based therapy	CAR-T expansion correlates with response; limited by TME	(115)
Brunner *et al*, 2025	YAP1-TGF-β1 axis targeting in CAFs	PDX models; genetic/pharmacological targeting	Combined targeting represses myCAF hallmarks; sensitizes to enzalutamide	Stromal YAP1-TGF-β1 axis as actionable vulnerability	Heterogeneity of CAF subtypes may affect efficacy	(39)
Song *et al*, 2024	YAP1 inhibition	In vivo mouse models	Induces CAF phenotype switching to tumor-suppressive; enhances $CD8^+$ T-cell infiltration	YAP1 inhibition as stromal-reprogramming strategy to improve ICI response	Phenotype switching may be context-dependent	(12)

Table III. Continued.

Authors, year	Therapeutic strategy/target	Study type/model	Key findings	Clinical/translational implications	Limitations/challenges	(Refs.)
Wang *et al*, 2023	Antiandrogen-induced stromal reprogramming	Preclinical models	Antiandrogen therapy paradoxically reprograms CAFs to promote castration resistance	Highlights bidirectional stromal dynamics; need for combination approaches	Stromal plasticity may limit monotherapy efficacy	(116)
He *et al*, 2026	Senolytic/NF-κB2/p52 pathway blockade	PDX models; genetic models	Reverses TGF-β-driven vascular-stromal barrier; restores antiandrogen sensitivity	Targeting senescent CAF ecotypes can overcome protective niches	Senolytic strategies require careful safety evaluation	(117)
Knutson *et al*, 2024	ctDNA-based AR alteration monitoring	Liquid biopsy (ctDNA)	AR amplifications and AR-V7 detected; predict poor response to AR pathway inhibitors	ctDNA-based AR monitoring as clinically actionable biomarker	ctDNA detection may be limited by low tumor shedding	(118)
Sweeney *et al*, 2024	Longitudinal ctDNA assessment	Liquid biopsy (ctDNA)	ctDNA dynamics provide earlier resistance detection and more accurate PFS prediction than PSA alone	ctDNA monitoring adds value to PSA for treatment monitoring	Requires serial sampling and standardized assays	(119)
Buteau *et al*, 2022	PSMA-PET and FDG-PET imaging biomarkers	Phase 2 biomarker analysis (TheraP)	Baseline PSMA-PET tumor volume and FDG-PET positivity independently predict OS	Imaging biomarkers guide patient selection for ^{177}Lu-PSMA-617 vs. cabazitaxel	Requires access to advanced PET imaging; cost-intensive	(120)
Armstrong *et al*, 2025	AI-based digital pathology biomarker	Phase 3 trial validation	Predicts benefit from long-term hormonal therapy and radiotherapy	Computational pathology enables automated patient stratification	Requires validation across diverse populations and platforms	(121)

Therapeutic strategies overcoming spatiotemporal resistance require biomarker-guided patient selection; combination approaches targeting ICI, stromal YAP1-TGF-β1 axis, and senescent CAF ecotypes show promise, while CAR-T/BiTE and liquid biopsy-based monitoring are advancing clinical translation. AE, adverse event; AI, artificial intelligence; AR, androgen receptor; AR-V7, androgen receptor splice variant 7; BAT, bipolar androgen therapy; BiTE, bispecific T-cell engager; CAF, cancer-associated fibroblast; CAR, chimeric antigen receptor; CRS, cytokine release syndrome; ctDNA, circulating tumor DNA; FDG, fluorodeoxyglucose; ICI, immune checkpoint inhibitor; myCAF, myofibroblastic CAF; NF-κB, nuclear factor-kappa B; ORR, objective response rate; OS, overall survival; PDX, patient-derived xenograft; PET, positron emission tomography; PFS, progression-free survival; PSA, prostate-specific antigen; PSA50, ≥50% decline in PSA; PSMA, prostate-specific membrane antigen; TGF-β, transforming growth factor-beta; TME, tumor microenvironment; YAP1, Yes-associated protein 1.

checkpoint blockade. Collectively, these studies indicate that ICIs in mCRPC are most effective when guided by predictive biomarkers (CDK12 status, immunogenic signatures) or combined with agents that remodel the TME.

PKMYT1 inhibition as a novel immune-modulatory strategy. Recent preclinical evidence has identified PKMYT1 as an emerging therapeutic vulnerability in mCRPC with direct relevance to spatiotemporal immune co-evolution. Gao *et al* (14) demonstrated that targeting PKMYT1 enhances antitumor immune responses to PD-L1 blockade in castration-resistant prostate cancer models. Mechanistically, PKMYT1 inhibition promotes $CD8^+$ T-cell infiltration into the tumor microenvironment and reduces the accumulation of immunosuppressive myeloid populations, thereby converting immunologically 'cold' tumors into 'hot' ones amenable to checkpoint blockade. This immune-modulatory effect is particularly relevant within the spatiotemporal co-evolution framework, as PKMYT1 inhibition appears to disrupt the fibroblast-dominated immune exclusion zones that characterize advanced mCRPC, potentially enabling T-cell access to tumor nests that would otherwise remain protected. The combination of PKMYT1 inhibitors with PD-L1 blockade has shown synergistic antitumor efficacy in preclinical models, suggesting that this approach may overcome both cell-intrinsic resistance and microenvironmental barriers (14). These findings position PKMYT1 as a promising target for vertically integrated combination strategies that simultaneously address tumor cell proliferation and immune evasion within the evolving mCRPC ecosystem.

Bispecific T-cell engagers (BiTEs) and chimeric antigen receptor (CAR)-macrophages in prostate cancer immunotherapy. BiTEs and CAR-based therapies represent emerging modalities for redirecting immune effector cells against PCa. In a phase 1 study of acapatamab, a half-life extended PSMA-targeting BiTE, Dorff *et al* (112) reported that the agent was well tolerated at lower dose levels and induced PSA declines in 35% of patients with heavily pretreated mCRPC, though cytokine release syndrome and neurological toxicities were dose-limiting, highlighting the need for careful dose optimization. In the phase 1 study of JNJ-80038114, a PSMA×CD3 bispecific antibody, Hudson *et al* (113) observed limited clinical activity, with only 12% of patients achieving a confirmed PSA response and treatment was complicated by neurologic adverse events and cytokine release syndrome, suggesting that next-generation formats with attenuated CD3 affinity may be required to improve the therapeutic index. For CAR-based approaches, Narayan *et al* (114) conducted a phase 1 trial of PSMA-targeting TGF-β-insensitive armored CAR T-cells in mCRPC, demonstrating that the construct was safe and induced PSA declines in 40% of patients, with one confirmed partial response; the armored design, which overcomes TGF-β -mediated immune suppression, appeared to enhance CAR T-cell persistence in the immunosuppressive TME. Additionally, Dorff *et al* (115) reported results from a phase 1 trial of PSCA-CAR T-cells in mCRPC, showing that the approach was feasible and induced objective responses in a subset of patients, though CAR T-cell expansion correlated with response and was limited by the hostile TME. These studies underscore that BiTEs and CAR-based therapies hold promise but require optimization of target selection, TME conditioning, and toxicity management.

Stroma-targeting strategies: CAF reprogramming, depletion and the YAP1-TGF-β axis. CAFs are central to therapy resistance and immune exclusion, presenting actionable vulnerabilities in mCRPC. Mechanistically, Brunner *et al* (39) established that the NFκB-TGF-β1-YAP1 axis drives *AR* loss in myofibroblastic CAFs and combined pharmacological targeting of this axis synergistically repressed protumorigenic hallmarks while sensitizing cells to enzalutamide. In parallel, YAP1 inhibition was shown to induce a phenotypic switch of CAFs toward a tumor-suppressive state, consequently enhancing $CD8^+$ T-cell infiltration and improving responses to immune checkpoint blockade (12). However, stromal dynamics are inherently bidirectional, as antiandrogen therapy can paradoxically reprogram CAFs to promote castration resistance (116). Furthermore, therapeutic pressure has been found to drive the evolution of senescent CAF ecotypes that establish protective niches, thereby constraining treatment efficacy (117). Collectively, these observations indicate that while disrupting YAP1-TGF-β1 signaling and depleting pathogenic CAFs represent promising approaches, the pronounced plasticity and functional heterogeneity of CAF subtypes necessitate subtype-specific targeting strategies to mitigate unintended protumorigenic consequences.

Biomarkers for patient stratification and resistance monitoring: tissue-based and liquid biopsy approaches. Biomarker-driven patient stratification is essential for optimizing therapeutic strategies in mCRPC. Based on the spatiotemporal co-evolution framework, biomarkers can be reclassified into two complementary categories: Temporal biomarkers, which monitor resistance evolution over time and inform adaptive treatment sequencing; and spatial biomarkers, which reflect intratumoral heterogeneity and niche architecture to guide patient selection.

Temporal biomarkers include longitudinal ctDNA assessment for AR alterations (including AR amplifications and AR-V7 expression), which predict poor response to AR pathway inhibitors and provide earlier detection of resistance emergence than PSA alone (118,119). Spatial biomarkers encompass imaging-based parameters such as baseline prostate-specific membrane antigen positron emission tomography (PSMA-PET) total tumor volume and fluorodeoxyglucose positron emission tomography (FDG-PET) positivity, which independently predict overall survival and guide patient selection for ^{177}Lu-PSMA-617 vs. cabazitaxel (120), as well as AI-based digital pathology signatures that predict benefit from long-term hormonal therapy and radiotherapy (121). Integrating these temporal and spatial biomarker classes enables precision selection of therapies and dynamic monitoring of resistance evolution. Knutson *et al* (118) demonstrated that AR alterations can be reliably detected in ctDNA and that their presence predicts poor response to AR pathway inhibitors. Sweeney *et al* (119) reported that longitudinal ctDNA assessment for treatment monitoring adds value to PSA in mCRPC, with ctDNA dynamics providing earlier detection of resistance emergence and more accurate prediction of progression-free survival than PSA alone. For PSMA-targeted radioligand

therapy, Buteau *et al* (120) analyzed biomarker data from the TheraP trial and showed that baseline PSMA-PET total tumor volume and fluorodeoxyglucose-PET positivity independently predicts overall survival and that these imaging biomarkers can guide patient selection for ^{177}Lu-PSMA-617 vs. cabazitaxel. In the immunotherapy setting, Armstrong *et al* (121) developed a digital pathology-based AI biomarker that predicted benefit from long-term hormonal therapy and radiotherapy across multiple phase 3 trials, demonstrating the potential of computational pathology for patient stratification. These studies underscore that integrating tissue-based (genomic classifiers, AR-V7), liquid biopsy (ctDNA, circulating tumor cells) and imaging biomarkers (PSMA-PET) can enable precision selection of therapies and dynamic monitoring of resistance evolution.

Adaptive therapy: Evolutionary-informed treatment scheduling. Adaptive therapy represents a paradigm shift from maximal cytotoxic dosing to a strategy that deliberately maintains residual tumor burden to preserve competitive suppression of resistant clones (122). This approach directly translates the spatiotemporal co-evolution framework into clinical application by exploiting the ecological principle that treatment-sensitive cells, when retained, outcompete resistant populations. Mathematical modelling has provided the theoretical foundation: Gallaher *et al* (122) developed models demonstrating that optimized adaptive schedules can prolong time to progression compared with continuous therapy. Early clinical proof-of-concept has emerged from a case report by Gatenby *et al* (123), who demonstrated that directed evolution principles restored castrate sensitivity in a patient with mCRPC using PSA/testosterone ratio as a biomarker for population dynamics, achieving three stable cycles of response. The clinical translation is now being evaluated in large-scale prospective trials, including the randomized phase III RECIPROCAL trial (NCT07200830) testing PSA-adaptive dosing of ^{177}Lu-PSMA radioligand therapy vs. standard fixed-interval dosing in patients with mCRPC (2,5). These developments position adaptive therapy as a clinically actionable strategy that directly embodies the spatiotemporal co-evolution framework, offering a roadmap for converting mCRPC into a chronically manageable condition through precisely timed, evolution-informed interventions.

8. Future perspectives

The tumor-immune spatiotemporal co-evolution paradigm reframes mCRPC resistance as an ecosystem-level adaptation unfolding across both temporal and spatial dimensions. This conceptual shift carries profound implications for future research and clinical practice. The spatial architecture of the TME, defined by the cellular networks of Tregs, MDSCs, TAMs and CAFs that actively silence antitumor immunity, must be integrated into routine clinical trial design. Andersen *et al* (124) revealed strong associations between SFRP4 and extracellular matrix remodeling in PCa, suggesting that spatial biomarkers may guide patient stratification while Blanke *et al* (125) defined CAFs subtypes that independently predict patient outcomes. These spatially resolved signatures, when combined with the temporal dynamics of clonal evolution documented by Zivanovic *et al* (126), offer a comprehensive view of the ecological drivers of resistance that can inform biopsy strategies and treatment selection.

However, several technical limitations currently constrain the clinical translation of spatial transcriptomics in mCRPC. First, most studies are limited by small sample sizes, which restricts statistical power for identifying robust spatial signatures associated with therapy response (35,56,65). Second, the lack of longitudinally matched specimens, that is, tumor samples collected before, during and after therapy from the same patient, prevents direct tracking of spatiotemporal evolution under therapeutic selection pressure, limiting current understanding to cross-sectional snapshots rather than dynamic trajectories (27,59). Third, current spatial resolution, while improving, remains insufficient to resolve single-cell interactions within complex niches; most platforms capture spots containing multiple cells, obscuring the precise spatial relationships between individual immune, stromal and tumor cells (56,65). Fourth, integrating spatial transcriptomics with other omics layers (metabolomics, proteomics, and longitudinal liquid biopsy data) remains technically challenging and computationally intensive (27,127). Addressing these limitations will require multi-center consortia to collect larger, longitudinally sampled cohorts; development of higher-resolution spatial technologies capable of single-cell or near-single-cell resolution; and standardized analytical pipelines for multi-omics integration.

The recognition that pre-existing castration-tolerant progenitors and adaptive resistance mechanisms operate from the earliest stages of therapy underscores the urgent need for dynamic monitoring and evolutionary-informed treatment sequencing. Mathematical modelling has emerged as a powerful tool to address this challenge. Gallaher *et al* (122) developed models of intermetastatic and intrametastatic heterogeneity that can simulate adaptive therapy cycling dynamics, providing a quantitative framework for optimizing treatment schedules. A case report by Gatenby *et al* (123) demonstrated that directed evolution principles restored castrate sensitivity in a patient with metastatic castration-resistant PCa, providing proof-of-concept that evolutionary-informed therapy can be clinically implemented. Concurrently, the development and refinement of humanized mouse models and patient-derived organoids, as highlighted by Beshiri *et al* (128), will be critical for bridging the gap between preclinical discoveries and clinical translation. These models have already been applied to evaluate novel immunotherapies, including a dnTGF-β RII-armored STEAP2-targeting CAR-T cell therapy that demonstrated potent antitumor activity in PCa models (129).

The cellular drivers of therapy resistance detailed throughout the present review, particularly CAF heterogeneity, TAM plasticity and neuroendocrine lineage plasticity, point toward stroma-reprogramming and lineage-targeting strategies as promising therapeutic frontiers. The fibroblast-dominated immune exclusion zones that physically bar $CD8^+$ T cells from tumor nests represent a major barrier to immunotherapy efficacy and emerging evidence indicates that disrupting these stromal barriers can restore immune access. Song *et al* (12) showed that YAP1 inhibition induces phenotype switching of CAFs to a tumor-suppressive state, offering a potential strategy to enhance immunotherapy

efficacy. Brunner *et al* (39) demonstrated that targeting the YAP1-TGF-β1 axis in CAFs can reverse *AR* loss and restore treatment sensitivity, highlighting the therapeutic potential of disrupting stromal-immune crosstalk. Severson *et al* (13) further demonstrated that epigenetic profiling can identify markers of endocrine resistance and reveal therapeutic options for metastatic castration-resistant PCa patients, linking epigenomic alterations to clinical outcome and providing a rationale for combination strategies that include epigenetic modulators.

The metabolic and therapy-induced immune remodeling described in the present review highlight the importance of integrating multi-omics data to capture the full complexity of the TME. The application of artificial intelligence and machine learning to integrate single-cell transcriptomics, spatial metabolomics, and longitudinal liquid biopsy data will accelerate biomarker discovery and enable real-time monitoring of resistance evolution (127,130). Priority directions for future research include: establishing prospective, multi-center cohorts with longitudinally collected tumor specimens to track spatiotemporal evolution in real time; developing and validating spatial biomarkers that predict response to ICIs, stroma-targeting agents, and adaptive therapy; integrating spatial transcriptomics with single-cell and liquid biopsy data to build comprehensive ecological models of resistance; and designing clinical trials that incorporate adaptive treatment algorithms and dynamic biomarker monitoring to test the spatiotemporal co-evolution framework prospectively. This integrative approach is essential for implementing the 'Dynamic Monitoring-Mechanistic Parsing-Synergistic Intervention' framework, which advocates for longitudinal ecological auditing of the TME to rationally guide mechanistically orthogonal combination therapies. The convergence of these computational tools with emerging therapeutic modalities, including bispecific T-cell engagers, CAR-macrophages and stroma-targeting approaches, offers unprecedented opportunities for precision ecological intervention.

The successful translation of the spatiotemporal co-evolution paradigm into clinical practice will require prospective trials that incorporate dynamic biomarker monitoring and adaptive treatment algorithms. The therapeutic strategies outlined in this review, from immune checkpoint blockade guided by CDK12 and immunogenic signatures to stroma-targeting approaches that disrupt the YAP1-TGF-β1 axis, must be validated in biomarker-stratified, longitudinally sampled cohorts. Ultimately, the goal is to transform mCRPC from a uniformly fatal disease into a chronically manageable condition through precise, temporally sequenced, and ecologically informed interventions. As demonstrated by the studies reviewed here, the tools and concepts are now available; the challenge lies in their systematic integration into clinical trial design and patient care.

9. Conclusions

The tumor-immune spatiotemporal co-evolution paradigm reframes mCRPC resistance as an ecosystem-level adaptive process governed by dynamic interactions among tumor cells, stromal fibroblasts and immune populations across both temporal and spatial dimensions. This framework underscores that effective therapy must move beyond static, reductionist models toward dynamic monitoring and mechanistically rational combination strategies. Embracing this ecological perspective offers a transformative roadmap for converting mCRPC into a chronically manageable condition through precisely timed, spatially informed interventions that disrupt co-evolutionary trajectories before resistance becomes entrenched.

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LY and XS contributed to the conception and design of the review, performed the literature search and data extraction and drafted the initial manuscript. PS participated in the critical revision of the manuscript for important intellectual content, provided supervision and guidance on the overall framework and interpretation of the included evidence, and gave final approval of the version to be published. All authors read and approved the final manuscript. Data authentication is not applicable.

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Competing interests

The authors declare that they have no competing interests.

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