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# Molecular Underpinnings Governing Genetic Complexity of *ETS*-Fusion-Negative Prostate Cancer

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## Abstract

Inter- and intra-patient molecular heterogeneity of primary and metastatic prostate cancer (PCa) confers variable clinical outcome and poses a formidable challenge in disease management. High-throughput integrative genomics and functional approaches have untangled the complexity involved in this disease and revealed a spectrum of diverse aberrations prevalent in various molecular subtypes, including *ETS* fusion negative. Emerging evidence indicates that *SPINK1* upregulation, mutations in epigenetic regulators or chromatin modifiers, and *SPOP* are associated with the *ETS*-fusion negative subtype. Additionally, patients with defects in a DNA-repair pathway respond to poly-(ADP-ribose)-polymerase (PARP) inhibition therapies. Furthermore, a new class of immunogenic subtype defined by *CDK12* biallelic loss has also been identified in *ETS*-fusion-negative cases. This review focuses on the emerging molecular underpinnings driving key oncogenic aberrations and advancements in therapeutic strategies of this disease.

## Molecular Heterogeneity of Fusion-Positive and Fusion-Negative PCa

Prostate adenocarcinoma represents a heterogeneous collection of malignancies with diverse molecular frameworks. Revolutionary advances in high-throughput sequencing technologies revealed extensive molecular heterogeneity at genomic, epigenomic, transcriptomic, and proteomic levels, which laid the foundation of molecular stratification of this disease [1]. Half of the molecular etiology of PCa is driven by recurrent genetic rearrangements involving E26-transformation-specific (ETS) transcription factors, hence classified as *ETS* fusion positive. Under this category, several members of the *ETS* family, namely, *ERG*, *ETV1*, *ETV4*, and *FLI1* are known to be aberrantly overexpressed [2]. Of these, *TMPRSS2*–*ERG* gene fusion involving the 50′-untranslated region of an androgen-regulated transmembrane protease serine-2 (*TMPRSS2*) and homolog v-Ets Erythroblastosis virus E26-oncogene (*ERG*) is the most frequent alteration affecting about ~50% of the total PCa cases [3,4].

Comprehensive genomic profiling has further unraveled diverse subclasses of *ETS*-fusion-positive and *ETS*-fusion-negative PCa. Molecular alterations involving gene rearrangements, **copy number alterations (CNAs**, see Glossary), and structural variants such as somatic mutations, insertions/deletion (indels), **tandem duplications**, and interchromosomal

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translocations are the key molecular drivers involved in the pathogenesis of this disease [5]. Outlier expression of *SPINK1*, RAF kinase fusions, deletions in *CHD1* and *MAP3K7*; mutations in *SPOP*, *FOXA1* and *IDH1* have been reported to be mutually exclusive to the *ETS* fusions, and categorized under *ETS*-fusion-negative subtypes [5–9]. Recently, new subclasses of the fusion-negative subtype, with somatic and pathogenic germline mutations in **DNA damage and response (DDR) pathways**, epigenetic modifiers, and members of the spliceosome machinery have been added [5,10,11]. Moreover, CNAs involving amplification of genomic loci comprising oncogenes, namely *MYC* and *PVT1;* deletions in tumor suppressors such as *NKX3.1*, *PTEN*, *RB1*, and *TP53* cooperate with above-mentioned alterations in promoting tumor progression [6,11,12]. In this review, we focus on diverse molecular heterogeneity within the fusion-negative subtype, its clinical significance, and implication in designing novel therapeutic strategies.

## Opportunities and Challenges in Targeting the *ETS*-Fusion-Positive Subtype

Aberrant expression of *ETS* fusion activates an invasive transcriptional program, inducing prostatic intraepithelial neoplasia (mPIN) in transgenic mice, and is often associated with poor clinical outcomes [13–16]. Moreover, *ERG* fusion protein is known to inhibit androgen receptor (AR) signaling and its occupancy on AR target genes, results in increased EZH2-mediated H3K27 methyltransferase activity, which potentiates the stem-cell-like dedifferentiation program [17]. Since *ETS* fusions are under transcriptional control of AR signaling, androgen-deprivation therapy (ADT) is the preferred therapeutic strategy for fusion-positive cases. Despite recent advances in ADT, most patients develop *de novo* resistance to second-generation FDA-approved antiandrogens, such as abiraterone and enzalutamide, due to AR amplification, mutations, and AR splice variant (AR-V7), with subsequent progression to metastatic castration-resistant PCa (mCRPC) [18–20]. Although *ETS*-fusion-positive PCa represents the major molecular subtype, therapies targeting these transcription factors remain challenging. Alternatively, therapeutic modalities against molecular cofactors that modulate the transcriptional activity of *ETS* factors represent a more viable approach [21].

Poly-(ADP-ribose) polymerase 1 (PARP1) and DNA protein kinases (DNA-PKcs) are known to physically interact with ERG-fusion protein and induce DNA double-strand breaks. Moreover, cell-based assays and preclinical studies using *ERG*-positive PCa xenografts exhibit an effective response to the pharmacological PARP1 inhibitor (PARPi), olaparib [22]. Unfortunately, the recent clinical trial (NCT01576172[i]), using PARPi (veliparib) failed to show any response in fusion-positive PCa patients (Box 1) [23]. Recently, inhibitors against **bromodomain and extra-terminal (BET)** family proteins (BRD2/3/4, critical AR coregulators), were shown to block BET-protein recruitment to the AR-target gene loci, hence negatively regulating ERG-mediated oncogenesis [24]. Unlike second-generation antiandrogens, BET inhibitors (BETis) regulate RNA processing and AR-V7 alternative splicing, resulting in its downregulation, hence highlighting the clinical utility

[i] https://clinicaltrials.gov NCT01576172

of BETis for abiraterone- or enzalutamide-resistant castration-resistant prostate cancer (CRPC) patients [25–27]. Currently, Phase I clinical trials (NCT02711956[ii] and NCT03150056[iii]) using BETis are underway for CRPC patients, although its clinical efficacy remains a matter of conjecture. Yet another promising therapeutic strategy targeting oncogenic *ERG* fusion involves ERG-inhibitory peptidomimetics, which results in proteolytic degradation of ERG-fusion protein, and reduced oncogenic activity [28]. Since most small-molecule inhibitors result in drug resistance and restricted therapeutic durability, a newer class of small molecule protein degraders such as proteolysis targeting chimeras (PROTACs), that target hitherto undruggable proteins (such as AR, BET, ERG, and AR-V7) by their selective proteasomal degradation are presently under clinical investigation (Box 2).

## Mechanistic Insight into Fusion-Negative *SPINK1*-Positive PCa

The etiology of the *ETS*-fusion-positive subtype has been well characterized, while little is known about the molecular aberrations driving the *ETS*-fusion-negative cancers. About 10–15% of PCa patients show outlier *SPINK1* expression, exclusively in the fusion-negative subtype [29]. Although several independent studies indicate that increased serine peptidase inhibitor, Kazal type-1 (SPINK1) levels are associated with shorter **biochemical recurrence** and a rapid progression to the castration-resistant stage [30–32]. However, the prognostic role of *SPINK1* in primary PCa remains controversial [33–35].

*SPINK1* acts as an autocrine/paracrine factor and mediates its downstream oncogenic effects partly through epidermal growth factor receptor (EGFR) interaction. Monoclonal antibodies against *SPINK1* or EGFR (e.g., cetuximab) administered in mice with *SPINK1*-positive tumor xenografts showed a modest decrease in tumor burden [36], although the clinical significance of EGFR inhibitors in mCRPC patients remains debatable [37,38]. Recently, hepatocyte nuclear factors HNF4G/HNF1A were shown to activate a gastrointestinal-lineage transcriptome in PCa, resulting in upregulation of PCa-associated gastrointestinal genes (PCa-GIs), including *SPINK1* [39]. It was shown that HNF1A directly binds to the *SPINK1* promoter and positively regulates its expression, while HNF4G upregulates the PCa-GI signature upon androgen deprivation, and thereby supports CRPC progression [39]. Nevertheless, an in-depth exploration of genetic/epigenetic molecular circuitries involved in *SPINK1* upregulation and ways to effectively target this subtype remains unaddressed.

A recent study established the molecular basis of *SPINK1* upregulation in PCa, wherein miRNA-338-5p/miRNA-421 regulate *SPINK1* post-transcriptionally and abrogate *SPINK1*-mediated oncogenicity [40]. Intriguingly, the majority of *SPINK1*-positive PCa patients exhibit increased expression of EZH2, a member of the Polycomb repressive complex, which epigenetically represses miRNA-338-5p/miRNA-421 resulting in *SPINK1* upregulation. Moreover, *SPINK1*-positive tumors also exhibit increased CpG-methylation marks on the regulatory regions of miRNA-338-5p/miRNA-421, thereby indicating the utility of epigenetic drugs in restoring the expression of these miRNAs (Figure 1). In concordance with earlier findings, the recent reports showed higher methylation events in

[ii] https://clinicaltrials.gov NCT02711956
[iii] https://clinicaltrials.gov NCT03150056

fusion-negative compared with fusion-positive PCa [5,41]. Conclusively, the use of epigenetic drugs might have potential for the *SPINK1*-positive subtype, and reduced miRNA-338-5p/-421 expression might possibly serve as selection criteria for these patients [40,42].

An unpublished study shows a possible role of androgen signaling in the transcriptional regulation of *SPINK1* in PCa, wherein AR and its corepressor, REST (RE1-silencing transcription factor) may function as a transcriptional repressor of *SPINK1*, and antiandrogen treatment could relieve AR-mediated repression, leading to *SPINK1* upregulation (Figure 1) [43]. Previously, Paju *et al.* also observed a decrease in *SPINK1* expression upon androgen stimulation in 22RV1 cells [44]. Moreover, an inverse association between *SPINK1* and AR expression was reported in multiple independent PCa cohorts, and these fidings are in line with the studies, where lower AR expression was observed in *SPINK1*-positive tumors [33,45]. Furthermore, a possible role of lineage programming factor sex determining region Y (SRY) box 2 (SOX2) in *SPINK1* upregulation has been suggested in long-term androgen deprived LNCaP cells [43]. Another possible mechanism of elevated *SPINK1* levels in patients who underwent ADT could be attributed to an increase in the expression of the PCa-GI gene signature. Therefore, taking clues from these independent studies, we emphasize that *SPINK1* is an androgen-repressed gene, and ADT, using bicalutamide/enzalutamide, might aggravate the clinical outcomes of patients with advanced disease.

## Genomic Aberrations Associated with *ETS*-Fusion-Negative PCa

In the past decade, whole genome, exome, and transcriptome integrative sequencing analysis have paved the way to further categorize primary and metastatic PCa patients based on co-occurring and mutually exclusive genomic aberrations associated with the *ETS*-fusion-negative subtype. While primary PCa genomes are largely diploid with a lower mutational burden (~0.94 average mutations/Mb), metastatic tumors often show extensive aneuploidy, loss of heterozygosity (LOH), and a significant increase (~4.4 average mutations/Mb) in actionable mutations, which implicate the use of targeted therapeutics either through clinical trials or FDA approval [5,6,8,46]. Here, we discuss the molecular underpinnings of distinct subclasses belonging to *ETS*-fusion-negative PCa and outline the recent advances in developing therapeutic interventions aimed to target these subclasses (Table 1 and Figure 2).

## RAF Kinase Fusions in *ETS*-Fusion-Negative PCa

Paired-end transcriptome sequencing of *ETS*-fusion-negative PCa identified actionable genetic rearrangements involving RAF-kinase family members, namely *SLC45A3-BRAF* and *ESRP1-RAF1* recurrent in about ~2% of advanced PCa cases [7]. Ectopic expression of both chimeras in prostate epithelial cells show an increase in oncogenic properties, which exhibits sensitivity to RAF and MEK inhibitors [7], emphasizing that RAF-fusion-positive patients may respond to RAF kinase inhibitors. Unlike Caucasian PCa patients, an increased frequency (~4–6%) of *RAF* fusions was reported in an Indian cohort [4]. Although of low recurrence, screening of these actionable RAF alterations could be beneficial in disease management of RAF-fusion-positive patients.

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## *SPOP* Mutations in PCa

Speckle-type POZ protein (SPOP) is a member of the MATH-BTB protein family and contains an N-terminal meprin and TRAF homology (MATH) domain, which binds to target substrates by recognizing degron motifs and a C-terminal BTB domain responsible for the binding of Cullin 3-RING box-1 protein, to form a functional E3-ubiquitin ligase complex [47]. In localized and advanced prostate tumors, *SPOP* has been found to be frequently altered in ~15% and ~6% of PCa patients, respectively. Patients harboring somatic mutations in the substrate-binding cleft domain of *SPOP* (p.Y87C, p.F102C, p.W131G, and p.F133V), show mutual exclusivity to *ETS* fusions [5,8]. Wild-type *SPOP* (*SPOP*$^{wt}$) is known to trigger polyubiquitination and proteasomal degradation of target proteins such as oncogenic coactivators TRIM24, chromatin-associated genes DEK [48], c-MYC [49], and AR [48], suggesting its tumor-suppressive role. Recent studies have shown that *SPOP*$^{wt}$ tumors are more sensitive to BET-domain inhibitors because of the ubiquitin-mediated proteasomal degradation of BET proteins (BRD2/3/4) [49]. PCa subtypes harboring mutations in *SPOP* (*SPOP*$^{mut}$) show impaired expression of BET proteins (specifically BRD4), resulting in their accumulation, thus conferring resistance to BET inhibitors [49–51]. Moreover, transcriptome and BRD4 cistrome analyses of *SPOP*$^{mut}$ cancer cell lines or organoids revealed enhanced expression of genes involved in AR signaling, cholesterol-biosynthesis pathways, and activation of ATK–mTOR signaling, due to BRD4 stabilization, thereby sensitizing *SPOP*$^{mut}$ cells to the AKT inhibitor, ipatasertib [49,52]. Considering the sensitivity of *SPOP*$^{mut}$ tumors to AKT inhibitors, several of these are already in clinical trials, hence emphasizing screening of *SPOP* mutations for guided precision medicine.

## *CHD1* Deletions Associates with *SPOP* Mutations *ETS*-Fusion-Negative PCa

Several studies have revealed that *SPOP*$^{mut}$ tumors are enriched for recurrent somatic deletions of 5q21 and 6q21 loci, known to harbor a chromatin-modifying enzyme, chromodomain helicase DNA-binding protein 1 (CHD1) and tumor suppressors Forkhead O transcription factor and B-lymphocyte-induced maturation protein 1 [8,53,54]. In particular, *CHD1* deletions (*CHD1*$^{del}$) are recurrent in ~15% of PCa cases, often associated with increased risk of biochemical recurrence, and inversely correlated with *ERG* fusion [5,8,55]. In normal tissue, *CHD1* occupies the promoters of actively transcribed genes and facilitates transcriptional initiation of tumor-suppressor genes by maintaining nucleosome turnover [56,57]. However, recently, the interactome of *CHD1* in a normal mouse prostate model established promoter-independent roles, where it occupied prostate-specific enhancers along with AR and its cofactors. Intriguingly, *CHD1*-deficient PCa cells show a redistribution of AR cistrome to HOXB13-enriched sites, which resembles the AR cistrome of human prostate tumors [58], driving a subtype-specific oncogenic transcriptional network with elevated AR activity; a phenomenon critical for PCa pathogenesis [59]. Moreover, a *CHD1* knockout mouse model also showed increased activity of the nonhomologous end joining (NHEJ) DNA-repair pathway, which elicits hypersensitivity to PARPi and DNA-damaging agents [60]. Furthermore, *CHD1*$^{del}$ tumors associate frequently with mutations in the *SPOP–BTB* domain, and inversely correlate with *ERG* fusion in ~25% cases and hence, are classified as *ERG*-/*SPOP*$^{mut}$/*CHD1*$^{del}$ PCa subtype. Both *SPOP*$^{mut}$ and *CHD1*$^{del}$ subclasses independently exhibit increased AR activity, therefore, PCa patients positive for *SPOP*$^{mut}$/

*CHD1*$^{del}$ show enhanced sensitivity to abiraterone therapy [61]. Also, this subtype exhibits elevated levels of DNA methylation, and frequent *SPINK1* outlier expression, thereby representing a key subtype of PCa molecular taxonomy [5].

## Co-occurrence of *CHD1* and *MAP3K7* Deletions in *ETS*-Fusion-Negative PCa

Chromosomal deletion of 6q12-22 loci forms the second most deleted genomic region in ~22% of primary and ~40% of metastatic PCa tumors [9,62]. Genome mapping revealed a marginal deleted 6q15 region (3–5 Mb long), encoding tumor-suppressor *MAP3K7* [9]. Heterozygous deletion (~18.48%) of *MAP3K7* exclusively in *ETS*-fusion-negative cases has been associated with a high Gleason score, tumor grade, lymph node metastasis, and shorter biochemical recurrence [9,63]. A comparative analysis of multiple clinical PCa genomic datasets [1,6,8,64] identified codeletion of both *MAPK37* and *CHD1* in localized (10–20%) and metastatic (20–25%) PCa cases [65], with overall poor disease-free survival. Dual knockdown of *MAP3K7–CHD1* in LNCaP xenografts showed increased tumor growth and overall poor survival compared with knockdown of an individual gene. Moreover, cosuppression of *Map3k7–Chd1* in mouse prostate critically disrupts normal prostate lineage differentiation, marked by a decrease in luminal cells, with a significant loss of AR, and a trend toward neuroendocrine differentiation marked by increased synaptophysin (SYP) and chromogranin (CHGA) levels. Tumors harboring *MAP3K7*$^{del}$/*CHD1*$^{del}$ represent a distinct PCa subtype with neuroendocrine and neural features [63,65], thus therapeutic interventions targeting neuroendocrine PCa [66] might prove effective for this subset of patients.

## *FOXA1* Mutations Define a Distinct Molecular Subtype

Multiple independent cohorts identified recurrent mutations in the forkhead protein, *FOXA1*, in ~4% of primary and metastatic PCa, which exhibits a mutually exclusive pattern with other genomic alterations [5,6,8,11]. *FOXA1*, being a pioneering factor facilitates AR recruitment by opening up compact chromatin, and plays a critical role in mediating androgen signaling [67,68]. Cases with *FOXA1* mutations show several **truncating mutations** adjacent to the C-terminal transactivating domain and missense mutations in the forkhead domain, affecting the *FOXA1* winged-helix DNA-binding domain. These forkhead mutations occur in the residues that do not directly interact with DNA; therefore, they do not alter its DNA-binding functions, but rather disrupts its interactions with other chromatin-bound cofactors [5]. Overexpression of C-terminal *FOXA1* mutants has been shown to increase cell proliferation, migration, and mouse xenograft growth [6,68]. While earlier studies focused only on *FOXA1* protein-coding domains, two recent independent studies using whole genome and untranslated regions (UTRs) sequencing revealed tandem duplications (~14%) and 3′-UTR indels (~12%) of *FOXA1* in mCRPC cases. However, the detailed functional and clinical significance of these aberrations remains unexplored [69,70]. Furthermore, the screening of aberrations associated with *FOXA1* might be useful in prognosis and clinical decision-making.

## Mutation Burden in the Epigenetic Regulators of *ETS*-Fusion-Negative PCa

Overall increased DNA-methylation events have been reported in advanced-stage and fusion-negative PCa patients, when compared with benign and fusion-positive cases, respectively [41,71]. The epigenetic profiles associated with primary PCa show substantial heterogeneity [5,41,71]. Recently, The Cancer Genome Atlas (TCGA) identified a distinct genomic subtype (~1%) exclusively present in *ETS*-fusion-negative PCa cases, defined by hotspot *IDH1* R132 mutations associated with lower frequency of CNAs and elevated genome-wide hypermethylation [5,72]. Isocitrate dehydrogenase 1 and 2 (IDH1/2) are multifunctional enzymes that maintain cellular epigenetics and metabolism by catalyzing isocitrate to α-ketoglutarate (α-KG) [73]. However, the mutated form of *IDH1* catalyzes the formation of oncometabolite D-2-hydroxyglutarate (D-2HG) from α-KG, which in turn deregulates cellular epigenetics and blocks differentiation, thereby driving the progression of multiple human malignancies [74]. Small-molecule inhibitors, namely AG-221 and AG-120 targeting IDH1 mutation have shown promising preclinical results, and are currently in Phase I clinical trials (NCT02074839[iv], NCT02073994[v]) for the advanced stage hematological and solid cancers [75]. Furthermore, these mutations are not observed in any mCRPC cohorts [11,46], suggesting their role in early onset of disease. Therefore, examining *IDH1* mutations or detecting D-2HG oncometabolite levels in plasma will be instrumental in identifying PCa patients with these aberrations.

Of late, a new subclass of *ETS*-fusion-negative tumors defined by truncating mutations in genes coding for chromatin remodelers (~20%) and spliceosome-complex members (~4%) were identified in a large cohort of primary and metastatic PCa cases [10]. Mutations in chromatin remodelers include epigenetic modifies (~15% of the cases) such as *KMT2C*, *KMT2D*, or *KDM6A*, and members of the **SWI/SNF nucleosome-remodeling complex** (~5%) such as *ARID1A*, *ARID4A*, *ARID2*, and *SMARCA1* were identified. Notably, ~4% of the cases show hotspot mutations in genes involved in spliceosome machinery such as *SF3B1* and *U2AF1*. Moreover, ~12% of the PCa patients harbor mutations in ubiquitin–proteasome and ligase family members, such as *USP28*, *USP7*, *CUL3*, and *SPOP*, which affects proteasomal degradation of several oncogenic regulators such as AR and its splice variants and coactivators [10]. Although the functional and clinical relevance of these mutations remains to be elucidated, the preliminary findings from this study implicate an essential role of these epigenetic modifiers and spliceosome machinery in the pathogenesis of this disease.

## Aberrations Associated with Novel Classes of DNA-Repair Pathways

Evolution has selected interconnected networks of DDR pathways, which ensures genomic integrity and cell survival after genotoxic insults. These critical pathways include the base excision repair (BER), nucleotide excision repair (NER), mismatch repair (MMR), and homologous recombination (HR), and NHEJ, which are typically involved in the removal of mismatches, indels, and double-stranded breaks induced during DNA replication. The

[iv] https://clinicaltrials.gov NCT02074839
[v] https://clinicaltrials.gov NCT02073994

somatic/germline aberrations, including SNPs and indels in DDR-pathway genes, have now been redefined as a ‘double-edged sword’ since an increase in the mutational burden of DDR pathways serves as a mechanism by which tumors generate secondary oncogenic drivers. In contrast, these mutations also make tumors more vulnerable to specific therapies targeting DNA-repair genes [76].

Whole exome and targeted deep-sequencing of advanced and metastatic PCa revealed **hypermutated genomes**, harboring mutations in MMR genes and associated microsatellite instability (~12%), with a few patients displaying complex structural rearrangements in *MSH2* and *MSH6* (MutS protein homologs) and focal homozygous deletion of *MSH2* and *BRCA2* [6,77,78]. Moreover, using multiomics platforms, loss-of-function mutations associated with several genes involved in DDR pathways, mostly affecting the HR pathway, were reported [5]. Of these aberrations, germline **frameshift mutations** in *BRCA1*, C-terminal truncating mutations (K3326*) in *BRCA2*, homozygous focal deletions in *CDK12*, heterozygous deletion in *RAD51C*, nonsense mutations in *ATM* and *FANCD2*, a key regulator of the Fanconi anemia pathway were found in ~19% of primary prostate tumors [5]. While an increased mutational burden, comprising pathogenic germline (~8%) and somatic aberrations (~23%) in DDR genes have been associated with mCRPC. Of these, *BRCA2* is the most altered gene in tumors with germline (~5.3%) and somatic biallelic (~12.7%) losses, followed by biallelic loss of *ATM* and somatic mutations *BRCA1*, *CDK12*, *FNACA*, and *RAD51B/C* [46,79].

Since aberrations associated with HR-defective genes are shown to be actionable, tumors with such defects are clinically more vulnerable to PARPi or platinum-based chemotherapy [80–82]. The recent Phase II clinical trials using the PARPis olaparib (NCT01682772[vi]) and veliparib (NCT01576172[i]) for advanced stage mCRPC showed impressive clinical outcomes with 20–30% of patients who harbored DDR defects responding to these therapies (Boxes 2 and 3) [23,83]. A recent follow-up clinical trial using olaparib on the other side identified divergent evolution of resistant tumor subclones with secondary mutations (Box 3), as a resistance mechanism to overcome PARPi-therapy-induced selective pressure [84]. This emphasizes that management strategies involving sequential monitoring of patients are critical during clinical trials to detect any therapy-induced early genetic changes (Box 3). Furthermore, emerging lines of evidence indicate that MMR-deficient tumors possess an active immune microenvironment, with robust immune-checkpoint ligand expression, such as, that of programmed death-1 (PD-1), programmed death ligand-1 (PDL1), cytotoxic T-lymphocyte-associated protein-4 (CTLA-4), increased tumor-specific **neoantigens**, and **tumor infiltrating T lymphocytes**, forming the basis of clinical investigation of immunotherapies for this subset (Box 3) [85,86].

[vi] https://clinicaltrials.gov NCT01682772

## Biallelic Loss of *CDK12* Represents a Distinct Immunogenic Class of *ETS*-Fusion-Negative PCa

Comprehensive sequencing analysis of multisite biopsies of 360 metastatic PCa patients (CRPC360) and a primary TCGA dataset identified a novel subtype, marked by the biallelic loss of *CDK12*, recurrent in ~1.2% and ~7% of primary and mCRPC cases, respectively [87]. This subtype shows mutual exclusivity to tumors harboring *ETS* fusions, DDR deficiencies, and *SPOP* mutations, thus pointing toward distinct tumor evolutionary patterns. Previously, *CDK12* has been implicated in regulating the expression of several DDR genes [88,89], and was shown to be mutated in advanced CRPC patients [6,11,90]. Comparative genome analysis of *CDK12*-mutation-positive tumors suggests that *CDK12* mutants are largely diploid, with the highest number of differentially expressed genes, higher gene-fusions incidences, and focal tandem duplications. Additionally, these *CDK12*-mutant tumors show increased expression of chemokines such as CCL18 and CXCL8, which support dendritic cell migration into the tumor microenvironment and an overall increase in neoantigens and T cell infiltration, marked by increased CD3 levels [87].

Furthermore, a retrospective clinical analysis of MI-ONCOSEQ enrolled patients revealed that four of 11 *CDK12*-mutant cases show a modest decrease in prostate-specific antigen (PSA) levels after anti-PD1 monotherapy. Additionally, one patient with prior lymph node metastases showed a robust decrease in $CD3^+$ staining in the lymph node metastatic biopsy and a concomitant decline in disease burden [87]. A prospective Phase II clinical study (NCT03570619[vii]) evaluating the combined effects of anti-PD-1 and anti-CTLA-4 immunotherapies for *CDK12*-mutant mCRPC patients is ongoing. Yet another, Phase III clinical trial (NCT02054806[viii]) with anti-PD1 monotherapy, revealed an increase in progression-free and overall survival in advanced PD-L1-positive mCRPC patients [91]. Taken together, these clinicogenomics trials using evidence-based sequencing approaches and combinatorial therapeutic strategies will support the development of precision therapies and better disease management [92].

## Concluding Remarks

The rapid advancement in sequencing technologies and integrative data analyses has advanced the field of PCa by revealing hitherto unknown molecular key players specific to the *ETS*-fusion-negative subtype. Comparative molecular studies of metastatic and primary PCa cohorts have identified a plethora of actionable and meaningful genetic mutations associated with AR signaling, **epigenetic regulators**, DDR pathway, immunogenic subclasses, polyubiquitin, and spliceosome machinery. Despite the detailed molecular taxonomy of the PCa genome, ~26% of all tumors appeared to be driven by still occult molecular abnormalities/aberrations, which need future investigations [5]. Nonetheless, most of these studies are limited to a single tumor focus, whereas most prostate tumors that are multifocal demonstrate molecular heterogeneity across different foci within the prostate gland. Such intratumoral heterogeneity should be considered for future genomics or clinical

[vii] https://clinicaltrials.gov NCT03570619
[viii] https://clinicaltrials.gov NCT02054806

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studies, as well as for designing gene signature panels and novel therapeutic strategies (see Clinician's Corner). Moreover, improved diagnostic technologies are required to detect pre-existing resistant mutations in heterogeneous tumors, followed by evidence-based tailored therapeutic approaches, which might circumvent the growth of drug-resistant clones. As stated by Adam Dicker, 'there is a lot more we need to figure out in the realm of prostate cancer, but it's the beginning of a road map for precision oncology, and before this, we didn't have a road map' [93]. Therefore, future cancer research should focus on integrating critical information pertaining to genetic aberrations and mutational profiles with artificial-intelligence-aided digital pathology and automated-image analysis to predict the clinical and therapeutic outcomes in a subtype-specific manner (see Outstanding Questions). Taken together, these integrative transdisciplinary efforts will enhance our understanding of the pathobiology of prostate cancer and facilitate the development of sensitive and robust predictive biomarkers for precision cancer medicine.

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## References

1. Taylor BS, et al. Integrative genomic profiling of human prostate cancer. Cancer cell. 2010; 18:11–22. [PubMed: 20579941]
2. Hollenhorst PC, et al. Genomic and biochemical insights into the specificity of *ETS* transcription factors. Annu Rev Biochem. 2011; 80:7–471.
3. Tomlins SA, et al. Recurrent fusion of TMPRSS2 and *ETS* transcription factor genes in prostate cancer. Science. 2005; 310:644–648. [PubMed: 16254181]
4. Ateeq B, et al. Molecular profiling of *ETS* and non-*ETS* aberrations in prostate cancer patients from northern India. Prostate. 2015; 75:1051–1062. [PubMed: 25809148]
5. Abeshouse A, et al. The molecular taxonomy of primary prostate cancer. Cell. 2015; 163:1011–1025. [PubMed: 26544944]
6. Grasso CS, et al. The mutational landscape of lethal castration-resistant prostate cancer. Nature. 2012; 487:239. [PubMed: 22722839]
7. Palanisamy N, et al. Rearrangements of the *RAF* kinase pathway in prostate cancer, gastric cancer and melanoma. Nat Med. 2010; 16:793. [PubMed: 20526349]
8. Barbieri CE, et al. Exome sequencing identifies recurrent *SPOP*, *FOXA1* and MED12 mutations in prostate cancer. Nat Genet. 2012; 44:685. [PubMed: 22610119]
9. Liu W, et al. Deletion of a small consensus region at 6q15, including the *MAP3K7* gene, is significantly associated with high-grade prostate cancers. Clin Cancer Res. 2007; 13:5028–5033. [PubMed: 17785553]
10. Armenia J, et al. The long tail of oncogenic drivers in prostate cancer. Nat Genet. 2018; 50:645. [PubMed: 29610475]
11. Robinson D, et al. Integrative clinical genomics of advanced prostate cancer. Cell. 2015; 161:1215–1228. [PubMed: 26000489]
12. Hieronymus H, et al. Copy number alteration burden predicts prostate cancer relapse. Proc Natl Acad Sci. 2014; 111:11139–11144. [PubMed: 25024180]
13. Demichelis F, et al. TMPRSS2: ERG gene fusion associated with lethal prostate cancer in a watchful waiting cohort. Oncogene. 2007; 26:4596. [PubMed: 17237811]

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14. Nam R, et al. Expression of the TMPRSS2: ERG fusion gene predicts cancer recurrence after surgery for localised prostate cancer. Br J Cancer. 2007; 97:1690. [PubMed: 17971772]
15. Klezovitch O, et al. A causal role for ERG in neoplastic transformation of prostate epithelium. Proc Natl Acad Sci. 2008; 105:2105–2110. [PubMed: 18245377]
16. Tomlins SA, et al. Distinct classes of chromosomal rearrangements create oncogenic *ETS* gene fusions in prostate cancer. Nature. 2007; 448:595. [PubMed: 17671502]
17. Yu J, et al. An integrated network of androgen receptor, polycomb, and TMPRSS2-ERG gene fusions in prostate cancer progression. Cancer cell. 2010; 17:443–454. [PubMed: 20478527]
18. Antonarakis ES, et al. AR-V7 and resistance to enzalutamide and abiraterone in prostate cancer. N Engl J Med. 2014; 371:1028–1038. [PubMed: 25184630]
19. Chen CD, et al. Molecular determinants of resistance to antiandrogen therapy. Nat Med. 2004; 10:33–39. [PubMed: 14702632]
20. Karantanos T, et al. Prostate cancer progression after androgen deprivation therapy: mechanisms of castrate resistance and novel therapeutic approaches. Oncogene. 2013; 32:5501–5511. [PubMed: 23752182]
21. Darnell JE Jr. Transcription factors as targets for cancer therapy. Nat Rev Cancer. 2002; 2:740. [PubMed: 12360277]
22. Brenner JC, et al. Mechanistic rationale for inhibition of poly (ADP-ribose) polymerase in *ETS* gene fusion-positive prostate cancer. Cancer cell. 2011; 19:664–678. [PubMed: 21575865]
23. Hussain M, et al. Targeting androgen receptor and DNA repair in metastatic castration-resistant prostate cancer: results from NCI 9012. J Clin Oncol. 2018; 36:991. [PubMed: 29261439]
24. Asangani IA, et al. Therapeutic targeting of BET bromodomain proteins in castration-resistant prostate cancer. Nature. 2014; 510:278. [PubMed: 24759320]
25. Welti J, et al. Targeting bromodomain and extra-terminal (BET) family proteins in castration-resistant prostate cancer (CRPC). Clin Cancer Res. 2018; 24:3149–3162. [PubMed: 29555663]
26. Liu LL, et al. Mechanisms of the androgen receptor splicing in prostate cancer cells. Oncogene. 2014; 33:3140–3150. [PubMed: 23851510]
27. Asangani IA, et al. BET bromodomain inhibitors enhance efficacy and disrupt resistance to AR antagonists in the treatment of prostate cancer. Mol Cancer Res. 2016; 14:324–331. [PubMed: 26792867]
28. Wang X, et al. Development of peptidomimetic inhibitors of the ERG gene fusion product in prostate cancer. Cancer cell. 2017; 31:532–548. e7. [PubMed: 28344039]
29. Tomlins SA, et al. The role of *SPINK1* in *ETS* rearrangement-negative prostate cancers. Cancer cell. 2008; 13:519–528. [PubMed: 18538735]
30. Leinonen KA, et al. Association of *SPINK1* expression and TMPRSS2: ERG fusion with prognosis in endocrine-treated prostate cancer. Clin Cancer Res. 2010; 16:2845–2851. [PubMed: 20442300]
31. Pan X, et al. The expression profile and prognostic value of *SPINK1* in initially diagnosed bone metastatic prostate cancer. Prostate. 2016; 76:823–833. [PubMed: 27159572]
32. Johnson MH, et al. *SPINK1* defines a molecular subtype of prostate cancer in men with more rapid progression in an at risk, natural history radical prostatectomy cohort. J Urol. 2016; 196:1436–1444. [PubMed: 27238617]
33. Flavin R, et al. *SPINK1* protein expression and prostate cancer progression. Clin Cancer Res. 2014; 20:4904–4911. [PubMed: 24687926]
34. Lippolis G, et al. A high-density tissue microarray from patients with clinically localized prostate cancer reveals ERG and TATI exclusivity in tumor cells. Prostate Cancer Prostatic Dis. 2013; 16:145. [PubMed: 23459095]
35. Grupp K, et al. *SPINK1* expression is tightly linked to 6q15- and 5q21-deleted ERG-fusion negative prostate cancers but unrelated to PSA recurrence. Prostate. 2013; 73:1690–1698. [PubMed: 23843146]
36. Ateeq B, et al. Therapeutic targeting of *SPINK1*-positive prostate cancer. Sci Transl Med. 2011; 3:72ra17.

Europe PMC Funders Author Manuscripts


37. Slovin SF, et al. Anti-epidermal growth factor receptor monoclonal antibody cetuximab plus doxorubicin in the treatment of metastatic castration-resistant prostate cancer. Clin Genitourin Cancer. 2009; 7:E77–E82. [PubMed: 19815486]
38. Nabhan C, et al. Erlotinib has moderate single-agent activity in chemotherapy-naive castration-resistant prostate cancer: final results of a phase II trial. Urology. 2009; 74:665–671. [PubMed: 19616281]
39. Shukla S, et al. Aberrant activation of a gastrointestinal transcriptional circuit in prostate cancer mediates castration resistance. Cancer cell. 2017; 32:792–806. e7. [PubMed: 29153843]
40. Bhatia V, et al. Epigenetic silencing of miRNA-338-5p and miRNA-421 drives *SPINK1*-positive prostate cancer. Clin Cancer Res. 2019; 25:2755–2768. [PubMed: 30587549]
41. Börno ST, et al. Genome-wide DNA methylation events in TMPRSS2–ERG fusion-negative prostate cancers implicate an EZH2-dependent mechanism with miR-26a hypermethylation. Cancer Discov. 2012; 2:1024–1035. [PubMed: 22930729]
42. Bjartell A. New hope in prostate cancer precision medicine? miRNA replacement and epigenetics. Clin Cancer Res. 2019; 25:2679–2681. [PubMed: 30808772]
43. Tiwari R, et al. Androgen deprivation upregulates *SPINK1* expression and potentiates cellular plasticity in prostate cancer. bioRxiv. 2019 Feb 27.2019doi: 10.1101/562652
44. Paju A, et al. Increased expression of tumor-associated trypsin inhibitor, TATI, in prostate cancer and in androgen-independent 22Rv1 cells. Eur Urol. 2007; 52:1670–1679. [PubMed: 17306443]
45. Faisal FA, et al. Racial variations in prostate cancer molecular subtypes and androgen receptor signaling reflect anatomic tumor location. Eur Urol. 2016; 70:14–17. [PubMed: 26443432]
46. Robinson DR, et al. Integrative clinical genomics of metastatic cancer. Nature. 2017; 548:297. [PubMed: 28783718]
47. Zhuang M, et al. Structures of *SPOP*-substrate complexes: insights into molecular architectures of BTB-Cul3 ubiquitin ligases. Mol Cell. 2009; 36:39–50. [PubMed: 19818708]
48. Theurillat J-PP, et al. Ubiquitylome analysis identifies dysregulation of effector substrates in *SPOP*-mutant prostate cancer. Science. 2014; 346:85–89. [PubMed: 25278611]
49. Geng C, et al. *SPOP* regulates prostate epithelial cell proliferation and promotes ubiquitination and turnover of c-MYC oncoprotein. Oncogene. 2017; 36:4767. [PubMed: 28414305]
50. Dai X, et al. Prostate cancer-associated *SPOP* mutations confer resistance to BET inhibitors through stabilization of BRD4. Nat Med. 2017; 23:1063. [PubMed: 28805820]
51. Janouskova H, et al. Opposing effects of cancer-type-specific *SPOP* mutants on BET protein degradation and sensitivity to BET inhibitors. Nat Med. 2017; 23:1046. [PubMed: 28805821]
52. Blattner M, et al. *SPOP* mutation drives prostate tumorigenesis in vivo through coordinate regulation of PI3K/mTOR and AR signaling. Cancer cell. 2017; 31:436–451. [PubMed: 28292441]
53. Mandelbaum J, et al. BLIMP1 is a tumor suppressor gene frequently disrupted in activated B cell-like diffuse large B cell lymphoma. Cancer cell. 2010; 18:568–579. [PubMed: 21156281]
54. Yang J-Y, Hung M-C. A new fork for clinical application: targeting forkhead transcription factors in cancer. Clin Cancer Res. 2009; 15:752–757. [PubMed: 19188143]
55. Burkhardt L, et al. *CHD1* is a 5q21 tumor suppressor required for ERG rearrangement in prostate cancer. Cancer Res. 2013; 73:2795–2805. [PubMed: 23492366]
56. Gaspar-Maia A, et al. *Chd1* regulates open chromatin and pluripotency of embryonic stem cells. Nature. 2009; 460:863. [PubMed: 19587682]
57. Simic R, et al. Chromatin remodeling protein *Chd1* interacts with transcription elongation factors and localizes to transcribed genes. EMBO J. 2003; 22:1846–1856. [PubMed: 12682017]
58. Pomerantz MM, et al. The androgen receptor cistrome is extensively reprogrammed in human prostate tumorigenesis. Nat Genet. 2015; 47:1346. [PubMed: 26457646]
59. Augello MA, et al. *CHD1* loss alters AR binding at lineage-specific enhancers and modulates distinct transcriptional programs to drive prostate tumorigenesis. Cancer Cell. 2019; 35:603–617. [PubMed: 30930119]
60. Shenoy T, et al. *CHD1* loss sensitizes prostate cancer to DNA damaging therapy by promoting error-prone double-strand break repair. Ann Oncol. 2017; 28:1495–1507. [PubMed: 28383660]

Europe PMC Funders Author Manuscripts


61. Boysen G, et al. *SPOP*-mutated/CHD1-deleted lethal prostate cancer and abiraterone sensitivity. Clin Cancer Res. 2018; 24:5585–5593. [PubMed: 30068710]
62. Sun J, et al. DNA copy number alterations in prostate cancers: a combined analysis of published CGH studies. Prostate. 2007; 67:692–700. [PubMed: 17342750]
63. Kluth M, et al. Genomic deletion of *MAP3K7* at 6q12-22 is associated with early PSA recurrence in prostate cancer and absence of TMPRSS2: ERG fusions. Mod Pathol. 2013; 26:975. [PubMed: 23370768]
64. Baca SC, et al. Punctuated evolution of prostate cancer genomes. Cell. 2013; 153:666–677. [PubMed: 23622249]
65. Rodrigues LU, et al. Coordinate loss of *MAP3K7* and *CHD1* promotes aggressive prostate cancer. Cancer Res. 2015; 75:1021–1034. [PubMed: 25770290]
66. Puca L, et al. Neuroendocrine differentiation in prostate cancer: emerging biology, models, and therapies. Cold Spring Harb Perspect Med. 2019 Feb 1.9doi: 10.1101/cshperspect.a030593
67. He HH, et al. Nucleosome dynamics define transcriptional enhancers. Nat Genet. 2010; 42:343. [PubMed: 20208536]
68. Jin H-J, et al. Androgen receptorindependent function of FoxA1 in prostate cancer metastasis. Cancer Res. 2013; 73:3725–3736. [PubMed: 23539448]
69. Annala M, et al. Frequent mutation of the *FOXA1* untranslated region in prostate cancer. Commun Biol. 2018; 1:122. [PubMed: 30272002]
70. Quigley DA, et al. Genomic hallmarks and structural variation in metastatic prostate cancer. Cell. 2018; 174:758–769. e9. [PubMed: 30033370]
71. Mahapatra S, et al. Global methylation profiling for risk prediction of prostate cancer. Clin Cancer Res. 2012; 18:2882–2895. [PubMed: 22589488]
72. Hinsch A, et al. Immunohistochemically detected IDH1 R132H mutation is rare and mostly heterogeneous in prostate cancer. World J Urol. 2018; 36:877–882. [PubMed: 29427004]
73. Molenaar RJ, et al. Wild-type and mutated IDH1/2 enzymes and therapy responses. Oncogene. 2018; 37:5810. [PubMed: 30140044]
74. Dang L, et al. Cancer-associated *IDH1* mutations produce 2-hydroxyglutarate. Nature. 2009; 462:739. [PubMed: 19935646]
75. Yen K, et al. Cancer-associated IDH mutations: biomarker and therapeutic opportunities. Oncogene. 2010; 29:6409. [PubMed: 20972461]
76. Schiewer MJ, Knudsen KE. DNA damage response in prostate cancer. Cold Spring Harb Perspect Med. 2019; 9:a030486. [PubMed: 29530944]
77. Pritchard CC, et al. Complex MSH2 and MSH6 mutations in hypermutated microsatellite unstable advanced prostate cancer. Nat Commun. 2014; 5:4988. [PubMed: 25255306]
78. Kumar A, et al. Exome sequencing identifies a spectrum of mutation frequencies in advanced and lethal prostate cancers. Proc Natl Acad Sci. 2011; 108:17087–17092. [PubMed: 21949389]
79. Pritchard CC, et al. Inherited DNA-repair gene mutations in men with metastatic prostate cancer. N Engl J Med. 2016; 375:443–453. [PubMed: 27433846]
80. Fong PC, et al. Inhibition of poly (ADP-ribose) polymerase in tumors from BRCA mutation carriers. N Engl J Med. 2009; 361:123–134. [PubMed: 19553641]
81. Kaufman B, et al. Olaparib monotherapy in patients with advanced cancer and a germline BRCA1/2 mutation. J Clin Oncol Off J Am Soc Clin Oncol. 2015; 33:244.
82. Bryant HE, et al. Specific killing of BRCA2-deficient tumours with inhibitors of poly (ADP-ribose) polymerase. Nature. 2005; 434:913. [PubMed: 15829966]
83. Mateo J, et al. DNA-repair defects and olaparib in metastatic prostate cancer. N Engl J Med. 2015; 373:1697–1708. [PubMed: 26510020]
84. Goodall J, et al. Circulating cell-free DNA to guide prostate cancer treatment with PARP inhibition. Cancer Discov. 2017; 7:1006–1017. [PubMed: 28450425]
85. Le DT, et al. PD-1 blockade in tumors with mismatch-repair deficiency. N Engl J Med. 2015; 372:2509–2520. [PubMed: 26028255]
86. Le DT, et al. Mismatch repair deficiency predicts response of solid tumors to PD-1 blockade. Science. 2017; 357:409–413. [PubMed: 28596308]

87. Wu Y-M, et al. Inactivation of *CDK12* delineates a distinct immunogenic class of advanced prostate cancer. Cell. 2018; 173:1770–1782. e14. [PubMed: 29906450]

88. Blazek D, et al. The Cyclin K/Cdk12 complex maintains genomic stability via regulation of expression of DNA damage response genes. Genes Dev. 2011; 25:2158–2172. [PubMed: 22012619]

89. Juan H, et al. *Cdk12* is essential for embryonic development and the maintenance of genomic stability. Cell Death Differ. 2016; 23:1038. [PubMed: 26658019]

90. Viswanathan SR, et al. Structural alterations driving castration-resistant prostate cancer revealed by linked-read genome sequencing. Cell. 2018; 174:433–447. e19. [PubMed: 29909985]

91. Hansen A, et al. Pembrolizumab for advanced prostate adenocarcinoma: findings of the KEYNOTE-028 study. Ann Oncol. 2018; 29:1807–1813. [PubMed: 29992241]

92. Cordes LM, et al. Perspectives on the clinical development of immunotherapy in prostate cancer. Asian J Androl. 2018; 20:253–259. [PubMed: 29582792]

93. Caruso C. *CDK12* changes telling in prostate cancer. Cancer Discov. 2018; 8:1055.

94. Polkinghorn WR, et al. Androgen receptor signaling regulates DNA repair in prostate cancers. Cancer Discov. 2013; 3:1245–1253. [PubMed: 24027196]

95. Schiewer MJ, et al. Dual roles of PARP-1 promote cancer growth and progression. Cancer Discov. 2012; 2:1134–1149. [PubMed: 22993403]

96. Sakamoto KM, et al. Protacs: chimeric molecules that target proteins to the Skp1-Cullin-F box complex for ubiquitination and degradation. Proc Natl Acad Sci U S A. 2001; 98:8554–8559. [PubMed: 11438690]

97. Raina K, et al. PROTAC-induced BET protein degradation as a therapy for castration-resistant prostate cancer. Proc Natl Acad Sci USA. 2016; 113:7124–7129. [PubMed: 27274052]

98. Sakamoto KM, et al. Development of Protacs to target cancer-promoting proteins for ubiquitination and degradation. Mol Cell Proteomics. 2003; 2:1350–1358. [PubMed: 14525958]

99. Han X, et al. Discovery of ARD-69 as a highly potent proteolysis targeting chimera (PROTAC) degrader of androgen receptor (AR) for the treatment of prostate cancer. J Med Chem. 2019; 62:941–964. [PubMed: 30629437]

100. Salami J, et al. Androgen receptor degradation by the proteolysis-targeting chimera ARCC-4 outperforms enzalutamide in cellular models of prostate cancer drug resistance. Commun Biol. 2018; 1:100. [PubMed: 30271980]

101. Neklesa T, et al. ARV-110: an androgen receptor PROTAC degrader for prostate cancer, AACR. 2018

102. Helleday, T. PARP inhibitor receives FDA breakthrough therapy designation in castration resistant prostate cancer: beyond germline BRCA mutations. Oxford University Press; 2016.

103. Linch M, et al. Intratumoural evolutionary landscape of high-risk prostate cancer: the PROGENY study of genomic and immune parameters. Ann Oncol. 2017; 28:2472–2480. [PubMed: 28961847]

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**Box 1**

### Clinical Failure of PARPi Therapy for *ETS*-Fusion-Positive PCa

Androgen signaling regulates a network of genes involved in DNA repair; thus, ADT could synergize response to PARP inhibition and radiotherapy [94,95]. A recent prospective clinical trial NCI-9012 (NCT01576172[i]) evaluating the therapeutic potentials of PARPi veliparib in combination with antiandrogen (abiraterone acetate) for ETS-fusion-positive mCRPC patients failed to show any additive response when compared with antiandrogens alone. Moreover, no significant response such as a decrease in PSA or progression-free survival was recorded in ETS-fusion-positive patients [23]. Furthermore, clinical assessment of tumor biopsies using targeted exon sequencing revealed that ~25% of mCRPC patients harbor mutations in the genes involved in the HR DNA-repair pathway. These patients with DNA-repair defects showed an exceptional response to veliparib with an overall decrease in PSA (≥90%), and a prolonged median progression-free survival in comparison to the wild-type tumors (14.5 vs 8.1 months) [23]. This study implicates a therapeutic strategy that includes PARPis in combination with antiandrogens for patients with DDR defects; however, it failed to predict clinical efficacy for ETS-fusion-positive PCa patients.

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**Box 2**

### Clinical Significance of Protein Degraders: PROTACs

The PROTACs are chimeric bifunctional small molecules with two arms joined by a linker, where one arm recognizes a protein of interest, and the other, E3 ubiquitin ligase to form a ternary complex facilitating proteasome degradation of the target protein through the ubiquitin–proteasomal machinery [96]. Recent studies from multiple groups have utilized PROTACs for targeting oncogenic drivers, such as AR and its coactivator, BRD4 [97,98]. Multiple AR–PROTACs have been designed based on different classes of AR antagonists, E3-ligase degradation systems, and varying lengths of linkers. Of these, the most potent being ARCC-4, ARD-69, and ARV-110 which have been shown to effectively reduce (>90%) expression of AR as well as AR mutants, and attenuate their oncogenic effects in cancer cell lines as well as in enzalutamide-resistant preclinical mouse models [99–101]. Recently the first oral AR PROTAC, ARV-110 has entered a Phase I clinical trial (NCT03888612[ix]), which may have potential for mCRPC patients. Notably, a small molecule pan-BET protein inhibitor ARV-771, designed based on similar PROTAC chemistry, has shown a significant decrease in BRD4, AR full length, AR-V7, and ERG proteins. When compared with conventional BET inhibitors, ARV-771 results in a remarkable inhibition of tumor growth in preclinical CRPC mouse models and enzalutamide-resistant AR-V7-positive 22RV1 xenografts [97]. Taken together, these preclinical findings encourage clinical applicability of the PROTACs for advanced stage mCRPC patients.

[ix] https://clinicaltrials.gov NCT03888612

**Box 3**

### Clinical Studies Targeting DNA Damage and Response Defects in PCa

The clinical outcomes of the recently conducted Phase II trial (TOPARP-A: Trial of PARP inhibition (PARPi) in prostate cancer, NCT01682772[vi]) showed an impressive response to PARP inhibition. Advanced stage mCRPC patients ($n = 50$), who failed to respond to prior therapies such as antiandrogens, docetaxel, or cabazitaxel were enrolled; of these, 16 patients (~33%) responded to PARPi therapy. Importantly, 88% of these responders harbored homozygous deletions or mutations in *BRCA2* (seven of 16), *ATM* (four of 16), *FNACA* (2 of 16), and *CHEK2* (one of 16) [83]. Subsequently, FDA approved olaparib as a breakthrough therapy designation for treatment of mCRPC patients harboring *BRCA1/2* or *ATM* gene mutations [102]. However, a recent follow-up study of this trial, using cell-free DNA (cfDNA) isolated from the blood samples serially collected during the course of the trial, identified evolution of PARPi-therapy-resistant subclones.Whole-exome cfDNA sequencing of six responders, where samples were collected during disease progression and at resistance stage, identified two patients with prior germline *BRCA2* frameshift mutations, and newly acquired frameshift somatic deletions, hence restoring the reading frame of *BRCA2*. Additionally, two patients with nongermline *BRCA2* and *PALB2* mutations in the pretrial tumor biopsy showed a new inframe somatic deletion, thereby restoring *BRCA2* and *PALB2* reading frames, and a patient with *HDAC2* biallelic loss before treatment exhibited new *TP53* and *TSC2* mutations in the resistant-tumor biopsies during the trial [84]. These secondary mutations were acquired as a result of PARP inhibition, which indicate clonal evolution of resistant tumors to overcome therapy induced drug pressures.

Recent studies also indicate that mCRPC patients with hypermutated genomes are enriched for MMR defects, which warrants clinical exploration and treatment responses to immune-checkpoint inhibitors in MMR-deficient mCRPC patients [103]. Phase II clinical studies (NCT01876511[x]) evaluating the effect of immune-checkpoint inhibitor, pembrolizumab (anti-PD-1) for metastatic colorectal carcinoma patients with MMR deficiency have shown remarkable response with prolonged progression-free survival [85]. Phase I/II clinical trials (NCT03061539[xi]) evaluating combinatorial effects of anti-PD-1 and anti-CTLA-4 immunotherapies for mCRPC patients with immunogenic MMR- and DDR-mutational signatures are currently ongoing.

[x] https://clinicaltrials.gov NCT01876511
[xi] https://clinicaltrials.gov NCT03061539

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## Highlights

Several distinct subtypes marked by germline or somatic mutations, gene fusions, focal deletions, and amplifications have been identified in the *ETS*-fusion-negative subtype.

Novel regulatory mechanisms underlying increased *SPINK1* levels, include: (i) AR-mediated transcriptional repression of *SPINK1*; (ii) EZH2-mediated epigenetic silencing of miR-338-5p/miR-421; and (iii) negative post-transcriptional regulators of *SPINK1*.

Aberrations in epigenetic regulators or chromatin remodelers, ubiquitin, and spliceosome machineries represent novel subclasses of *ETS*-fusion-negative PCa.

Mutations in DNA damage and repair genes sensitize PCa cells to PARP inhibition, increases neoantigens and T cell infiltration hence sensitizing them towards immune checkpoint inhibitors.

Sequentially collected liquid biopsies support identification of therapy-resistant clones, and reveal the mechanism involved in tumor evolution.

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## Glossary

**Biochemical recurrence:** refers to a clinical condition marked by an increase in serum PSA levels in PCa patients who have undergone surgery or radiation therapy.

**Bromodomain and extra-terminal (BET) protein inhibitors:** small-molecule inhibitors which target the bromodomain of BET proteins hindering their interaction with acetylated histones, thereby inhibiting transcription of BRD target genes.

**Copy number alterations (CNAs):** structural variations including duplications, insertions/deletions (indels) of genes that impart phenotypic variations or causative events in genetic disorders or cancers.

**DNA damage and response (DDR) pathway defects:** DDR pathways maintain genomic stability by preventing duplication and propagation of DNA errors to the next generation. These defects initiate neoplastic transformations, neurodegenerative or cardiovascular disorders, and other heritable diseases.

**Epigenetic regulators:** a group of chromatin-remodeling enzymes such as histone acetyltransferases, deacetylases, methyltransferases, and DNA-methyltransferases that play a crucial role in modulating gene expression by orchestrating the chromatin state. Aberrations in these enzymes alter histone modifications and methylation patterns, which in turn disrupts the cellular homeostasis in human malignancies.

**Frameshift mutations:** these are caused by insertion/deletion of nucleotides that change the reading frame of the encoded protein, often resulting in a new or a nonfunctional protein.

**Hypermutated genomes:** individual tumor genome with about ≥300 somatic mutations (an outlier number) compared with its matched normal is considered to be hypermutated. Higher frequencies of mutations arise due to impairment of the DNA mismatch repair pathway.

**Immune checkpoint inhibitors:** cancer cells exhibit high PD-L1 expression, which interacts with immune checkpoint proteins, namely PD-1 expressed on T cells, resulting in immune escape. This class of drugs/inhibitors targets these immune checkpoint proteins allowing T cell-mediated cytotoxicity.

**Neoantigens:** newly formed antigens, generally truncated proteins, cause higher degree of antigenicity in tumor cells, hence facilitating immune cells to target and eliminate them. Tumors with high MMR defects or microsatellite instability (MSI) show higher number of neoantigens, making them suitable candidates for immunotherapy.

**SWI/SNF nucleosome-remodeling complex:** ATP-dependent nucleosome-remodeling complex, comprises a group of proteins that disrupt histone–DNA interactions by ATP hydrolysis, thereby making DNA accessible for transcription machinery and DNA repair.

**Tandem duplications:** intra-chromosomal duplication where a segment of DNA is duplicated, and placed on the same chromosomal arm next to the original segment. Tandem duplications are further divided into direct tandem, in which the gene order

remains the same as the original, and reverse tandem, where the duplicated segment order is reversed.

**Truncating mutations:** these are also known as nonsense mutations, caused by a change in DNA base pairs, leading to a premature stop codon that results in a truncated or nonfunctional protein.

**Tumor-infiltrating T cells:** infiltration and activation of immune cells, especially effector T lymphocytes into the tumor microenvironment. This phenomenon is a predictor for an effective response of T cell-based immunotherapies.



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## Clinician's Corner

Multiclonality and evolution of resistant tumor clones with secondary mutations due to druginduced selective pressures remain challenging and must be considered while designing therapeutic strategies and clinical trials.

Clinicogenomic studies using noninvasive liquid biopsies collected pre-treatment, on therapy, and resistant stages offer opportunities for early detection of molecular events that might rationalize resistance-induced changes in the tumors.

Preclinical and clinical evaluation of targeted therapies involving PROTACs, which utilizes endogenous proteasomal degradation machinery, represents a novel class of small molecule inhibitors that might effectively target oncogenic transcriptional activators/coactivators.

Screening of clinically relevant genomic aberrations involving actionable *RAF* fusions, *SPINK1* overexpression, *SPOP*, *FOXA1*, or *IDH1* mutations, deletions of *CHD1* or *MAP3K7*, DNA repair defects, or immunogenic *CDK12* mutations would guide the development of precision medicine for these distinct molecular subtypes.

The use of evidence-based sequencing approaches or combinatorial therapeutic strategies should be employed for patients harboring DNA repair defects along with immune activation (such as patients with mismatch repair defects) would predict better treatment response.

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## Outstanding Questions

How can genomics and functional genomics approaches be systematically integrated to fully understand the role of novel somatic or genetic molecular events in tumor initiation, progression, and therapy-induced drug resistance?

Not all molecular aberrations are drivers, many of these are mere passenger alterations, thus, how can we accurately identify these events for their clinical utility?

Can we effectively predict the clonal evolution of resistant tumor clones by employing serial evaluation of tumor biopsies collected during the course of therapeutic trial using sensitive techniques and integrative analysis, such as single cell sequencing?

Can artificial-intelligence-based approaches through the integration of digital pathological features with genomics, transcriptomics, and functional genomics data help to develop precision medicine for diverse cancer molecular subtypes?

How could unsuccessful clinical trials be utilized to improve our understanding and provide ways to overcome the mechanisms of therapy resistance?

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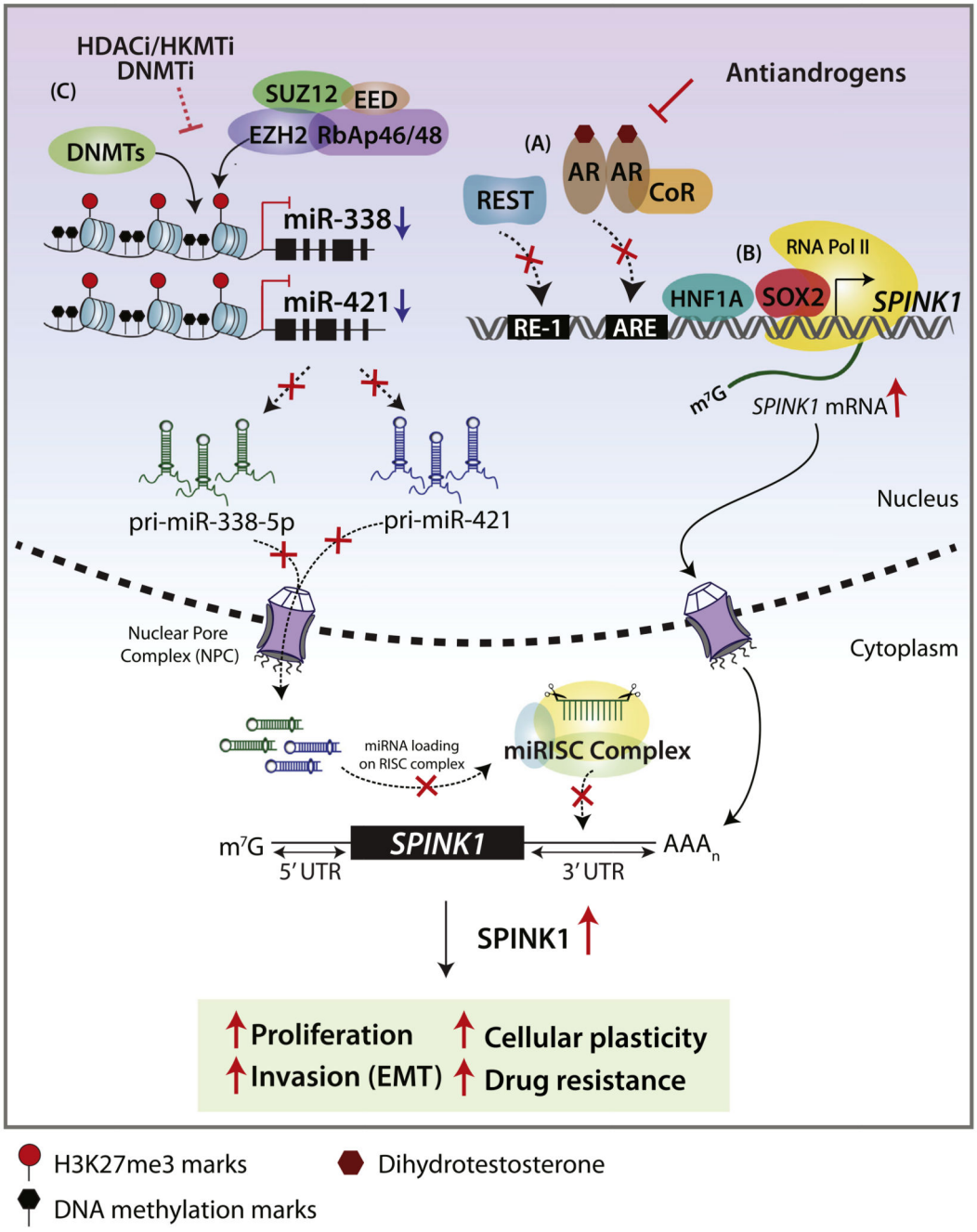


**Figure 1. Molecular Circuitries Involved in SPINK1 Upregulation in Prostate Cancer.**

A schematic showing (A) Androgen receptor (AR)-mediated transcriptional repression of *SPINK1*, wherein antiandrogen treatment may result in *SPINK1* upregulation (right). (B) SOX2 and HNF1A binds on the *SPINK1* promoter and positively regulates its expression (right). (C) Epigenetic repression of post-transcriptional regulators, miR-338-5p and miR-421, results in increased *SPINK1* expression and oncogenicity in prostate cancer (left). Abbreviations: ARE, androgen response element; CoR, corepressor; DNMTi, DNA methyltransferase inhibitor; EMT, epithelial–mesenchymal transition; EZH2, enhancer of

zeste homolog 2; HDACi, histone deacetylase inhibitor; HKMTi, histone lysine methyltransferase inhibitor; HNF1A, hepatocyte nuclear factor 1-α; miRISC, miRNA-induced silencing complex; REST, RE1 silencing transcription factor; SOX2, SRY (sex determining region Y) box 2; *SPINK1*, serine protease inhibitor, Kazal type 1; UTR, untranslated region.



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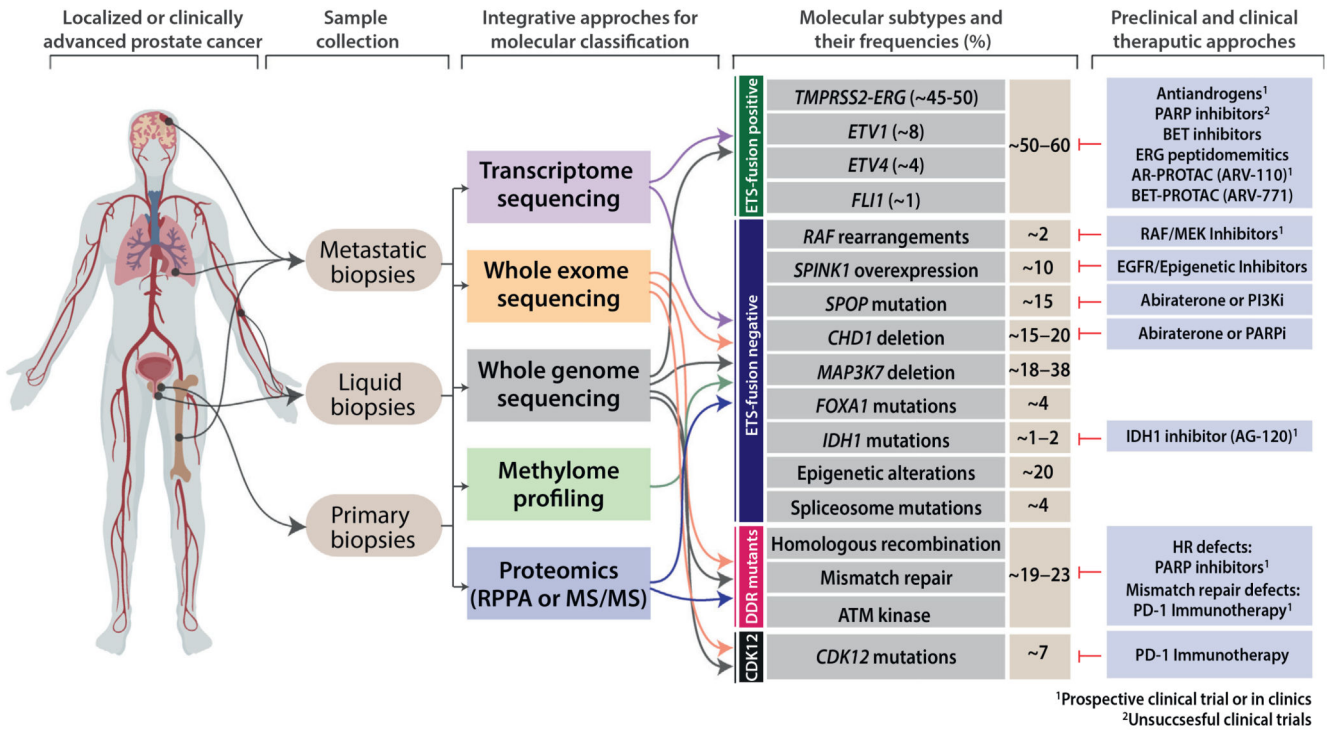


**Figure 2. Integrative Clinicogenomic Analysis of Molecular Aberrations in Prostate Cancer.**

A schematic representation depicting tissue or liquid biopsies taken from prostate cancer patients subjected to integrative sequencing analysis for molecular stratification, followed by implementation of evidence-based therapeutic interventions. Abbreviations: AR, androgen receptor; ATM, ataxia telangiectasia mutated kinase; BET, bromodomain and extra-terminal protein; EGFR, epidermal growth factor receptor; ERG, homolog v-Ets erythroblastosis virus E26-oncogene; HR, homologous recombination; IDH1, isocitrate dehydrogenase 1; PARPi, poly (ADP) ribose polymerase inhibitor; PD-1, programmed cell death protein 1; PI3Ki, phosphoinositide 3-kinase inhibitor; PROTAC, proteolysis targeting chimera; RPPA, reverse phase protein array.




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## Table 1

## Genetic Alterations and Therapeutic Interventions Targeting Different Molecular Subtypes in PCa

| Molecular subtype | Frequencies | Causal genetic alterations/ drivers | Proposed drug therapies | Clinical trials | Refs |
|---|---|---|---|---|---|
| *ETS*-fusion-positive PCa | | | | | |
| *TMPRSS2–ERG* | 45–50% | AR and its coactivator BET proteins regulate expression of these oncogenic fusion proteins *ETS* factor interacts with DNA repair enzyme PARP and DNA-PKs and upregulates DNA repair genes | Antiandrogens PARPis ERG peptidomimetics BET inhibitors PROTACs | PARPi- NCI 9012-NCT01576172[i] BETi-NCT02711956[ii], NCT03150056[iii] AR PROTACs-NCT03888612[ix] | [3,5,16,22–24,28,97,101] |
| *ETV-1* | ~8% | | | | |
| *ETV-5* | ~4% | | | | |
| *FLI-1* | ~1% | | | | |
| *ETS*-fusion-negative PCa | | | | | |
| *SPINK1* overexpression | 10–15% | AR signaling transcriptionally represses *SPINK1*, while SOX2 and HNF1A transcriptionally activate *SPINK1 SPINK1*-positive cases show increased EZH2 expression, which epigenetically silences miR-338-5p/-421 which negatively regulate *SPINK1* | EGFR inhibitors, Epigenetic inhibitors or miR-338-5p/-421 replacement therapies | — | [36,39,40,43] |
| *RAF* rearrangements | 2–6% | Upregulates downstream oncogenic MEK and ERK signaling | Sensitive to RAF kinase inhibitor sorafenib, and MEK inhibitors | — | [7] |
| *SPOP* mutations (*SPOP*$^{mut}$) | 6–15% | Increased expression of BET proteins, confers resistance to BET inhibitors Activates AKT and AR signaling. | Sensitive to abiraterone therapy or PI3K inhibition | — | [5,8,50,52,61] |
| *CHD1* deletions (CHD1$^{del}$) | 15–17% | Show increased AR activity Activates NHEJ repair pathway. | Sensitive to DNA damage agents | — | [6,59,60] |
| *SPOP*$^{mut}$/ *CHD1*$^{del}$ | ~25% | Show a higher response to AR inhibitors due to increase in AR activity | Sensitive to abiraterone therapy | — | [61] |
| *CHD1*$^{del}$/ *MAP3K7*$^{del}$ | 20–25% | Show loss of AR, and increased levels of SYP and CHGA, associates with neuroendocrine and neural features | — | — | [65] |
| *IDH1* mutations | ~1% | IDH1 R132 mutations; increase in oncometabolite D-2-hydroxyglutarate | AG-221 and AG-120 | NCT02074839[iv], NCT02073994[v] | [5,72,73] |
| *FOXA1* mutations | ~4% | Show C-terminal truncating and forkhead domain missense mutations, disrupting its interactions with other cofactors | — | — | [5,8,69] |
| Epigenetic regulators | ~15% | Mutations in *KMT2C* (7%), *KMT2D* (6%), *KDM6A* (3%), *KMT2A* (1.2%) | — | — | [10] |
| Chromatin remodelers | ~5% | Mutations in SWI/SNF nucleosome-remodeling members *ARID1A*, *ARID4A*, *ARID2*, *SMARCA1* | — | — | [10] |
| Splicing pathway | ~4% | Mutation in *SF3B1* (1.1%), *U2AF1* (0.5%), *GEMIN5* (0.5%), *TCERG1* (0.8%) and *PRPF8* (1.2%) | — | — | [10] |

| Molecular subtype | Frequencies | Causal genetic alterations/ drivers | Proposed drug therapies | Clinical trials | Refs |
|---|---|---|---|---|---|
| Ubiquitin–proteasome and ligase family | ~12% | Mutations in *USP28* (1.4%), *USP7* (1.2%), and *CUL3* (1.3%), also *SPOP* (9%) | — | — | [5,8,10] |
| DDR pathway alterations | 19–23% | Somatic and germline mutations in genes involved in, HR pathway (~19%): *BRCA1, BRCA2, RAD51C, FANCD2, ATM.* MMR pathway (8–12%): *MSH2, MSH6, MLH1* | HR defects: PARPi therapy such as olaparib or veliparib MMR defects Immunogenic therapies such as anti-PD-1 and anti-CTLA-4 therapy | HR DDR defects: NCT01682772[vi], NCT01576172[i], NCT02966587[xii] For MMR defects: NCT03061539[xi], NCT02484404[xiii] | [5,11,23,77,83–86,103] |
| *CDK12* mutations | 1.2–7% | Show frequent focal tandem duplications, increased expression of neoantigens, T cell infiltration, and dendritic cell migration to the tumor microenvironment. | Immune checkpoint inhibitors against PD-1 and CTLA-4 | IMPACT-NCT03570619[vii] | [5,87] |

[xii] https://clinicaltrials.gov NCT02966587

[xiii] https://clinicaltrials.gov NCT02484404