Emerging Variants of Castration-Resistant Prostate Cancer

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Abstract

Metastatic castration resistant prostate cancer (CRPC) is associated with substantial clinical, pathologic, and molecular heterogeneity. Most tumors remain driven by androgen receptor (AR) signaling, which has clinical implications for patient selection for AR-directed approaches. However, histologic and clinical resistance phenotypes can emerge after AR-inhibition, in which the tumors become less dependent on the AR. In this review we discuss prostate cancer variants including neuroendocrine (NEPC) and aggressive variant (AVPC) prostate cancers and their clinical implications. Improvements in the understanding of the biologic mechanisms and molecular features underlying prostate cancer variants may help prognostication and facilitate the development of novel therapeutic approaches for subclasses of patient with CRPC.

Keywords

Introduction

Prostate cancer arises as an androgen driven disease [1]. Therefore the mainstay of systemic therapy for patients with advanced disease is androgen deprivation therapy [2]. However despite significant responses, nearly all patients ultimately progress and castration resistance ensues [3]. Castration resistant prostate cancer (CRPC) therefore represents a stage in the continuum of the disease. CRPC is defined by consecutive rises in serum prostate specific antigen (PSA) levels or/and progression of metastatic spread in the setting of castrate levels of testosterone [4, 5]. Despite recent advances in the treatment of patients progressing with metastatic CRPC, average survival remains approximately three years [6]. Identification of more effective and/or precise therapeutic approaches is a clinical unmet need.

Disease progression in CRPC is most commonly driven by reactivation of AR signaling [7]. This has led to the clinical development and approval of potent AR-targeted therapies (ie., the cytochrome P450 17 inhibitor abiraterone acetate (ABI) and the AR antagonist

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enzalutamide (ENZ)) and several others in clinical development [8] for patients with metastatic CRPC. Disease progression after potent AR-therapies is still mediated by activated AR signaling in the majority of cases, and this can occur through secondary alterations involving the AR gene (eg., amplification or activating point mutations, AR splice variants), AR bypass or crosstalk mechanisms (eg,. glucocorticoid receptor, PI3K/AKT pathway activation), or other means (Figure). It has been increasingly recognized that a subset of CRPC tumors evade therapy through another mechanism in which the tumors become less dependent on AR signaling (ie., "androgen indifferent"), and this is associated with lineage plasticity, loss of luminal markers, and acquisition of neuroendocrine features (Figure).

Other approved therapeutic options for metastatic CRPC with demonstrated survival benefit in randomized Phase 3 trials include taxane chemotherapies (docetaxel and cabazitaxel), autologous cell-based immunotherapy with sipuleucel-T, and the radioisotope ^{223}radium [9]. Although these agents have significantly improved outcomes and quality of life for patients, there can be wide clinical variability in individual response between patients. Therefore there is a growing need for biomarkers to help improve patient selection and inform sequencing of systemic therapies [9]. Further, emerging therapeutics such as PARP inhibitors for CRPC have been shown to benefit only subsets of patients in early phase trials (eg., DNA repair alterations in the case of PARP [10]), highlighting the need for predictive biomarkers.

One challenge in the development of molecular biomarkers for advanced prostate cancer is the availability of tumor tissue [11]. Metastatic biopsies have been challenged by a predominance of sclerotic bone lesions, which are technically challenging and harbor limited viable tissues for extensive analyses. Nonetheless, in recent years through collaborative efforts and clinical trials, biopsies in metastatic prostate cancer have become feasible [12]. Tumor biopsy programs including those from the Stand Up To Cancer (SU2C)- Prostate Cancer Foundation (PCF) Dream Team clinical trials [13, 14] have revealed 1) genomic diversity between patients with CRPC; 2) acquisition and/or enrichment of molecular alterations in CRPC compared to primary prostate cancer; 3) emergence of pathologic variants including atypical and neuroendocrine phenotypes. These studies have provided insights into mechanisms of treatment resistance, intra- and inter-patient heterogeneity, and clonal evolution [15]. This review focuses on recent advances in our understanding of emerging clinical and pathologic variants in CRPC. Based on a growing number of biopsies being performed clinically, refinement of pathologic criteria for histological subtyping [16], and an expanded use of high-throughput technologies [11], the clinical, pathologic and molecular features CRPC variants have become better characterized. Their full characterization is a subject of ongoing investigation.

Neuroendocrine prostate cancer

The diagnosis of neuroendocrine prostate cancer (NEPC) is histologically defined, encompasses morphologies that overlap with neuroendocrine tumors arising from other primary sites (eg., lung, bladder), and may occur with mixed or overlapping features with prostate adenocarcinoma. The recent refinement of diagnostic terminology for NEPC came

after accumulation of new clinical data and a revised pathologic consensus for defining NEPC subtypes [16]. These subtypes include: a) usual prostate adenocarcinoma with neuroendocrine differentiation; b) adenocarcinoma with Paneth cell neuroendocrine differentiation; c) carcinoid tumor; d) small cell carcinoma; e) large cell neuroendocrine carcinoma; and f) mixed neuroendocrine carcinoma - acinar adenocarcinoma [7]. Immunohistochemical detection of neuroendocrine markers such as synaptophysin, chromogranin, and CD56 may help support the diagnosis of NEPC but are not required in the presence of morphologic characteristics. Pure small-cell neuroendocrine prostate carcinoma is a subtype of NEPC that has the most aggressive biologic behavior and poor outcomes. The clinical significance of other NEPC subtypes especially mixed or hybrid phenotypes is not well defined.

Although NEPC rarely arises *de novo* and accounts for less than 2% of patients at the time of diagnosis of prostate cancer [17], NEPC is enriched in the advanced disease setting and reported as high as 10-20% in CRPC [14, 18]. Clinically, NEPC is often suspected in patients that progress in the setting of low or moderately rising PSA levels (suggesting less AR driven disease) with a predominance of visceral and/or lytic bone metastases. Morphologically, NEPC shares features with other high grade neuroendocrine cancers, including presence of small cells with 'salt and pepper' chromatin, high mitotic count and nuclear molding [19]. In the majority of cases at least one neuroendocrine immunohistochemical (IHC) marker, such as neuron-specific enolase, synaptophysin, chromogranin, or CD56 stains positive. NEPC is often negative for classical luminal markers of prostatic glandular differentiation (eg., prostate-specific antigen (PSA) and prostatic acid phosphatase). Uncommonly, polypeptide hormones may be present (including ACTH, antidiuretic hormone (ADH), and corticotropin-releasing factor).

In ambiguous cases or in cases of small cell carcinoma of unknown primary, ERG break-apart fluorescence in situ hybridization (FISH) may be used clinically to evaluate for the presence of the prostate cancer-specific ERG gene rearrangement. ERG rearrangement is present in approximately 50% of NEPC and is diagnostic of prostate cancer [20, 21], though a negative test does not rule out prostate origin. Patients with NEPC are often treated like other high-grade neuroendocrine tumors with platinum-based chemotherapy [22], though this treatment decision may depend on the clinical context especially in cases with mixed features. Although there are few published clinical series reporting clinical outcomes of patients with NEPC, median survival of small cell NEPC has been reported at 7 months [23] with patients with mixed adenocarcinoma-NEPC tumors having a more variable prognosis [24].

Early insights into the molecular background of NEPC came with the use of next generation RNA-sequencing comparing 7 NEPC tumors, 30 PC adenocarcinomas (PCA) and 5 benign prostate tissue samples [25]. The transcriptome of NEPC was distinct from PCA, with 936 genes differentially expressed. One notable finding was overexpression and amplification of the oncogenic transcription factor N-myc (MYCN). In multiple preclinical models N-myc has been found to be a key driver of the NEPC phenotype [25-27]. Overexpression of N-myc in the context of either PTEN loss or AKT overexpression results in the development of aggressive features, including histologic features of NEPC, low AR and low AR signaling,

up-regulation of NEPC markers, and resistance to AR therapies [26, 27]. Similar to what has been observed in N-myc-amplified neuroblastoma [28, 29], N-myc may be inhibited indirectly by targeting its allosteric protein partner Aurora kinase A (AURKA) using several of the available aurora kinase inhibitors (eg., alisertib, CD532) [28-30]. AURKA is a mitotic kinase and oncogene [31], also significantly overexpressed in NEPC [25]. A mechanistic link has also been demonstrated between mutated *TP53* and elevated levels of AURKA, through increased expression of miR25 and resultant inhibition of E3 ubiquitin ligase FBXW7-mediated AURKA degradation [32].

In an extended cohort evaluating metastatic biopsies from 81 patients with CRPC, including 51 with clinical and pathologic features of AR-driven adenocarcinoma and 30 with features of AR-indifferent NEPC [33], the two ends of the spectrum were compared in order to elucidate the molecular features that distinguish NEPC. Unexpectedly, despite distinct pathologic and clinical differences, whole exome sequencing (WES) revealed significant overlap in the mutational and copy number landscape between castration resistant adenocarcinoma and NEPC tumors, supporting clonal evolution of NEPC from an adenocarcinoma precursor. There was significant enrichment in *RB1* loss and *TP53* mutations and deletions in NEPC, alterations commonly observed in other high-grade neuroendocrine tumors [34]. In another study reported by Tan et al, *RB1* alterations were found in 90% of small cell NEPC (26 of 29 patients) [35]. In prostate cancer preclinical models, the combination of RB1 and TP53 drives the development of NEPC [36-39], suggesting that *RB1* and *TP53* contribute to the pathogenesis of NEPC. Extending upon these findings, Mu et al (38) and Ku et al (39) recently described in two elegant studies how loss of p53 and Rb1 can drive resistance to AR-antagonists in prostate cancer by promoting lineage plasticity and progression towards an androgen indifferent state – a state in which AR protein is still present, but tumors are less dependent on androgen signaling - mediated in part by SOX2, a lineage transcription factor also capable of reprogramming fibroblasts to pluripotent stem cells.

As opposed to their largely similar genomic profiles, the epigenomic landscape of NEPC is distinct compared to castration resistant adenocarcinoma [33]. Combined analysis of genome-wide CpG methylation with transcript data revealed epigenetically dysregulated pathways including those involved in epithelial mesenchymal transition, neuronal, stem cell like, and developmental pathways, suggesting a key role of the epigenome in modulating the NEPC phenotype. The histone methyltransferase EZH2 is also up-regulated in NEPC and EZH2-supressed target genes down-regulated. Treatment with EZH2 inhibitors demonstrated antitumor activity in NEPC models and modulated NEPC and AR signaling pathways (33, 39), pointing to a cellular plasticity that may be exploited therapeutically. A mechanistic link between N-myc and EZH2 has also been elucidated in that they cooperate to drive histone methylation, suppress AR signaling, and drive a NEPC phenotype [27].

Development of preclinical models is another important tool for *in vitro and in vivo* replication of disease subtypes, identification of biomarkers, and evaluation of novel treatments. In addition to the N-myc/PTEN [27], N-myc/AKT [26], and the TP53/RB1 model [36-39] systems that drive NEPC, enzalutamide resistant models [40, 41], patient-derived xenograft (PDX) models [42, 43] and patient-derived organoids [44, 45] may be

used to study drug resistance and the development of NEPC [42, 43]. Preclinical observations using these model systems combined with evaluation of additional patient samples have elucidated additional NEPC associated biologic pathways. The RE1-silencing transcription factor (REST), a neuronal signaling transcriptional repressor, is significantly downregulated in NEPC, mediated by the splicing regulator SRRM4. Overexpression of SRRM4 occurring in 50% of NEPC as opposed to only 3% of PCA cases (N=218) [43, 46, 47] and is a central regulator of alternative splicing events [48]. SRRM4 was found to be a potent inducer of neuroendocrine differentiation under conditions of AR pathway inhibition, accentuated by RB1 and p53 loss, acting upstream of several neuronal signaling and chromatin remodeling genes, including REST [48]. De-repression of the placental gene PEG10 also occurs during the NEPC transition, and is regulated by AR and the E2F/RB pathway. PEG10 promotes cell-cycle progression in the context of TP53 loss and regulates Snail expression via TGF-β signaling to promote invasion [49]. Loss of the androgen regulated ERG overexpression in the context of TMPRSS2-ERG fusion- positive NEPC has also been implicated in promoting de-differentiation, proliferation, resistance to AR therapies, and the NEPC phenotype [50]. In addition to AURKA, up-regulation of UBE2C and other mitotic genes as well as cyclin D1 (CCND1) overexpression also occurs in NEPC [51, 52]. Recently the neural transcription factor BRN2 was identified as a key driver of the NEPC phenotype, significantly overexpressed in NEPC, and inversely correlated with AR signaling in CRPC. Mechanistic studies have revealed that BRN2 is directly repressed by the AR and this can be lost under selective pressure of enzalutamide, thereby promoting the development of NEPC both in vitro and in vivo [40].

Aggressive variant or anaplastic prostate cancer

As metastatic biopsies are invasive and may be challenging to perform, clinical features suggestive of small cell/NEPC may be useful to select patients for platinum-based chemotherapy and potentially other NEPC-directed approaches. The aggressive variant prostate cancer (AVPC), formally termed as anaplastic prostate cancer, is clinically defined as CRPC with at least 1 of the following: 1) histologic evidence of small-cell NEPC (pure or mixed), 2) the presence of exclusively visceral metastases, 3) radiographically predominant lytic bone metastases by plain x-ray or CT scan, 4) bulky (5 cm) lymphadenopathy or bulky (5 cm) high-grade (Gleason 8) tumor mass in prostate/pelvis. 5) low PSA (10 ng/mL) at initial presentation (prior to ADT or at symptomatic progression in the castrate setting) plus high volume (20) bone metastases, 6) presence of neuroendocrine markers on histology (positive staining of chromogranin A or synaptophysin) or in serum (abnormal high serum levels for chromogranin A or gastrin-releasing peptide (GRP)) at initial diagnosis or at progression, plus any of the following in the absence of other causes: (a) elevated serum LDH ($2 \times$ ULN), (b) malignant hypercalcemia, (c) elevated serum CEA ($2 \times$ ULN), 7) short interval (6 months) to androgen-independent progression following the initiation of hormonal therapy with or without the presence of neuroendocrine markers [53]. These features have been used as inclusion criteria for patient enrollment in clinical trials incorporating platinum chemotherapy. In a prospective Phase 2 clinical trial of carboplatin and docetaxel (CD) with cisplatin-etoposide (EP) upon progression, 120 patients with at least one out of the seven clinical features were enrolled [53]. The primary objectives were

to estimate the response rate and time to progression while secondary objectives included the collection of tissues for investigating the underlying biology of this subset, the toxicity of the regimens and overall survival [53]. The high response rates (65.4% and 33.8% after four cycles of CD and EP, respectively) and the significant associations of certain clinical criteria with outcomes (ie., bulky disease, serum CEA) led to the exploration of a molecular signature within this cohort [54]. Targeted genomic analysis in 36 trial participants and 8 patient tumor xenografts (PDX) revealed 8q amplification (MYC), and RB1 and PTEN copy number losses (CNL) as the most frequent events in approximately 50% of samples [54]. More recently the combination cabazitaxel/carboplatin (CAB/CARB) in clinically defined AVPC patients demonstrated a significant 3-month PFS benefit compared to cabazitaxel (CAB) alone (3.8 months with CAB vs. 5.6 months with CAB/CARB) and a trend towards survival improvement (17.4 months with CAB vs. 19.2 with CAB/CARB). Approximately half (42/79) of CAB patients and a quarter (19/81) of CAB/CARB patients received additional salvage platinum-based therapy after progression [55]. Combined alterations in RB1, TP53, and/or PTEN were more frequent in the AVPC cohort as compared to an unselected CRPC cohort [13] and The Cancer Genome Atlas (TCGA) datasets [54]. Overall these data suggest that a subset of CRPC defined by aggressive clinical features may be more amenable to platinum, potentially based on the molecular overlap with NEPC. These studies as well as the high frequency of germline and somatic DNA repair alterations in CRPC [55, 56], warrant further exploration of platinum chemotherapy for a biomarker - selected or clinically enriched subset of patients with CRPC.

Intermediate Atypical prostate cancer

The West Coast Stand Up To Cancer (SU2C)- Prostate Cancer Foundation (PCF) Dream Team recently presented data from a prospective metastatic biopsy trial of patients with CRPC with primary and acquired resistance to abiraterone and/or enzalutamide. One unexpected finding after evaluation of the first 226 cases was a high frequency of histologic variants including small cell NEPC, mixed NEPC/adenocarcinoma, intermediate atypical prostate cancer (IAC) variants [57]. The molecular classification and prognostic role of these variants are yet to be fully reported, but there is some suggestion that there may be a transition state in CRPC in which patients develop mixed or overlapping pathologic and molecular features with NEPC.

In a related study, a 91 basal stem cell gene signature, termed CD49f Hi, was generated after purification and transcriptional profiling of basal and luminal epithelial cells from primary prostate tumors and adjacent benign tissue [58]. The signature, comprising E2F target genes, was particularly enriched in small-cell NEPC when applied to the TCGA, Weill Cornell and West Coast SU2C-PCF datasets, as compared to adenocarcinoma and IAC, implying the presence of a conserved transcriptional program between normal prostate basal stem cells and small-cell NEPC [58]. Overall, it is expected that as molecular data accumulate, clinical and pathologic recommendations regarding classification of tumors may be refined to address variability in reporting and areas of uncertainty regarding morphologies with focal neuroendocrine differentiation of primary prostate cancer, treatment-induced changes, pure small cell or large cell carcinoma of the prostate, and other variant or hybrid phenotypes [16, 59].

Other rare histological variants

Another histologic variant with aggressive clinical behavior is ductal adenocarcinoma. Its histologic appearance is similar to endometrial carcinoma [60]. Clinical characteristics involve presentation with a high Gleason score, local symptoms including intractable urinary symptoms, often atypical visceral metastases, and poor response to hormonal and radiotherapy [60, 61]. TMPRSS2:ERG fusion is reported at similar frequency (50%) as typical prostate acinar adenocarcinomas [62] supporting a same cell of origin. Notably there is an enrichment in mismatch repair alterations and a hyper-mutated phenotype in ductal adenocarcinoma [63], which has both biologic and potential treatment implications and warrants further investigation. Other rare histologic variants in prostate cancer include adenosquamous, mucinous, prostate leiyomyosarcomas, cystosarcoma phyllodes, carcinosarcoma, radiation associated sarcoma, or primary lymphoma of the prostate [64]. Clinical (survival), histological and molecular characteristics of the most common CRPC variants are summarized in Table 1.

Other clinical phenotypes of aggressiveness

There are other clinical characteristics with prognostic value in CRPC, such as circulating tumor cell count and sites of metastases. For instance, lymph node disease has been associated with longer median survival compared to bone and visceral (particularly liver) metastases (31.6 months vs. 21.3 months vs. 13.5 months, respectively) [65]. Other clinical characteristics that have independently been associated with worse outcomes are anemia, pain not responding to treatment, less than 30 or 50% PSA decline with therapy, and elevated lactate dehydrogenase (LDH) [66]. Recently, an updated prognostication for CRPC patients has identified three risk groups, low, intermediate and high, on the basis of 8 prognostic factors, including Eastern Cooperative Oncology Group performance status, disease site, LDH, opioid analgesic use, albumin, hemoglobin, prostate-specific antigen, and alkaline phosphatase [67]. The molecular features underlying these risk groups and their respective drivers of tumor aggressiveness require further characterization.

Molecular tools for patient selection

The clinical utility of molecular biomarkers to identify emerging variants and improve patient selection for therapy is an area of active investigation. The use of molecular criteria to identify NEPC and other clinical or pathologic variants may help overcome some of the current issues with pathologic heterogeneity and pave the way for non-invasive testing. As with any biomarker, application and validation of tissue-based and potentially liquid biomarkers will require prospective testing and extensive clinical validation. Circulating tumor cells (CTCs) have shown promise beyond enumeration in metastatic CRPC [68, 69] and have demonstrated feasibility for molecular investigation across various platforms. For instance, the presence of nuclear AR expression and active AR signaling ("AR-mixed" CTCs and "AR-on" cells) despite treatment with ARPI therapies (ABI, ENZ) has demonstrated prognostic value [70, 71]. At the gene level, differential expression of 282 genes between samples with ≥5 CTCs versus <5 CTCs was demonstrated, with significant prognostic ability of a two-gene model [72]. Concordance of PTEN status between CTCs

and fresh tumor tissue is promising of the ability of CTC profiling to reflect tumor mutational status [73]. In addition, AR-V7 splice variant detection in CTCs may predict better response to taxanes compared to ARPIs [74, 75]. A NEPC classifier composed of protein and morphological characteristics including low or absent AR expression, lower cytokeratin expression, and smaller morphology, has also been proposed [76]. Circulating tumor DNA (ctDNA), including evaluation of gene copy number and mutation status is also feasible and has shown utility in clinical cohorts for predicting response to either ABT or ENZ [77-80]. Investigation of ctDNA as a platform to evaluate NEPC genomic changes [33] for non-invasive detection is also an area of ongoing research. Urine is another source of tumor cell DNA and was also recently exploited for whole genome sequencing at different stages, before or after ADT in hormone sensitive disease as well as post-docetaxel chemotherapy in CRPC patients [81]. Urine [82] or blood exosome, oncosomes [83], or microvesicles (extracellular vesicles) have also been introduced as a non-invasive platform for studying gene expression changes reflective of primary or metastatic prostate tissue. How these alternative technologies may apply to the detection of CRPC variants and/or other resistance pathways and the best timing and cohorts to evaluate warrant further investigation.

Novel therapeutic targets for CRPC variants

During the time of initial molecular data accumulation on the implication of AURKA and MYCN in the pathogenesis of NEPC, a phase II trial evaluated the Aurora kinase inhibitor danusertib in 88 metastatic CRPC patients but failed to achieve its primary end point of PSA response and showed minimal objective tumor responses with stable disease rates of 18.6% and 34.2% respectively, in the two different dose arms of the study [84]. However, the unselected population of the study and the non-surprising fact of poor performance of PSA as a biomarker in the androgen-independent NEPC subtype of the disease encouraged the initiation of an ongoing phase II trial of alisertib, a selective AURKA inhibitor in an enriched population of NEPC (NCT01799278). Given the physical interaction between AURKA and MYCN and their consistent and concurrent up-regulation in NEPC [25-27], it is anticipated that novel strategies to target MYCN or the AURKA/MYCN complex may also be effective. Pharmacologic inhibition of MYC family transcription factors is challenging, however a direct inhibition strategy with DCR-MYC, which is a Dicer-substrate small, interfering double-stranded RNA (si-RNA) targeted to the MYC oncogene is currently being tested (NCT02110563). Other approaches of targeting MYC family genes have focused on inhibiting bromodomains of putative co-activator proteins implicated in its transcriptional initiation and elongation (BET) [85]. Inhibition of the N-terminal bromodomain of bromodomain-containing protein 4 (BRD4) has also implications for AR signaling given BRD4 interaction with the N-terminal domain of AR [86]. Approaches for targeting of other emerging key drivers of NEPC such as BRN2 [40] warrant further investigation.

The epigenomic landscape is a critical area of change in NEPC, particularly, but not exclusively, pertaining to polycomb group (PcG) protein-mediated epigenetic silencing, leading to a de-differentiated phenotype [27, 33, 87]. Two such PcG family members, CBX2 and EZH2 were the most overexpressed in comparative gene expression analysis in an *in*

vivo model of ADT-induced NEPC with clinical NEPC cohort validation, and found to be associated with a NE-associated repression signature (NEARS) of 185 genes, conferring a more aggressive clinical phenotype and progression [88]. Selective inhibition of EZH2 in *in vitro* and *in vivo* models also resulted in modulation of AR signaling and/or down-regulation of NEPC-associated genes [26, 33, 39, 49]. DNA methylation profiles are also distinct between castration resistant adenocarcinoma and NEPC, associated with differentially methylated promoters and downstream transcriptional changes [33, 89]. As a result, epigenetic strategies with use of EZH2 inhibitors (EPZ-6438, GSK2816126) and DNA methyltransferase (DNMT) inhibitors (azacitidine) are currently under phase I/II testing.

As patients with NEPC and AVPC are often treated with similar regimens as small cell lung cancer (ie. platinum), emerging targets for small cell lung cancer (SCLC) may have relevance for CRPC variants. The antibody drug target SC16LD6.5 (rovalpituzumab) targets the notch ligand delta-like 3 (DLL3), a cell surface protein aberrantly expressed in SCLC [90]. Recent early phase studies have demonstrated antitumor activity of rovalpituzumab in patients with recurrent SCLC [91]. A Phase 1 trial investigating rovalpituzumab in other neuroendocrine tumors, including a dedicated NEPC arm, is now underway (NCT02709889). Based on the emerging role of anti-PD1 therapy in SCLC [92, 93], high frequency of mismatch repair defects in ductal adenocarcinoma [63], and new data suggesting a subset of enzalutamide resistant CRPC may benefit from the addition of checkpoint inhibition [94], further investigation of immunotherapy for CRPC variants is also warranted.

Conclusions

The identification and characterization of clinical and pathologic variants of CRPC have provided new insights into the spectrum of CRPC, have facilitated preclinical studies exploring the biology of AR-indifferent CRPC, and have led to the development of new biomarker-enriched clinical trials [95]. A collective effort and use of common language in terms of standardization of methods applied and for the clinical or/and molecular categorization of metastatic CRPC patients is required and will help facilitate the design of future prospective studies. These efforts may ultimately help define the most appropriate choice and sequencing of treatments in order to maximize patient responses and improve clinical outcomes.

Acknowledgments

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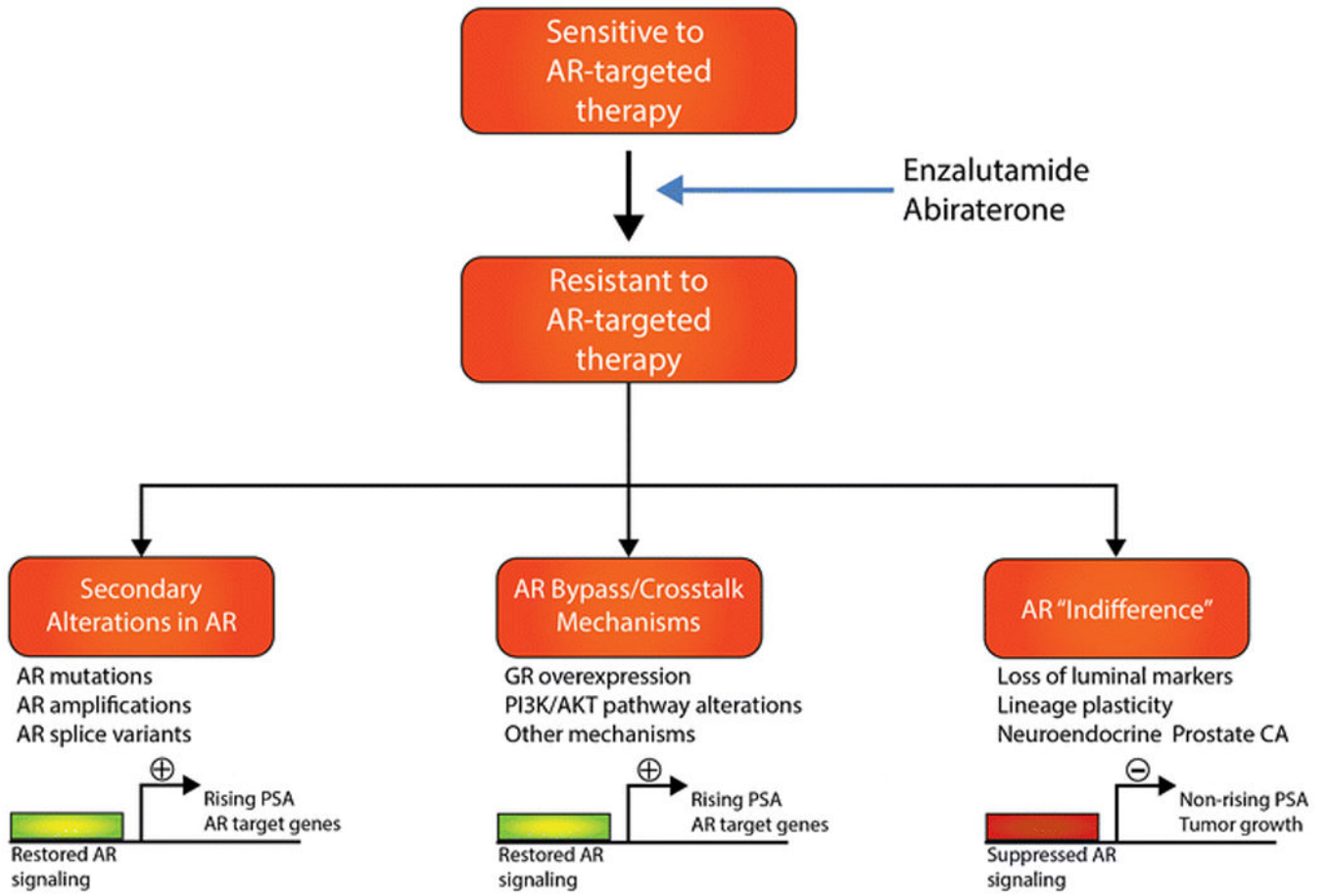
Figure 1. Schematic illustrating the three main classes of treatment resistance to AR targeted therapies in prostate cancer

Table 1

Clinical, histological and molecular characteristics of most common CRPC variants

CRPC variant	Survival	Histology	Molecular aberrations	Ref
Neuroendocrine (NEPC)	1-2 years	chromogranin (+/-),synaptophysin (+/-), AR low/absent	low AR signaling, RB1 loss, TP53 loss, REST, high MYCN, AURKA, EZH2, PEG10, SRRM4, DLL3, epigenetic	[19, 23-27, 33, 49, 50, 52, 60]
Small cell carcinoma	7 months	Small blue/oat cells		
"AR indifferent" castration resistant adenocarcinoma	To be defined	Adenocarcinoma, AR (+),	AR signaling low, RB1 loss, TP53 loss, high EZH2	[33, 38, 39]
Intermediate atypical	To be defined	To be defined	Mixed/overlapping molecular features with adenocarcinoma and NEPC	[14, 57]
Aggressive variant	9-17 months	Heterogeneous (Adenocarcinoma or NEPC)	RB1, TP53, PTEN loss	[51, 53-55]
Ductal	84 months	High Gleason, endometrioid	MMR gene alterations/hypermutation	[60, 61, 63]