Review

Precision Targeting in Metastatic Prostate Cancer: Molecular Insights to Therapeutic Frontiers

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Article

Precision Targeting in Metastatic Prostate Cancer: Molecular Insights to Therapeutic Frontiers

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Abstract: Metastatic prostate cancer (mPCa) remains a significant global health concern and cause of cancer-related mortality in men. Advances in molecular profiling have illuminated the critical drivers of disease progression and therapeutic resistance, notably within the androgen receptor (AR) axis, DNA damage repair (DDR) pathways, and PI3K/AKT/mTOR network. Despite the established benefits of hormone therapy, chemotherapy, and bone-targeting agents, mPCa commonly evolves into a treatment-resistant state, typified by intrapatient heterogeneity and clonal evolution. Recent breakthroughs have highlighted the importance of identifying actionable genetic alterations such as BRCA2 or ATM defects that render tumors sensitive to poly-ADP ribose polymerase (PARP) inhibitors. Parallel efforts have refined imaging—particularly prostate-specific membrane antigen (PSMA) PET-CT—to detect and localize metastatic lesions with high sensitivity, thereby guiding patient selection for PSMA-targeted radioligand therapies (^{177}Lu-PSMA, ^{225}Ac-PSMA). Multi-omics innovations, including liquid biopsy technologies, enable real-time tracking of emergent AR splice variants (e.g., AR-V7) or reversion mutations, supporting adaptive therapy paradigms. Nonetheless, the complexity of mPCa necessitates combination strategies—for example, pairing AR inhibition with PI3K/AKT blockade or PARP inhibitors—to intercept tumor plasticity. Immuno-oncology approaches remain challenging in unselected patients; however, subsets with mismatch repair deficiency (MSI-high) or neuroendocrine phenotypes may benefit from immune checkpoint blockade or targeted epigenetic interventions. We present these pivotal advances and discuss how biomarker-guided integrative treatments can improve the management of mPCa.

Keywords: metastatic prostate cancer; precision medicine; PARP inhibitors; PSMA-targeted therapy; tumor microenvironment

1. Introduction

Prostate cancer (PCa) is one of the most commonly diagnosed malignancies in men worldwide, with over 1.4 million new cases and more than 375,000 deaths annually [1]. North America, Europe, and Australia report particularly high incidence rates, reflecting genetic factors, lifestyle, and screening practices [2]. While localized or locally advanced disease can often be managed with surgery or radiation therapy, a notable percentage of patients present with metastatic disease or progress to metastasis, notably to the bones, lymph nodes, lungs, or liver [1,2]. Once PCa metastasizes, the five-year survival rate remains poor, especially in metastatic castration-resistant PCa (mCRPC) [3].

Over the past two decades, next-generation androgen receptor (AR) pathway inhibitors, taxane-based chemotherapy, bone-targeted therapies, and prostate-specific membrane antigen (PSMA)-

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directed radioligand therapies have resulted in increased survival. Nevertheless, treatment resistance frequently arises, driven by AR splice variants, lineage plasticity, and upregulation of survival pathways [4]. The emergence of precision oncology is driven by high-throughput DNA/RNA sequencing, single-cell omics, and other advanced technologies [5]. Landmark projects (TCGA, SU2C/PCF Dream Teams) have identified alterations in DNA damage r repair (DDR) genes (BRCA2, ATM, and CHEK2), the PI3K/AKT/mTOR pathway (often via PTEN loss), and AR amplifications/mutations [6]. The clinical relevance is evident in the success of poly-ADP ribose polymerase (PARP) inhibitors (olaparib, rucaparib) in mCRPC patients harboring DDR mutations [7–9].

AR splice variant detection, particularly of AR-V7, has emerged as a biomarker of resistance to AR-targeted therapies [10]. PSMA PET-CT enables the earlier detection of micrometastatic disease and guides PSMA-targeted radioligand therapy [11].

However, metastatic PCa remains highly heterogeneous [12,13]. Clonal evolution and an immunosuppressive tumor microenvironment (TME) impede durable responses, emphasizing the need for TME reprogramming [14–19].

Multidisciplinary strategies integrating genomic data, advanced imaging, adaptive trials, and real-world evidence aim to refine patient stratification, identify actionable targets, and optimize therapy sequences [20–24].

As PCa management becomes increasingly complex, owing to the rapid expansion of precision oncology tools, novel therapeutic agents, and dynamic insights into the TME, an up-to-date synthesis of these advancements is vital. A timely review provides clinicians and researchers with the necessary context to integrate emerging data into evidence-based practice, help overcome resistance mechanisms, and realize the promise of truly personalized, potentially transformative care.

This review discusses the molecular underpinnings of disease progression, key diagnostic and stratification methods (e.g., next-generation sequencing (NGS) liquid biopsies), current and emerging therapeutics (AR inhibitors, PARP inhibitors, immunotherapies, radioligand therapies), and future directions for TME modulation and artificial intelligence (AI)-driven approaches. Ultimately, this comprehensive synthesis aimed to improve outcomes and explore the potential for long-term disease control—even cure—in carefully defined molecular subsets.

2. Molecular Pathophysiology of Metastatic Prostate Cancer

Several key molecular alterations drive the progression and therapeutic resistance of metastatic prostate cancer, including AR amplification/splice variants, PTEN loss, and DNA damage repair (DDR) defects. These alterations are summarized in Table 1 along with their clinical implications and potential therapeutic avenues.

Table 1. Major Molecular Alterations in Metastatic Prostate Cancer and Their Clinical Implications.

Molecular Target or Genetic Alteration	Key Mechanism/Function	Clinical Features	Clinical Utility
AR Amplification / AR Splice Variants (e.g., AR-V7)	Sustained AR signaling under low-androgen conditions; ligand-independent activation	Poor response or resistance to AR-targeted therapies; commonly seen in mCRPC	Predicts resistance to enzalutamide or abiraterone; potential biomarker for treatment selection
PTEN Loss	Hyperactivation of the PI3K/AKT/mTOR pathway; cross-talk with AR signaling	Associated with high-grade tumors and	May guide PI3K/AKT/mTOR inhibitor-based combination trials;

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		aggressive clinical course	potential prognostic indicator
DDR Defects (e.g., BRCA2, ATM)	Impaired DNA repair and increased genomic instability; vulnerability to PARP inhibition	More aggressive behavior if untreated; better response to PARP inhibitors	Companion diagnostics for PARP inhibitors; synthetic lethality-based therapy targeting
TMPRSS2-ERG Fusion	ETS transcription factor (ERG) overexpression; promotes invasion, EMT, and genomic instability	High prevalence in localized prostate cancer; variable association with outcomes in mPCa	Potential prognostic marker in combination with other alterations (e.g., PTEN)
PI3K/AKT/mTOR Mutations	Aberrant cell proliferation and survival; metabolic reprogramming	Often co-occurs with AR pathway alterations; contributes to therapeutic resistance	Under investigation in clinical trials targeting AKT and mTOR; potential combination strategy with AR inhibitors
TP53 / RB1 Co-mutations	Disruption of cell-cycle checkpoints; may facilitate lineage plasticity or neuroendocrine differentiation	Common in advanced mPCa; associated with poor prognosis	Emerging biomarker for early switch to chemotherapy or combination therapies

Abbreviations: AKT, Protein Kinase B; AR, Androgen Receptor; AR-V7, Androgen Receptor Splice Variant 7; ATM, Ataxia Telangiectasia Mutated; BRCA2, Breast Cancer Susceptibility Gene 2; DDR, DNA Damage Repair; EMT, Epithelial–Mesenchymal Transition; ERG, ETS-Related Gene; ETS, E26 Transformation-Specific; mCRPC, Metastatic Castration-Resistant Prostate Cancer; mPCa, Metastatic Prostate Cancer; mTOR, Mechanistic Target of Rapamycin; PARP, Poly(ADP-ribose) Polymerase; PI3K, Phosphoinositide 3-Kinase; PTEN, Phosphatase and Tensin Homolog; RB1, Retinoblastoma 1; TMPRSS2, Transmembrane Protease, Serine 2; TP53, Tumor Protein p53.

2.1. Centrality of the Androgen Receptor (AR) Axis

2.1.1. Historical Underpinnings and Core AR Functions

In the 1940s, Charles Huggins provided seminal evidence that prostate tumors regress under surgical castration or estrogen therapy, establishing androgen dependence as a fundamental driver of PCa progression [25]. This discovery remains the cornerstone of therapy for advanced or high-risk PCa for which androgen deprivation therapy (ADT) continues to be the mainstay. Although early studies could not fully harness modern molecular techniques, they laid the groundwork for understanding the pivotal "testosterone–dihydrotestosterone (DHT)–AR" signaling axis in both normal and malignant prostate cells.

AR is a ligand-dependent transcription factor comprising an N-terminal transactivation domain (NTD), DNA-binding domain (DBD), hinge region, and ligand-binding domain (LBD). In normal prostate cells, testosterone is converted to DHT, which binds the AR-LBD, triggers a conformational change, and facilitates nuclear translocation. Once in the nucleus, AR binds to androgen response elements (AREs) and recruits cofactors (e.g., p300/CBP and SRC-1) to regulate genes that promote proliferation, survival, and secretory functions.

Early work with first-generation anti-androgens (e.g., flutamide) showed temporary growth control but soon revealed a rapid onset of resistance driven by compensatory alterations within AR

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signaling [26,27]. These findings underscore the remarkable adaptability of the AR pathway and highlight the clinical challenges associated with CRPC. While confirming that the central role of AR was once considered sufficient for clinical effect, advanced molecular and genomic technologies now enable deeper insights into how AR signaling becomes dysregulated or adapts to therapeutic pressure.

Early in vivo tumor regrowth assays demonstrated the importance of AR signaling; however, by modern standards, they lacked the robust molecular stratification required to capture the heterogeneity among different patient subgroups [28]. Consequently, historical clinical trials have provided only partial insights into the full spectrum of AR mutations, splice variants, and crosstalk with growth factor pathways. Over time, the integration of genomic, transcriptomic, and proteo-mic techniques has yielded more nuanced patient-specific treatment approaches, continually raising the pivotal clinical and scientific question of how best to inhibit AR-related tumor progression [29].

2.1.2. Routes to AR Reactivation Under Therapeutic Pressure

During prolonged androgen deprivation therapy (ADT) in prostate cancer, strong selective pressure within a low adrogen environment favors the survival of tumor cell clones that maintain androgen receptor (AR) activity, ultimately leading to castration-resistant prostate cancer (CRPC). In this setting, AR gene amplification and overexpression enable tumors to respond to even minimal levels of androgen or weak agonists, while mutations in the receptor's ligand-binding domain (LBD), such as T877A or F876L, can convert certain anti-androgens into partial agonists that pro-mote tumor growth [30]. Additionally, growth factor pathways like IGF-1R and EGFR can activate AR in a ligand-independent manner, and enzymes such as CYP17A1 or AKR1C3 facilitate intratumoral androgen synthesis, providing an alternative route for tumor cells to circumvent low-androgen conditions. Abiraterone's effectiveness stems in part from its ability to inhibit CYP17A1 and block this endogenous androgen production. Furthermore, AR splice variants (e.g., AR-V7) remain constitutively active even without ligand binding, rendering them resistant to therapies targeting the LBD, such as enzalutamide [31]. The fact that second-generation AR-directed drugs (e.g., enzalutamide, abiraterone) confer survival benefits in CRPC underscores the persistent dependence of most prostate cancers on the AR axis, even after ADT failure [30]. Multiple mechanisms contribute to AR reactivation in castration-resistant prostate cancer, including ligand-binding domain mutations, AR amplification, and the emergence of constitutively active splice variants. Figure 1 illustrates these pathways, highlighting how each can lead to resistance against second-generation AR-targeted therapies.

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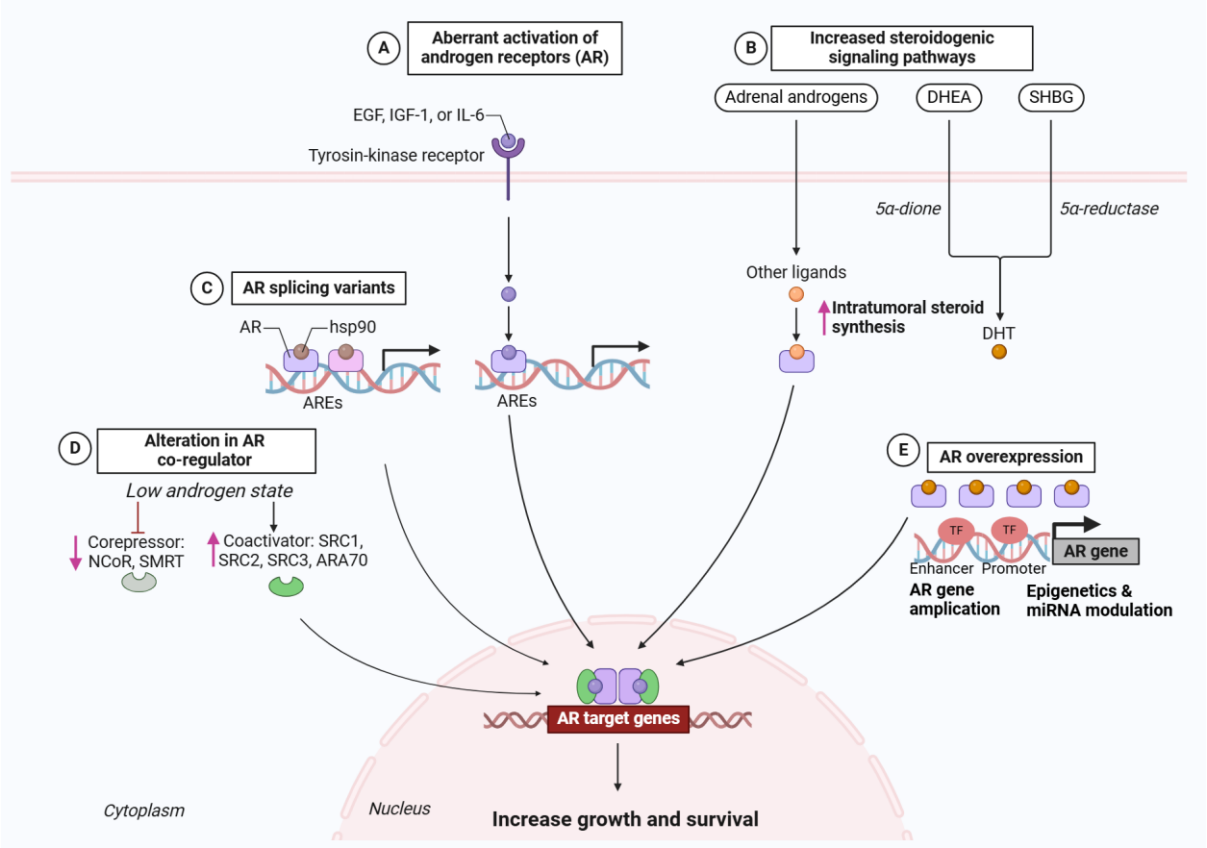

Figure 1. Mechanisms of AR Reactivation and Resistance. This schematic illustrates multiple pathways by which AR signaling can be reactivated in prostate cancer, ultimately driving therapy resistance. (A) Aberrant AR Activation: Growth factors such as EGF, IGF-1, or IL-6 can transactivate the AR via tyrosine-kinase receptors, enabling AR signaling even under low-androgen conditions. (B) Increased Steroidogenic Signaling Pathways: Adrenal androgens and their precursors (e.g., DHEA) are converted intratumorally to DHT through 5α-reductase activity. Elevated levels of SHBG and other ligands also contribute to AR activation. (C) AR Splicing Variants: Certain variants (e.g., AR-V7) lack the ligand-binding domain, allowing constitutive AR target gene activation independently of androgen binding. (D) Alterations in AR Co-Regulators: Decreased expression of co-repressors (e.g., NCoR and SMRT) or increased expression of coactivators (e.g., SRC1, SRC2, SRC3, and ARA70) can further enhance AR-mediated transcription. (E) AR Overexpression: AR gene amplification, epigenetic modifications, and miRNA dysregulation can lead to AR overexpression, thus amplifying AR signaling and promoting tumor growth. Together, these mechanisms converge to maintain or upregulate AR signaling despite therapeutic interventions, resulting in increased cancer cell survival and disease progression. Abbreviations: ARA70, Androgen Receptor Coactivator 70; AR, Androgen Receptor; DHEA: Dehydroepiandrosterone; DHT, Dihydrotestosterone; EGF, Epidermal Growth Factor; IGF-1, Insulin-Like Growth Factor-1; IL-6, Interleukin-6; miRNA, MicroRNA; NCoR, Nuclear Receptor Co-Repressor; SHBG, Sex Hormone-Binding Globulin; SMRT, Silencing Mediator for Retinoid or Thyroid Hormone Receptor; SRC, Steroid Receptor Coactivator. Created with BioRender.com.

Research is now extending toward novel AR inhibition approaches, including N-terminal domain (NTD) inhibitors [32] and proteolysis-targeting chimeras (PROTACs) that degrade both full-length AR and splice variants [33]. Combination regimens that merge AR-targeted therapies with PI3K/AKT inhibitors, epigenetic modulators, or immunotherapeutic agents are being explored to address the multifaceted resistance mechanisms in CRPC [34,35]. While metastatic tumor samples and cell line models have been critical to understanding how AR reactivation occurs, they often lack the ability to fully replicate the complexity and heterogeneity of the clinical tumor microenvironment (TME), including immune and stromal factors [36]. This limitation highlights the need for refined

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multi-omic profiling and more sophisticated ex vivo and organoid models that can more accurately reflect individual patient contexts [36,37].

Ultimately, AR signaling remains a major determinant of long-term prostate cancer control, and unraveling patient-specific AR mutations and TME interactions may reveal predictive markers for therapeutic resistance as well as guide personalized combination regimens. Epigenetic modifications, such as methylation or acetylation, may further regulate AR splice variant expression, offering fresh targets and diagnostic biomarkers for treatment-resistant disease [29]. A deeper understanding of AR reactivation via multiple pathways is essential for developing layered inhibition strategies and precision approaches, both of which are crucial for overcoming treatment resistance and improving patient outcomes [35].

2.2. PARP Inhibitors and the Synthetic Lethality Paradigm (Molecular Pathophysiology Focus)

2.2.1. Historical Context and Foundational Insights

PARP enzymes are central to the repair of single-stranded DNA breaks. The concept of synthetic lethality, initially demonstrated in BRCA1/2-mutant breast and ovarian cancers, provides a new perspective on how defective homolo-gous recombination (HR) renders tumor cells exceptionally vulnerable to PARP disruption [38]. Early evidence of this vulnerability, which showed a pronounced reliance on PARP when HR pathways were compromised, shifted the broader DDR field to investigate whether similar liabilities exist in other malignancies, including PCa [39].

The demonstration of synthetic lethality has moved beyond the classical one-gene/one-enzyme paradigm, showing that blocking a compensatory repair route, such as PARP, can selectively kill HR-deficient cells. These foundational insights have spurred extensive research into DDR pathways, revealing that BRCA1/2 mutations are only one facet of a larger network of potential repair defects [40].

2.2.2. Core Molecular Mechanisms in Prostate Cancer

Prostate tumors may harbor a spectrum of DDR alterations beyond BRCA1/2, including ATM or other HR-associated genes [41]. Normally, PARP mediates the repair of single-strand breaks. When PARP activity is inhibited, unrepaired single-strand breaks collapse into double-strand breaks (DSBs). Cells equipped with robust HR machinery typically resolve DSBs. In contrast, HR-deficient cells fail to complete their repair, leading to catastrophic DNA damage, and ultimately, cell death [42]. BRCA2- or ATM-deficient prostate tumors exhibit heightened sensitivity to PARP inhibitors via synthetic lethality. Figure 2 summarizes the core mechanism by which PARP blockade leads to irreparable double-strand breaks in DDR-deficient cells, ultimately causing cell death.

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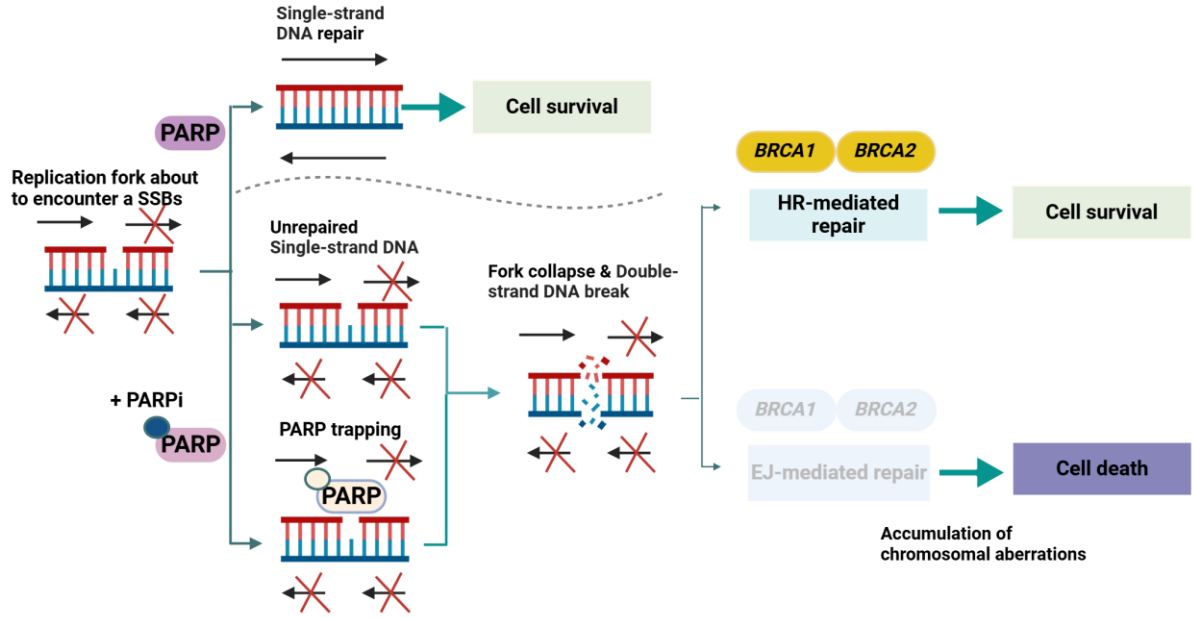

Figure 2. PARP Inhibitors and the Synthetic Lethality Paradigm. Initially, it was believed that PARP inhibitors worked by blocking PARylation and causing cytotoxicity. However, it was later discovered that the primary reason for tumor cell death was the trapping of the PARP1 enzyme at DNA lesions. When DNA damage leads to SSBs, PARP1 is responsible for their accurate repair. However, when PARP1 becomes trapped, it poses a threat to replication forks during the S phase of the cell cycle. This ultimately results in the collapse of the replication fork and the creation of double-strand breaks. In cells with functional BRCA genes, homologous recombination (HR) can repair these breaks without errors. On the other hand, cells lacking BRCA1/2 are deficient in HR and rely on error-prone DNA EJ pathways, such as classical non-homologous EJ or alternative EJ, to fix the double-strand breaks caused by the collapse of replication forks. This triggers the accumulation of chromosomal abnormalities and cell death through mitotic catastrophe. Abbreviations: BRCA1 / BRCA2, Breast Cancer Susceptibility Gene 1 / 2; DSB, Double-Strand Break; EJ, End-Joining; HR, Homologous Recombination; PARP, Poly(ADP-ribose) Polymerase; PARPi, PARP Inhibitor (Poly(ADP-ribose) Polymerase Inhibitor); SSB, Single-Strand Break. Created with BioRender.com.

While BRCA2- and ATM-mutated prostate tumors often exhibit more pronounced susceptibility to PARP blockade, some non-BRCA cases also display partial responses [43]. The reasons for this are not completely understood, suggesting that the DDR landscape in PCa is more intricate than initially assumed [40]. Additional genetic or epigenetic defects, potentially in Fanconi anemia genes, other HR components, or even in replication stress–related pathways, may confer comparable vulnerabilities. Ongoing research highlights a complex network of repair proteins that modulates the response of tumors to DNA damage. Variations in the TME, mutational load, and epigenetic regulation could further shape DDR competence, explaining partial or atypical responses [41].

2.2.3. Adaptive and Resistance Mechanisms

Despite the clear link between HR defects and increased PARP sensitivity, prostate tumors can acquire resistance over time. One well-characterized route involves reversion mutations in which previously inactivated HR genes (e.g., BRCA2) regain partial or full function. In other cases, alternative repair pathways, such as non-homologous end joining (NHEJ) or translation DNA synthesis, may be upregulated, compensating for the blocked PARP axis and enabling tumor cells to survive. Restoration of HR capacity undercuts the premise of synthetic lethality and complicates long-term control [44]. Additionally, the inherent redundancy of the DDR network underscores the

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need for a comprehensive molecular profile of each tumor, rather than relying on a single mutational marker [45].

2.2.4. Research Methodologies and Knowledge Gaps

Large-scale genomic profiling, including whole-exome and whole-genome sequencing, along with multi-omic ap-proaches that integrate transcriptomics, proteomics, and epigenomics, has significantly advanced our understanding of DNA damage repair (DDR) perturbations in prostate cancer [6]. Nonetheless, important gaps remain in the current literature [43]. Biomarker panels tend to center on BRCA1/2 and a limited set of DDR genes, potentially overlooking less common or functionally redundant pathways [45]. In addition, prostate tumors often exhibit marked heterogeneity both among patients and across different metastatic sites within a single patient, complicating the interpretation of DDR defects [6]. A further challenge arises when tumors show DDR-related abnormalities primarily at the functional level—such as replication stress—rather than through clear genomic alterations, meaning they may evade detection by standard gene panels.

Critical appraisal of existing evidence demonstrates that large-scale, multi-institutional genomic studies have consist-ently linked BRCA2 and ATM mutations with DDR dysfunction, yet data on noncanonical DDR alterations frequently come from small cohorts or exploratory analyses, limiting their broader applicability [45]. While some researchers emphasize BRCA gene alterations as the primary drivers of PARP inhibitor sensitivity, others advocate a more comprehensive perspective that accounts for epigenetic factors and secondary DNA repair pathways [46]. This debate underscores the inherent complexity of tumor biology and highlights the pressing need for holistic approaches that integrate genomics, epigenomics, and functional assays to better characterize and target DDR dysregulation in prostate cancer.

2.2.5. Forward-Looking Perspectives in Molecular Pathophysiology

Next-generation technologies are transforming the understanding of DNA damage repair (DDR) and synthetic lethality in prostate cancer by moving beyond traditional gene mutation models. These approaches collectively enrich the classification of prostate tumors, revealing vulnerabilities that expand the paradigm of synthetic lethality. In parallel, the historical success of PARP inhibition in BRCA-mutant breast and ovarian cancers underscores how targeting single-strand break repair can be lethal in cells already compromised in homologous recombination, a principle now applied to prostate cancer with a growing list of DDR defects [47]. The discovery of reversion mutations and alternative repair routes highlights the complexity of the DDR landscape, driving efforts to refine molecular classifications and rational therapeutic strategies [48]. As advanced genomic techniques, functional assays, and computational modeling continue to evolve, they promise to illuminate new mechanisms of DNA repair vulnerability in prostate cancer, ultimately enabling more precise interventions and improved clinical outcomes [47].

2.3. TMPRSS2-ERG Fusions and Oncogenic Transcription Factors (Molecular Pathophysiology Focus)

2.3.1. Historical to Current Understanding

The discovery of TMPRSS2-ERG fusions marked a milestone in deciphering how androgen-responsive promoters drive aberrant transcriptional programs in PCa. Early studies established that ERG, which belongs to the ETS transcription factor family, is overexpressed when placed under the regulatory control of the AR-responsive TMPRSS2 promoter [49]. This rearrangement was initially considered a distinct molecular subtype of prostate cancer.

Over the past decade, numerous studies have examined how TMPRSS2-ERG–positive tumors intersect with other molecular subtypes; however, the precise prognostic significance of these fusions remains debatable. Historically, the discovery of these fusions has provided a novel example of how AR signaling can be co-opted, revealing the broader principles of oncogenic transcription in prostate

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cancer. Although ERG fusions are frequently detected, their direct role in driving tumor aggressiveness remains controversial, with conflicting reports on clinical outcome correlations [49].

2.3.2. Mechanistic Insights

ERG modulates numerous gene expression pathways implicated in epithelial–mesenchymal transition (EMT) and ge-nomic instability [50]. Under TMPRSS2 control, ERG is androgen regulated, reinforcing a feed-forward loop in which AR signaling indirectly augments oncogenic transcription [51]. Notably, co-alterations, most prominently PTEN loss, can synergize with ERG overexpression, potentiating pathways that promote proliferation, invasion, and possibly metastasis [50]. TMPRSS2-ERG fusions result in androgen-responsive overexpression of ERG, promoting invasive and pro-oncogenic gene programs. Figure 3 schematically depicts how the TMPRSS2 promoter drives ERG transcription, thereby linking AR signaling to enhanced tumor invasiveness.

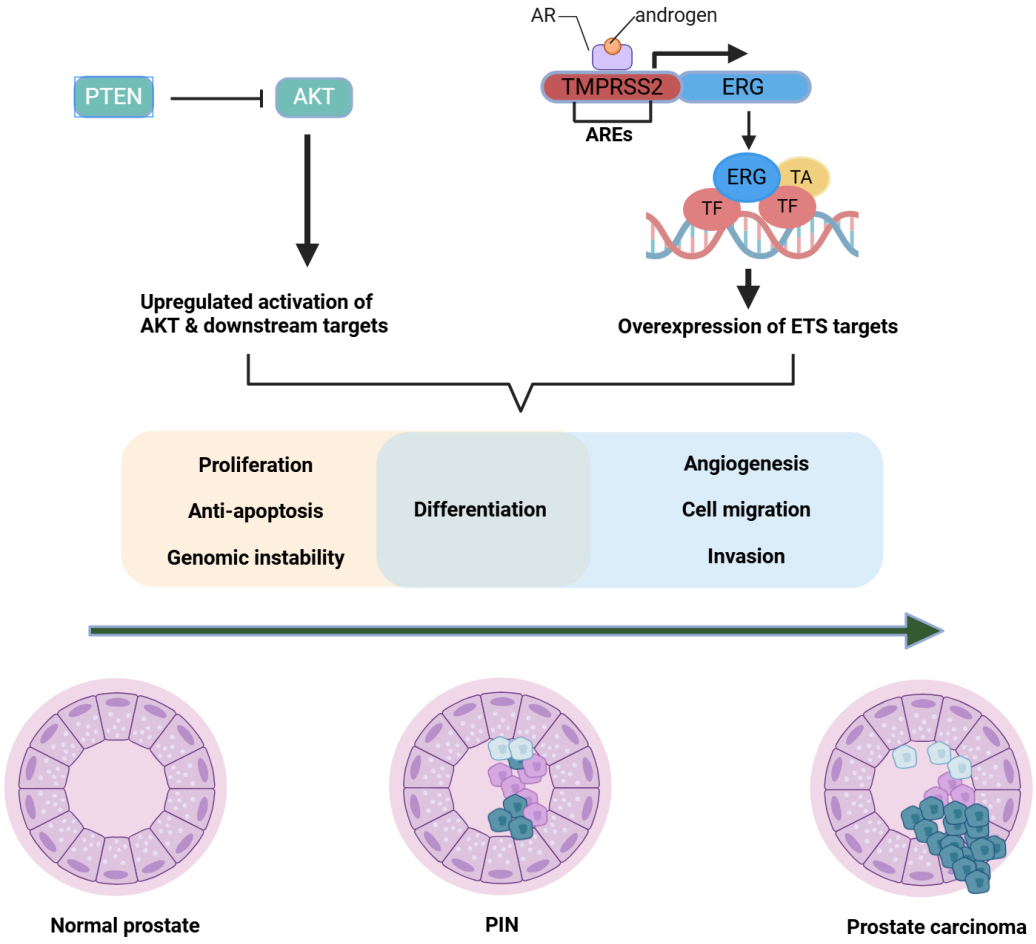

Figure 3. TMPRSS2-ERG Gene Fusion and AR-Driven Oncogenic Transcription. Loss of PTEN and concomitant activation of AKT could act in partnership with the TMPRSS2-ERG fusion protein to promote progression to prostate cancer through downstream pathways that increase the selective advantage of premalignant prostatic intraepithelial neoplasia (PIN) cells. Abbreviations: AKT, Protein Kinase B; AREs, Androgen Response Elements; ERG, ETS-Related Gene; ETS, E26 Transformation-Specific; PIN, Prostatic Intraepithelial Neoplasia; PTEN, Phosphatase and Tensin Homolog; TA, Transactivation Domain; TF, Transcription Factor; TMPRSS2, ransmembrane Protease, Serine 2. Created with BioRender.com.

A critical challenge lies in the absence of well-defined ERG domains that are amenable to direct pharmacological inhibi-tion. This molecular architecture complicates the efforts to suppress ERG's oncogenic activity. Consequently, ERG co-regulators, such as BRD4 and DNA-PK, have garnered

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attention as alternative therapeutic interventions, although these directions remain under investigation at the mechanistic level rather than as established clinical strategies. ERG's ability to modulate genes associated with invasion, EMT, and DNA repair demonstrates its integration with other onco-genic signals in a context-dependent manner [51]. Furthermore, PTEN deficiency often co-occurs with ERG overexpression, accentuating its protumorigenic effects [50].

2.3.3. Methodological Limitations

In many early cohort studies, patients were not uniformly profiled for additional molecular events beyond TMPRSS2-ERG status. This lack of a comprehensive molecular annotation confounds efforts to parse the specific contribution of ERG to tumor aggressiveness, especially in the presence of PTEN loss or other concurrent mutations [52].

TMPRSS2-ERG–positive cell lines and xenograft models often fail to capture the TME and intrapatient heterogeneity of clinical disease. Furthermore, the reliance on overexpression systems can exaggerate or misrepresent physiological ERG levels, complicating efforts to draw definitive conclusions [53]. Although correlative data suggest an association between ERG fusion and certain tumor phenotypes, the strengths of these associations vary widely. Differing assay sensitivities and patient selection criteria, such as Gleason score distributions and prior therapies, further contribute to the contradictory findings regarding prognosis [54].

2.3.4. Clinical or Scientific Significance

From a molecular pathophysiological standpoint, TMPRSS2-ERG fusions illustrate how a normal physiological path-way, AR signaling, can be subverted to drive oncogenic transcription. Notably, ERG overexpression upregulates inva-sion and EMT-related genes and potentially modulates PI3K/AKT signaling, DDR networks, and epigenetic regulators. This interplay underscores the complexity of PCa biology, as single genetic events such as TMPRSS2-ERG fusion can rewire multiple downstream processes, often amplifying additional oncogenic hits such as PTEN loss. Understanding this intricate crosstalk highlights how AR signaling interacts with transcription factors to shape the gene networks that are fundamental to cancer progression. Furthermore, insights into how TMPRSS2-ERG integrates into broader molecu-lar signatures offer the potential to refine patient stratification beyond traditional clinical parameters, emphasizing its importance as a biomarker and therapeutic target [55].

2.3.5. Comparisons, Divergent Findings, and Future Outlook

While some groups have linked TMPRSS2-ERG positivity to unfavorable outcomes, others have observed no significant prognostic correlations. These discrepancies likely stem from differences in assay methodologies, such as FISH, PCR, and RNA-seq, which can influence the detection sensitivity. Additionally, patient selection bias and coexisting genetic or epigenetic alterations that modify the impact of ERG fusions further contribute to inconsistent findings [56].

These advancements exemplify how TMPRSS2-ERG hijacks AR-responsive elements and often coexists with other key aberrations such as PTEN loss. However, unresolved questions remain regarding the precise links between ERG fusion status and disease aggressiveness, as well as the optimal molecular context for targeting ERG or its co-regulators. These ongoing investigations are critical to refine our understanding of ERG's functional effect on the pathophysiology of prostate cancer [55].

Ultimately, TMPRSS2-ERG fusions represent a central node in the molecular architecture of prostate cancer, demonstrating how transcription factors hijack androgen-responsive promoters to enhance oncogenesis [50]. Historically recognized for their prevalence, these fusions are now integrated into other pathways that govern genomic stability, cell motility, and survival. However, variability in clinical outcomes and methodological constraints underscore the need for robust

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integrative molecular analyses. Ultimately, clarifying ERG's full impact on prostate cancer pathophysiology remains an active area of investigation [55].

2.4. PTEN Loss, PI3K/AKT/mTOR Hyperactivation, and Crosstalk with AR (Molecular Pathophysiology Focus)

2.4.1. Historical to Current Perspective

PTEN, one of the most frequently inactivated tumor suppressors in prostate cancer, initially gained attention because of its role in the negative regulation of cell survival pathways. Early studies on genetically engineered mouse models con-firmed that PTEN loss accelerates tumorigenesis in the prostate, solidifying its importance in preserving normal epithelial homeostasis.

Accumulating clinical data have linked PTEN deficiency to high-grade tumors and aggressive disease phenotypes. Nevertheless, the broader landscape of prostate cancer genomics has shown that PTEN status often inter-twines with additional mutations, such as in TP53, making straightforward prognostic or mechanistic conclusions more complex. Historically, PTEN has been recognized as a central gatekeeper that inhibits oncogenic cell growth. Modern genomic screens, however, underscore not only the high frequency of PTEN deletions, but also their synergy with other driver lesions, emphasizing the interconnected nature of genetic alterations in prostate cancer [57].

2.4.2. Mechanistic Insights

PTEN dephosphorylates phosphatidylinositol (3,4,5)-trisphosphate (PIP3), thereby limiting AKT activation. When PTEN is deficient, PI3K/AKT/mTOR signaling becomes hyperactive, fueling uncontrolled cell growth, metabolism, and survival. In prostate cancer, this cascade may bolster the adaptation to low-androgen conditions by offering alternative survival routes [58].

Studies demonstrate that PTEN-deficient tumors often exhibit compensatory modulation of the AR pathway. When AR signaling is suppressed, PI3K/AKT activity increases, and vice versa, indicating a bidirectional feedback loop. This in-terplay exemplifies the convergence of multiple pathways in prostate cancer, which reinforce each other when therapeu-tically inhibited. Hyperactivation of PI3K/AKT/mTOR can shift metabolic profiles to support rapid proliferation, while compensatory loops between the AR and PI3K/AKT/mTOR pathways demonstrate tandem behavior, where inhibition of one pathway frequently leads to the upregulation of the other [59]. The intricate relationship between androgen receptor signaling and the PI3K/AKT/mTOR pathway in advanced prostate cancer is depicted in Figure 5. Loss of PTEN function, for instance, drives AKT hyperactivation, which can become more pronounced under conditions of AR inhibition, underscoring the need for combination therapeutic strategies.

2.4.3. Methodological Constraints

Much of our knowledge of PTEN deficiency stems from cell lines or xenografts with distinct PTEN statuses. Although informative, these models lack the full intrapatient heterogeneity of clinical diseases. For instance, they may not capture the dynamic interplay between PTEN loss, AR signaling, and additional oncogenic hits across different prostate tumor subclones.

Single-cell sequencing and patient-derived organoids have the potential to dissect PTEN-mediated molecular events in authentic tumor settings. However, these platforms are still evolving and variations in culture conditions or sequencing depths can limit their reproducibility or obscure rare subpopulations. Despite the robust evidence emerging from specif-ic experimental systems, these findings may not fully capture the clinical spectrum of PCa. Integrating transcriptomics, proteomics, and epigenomics could provide a more comprehensive understanding of the nuanced roles of PTEN loss in different TMEs [60].

2.4.4. Clinical or Scientific Significance

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From a molecular pathophysiological standpoint, PTEN deficiency is the cornerstone for understanding how PCa cells escape normal growth restraints, as patients with PTEN-deficient tumors often exhibit more aggressive histopathologi-cal features—although co-mutations, such as those involving TP53, can sometimes overshadow the effect of PTEN loss [61]. Moreover, the absence of PTEN demonstrates how dysregulation of a single node within the PI3K/AKT/mTOR path-way can orchestrate wide-ranging changes in cell survival and androgen receptor feedback loops, highlighting the in-tricate complexity of PCa signaling networks [62]. These findings underscore that aberrations in a single tumor suppressor can reshape multiple survival and proliferative pathways, while also extending their broader relevance to other malig-nancies in which PTEN is similarly compromised.

2.4.5. Contrasting Evidence and Future Directions

While some studies strongly associate PTEN loss with poor prognosis, others suggest that additional genetic altera-tions such as TP53 or RB1 inactivation may have a more dominant influence on outcomes. Consequently, PTEN status alone does not always provide clear prognostic discrimination.

PTEN deletion fundamentally disrupts cell cycle control and fosters the hyperactivation of PI3K/AKT/mTOR, illustrating its critical role in prostate cancer pathogenesis [63]. However, open questions remain regarding how PTEN inter-sects with other frequently mutated pathways, and whether PTEN status alone reliably predicts clinical behavior. Historically viewed as a gatekeeper for normal cell cycle regulation, PTEN is now recognized for its broader influence on processes such as metabolic reprogramming and AR pathway crosstalk. The presence of co-mutations and compensatory signaling loops complicates the unidimensional view of PTEN deficiency. Further elucidation of PTEN's role within the broader oncogenic network is pivotal for advancing the understanding of prostate cancer pathogenesis.

2.5. Tumor Heterogeneity, Clonal Evolution, and Lineage Plasticity

2.5.1. Historical to Current Understanding

Prostate cancer exhibits profound heterogeneity with variations among metastatic lesions in the same patient that evolve over time [64]. Early explanations of PCa progression focused on single "driver mutations" but failed to encompass the disease's wide range of clinical behaviors. The advent of multiregion sequencing has revealed that individual meta-static sites often harbor distinct genetic alterations, challenging the notion of a uniform clonal driver [65]. Recent single-cell analyses have emphasized the dynamic nature of clonal populations as they adapt to selective pressures, highlighting the interplay between genetic diversity and environmental factors.

2.5.2. Mechanistic Insights on Clonal Dynamics

Key molecular drivers such as persistent AR signaling, DDR defects, and PTEN loss independently confer survival ben-efits in different therapeutic contexts [61,63]. Over time, subclones harboring AR splice variants or traits of treatment-induced neuroendocrine PCa (t-NEPC) can outcompete other populations, resulting in a shifting hierarchy of dominant clones shaped by specific environmental or pharmacological pressures [66]. AR signaling remains a cornerstone of prostate cancer biology; however, subclones can adapt through ligand-independent AR variants [65]. Additionally, alterations in the DDR or PI3K/AKT/mTOR pathway may emerge or expand under therapy, reinforcing resistant phenotypes. Some cells exhibit lineage plasticity, adopting t-NEPC characteristics, and transitioning to a small-cell–like phenotype to evade AR-targeted approaches [66]. The evolution of prostate cancer under therapeutic pressure can result in diverse subclonal populations, some of which acquire neuroendocrine features. Figure 4 illustrates this progression from an AR-driven phenotype to treatment-emergent neuroendocrine prostate cancer, highlighting key genetic events like RB1 and TP53 loss.

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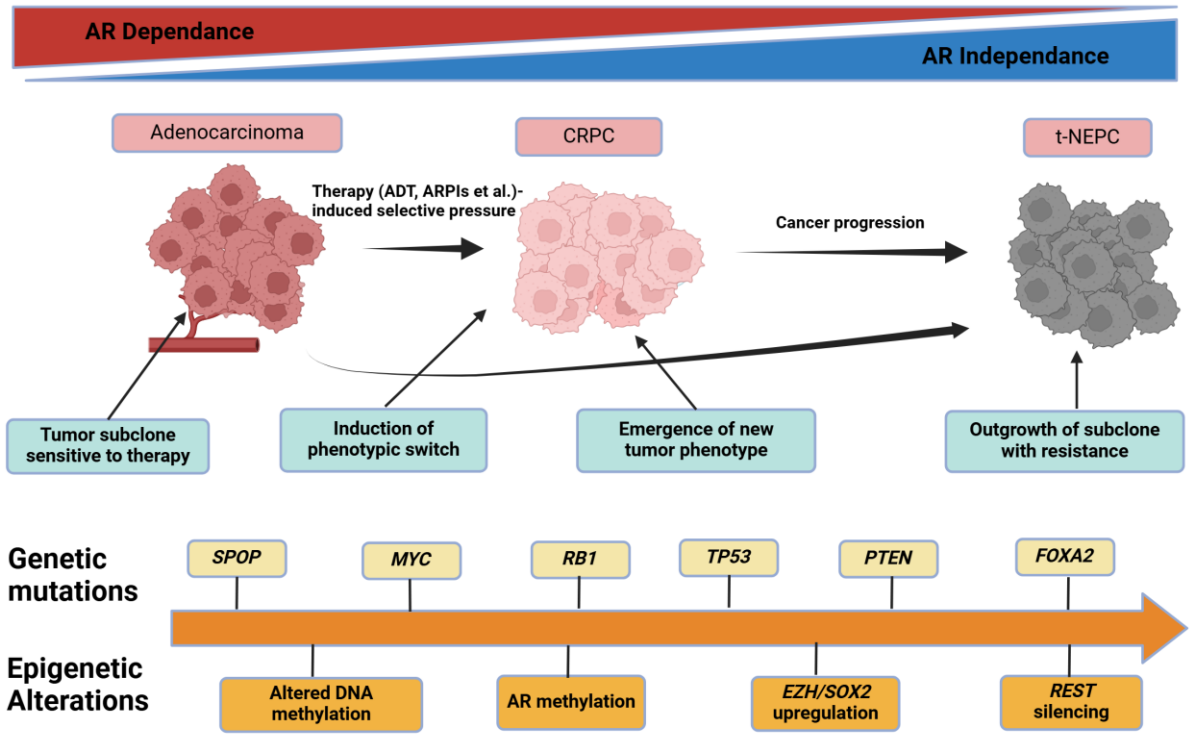

Figure 4. Tumor Heterogeneity, Clonal Evolution, and Treatment-Induced Neuroendocrine Prostate Cancer. This schematic illustrates how prostate adenocarcinoma evolves under the selective pressure of ADT and ARPIs, leading to the emergence of CRPC and, ultimately, t-NEPC. Initially, therapy-sensitive subclones may be outcompeted by therapy-resistant populations, driving a phenotypic switch and the appearance of new tumor subtypes. Over time, resistant subclones predominate, resulting in more aggressive disease states such as NEPC. Underlying these transitions are key genetic mutations (e.g., in SPOP, MYC, RB1, TP53, PTEN, FOXA2) and epigenetic alterations (e.g., altered DNA methylation, AR methylation, EZH2/SOX2 upregulation, REST silencing), which together promote tumor heterogeneity, clonal expansion, and therapeutic resistance. Abbreviations: ADT, Androgen Deprivation Therapy; ARPI, Androgen Receptor Pathway Inhibitor; CRPC, Castration-Resistant Prostate Cancer; MYC, MYC Proto-Oncogene; RB1, Retinoblastoma 1; SPOP, Speckle-Type POZ Protein; t-NEPC, treatment induced Neuroendocrine Prostate Cancer; TP53, Tumor Protein 53; PTEN, Phosphatase and Tensin Homolog; FOXA2, Forkhead Box A2; EZH2, Enhancer of Zeste Homolog 2 (Histone Methyltransferase); SOX2, Sex Determining Region Y (SRY)-Box Transcription Factor 2; REST, RE1 Silencing Transcription Factor. Created with BioRender.com.

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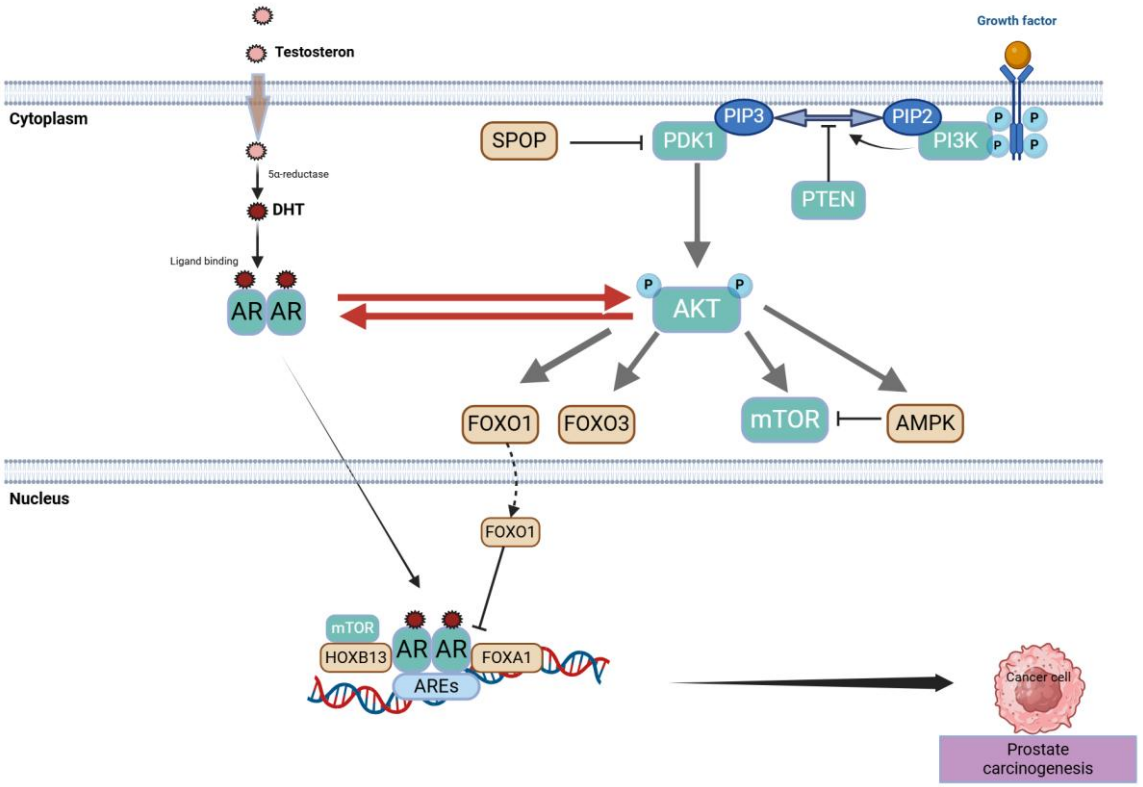

Figure 5. AR Axis and PI3K/AKT/mTOR Crosstalk. This schematic illustrates the reciprocal interactions between the AR signaling axis and the PI3K/AKT/mTOR pathway in prostate cancer. Testosterone is converted to DHT by 5α-reductase, and DHT-activated AR translocates into the nucleus to regulate gene transcription in collaboration with cofactors (e.g., FOXA1, HOXB13). Concurrently, growth factor signals drive PI3K-dependent production of PIP3 from PIP2, activating PDK1 and AKT. AKT phosphorylates multiple targets, including mTOR, promoting cell growth and survival. Tumor suppressors such as PTEN negatively regulate this pathway by converting PIP3 back to PIP2, while SPOP influences protein turnover within the pathway. Transcription factors FOXO1 and FOXO3 are regulated by both AR and AKT, integrating signals from each pathway. Crosstalk between AR and PI3K/AKT/mTOR signaling underpins key processes in prostate carcinogenesis, including proliferation, survival, and therapeutic resistance. Abbreviation: AKT, AKT Serine/Threonine Kinase; AMPK, AMP-Activated Protein Kinase; AR, Androgen Receptor; AREs, Androgen Response Elements; DHT, Dihydrotestosterone; FOXA1, Forkhead Box A1; FOXO1, Forkhead Box O1; FOXO3, Forkhead Box O3; HOXB13, Homeobox B13; mTOR, Mechanistic Target of Rapamycin (mTOR); PDK1, Phosphoinositide-Dependent Kinase-1; PIP2, Phosphatidylinositol 4,5-Bisphosphate; PIP3, Phosphatidylinositol (3,4,5)-Trisphosphate; PTEN, Phosphatase and Tensin Homolog; SPOP, Speckle-Type POZ Protein. Created with BioRender.com.

2.5.3. Methodological Constraints

Monitoring clonal evolution in real-time poses significant challenges owing to the inherent limitations of current meth-odologies. Traditional biopsies are often invasive and can be technically and ethically difficult to repeat, particularly in metastatic settings. Circulating tumor DNA (ctDNA) offers a less invasive alternative, providing a window for genomic alterations; however, rigorous validation is required to ensure its reliability and sensitivity [67]. Many studies have relied on single time points, which makes it difficult to track dynamic shifts in a subclonal architecture [68]. Although high-throughput sequencing has significantly improved our understanding, real-time longitudinal insights remain limited [64]. Moreover, single snapshots of the disease may fail to capture emerging or minority clones that eventually dominate therapy, introducing potential biases into the data [69].

2.5.4. Clinical or Scientific Significance

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Acknowledging tumor heterogeneity and clonal evolution is pivotal for understanding the molecular pathophysiology of PCa. Heterogeneity leads to varied responses to the same therapy, necessitating individualized treatment strategies [69]. Subclonal expansion often aligns with genetic or epigenetic changes such as AR splice variants or neuroendocrine differentiation, enabling therapies to escape. Furthermore, the shift to t-NEPC exemplifies how some prostate tumors can adopt a small-cell–like phenotype, resembling other neuroendocrine malignancies both molecularly and histologically, with significant implications for prognosis and therapy design [70]. Recognizing phenotypic shifts improves risk stratification and illustrates how tumor cells adaptively rewire their signaling pathways under therapeutic stress, shedding light on their intricate biology[71].

2.5.5. Comparisons and Divergent Data

Discrepancies often arise regarding the initiating factors of t-NEPCs or other aggressive phenotypes. Some researchers emphasize RB1 or TP53 inactivation as essential early events, while others highlight the role of methylation patterns and chromatin remodeling, which may subtly shape the transition [70,71]. These contrasting perspectives underscore the need for harmonized study designs that incorporate both genetic and epigenetic profiling. Resolving whether specific muta-tions universally precede neuroendocrine transformation or broader epigenetic reprogramming drives this shift remains a critical question [70]. Despite these differences, there is agreement on the importance of the synergy between genomic lesions and epigenetic states. However, debate continues over the relative contribution of each factor and the precise sequence of events in clonal shifts [72].

2.5.6. Future Outlook

Single-cell omics and longitudinal ctDNA analyses provide higher-resolution tracking of subclonal populations, and when combined with advanced imaging techniques—such as radiomic approaches that capture phenotypic diversity—these strategies illuminate the real-time evolution of clones [67]. Furthermore, pattern recognition and predictive modeling can forecast the emergence of aggressive subclones prior to overt clinical progression, thereby offering an earlier window for intervention [64]. Collectively, tumor heterogeneity, clonal evolution, and lineage plasticity underscore the complexity of prostate cancer, as historical multi region studies have dismantled the simplistic notion of a single-driver event and single-cell analyses have reinforced that evolving subclones exploit distinct molecular pathways—from androgen receptor variants to transitions toward treatment-emergent neuroendocrine prostate cancer—to over-come therapeutic barriers [68]. Future efforts that harness real-time genomic monitoring and high-resolution single-cell methods will help clarify the precise underpinnings of these adaptive processes, ultimately guiding more nuanced disease stratification and potentially intercepting lethal disease progression at earlier stages [73].

2.6. Tumor Microenvironment (TME) and Immune Dynamics (Molecular Pathophysiology Focus)

2.6.1. Historical to Current Understanding

The TME in advanced PCa comprises a complex network of cancer cells, stromal elements, immune populations, and secretory factors. Historically, most studies have focused on stromal fibroblasts and angiogenesis. More nuanced im-munological studies have revealed a spectrum of tumor-associated immune cells, including myeloid-derived suppres-sor cells (MDSCs), tumor-associated macrophages (TAMs), and regulatory T cells (Tregs), which collectively impair cy-totoxic T-cell activity. These findings transformed the view of prostate cancer from a purely “cold” tumor to one adept at deploying multiple immunosuppressive strategies, emphasizing the dynamic interplay of stromal and immune ele-ments in shaping disease progression [74,75].

2.6.2. Mechanistic Insights

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PCa cells secrete key immunosuppressive cytokines, such as TGF-β and IL-10, and frequently express immune checkpoints like PD-L1, collectively dampening T-cell–mediated responses [75,76]. In addition, myeloid-derived suppressor cells (MDSCs) and tumor-associated macrophages (TAMs) infiltrate the tumor microenvironment, releasing further factors that stifle cytotoxic lymphocytes and thereby preserve a pro-tumor milieu [77]. In the context of bone metastasis, osteoblastic lesions disrupt normal bone remodeling, creating a niche that compromises immune surveillance [78]. This unique interaction between tumor cells and the bone environment fosters local immunosuppression, enabling metastatic lesions to evade host defenses. Moreover, interactions between PCa cells and the surrounding stromal and immune compartments further influence disease progression, as tumors adaptively reconfigure the cytokine landscape and immune checkpoint expression in response to selective pressure, highlighting the dynamic nature of immune evasion mechanisms [69]. Although prostate cancer has historically been categorized as an immunologically 'cold' tumor, multiple immunosuppressive factors and cells orchestrate a complex TME. Figure 6 outlines the spatial distribution of key immune cells, such as MDSCs and Tregs, and emphasizes how they inhibit antitumor responses.

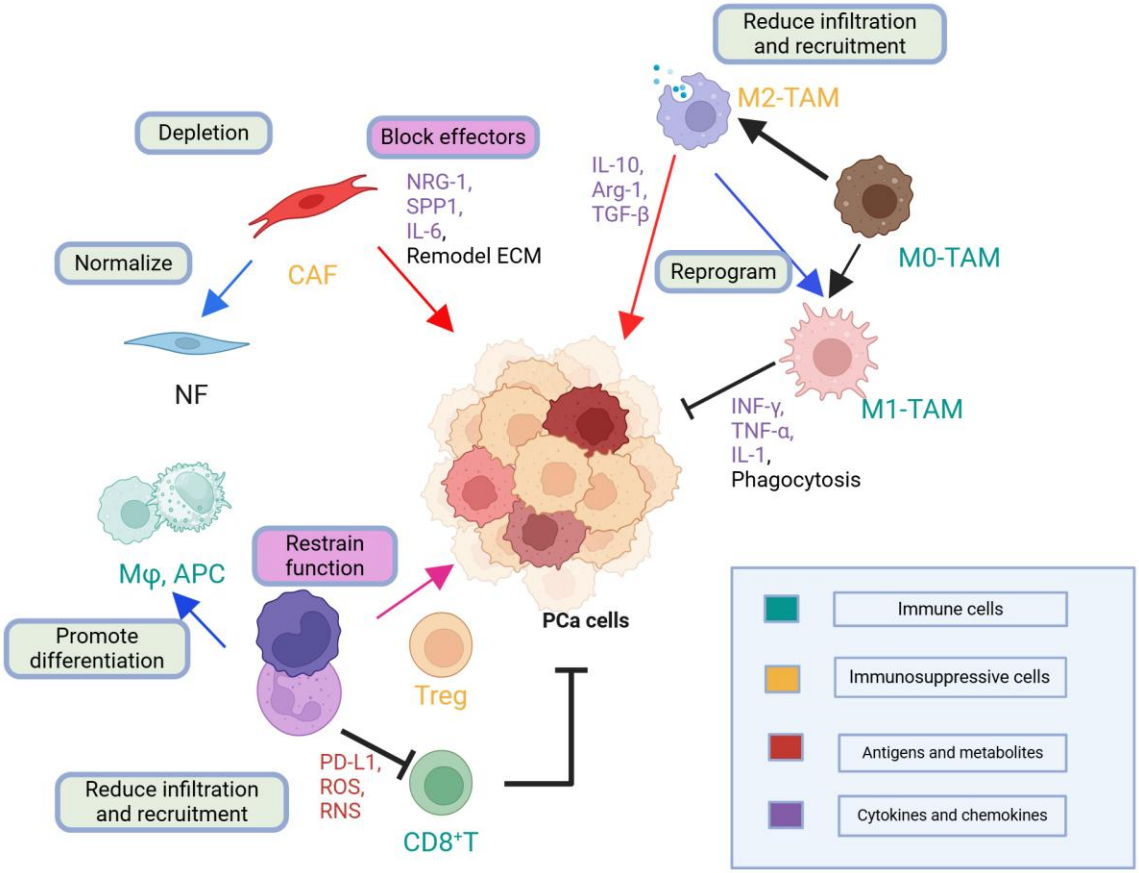

Figure 6. Immune Landscape and TME in Prostate Cancer. This schematic highlights the complex interactions among PCa cells, immune cells, and stromal components within the TME. NFs help maintain tissue homeostasis, whereas CAFs secrete factors such as NRG-1, SPP1, and IL-6 that remodel the ECM and support tumor progression. TAMs originate from M0 precursors and can polarize into M1 or M2 phenotypes: M1-TAMs secrete pro-inflammatory cytokines (e.g., IFN-γ, TNF-α, IL-1) that facilitate antitumor immunity, whereas M2-TAMs produce immunosuppressive mediators (e.g., IL-10, Arg-1, TGF-β), enhancing tumor growth and immune evasion. Tregs further suppress antitumor responses by inhibiting $CD8^+$ T-cell activity through mechanisms involving PD-L1, ROS, and other immunosuppressive factors. Collectively, these dynamic cellular and molecular interactions shape the immune milieu in prostate cancer, driving disease progression and influencing therapeutic responses. Abbreviation: APC, Antigen-Presenting Cell; Arg-1, Arginase-1; CAF, Cancer-Associated Fibroblast; D8+ T, CD8+ T Cell; ECM, Extracellular Matrix; IFN-γ, Interferon-gamma; IL-1, Interleukin-1; IL-10,

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Interleukin-10; M0-TAM, M0 Tumor-Associated Macrophage; M1-TAM, M1 Tumor-Associated Macrophage; M2-TAM, M2 Tumor-Associated Macrophage; Mφ, Macrophage; NF, Normal Fibroblast; NRG-1, Neuregulin-1; Pca, Prostate Cancer; PD-L1, Programmed Death-Ligand 1; RNS, Reactive Nitrogen Species; ROS, Reactive Oxygen Species; SPP1, Secreted Phosphoprotein 1; TGF-β, Transforming Growth Factor-beta; TNF-α, Tumor Necrosis Factor-alpha; Treg, Regulatory T Cell. Created with BioRender.com.

2.6.3. Methodological Constraints

Investigating the interplay between PCa cells and the immune system is a significant challenge. Standard 2D cell cul-tures fail to replicate the complexity of the bone microenvironment and its immune components [78]. While xenograft and organoid systems capture some tumor-stroma interactions, they lack an intact human immune system [79]. Furthermore, assessing the dynamic changes in immune cell populations over time remains difficult without repeated tissue or liquid biopsies.

Insights from patient-derived xenografts and organoids provide partial authenticity but still fail to reflect real-time im-mune dynamics. The complexity of the immune landscape also makes it challenging to distinguish which immuno-suppressive elements are the primary drivers versus passive bystanders, necessitating more sophisticated preclinical models and longitudinal studies in patients [80].

2.6.4. Clinical or Scientific Significance

From a molecular pathophysiological perspective, understanding the immune interplay within the prostate TME is cru-cial. Immune-related biomarkers can help to stratify patients more accurately and identify those who may respond bet-ter to immunomodulatory interventions [81]. Evidence also suggests that DDR defects or shifts in AR signaling may influence immune cell recruitment and functionality, revealing a complex interaction between oncogenic pathways and the immune system [82].

Clarifying the molecular mediators of immune escape can help to elucidate how prostate tumors resist or remain dormant under immune pressure. Additionally, the TME reflects an intricate network of signals from the AR pathways to PI3K/AKT, which collectively shape tumor-immune dynamics and underscore the potential for integrated therapeu-tic strategies. Prostate cancer progression and therapeutic resistance are significantly influenced by the surrounding TME, which includes a variety of stromal and immune components [83]. Table 5 summarizes the key TME players, their secreted factors, and the mechanisms by which they contribute to immune suppression and cancer progression.

2.6.5. Comparisons and Divergent Data

Clinical trials evaluating immunotherapeutic approaches for prostate cancer have yielded mixed results. Some studies have highlighted the advantages of blocking MDSC recruitment or combining immunotherapy with DDR-targeted strategies, which partially improve immune responsiveness [84]. However, other trials have reported limited response rates, often attributing these modest outcomes to heterogeneous patient populations, variations in immune profiling methods, and use of endpoints that fail to capture delayed immunological benefits [85].

The TME of prostate cancer is broadly recognized as immunosuppressive, suggesting that successful therapeutic strate-gies require multi-faceted approaches. Despite this agreement, debates persist regarding which immune cell types—MDSCs, Tregs, or TAMs—present the most viable targets and how best to evaluate their clinical activity.

2.6.6. Future Outlook

Advancements in single-cell immunophenotyping and high-resolution imaging provide deeper insights into the composition and spatial organization of immune cells within the tumor

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microenvironment (TME). These insights underscore that the prostate TME is neither as inert nor as straightforward as previously thought; rather, a combination of immunosuppressive cytokines, regulatory immune cells, and a unique bone niche orchestrates an environment that shelters cancer cells from robust immune clearance. While existing models offer crucial clues, they remain imperfect in recapitulating the full complexity of human disease, and future advances in in situ single-cell techniques, next-generation 3D organotypic systems, and integrative analyses are set to sharpen our understanding of immune-tumor crosstalk, ultimately facilitating better molecular stratification and improved therapeutic approaches tailored to the immune landscape of prostate cancer [80,86].

Table 3. Key Molecular Diagnostic Panels and Recommended Biomarkers in Prostate Cancer.

Diagnostic Panel/Biomarker	Testing Method	Clinical Significance	Limitations/Considerations
DDR-Focused Panel (BRCA1/2, ATM, etc.)	- Targeted NGS or expanded gene panels - Germline vs. somatic testing	- Identifies candidates for PARP inhibitors and platinum-based therapies - May inform familial genetic risk	- Cost and limited access in some regions - May miss epigenetic alterations
AR Splice Variants (e.g., AR-V7)	- RT-PCR or ddPCR on CTCs - Tissue-based RNA assays	- Predicts resistance to enzalutamide or abiraterone - Can guide switch to chemotherapy or other targeted agents	- Variable sensitivity depending on assay - Not yet universally available or standardized
PTEN / PI3K / AKT	- IHC, FISH - Targeted sequencing for hotspot mutations	- Potential biomarker for AKT/mTOR inhibitors - May correlate with disease aggressiveness	- Limited predictive validation in some trials - Reimbursement issues in certain regions
TP53 / RB1	- Targeted NGS or WES/WGS - IHC for protein loss	- Associated with poor prognosis - May indicate early progression toward neuroendocrine differentiation	- Rarely used in routine practice - Data interpretation can be complex (co-occurring events)
TMPRSS2-ERG Fusion	- FISH, RT-PCR, or RNA-seq	- Possible prognostic marker when combined with other aberrations (e.g., PTEN)	- Prognostic impact remains debated - May not be actionable with current therapies

Abbreviations: AKT, Protein Kinase B; AR-V7, Androgen Receptor Splice Variant 7; ATM, Ataxia Telangiectasia Mutated; BRCA1, Breast Cancer Susceptibility Gene 1; BRCA2, Breast Cancer Susceptibility Gene 2; CTCs, Circulating Tumor Cells; DDR, DNA Damage Repair; ddPCR, droplet digital Polymerase Chain Reaction; FISH, Fluorescence In Situ Hybridization; IHC, Immunohistochemistry; NGS, Next-Generation Sequencing; PI3K, Phosphoinositide 3-Kinase; PTEN, Phosphatase and Tensin Homolog; RT-PCR, Reverse Transcription Polymerase Chain Reaction; TMPRSS2, Transmembrane Protease, Serine 2; WES, Whole Exome Sequencing; WGS, Whole Genome Sequencing.

Table 4. Liquid Biopsy Modalities in Metastatic Prostate Cancer: Key Features and Clinical Applications.

Modality	Specimen Characteristics	Analytical Techniques	Clinical Applications	Advantages	Limitations

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ctDNA	- Cell-free DNA fragments shed by tumor cells - Detected in plasma or serum	- Targeted/Whole-Exome NGS - ddPCR	- Real-time monitoring of tumor burden - Detection of actionable mutations (e.g., BRCA2)	- Minimally invasive - Repeat sampling feasible - Reflects genomic heterogeneity	- Low abundance in early disease - Sensitivity depends on tumor fraction - Assay costs and standardization issues
CTCs	- Intact, viable tumor cells in the bloodstream - May be enriched via immunomagnetic or size-based separation methods	- Immunophenotyping - Single-cell genomics/transcriptomics	- Prognostic biomarker (CTC count) - AR-V7 status for therapy guidance - Potential ex vivo drug testing	- Allows morphological and molecular analyses - Provides insight into specific cell populations	- Rare cells, labor-intensive - Limited sensitivity in low-volume disease - Heterogeneity among different CTC populations
Exosomes and Extracellular Vesicles	- Nano-scale vesicles containing proteins, RNA, and DNA - Released by tumor and stromal cells into bodily fluids	- RNA-seq, proteomics - Nanoparticle tracking - Advanced mass spectrometry	- May reveal early resistance signatures - Potential biomarkers for immune- and stromal interactions	- Reflects active secretory pathways - Can capture tumor–stromal communication	- Isolation protocols not standardized - Complexity of vesicle subtypes - Data interpretation is challenging

Abbreviations: AR-V7, Androgen Receptor Splice Variant 7; BRCA2, Breast Cancer 2; CTCs, Circulating Tumor Cells; ddPCR, Digital Droplet Polymerase Chain Reaction; Exosomes, Extracellular Vesicles; NGS, Next-Generation Sequencing; PCR, Polymerase Chain Reaction; RNA-seq, RNA Sequencing; ROS, Reactive Oxygen Species; ddPCR, Digital Droplet PCR.

Table 5. Major Clinical Trials of Targeted and Immunotherapeutic Approaches in Prostate Cancer.

Treatment or Combination	Primary Target/Mechanism	Trial Phase	Patient Population	Key Outcomes	Current Status	Reference
Olaparib vs. Abiraterone/Enzalutamide (PROfound)	PARP inhibition (DDR deficiency)	Phase III	mCRPC with HRR gene alteration	Improved radiographic PFS and OS in biomarker	Approved for HRR-mutated mCRPC	[171]

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			s (e.g., BRCA1/2)	-selected patients		
Ipatasertib + Abiraterone (IPATential150)	AKT inhibitor + AR axis blockade	Phase III	mCRPC, particularly with PTEN loss	Prolonged PFS in the PTEN-loss subgroup	Ongoing or completed; subset analyses continuing	[148]
177Lu-PSMA-617 + Standard of Care (VISION)	PSMA-targeted radioligand therapy	Phase III	Heavily pretreated mCRPC	Improved OS and PFS vs. standard care	Approved in multiple regions	[172]
Nivolumab + Ipilimumab (CheckMate 650)	Dual immune checkpoint blockade (PD-1, CTLA-4)	Phase II	mCRPC, previously treated	Moderate objective response; significant immune-related toxicity	Further refinement of combination strategies needed	[173]
Pembrolizumab (KEYNOTE-199)	PD-1 immune checkpoint blockade	Phase II	mCRPC with prior treatments	Modest response rates; better outcomes in MSI-H or DNA repair defects	Investigational in selected biomarker-defined subgroups	[174]
Apalutamide (SPARTAN)	Next-generation AR antagonist	Phase III	nmCRPC (non-metastatic CRPC)	Significantly improved metastasis-free survival (MFS)	Approved for nmCRPC	[175]

Abbreviations: AKT, AKT Serine/Threonine Kinase; AR, Androgen Receptor; BRCA1/2, Breast Cancer susceptibility genes 1 and 2; CTLA-4, Cytotoxic T-Lymphocyte Antigen 4; DDR, DNA Damage Repair; HRR, Homologous Recombination Repair; mCRPC, Metastatic Castration-Resistant Prostate Cancer; MFS, Metastasis-Free Survival; MSI-H, Microsatellite Instability-High; OS, Overall Survival; PARP, Poly (ADP-Ribose) Polymerase; PD-1, Programmed Death-1; PFS, Progression-Free Survival; PSMA, Prostate-Specific Membrane Antigen; PTEN, Phosphatase and Tensin Homolog.

3. Molecular Stratification and Diagnostic Advances

Technological breakthroughs in genomic, transcriptomic, and epigenomic profiling, combined with ever-evolving imaging modalities, have transformed the classification of mPCa. Historically, simpler diagnostic assays have offered limited insight into the molecular underpinnings of the disease; however, contemporary approaches allow for more refined stratification, enabling clinicians to match patients with targeted therapies against specific molecular aberrations [87]. Despite these advances, significant challenges remain, including limited global access, cost constraints, and varying guidelines for the implementation of advanced diagnostics [88].

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A variety of genomic and immunohistochemical assays have been adopted in clinical practice to identify actionable alterations in PCa, from BRCA2 and ATM mutations to AR-V7 status [89]. Table 3 summarizes the key diagnostic panels and recommended biomarkers, highlighting their respective clinical utilities and current limitations.

3.1. High-Resolution Molecular Profiling

Next-Generation Sequencing (NGS), Whole-Exome/Genome Sequencing (WES/WGS)

From a historical perspective, the introduction of targeted next-generation sequencing (NGS) panels—typically covering 50 to 500 genes—revolutionized cancer diagnostics by enabling rapid, cost-effective detection of actionable driver mutations such as BRCA2 or PTEN [90]. As these panels became increasingly comprehensive, the field evolved to incorporate whole-exome and whole-genome sequencing (WES/WGS) approaches, which have not only facilitated the identification of complex structural rearrangements and rare genetic variants but also signaled a paradigm shift from broad histopathological classification to precision oncology driven by molecular signatures [91].

In current clinical practice, many institutions have adopted a tiered testing strategy that begins with focused gene panels and escalates to WES/WGS when initial results are ambiguous or when a patient's clinical risk profile justifies a more in-depth inquiry [92]. While targeted NGS remains more accessible and directly actionable, WES/WGS often uncovers potentially meaningful but less common alterations, though the additional complexity can present interpretation challenges that are frequently addressed by cross-disciplinary molecular tumor boards [93]. Although some centers report high concordance between gene panels and broader sequencing approaches for common mutations, others note that extended analyses reveal rare variants that could influence therapeutic decision-making—discrepancies that likely stem from differences in sequencing depth, analytical pipelines, and patient selection criteria [94].

Looking to the future, as costs continue to decline and AI-driven analytics become more integrated, these next-generation techniques are expected to play an even more central role, although the extent to which WES/WGS will be routinely applied, especially in resource-limited settings, remains to be seen [95]. Complementing DNA-based approaches, transcriptomics via RNA sequencing has enhanced our understanding by identifying functionally active genes and alternative splicing events such as AR-V7 [96], while epigenomic assays like ATAC-seq and ChIP-seq have revealed regulatory elements and super-enhancers that may drive therapeutic resistance [97]. At the frontier of molecular profiling, single-cell sequencing captures subclonal diversity with unprecedented resolution, offering the promise of early detection of therapy resistance and dynamic treatment adaptation [98]. Nevertheless, the interpretation of epigenetic and transcriptomic data is often context-dependent and requires functional validation, with variability in tissue acquisition and RNA quality complicating cross-study comparisons [99]. For these methods to influence clinical decisions effectively, robust frameworks for data interpretation and standardized reporting are essential—an area that continues to evolve [100]. Despite the high cost and complexity that currently limit the routine use of single-cell sequencing, its potential to unveil emergent resistant clones in near-real time underscores its promise for the future of precision oncology [101].

3.2. Companion Diagnostics and Gene Panels

3.2.1. DDR-Focused Panels for PARP Inhibitor Selection

Historically, the discovery of BRCA1/2 mutations as potent drivers of tumorigenesis revolutionized breast and ovarian cancer treatment, and prostate cancer soon followed suit.

Today, commercial assays commonly include BRCA1, BRCA2, ATM, and other DNA damage repair (DDR) genes, reshaping diagnostic paradigms by enabling clinicians to identify patients who may benefit from PARP inhibitors.

U.S. Food and Drug Administration approval of these agents for metastatic castration-resistant prostate cancer has further underscored the necessity of systematic genetic testing [102]. Germline

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DDR mutations not only impact patients but also have implications for their relatives, emphasizing the ethical and practical importance of genetic counseling [103]. However, many patients still lack access to genetic testing due to cost or geographical barriers, and the predictive value of less common DDR mutations remains underexplored, highlighting a significant gap in the existing evidence [102]. By identifying DDR deficiencies, clinicians can more accurately administer PARP inhibitors—a prime example of stratified medicine—though the emergence of reversion mutations and evolving resistance patterns cautions against viewing these biomarkers as static or universally reliable [103].

3.2.2. AR Variant Detection and PTEN/PI3K Panels

In addition to the DNA damage repair (DDR) genes, tests for androgen receptor (AR) splice variants, such as AR-V7, and ligand-binding domain (LBD) mutations are proving useful in predicting responses to second-generation AR inhibitors [104]. Parallel testing of PTEN status or PIK3CA/AKT mutations, often conducted through immunohistochemistry or targeted sequencing, further refines patient eligibility for clinical trials evaluating PI3K/AKT inhibitors [63]. Despite challenges with reimbursement and varying guideline structures across regions, the shift toward precision oncology continues to drive the clinical adoption of these gene panels [105]. However, divergent evidence exists: some studies show a strong correlation between AR variants and a poor response to AR-targeted therapies, while others highlight the role of co-occurring alterations, such as TP53, in influencing therapeutic resistance [106]. This variability underscores the multifactorial nature of treatment resistance. Looking forward, AI-powered predictive models and CRISPR-based functional assays hold promise for deepening our understanding of how alterations in AR and PTEN intersect to affect therapeutic outcomes, potentially guiding more personalized treatment strategies [107].

In addition to assessing DDR genes, tests for AR splice variants (such as AR-V7) or LBD mutations have become essential for predicting responses to second-generation AR inhibitors, while parallel evaluations of PTEN status or PIK3CA/AKT mutations via immunohistochemistry or targeted sequencing further refine patient eligibility for clinical trials of PI3K/AKT inhibitors [108]. Despite variable reimbursement policies and differing guideline structures across regions, the trend toward precision oncology has propelled these gene panels into growing clinical use [90]. Notably, some studies have strongly correlated AR variants with poor responses to AR-targeted therapy, whereas others have emphasized the significance of co-occurring alterations such as TP53, underscoring the multifactorial nature of treatment resistance [52]. Looking forward, AI-powered predictive models and CRISPR-based functional assays may eventually refine our understanding of how alterations in AR and PTEN intersect to drive therapeutic outcomes [109].

3.3. Advanced Imaging: PSMA PET-CT and Beyond

Historically, conventional imaging techniques such as CT and bone scans often underestimated metastatic lesions due to limited resolution, but the introduction of PSMA PET-CT has made it possible to detect subclinical or oligometastatic disease with far greater accuracy, thus enabling more precise therapeutic interventions [110]. This breakthrough has proven especially valuable for patients with biochemical recurrence, where it informs localized salvage treatments and radioligand strategies [111]. Despite its high sensitivity, however, PSMA PET-CT can be less effective for identifying neuroendocrine or small cell variants, which typically exhibit low or absent PSMA expression—an important limitation when every lesion, no matter how small, should be considered for tailored management [112]. Moreover, medical centers without PSMA PET-CT technology risk compromising their ability to detect micro-metastases and provide equitable care, raising concerns about the accessibility gap [113].

Meanwhile, experimental tracers targeting integrins or fibroblast activation proteins (FAP) are under active investigation, reflecting the growing interest in visualizing multiple components of the tumor microenvironment [114]. Radiogenomics—the integration of imaging data and genomic profiles—further expands this landscape, offering the potential to monitor tumor biology in real time

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without invasive procedures [115]. Yet challenges persist, including the lack of standardized imaging protocols, limited tracer availability, and inconsistent reporting metrics [116]. The incorporation of AI and machine learning could help interpret complex radiogenomic datasets, potentially detecting early patterns of disease progression [117]. Nevertheless, cost and regulatory barriers continue to limit widespread adoption, underscoring the need to balance innovation with real-world feasibility. These developments highlight the evolution of prostate cancer diagnostics from rudimentary classification to increasingly sophisticated molecular and imaging-based approaches, which have already revolutionized patient stratification and outcome prediction. Although discrepancies in reported findings—regarding AR variants, DDR mutations, or imaging modalities—reflect the inherent complexity of treatment resistance, there is growing consensus that molecular stratification is critical for precision care [118].

Looking ahead, emerging technologies like CAR-T cell therapy show potential for highly specific antigen targeting, but on-target/off-tumor toxicity and immunosuppressive microenvironments remain formidable obstacles [119]. CRISPR-based gene editing, such as correcting germline BRCA2 mutations, also holds promise, but ensuring both safety and target specificity is paramount [120]. AI-driven analytics may eventually integrate genomic, imaging, and clinical data on a large scale to uncover novel insights, provided quality control of input data remains rigorous [121]. Overall, molecular stratification and innovative diagnostics are transforming the management of metastatic prostate cancer by refining disease classification, guiding individualized treatment choices, and detecting resistance at earlier stages, even as affordability, standardization, and regulatory issues persist [87]. Yet the synergy of multi-omic profiling, cutting-edge imaging, and computational tools points toward a future where genuinely patient-tailored therapy becomes the standard of care.

3.4. Liquid Biopsies in Metastatic Prostate Cancer

3.4.1. Historical Context and Technological Evolution

Liquid biopsy has emerged as a minimally invasive approach for capturing molecular information from metastatic prostate cancer, building on earlier research on circulating tumor cells (CTCs) and cell-free DNA (cfDNA) in other solid tumors [122]. Initial efforts in the early 2000s demonstrated that enumerating CTCs could provide prognostic insights; however, subsequent technological refinements expanded the scope to include ctDNA, exosomes, and other blood-based analytes [123]. Liquid biopsy strategies have gained significant traction in the management of metastatic prostate cancer, offering minimally invasive insights into tumor evolution and therapeutic resistance. Table 4 highlights the key liquid biopsy modalities, their respective analytical methods, and current clinical applications, providing a comparative overview of the advantages and limitations associated with each approach. These assays offer a window into tumor heterogeneity and dynamic clonal evolution, circumventing the challenges of repeated tissue biopsies at metastatic sites [124].

3.4.2. Methodological Approaches and Clinical Relevance

Advanced platforms isolate viable tumor cells from peripheral blood, enabling downstream analyses such as immunophenotyping, genomic sequencing, and functional assays. Although circulating tumor cell (CTC) enumeration has been linked to survival outcomes, emerging techniques now probe gene expression, androgen receptor variants, and epithelial–mesenchymal transition markers to gain deeper insights into resistance mechanisms [125]. Meanwhile, next-generation circulating tumor DNA (ctDNA) sequencing can detect actionable mutations (e.g., BRCA2, AR, and PIK3CA) and track tumor burden over time; longitudinal sampling helps identify emerging resistance pathways—particularly under selective pressure from AR-targeted therapies or PARP inhibitors—allowing clinicians to adapt treatments before overt clinical progression occurs [126]. In addition, exosomes containing tumor-derived proteins, RNA, and DNA have garnered interest for their potential to reflect the real-time state of metastatic lesions; although protocols for their isolation

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and characterization remain less standardized, exosome-based assays may complement or refine ctDNA analysis [127].

3.4.3. Critical Assessment and Significance

Liquid biopsies address many of the limitations of tissue biopsies, including sampling bias and procedural risks, by offering a noninvasive, real-time window into tumor biology—an approach that is especially relevant in metastatic prostate cancer, where subclonal differences among bone, lymph node, and visceral lesions can be significant [89]. Clinical trials have begun incorporating ctDNA- or CTC-based endpoints to stratify patients for experimental therapies; for example, the detection of AR-V7 in circulating tumor cells can inform decision-making on whether to continue with AR-targeted agents or switch to chemotherapeutic or other targeted strategies [128]. While some studies have demonstrated high concordance between mutations identified via liquid biopsy and those detected through tissue-based sequencing, others have revealed discrepancies that may arise from a low circulating tumor fraction or spatial heterogeneity, underscoring the need to harmonize assay sensitivity and specificity across different platforms [129].

3.4.4. Methodological Challenges

Variations in pre-analytical processing, assay design, and bioinformatics pipelines can yield inconsistent results, highlighting the need for a standardized framework for ctDNA and CTC collection, storage, and analysisa framework that is still under development [130]. Detecting low-frequency variants, such as rare mutations in cfDNA, demands ultra-deep sequencing coupled with rigorous quality control measures to minimize false positives, which is critical for identifying subclonal populations that may drive treatment resistance [24]. Moreover, despite the less invasive nature of liquid biopsies compared to tissue biopsies, high costs and barriers to insurance coverage continue to limit their widespread adoption, particularly in resource-limited settings [131].

3.4.5. Future Outlook

Machine learning algorithms may refine the interpretation of liquid biopsy data by correlating mutational patterns with imaging findings, clinical outcomes, and other biomarkers, potentially predicting impending relapse or identifying the optimal switch in therapy [132]. Combining cfDNA mutation analysis with transcriptomic or epigenomic signatures from exosomes may provide a more comprehensive understanding of tumor evolution [133]. Beyond the mere detection of mutations, next-generation platforms aim to culture CTCs ex vivo or use exosomal content to assess drug susceptibility and guide real-time therapeutic adjustments [134]. Thus, liquid biopsies represent a transformative diagnostic modality for mPCa, offering insights into tumor heterogeneity and resistance dynamics. As technology matures and clinical validation increases, blood-based assays may become integral to precision oncology, enabling agile and personalized treatment strategies [105]. Their success depends on standardized methodologies, robust validation in prospective trials, and thoughtful integration into existing clinical workflows.

4. Targeted Therapeutic Approaches

As PCa management has evolved, the integration of next-generation agents and combination strategies has reshaped clinical practice. Historically, therapies have been limited to nonspecific hormonal suppression and cytotoxic regimens; however, modern approaches aim to exploit specific molecular vulnerabilities. This section outlines major targeted treatments, focusing on their clinical utility, evidence, and prospects.

4.1. AR Axis–Centric Treatments

Enzalutamide, apalutamide, and darolutamide bind to the androgen receptor (AR) with high affinity, providing overall survival benefits in both hormone-sensitive and castration-resistant

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settings, while abiraterone, by blocking CYP17A1-mediated androgen biosynthesis, has shown similar efficacy in pivotal clinical trials [29]. Despite these advances, resistance remains inevitable in many patients, driven by AR mutations, AR-V7 splice variants, and upregulated bypass pathways [135]. Early AR blockade therapies revolutionized treatment but were limited by short-lived responses; in contrast, second-generation anti-androgens and abiraterone have significantly extended survival in large randomized trials [136]. Newer combination strategies are emerging, such as the use of PROTACs to harness the ubiquitin–proteasome system for degrading full-length AR and its variants, DBD inhibitors that circumvent resistance stemming from ligand-binding domain alterations, and epigenetic co-targeting approaches using LSD1 or BET inhibitors to modify AR-centric chromatin states, potentially delaying or preventing resistance [137]. In particular, PROTAC (Proteolysis-Targeting Chimeras) technology degrades target proteins via the ubiquitin–proteasome system, overcoming the limitations of traditional inhibitors by targeting "undruggable" proteins and reducing drug resistance [138]. In mCRPC, ARV-110 targets the AR and has demonstrated over 50% PSA reduction in AR-mutant patients with good tolerability, while ARV-766 shows broader efficacy with lower toxicity [138]. Additionally, BET (Bromodomain and Extra-Terminal) degraders such as ARV-771 inhibit BRD4—a key transcription factor in CRPC progression—leading to robust tumor regression [139]. Despite ongoing challenges in optimizing drug delivery and managing dose-dependent hook effects, PROTAC technology represents a promising strategy for treating drug-resistant prostate cancer, with potential applications in other malignancies. However, many trials supporting these combination regimens involve limited patient numbers, which makes it difficult to generalize toxicity profiles or long-term efficacy. Looking forward, ongoing efforts include AI-based analyses of AR structural changes to predict resistance patterns and guide drug design, while CRISPR technology may enable more precise modeling of AR mutations, thereby facilitating improved risk stratification and the development of personalized therapies [140,141].

4.2. DDR-Defect–Based Therapies: PARP Inhibitors and Beyond

Building on the concept that tumors with DDR deficiencies can be selectively killed, olaparib and rucaparib have gained approval for BRCA-mutated or other DDR-altered mCRPC, and the PROfound trial demonstrated that PARP inhibition can outperform certain AR-targeted therapies in biomarker-selected populations, underscoring the clinical importance of systematic genetic testing [142]. These findings have spurred interest in synergistic combinations, where AR blockade may amplify DNA damage and thereby enhance the cytotoxicity of PARP inhibitors, while ATR or DNA-PK inhibitors also show promise in intensifying therapeutic responses, albeit with higher toxicity [8]. Although BRCA2 mutations strongly predict benefit, the variable sensitivity of other DDR alterations highlights the need to optimize predictive biomarkers in order to prevent undue toxicity in non-responders [46]. Overall, PARP inhibitors represent a major step toward precision oncology by confirming that targeted therapy can be highly effective in genomically defined subgroups, yet the emergence of acquired resistance—such as through reversion mutations—underscores the dynamic interplay between treatment and tumor evolution [143]. In parallel, several DDR-targeted therapy trials are ongoing in prostate cancer, including a Phase 2 trial of carboplatin (NCT03148795) in HRR-mutated mCRPC that began enrollment in September 2023 and focuses on PSA reduction and progression-free survival [144], and the GUNS trial (NCT04812366), an adaptive umbrella study in high-risk localized disease that includes niraparib for DDR-deficient tumors [145]. Furthermore, the EvoPAR-Prostate01 trial (NCT06120491) is investigating the combination of Saruparib, a selective PARP1 inhibitor, with an androgen receptor pathway inhibitor in mHSPC, stratifying patients based on HRR mutation status [146]. Collectively, these efforts emphasize the ongoing pursuit of biomarker-driven and combination strategies to advance personalized therapies for prostate cancer.

4.3. Targeting PI3K/AKT/mTOR and WNT Pathways

PI3K/AKT/mTOR hyperactivation frequently coincides with PTEN loss, rendering it a prime target for drug development. CAPItello-281 (NCT04493853) is an ongoing Phase 3, randomized,

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double-blind trial evaluating the efficacy and safety of Capivasertib, a selective oral pan-AKT inhibitor, in combination with Abiraterone and ADT in patients with de novo mHSPC and PTEN loss, a deficiency common in prostate cancer that leads to PI3K/AKT pathway hyperactivation and resistance to AR inhibitors. The trial aims to enroll 1,012 patients, with rPFS as the primary endpoint and OS as a secondary endpoint. Preliminary results indicate that Capivasertib significantly improves rPFS compared to placebo, with a trend toward OS benefit, and the safety profile aligns with prior data; final results are expected by 2027 [147]. Clinical trials with AKT inhibitors such as ipatasertib and capivasertib, when combined with AR blockade, have demonstrated modest benefits, particularly in PTEN-deficient prostate cancers; however, adverse effects like hyperglycemia and dermatological toxicities limit the therapeutic window [148]. Emerging evidence implicates WNT pathway dysregulation in disease progression and neuroendocrine transdifferentiation under potent AR suppression, with preclinical data suggesting that inhibiting WNT ligand secretion or β-catenin may help prevent treatment-emergent neuroendocrine prostate cancer [149]. Additionally, epigenetic modulation through agents such as LSD1 or EZH2 inhibitors appears to preserve epithelial differentiation and delay the onset of aggressive phenotypes [150]. Although synergistic effects of multi-pathway inhibition (e.g., combining AR, AKT, and WNT targeting) have been reported, the scarcity of late-phase trials and the challenge of overlapping toxicities underscore the need for level I evidence from robust randomized studies .

4.4. Immuno-Oncology Approaches

Despite the transformative impact of checkpoint inhibitors in melanoma and lung cancer, prostate cancer has seen limited success, with benefits largely confined to select molecular subsets such as mismatch repair–deficient or CDK12-mutant tumors [151]. Experimental strategies—such as combining checkpoint blockade with radiation or tumor vaccines—aim to improve immune infiltration and overcome the immunologically "cold" tumor microenvironment [152]. In parallel, cellular therapies like CAR-T cells targeting PSMA or PSCA hold promise, although their potential is currently hindered by T-cell exhaustion and "on-target, off-tumor" toxicities; novel approaches that engineer T cells with dominant-negative PD-1 or IL-12 expression are being explored to enhance durability [153]. Additionally, efforts in myeloid reprogramming—such as blocking angiogenesis or targeting fibroblast activation protein (FAP)—seek to reshape the stromal environment to facilitate more effective T-cell responses [154]. These advances are particularly important because immunotherapy offers the potential for durable control in patients who fail conventional AR- or DDR-targeted treatments, yet comprehensive prospective trials are essential to establish optimal combinations, address toxicity management, and identify robust predictive biomarkers [155].

Building on this evolving immunotherapy landscape, STEAP1 (Six-Transmembrane Epithelial Antigen of Prostate 1) has emerged as a promising immunotherapeutic target for mCRPC due to its selective tumor expression and accessible cell-surface localization [153]. Bispecific T-cell engagers (BiTEs), such as Xaluritamig (AMG 509), simultaneously bind STEAP1 on cancer cells and CD3 on T-cells, bridging them into close proximity, resulting in direct and potent activation of cytotoxic T-cells to induce tumor cell killing [156]. In a recent phase I trial (NCT04221542), Xaluritamig demonstrated significant PSA reductions, partial clinical responses, and durable benefits exceeding six months in high-dose cohorts. However, immune-related toxicities, notably cytokine release syndrome (CRS), fatigue, and anemia, constrained dosing to a weekly maximum tolerated dose of 1.5 mg [157]. Further enhancing therapeutic efficacy, next-generation BiTE candidate BC261 demonstrated superior tumor eradication in preclinical models, achieving approximately 30-fold greater T-cell infiltration even against tumors with low STEAP1 expression [156]. BiTE therapies targeting STEAP1 offer advantages over CAR-T cells, including faster clinical availability, potent T-cell activation independent of prior immune priming, and combinational treatment potential. Nonetheless, overcoming immune-mediated toxicities and optimizing dosing regimens remain critical challenges that require additional clinical validation to integrate STEAP1-directed BiTE therapies fully into the prostate cancer treatment landscape [156].

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4.5. PSMA-Targeted Radioligand Therapy

PSMA is overexpressed in most prostate cancers, making it an attractive target for radioligand therapy. The VISION trial demonstrated that adding 177Lu-PSMA-617 to standard care for heavily pretreated metastatic castration-resistant prostate cancer yields a survival advantage, reflecting a new treatment horizon for advanced disease [158]. Alternative alpha-emitting agents, such as 225Ac-PSMA-617, induce more potent DNA damage but are associated with significant salivary gland toxicity [159]. PSMA-targeted radioligand therapy delivers cytotoxic radiation directly to prostate cancer cells while sparing normal tissues. As shown in Figure 7, patients undergo PSMA PET-CT imaging to confirm target expression before receiving 177Lu-PSMA-617 or related alpha-emitting agents.

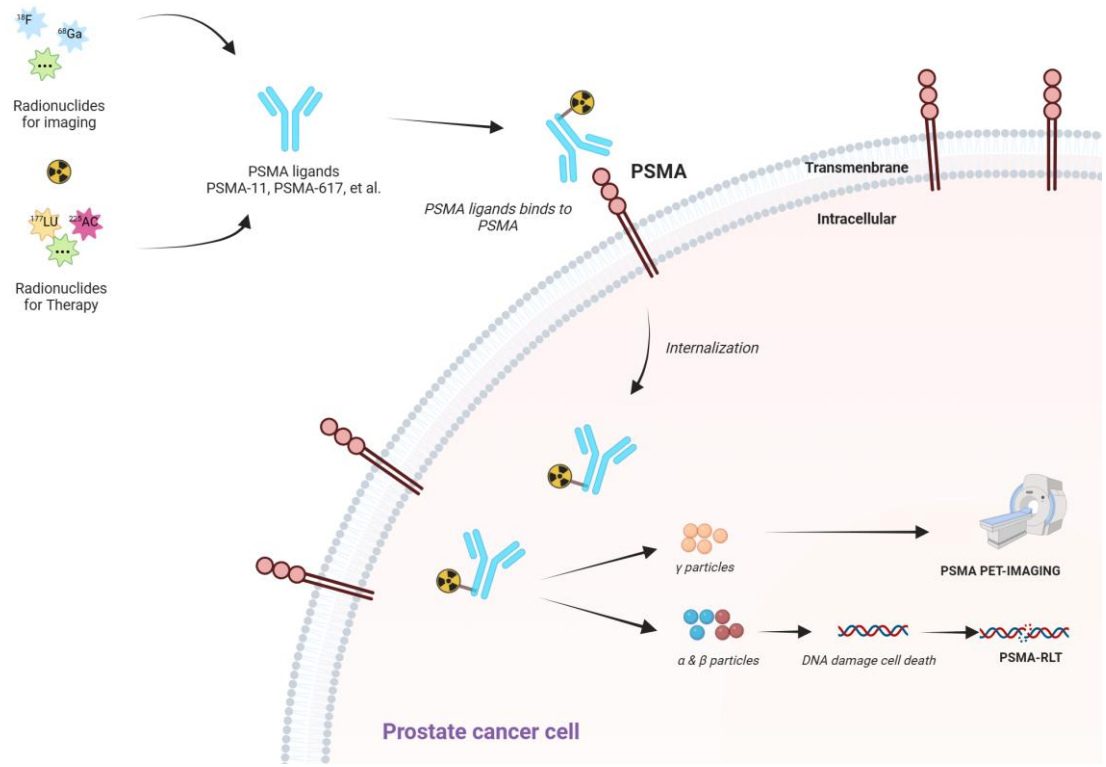

Figure 7. PSMA-Targeted Radioligand Therapy. This schematic illustrates the principle of PSMA-based imaging and therapy in prostate cancer. Radioligands (e.g., PSMA-11 for imaging and PSMA-617 for therapy) bind to the extracellular domain of PSMA on tumor cells. After binding, the complex undergoes internalization, delivering radioactive payloads into the cell. For imaging (PSMA-PET), positron-emitting isotopes enable visualization of tumor lesions, whereas therapeutic radionuclides (PSMA-RLT) emit alpha or beta particles that induce DNA damage and cancer cell death. This targeted approach exploits high PSMA expression on prostate cancer cells, offering both diagnostic and therapeutic benefits. Abbreviation: APC, Antigen-Presenting Cell; Arg-1, Arginase-1; CAF, Cancer-Associated Fibroblast; CD8+ T, CD8+ T Cell; ECM, Extracellular Matrix; IFN-γ, Interferon-gamma; IL-1, Interleukin-1; IL-10, Interleukin-10; M0-TAM, M0 Tumor-Associated Macrophage; M1-TAM, M1 Tumor-Associated Macrophage; M2-TAM, M2 Tumor-Associated Macrophage; Mφ, Macrophage; NF, Normal Fibroblast; NRG-1, Neuregulin-1; PCa, Prostate Cancer; PD-L1, Programmed Death-Ligand 1; PSMA, prostate-specific membrane antigen; RNS, Reactive Nitrogen Species; ROS, Reactive Oxygen Species; SPP1, Secreted Phosphoprotein 1; TGF-β, Transforming Growth Factor-beta; TNF-α, Tumor Necrosis Factor-alpha; Treg, Regulatory T Cell. Created with BioRender.com.

Several key trials are currently underway to evaluate PSMA-targeted radioligand therapy (PSMA-RLT) in prostate cancer. The CONVERGE-01 trial is investigating Ac-225 Rosopatamab Tetraxetan in PSMA-positive mCRPC to optimize dosing and evaluate efficacy, focusing on rPFS and

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OS. The PSMAction trial (Phase 2/3) is assessing Ac-PSMA-617 in mCRPC patients who have previously received Lu-177 therapy, with rPFS and OS as primary endpoints [160]. The Satisfaction Trial (NCT05983198) is evaluating the safety and efficacy of Ac-PSMA-R2 in both Lu-177-pretreated and Lu-177-naïve PSMA-positive mCRPC patients [161]. Meanwhile, the EVOLUTION trial is exploring the combination of Lu-177-PSMA-617 with immune checkpoint inhibitors (Ipilimumab + Nivolumab) to enhance therapeutic synergy [162]. Lastly, the ALPHABET trial is investigating Lu-177-PSMA-I&T in combination with Radium-223 to determine the maximum tolerated dose and PSA response rates [163]. Collectively, these studies aim to improve the efficacy of PSMA-RLT by leveraging alpha-emitting isotopes and combination strategies, offering promising advancements in the treatment of mCRPC.

Patient selection for these therapies relies on PSMA-PET imaging to confirm target expression, and there is growing interest in combining PSMA-targeted radioligand therapy with PARP inhibitors, AR blockade, or immunotherapies to synergistically enhance tumor cell death, although overlapping toxicities remain a concern [164]. Moreover, most radioligand therapy studies have focused on end-stage disease, leaving it unclear whether earlier interventions could yield greater survival gains; interim imaging or circulating biomarkers may help tailor doses and mitigate toxicity [165]. Radioligand therapy exemplifies precision radio-oncology by directly targeting tumor cells while sparing normal tissues, yet its widespread implementation is hindered by logistical challenges, cost, and the complexities of radiopharmaceutical handling [166].

Targeted therapies and combination regimens continue to redefine the management of metastatic prostate cancer, bridging historical insights into androgen receptor suppression with cutting-edge modalities such as PARP inhibition, PI3K/AKT blockade, immunotherapy, and PSMA-targeted radioligand therapy [167]. Multiple targeted and immunotherapeutic approaches are under investigation or have recently demonstrated clinical benefit in advanced prostate cancer, ranging from PARP inhibition in BRCA1/2-mutated disease to PSMA-targeted radioligand therapy in mCRPC [168]; Table 5 provides an overview of key trials and their outcomes, highlighting the progress and challenges associated with each strategy. While numerous trials have confirmed significant survival benefits, the heterogeneity across patient populations necessitates personalized strategies that are often guided by molecular diagnostics and advanced imaging techniques [169]. The field has evolved rapidly from basic hormonal manipulation to sophisticated targeted approaches, and disparate clinical trial outcomes often reflect differences in biomarker selection, trial design, and disease heterogeneity. There is a growing consensus that multi-omic profiling is vital for optimally matching treatment with tumor biology [170]. Ultimately, the therapeutic landscape of metastatic prostate cancer is dynamic and increasingly personalized, reflecting the convergence of robust clinical evidence, molecular diagnostics, and novel mechanistic insights. Long-term success will depend on equitable access, standardized protocols, and collaborative research aimed at overcoming current limitations and delivering durable patient benefits.

5. Conclusion and Future Perspectives

In this review, we summarize a broad range of findings that underscore the molecular and clinical complexities driving metastatic prostate cancer. At the molecular level, key themes include the centrality of AR signaling, the importance of DDR pathways (particularly BRCA1/2 and related genes), and the convergence of PI3K/AKT/mTOR and WNT signaling. Studies of the TME have further highlighted the immunosuppressive barriers encountered in advanced disease. In clinical settings, newer AR inhibitors, PARP inhibitors, radioligand therapies, and emerging immuno-oncological approaches have all contributed to improved outcomes; however, resistance remains pervasive. Resistance is exacerbated by the heterogeneous and evolving genetic and epigenetic landscapes of metastatic lesions, underscoring the need for refined molecular stratification and real-time monitoring. Although genomic and imaging technologies have expanded substantially, obstacles such as cost, access, and lack of standardized interpretation limit their full-scale adoption in diverse clinical settings.

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Conflicting evidence, particularly regarding biomarkers such as TMPRSS2-ERG fusions and AR splice variants, stems from methodological inconsistencies and highlights the necessity for harmonized protocols. By consolidating recent mechanistic discoveries and linking them to evolving clinical strategies, this review offers an integrated framework that spans the molecular, immunological, and therapeutic dimensions of mPCa. Clinically, the insights discussed—mapping core pathways, identifying known resistance mechanisms, and emphasizing the role of molecular diagnostics—provide strategic advantages for improving patient selection and optimizing therapeutic sequences.

Several opportunities and future agendas have emerged. First, synergistic clinical trials that systematically assess combination regimens (for instance, AR inhibition plus DDR-targeted or immunotherapeutic agents) could clarify not only the survival benefits but also the molecular trajectories of resistance. Secondly, longitudinal biomarker tracking, particularly through liquid biopsies, could reveal early resistance pathways and enable adaptive therapy switching before overt clinical progression. Third, future trial designs could benefit from multi-omic, prospective cohorts that unify genomic, transcriptomic, and imaging data along with standardized clinical endpoints, potentially reconciling conflicting biomarker outcomes. The incorporation of advanced computational tools, including AI-driven analytics, can further integrate these data streams, while CRISPR-based functional assays could unearth new molecular targets within the AR signaling and DDR pathways. In parallel, more refined cellular therapies, such as CAR-T and natural killer cell platforms, may eventually overcome immunosuppressive obstacles in PCa if issues of specificity and toxicity can be resolved. Finally, global collaborative efforts coordinated by academic institutions, industry partners, and regulatory bodies are essential for validating assay standards, sharing biobanks, and rapidly translating bench discoveries into bedside interventions. Therefore, the field of mPCa is at a moment of convergence, and the integration of multi-omic insights, expanding therapeutic approaches, and computational advances has laid the groundwork for more precise and durable treatment strategies. Addressing the current evidence gaps, refining trial methodologies, and fostering cross-sector partnerships will be pivotal to ensure that these innovations yield tangible life-extending benefits for patients in real-world practice.

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Abbreviations

The following abbreviations are used in this manuscript:

mPCa	metastatic prostate cancer
AR	androgen receptor
DDR	DNA damage repair
PARP	poly-ADP ribose polymerase
PSMA	prostate-specific membrane antigen
PCa	prostate cancer
mCRPC	metastatic castration-resistant prostate cancer
TME	tumor microenvironment
NGS	next-generation sequencing
ADT	androgen deprivation therapy
DHT	testosterone–dihydrotestosterone
NTD	N-terminal transactivation domain

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DBD	DNA-binding domain
LBD	ligand-binding domain
ARE	androgen response element
CRISPR	clustered regularly interspaced short palindromic repeats
PROTAC	proteolysis-targeting chimera
CAR-T	chimeric antigen receptor T
HR	homologous recombination
DSB	double-strand break
NHEJ	non-homologous end joining
EMT	epithelial–mesenchymal transition
PIP3	phosphatidylinositol (3,4,5)-trisphosphate
t-NEPC	treatment-induced neuroendocrine prostate cancer
ctDNA	circulating tumor DNA
MDSC	myeloid-derived suppressor cell
TAM	tumor-associated macrophage
Treg	regulatory T cell
WES/WGS	whole-exome/genome sequencing
FAP	fibroblast activation protein
CTC	circulating tumor cell
cfDNA	cell-free DNA
t-NEPC	treatment-emergent neuroendocrine prostate cancer

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