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A comprehensive review of genomic landscape, biomarkers and treatment sequencing in castration-resistant prostate cancer

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A comprehensive review of genomic landscape, biomarkers and treatment sequencing in castration-resistant prostate cancer**Abstract**

Hormone-naïve prostate cancer and its castration-resistant state (CRPC) are clinically and genetically heterogeneous diseases. From initiation of prostate carcinogenesis to its evolution towards therapeutic resistance, various combinations of genetic and epigenetic events occur. Schematically, progression to CRPC could be divided in two distinct pathways, either dependent or independent of the androgen receptor activity. Nevertheless, because the better knowledge of the genetic landscape of CRPC is under way, limited clinical applications are available at the moment, underlying the usefulness of prognostic and predictive biomarkers in daily practice. Despite the promising prognostic value of circulating tumor cells, no biomarker has been currently validated as a surrogate for overall survival in CRPC patients. Inversely, considerable interest has been generated with the recent finding of the splice variant AR-V7 that allows to predict resistance to abiraterone acetate and enzalutamide. However, other predictive biomarkers would be necessary to accurately guide personalized sequencing of CRPC treatment, which now includes numerous possibilities based on the six validated drugs, without accounting for those currently under investigation in the ongoing randomized controlled trials. As a consequence, only rational sequencing, which consists in choosing an agent that is not expected to have cross-resistance with previous therapy, can be currently advised.

Introduction

Hormone-naïve prostate cancer (HNPC) and its castration-resistant state (CRPC) are clinically and genetically heterogeneous diseases [1]. It is now well-established that some patients diagnosed with metastatic HNPC will rapidly progress to the lethal CRPC phenotype while others will show long-term and durable response to androgen deprivation therapy. Preliminary reports suggest that such a transition from HNPC to CRPC might be driven by a wide range of genetic events leading to distinct progression pathways either dependent or independent of the androgen receptor (AR) activity [1–4].

Because of the greater understanding of CRPC biology, docetaxel (DCT) based treatment landscape has dramatically changed over last years. New systemic therapies, including the androgen biosynthesis inhibitor abiraterone acetate (AA) [5,6] and the next-generation androgen receptor antagonist enzalutamide (ENZ) [7,8] have demonstrated to prolong overall survival (OS) in both post- and, more recently, pre-DCT settings (Figure 1). Along with AA and ENZ, the bone targeted agent alpha emitter Radium 223 [9] and the immunotherapeutic sipuleucel-T[10] are also effective treatment options for the management of chemotherapy-naïve patients while the taxoid cabazitaxel (CZT) [11] remains the standard of care for those pretreated with DCT. Sequencing all these innovative agents according to individual characteristics is now the challenge that physicians face in daily practice. Interestingly, there is intensive research to develop prognostic and predictive biomarkers for guiding clinical decision making and achieve optimal medical management of CRPC [12,13]. Therefore, our purpose was to describe the current knowledge of CRPC genomic landscape and to summarize the established and potential biomarkers as well as available evidence for treatment sequencing of patients diagnosed with metastatic disease.

From HNPC to CRPC

Genetic landscape of HNPC

The earliest molecular events commonly reported in human prostate carcinogenesis are the loss of 8p21 region including NKX3 locus, the CpG island promoter methylation of GSTP1 gene, and particularly, androgen driven gene fusions (ETS positive) such as TMPRSS2-ERG fusion gene [14]. Through this ETS positive pathway, deletions at 10q24 region including PTEN locus and gains at 8q24 region including cMyc locus have been shown to foster further disease progression [14].

Alternatively, mutations of the SPOP gene have been recently identified as a key genomic event involved in the natural history of HNPC without any androgen driven gene fusions [14]. Indeed, these mutations result in the inability of the tumor cells to bind and promote the degradation of SRC-3, leading to increased androgen signaling. Through this ETS negative pathway, further disease progression might be induced by the deletion or the silencing of CDH1 gene [14]. More evidence to support distinct ETS positive and negative pathways has been recently reported by Grasso et al, who demonstrated that deregulations of ETS2 were specifically involved in growth of tumors with TMPRSS2-ERG fusion gene whereas disruptions of CDH1 might define a subtype of fusion gene free tumors (Figure 2) [2].

Chromoplexy and chromotripsis models

From initiation of HNPC to its progression towards therapeutic resistance and death, various combinations of genetic and epigenetic events occur (Figures 3A and 3B). Next generation sequencing of prostate cancer prior to and following androgen deprivation therapy has dramatically helped to identify important androgen-regulated pathways or genes that may be reactivated in CRPC [15]. Specifically, chromosomal rearrangements, amplifications, deletions, or point mutations and DNA methylation alterations allow for emerging aggressive

clones [16]. These mechanisms involved in progression to the lethal stages are usually described according to the chromoplexy model, which drives the punctuated progression of HNPC and induces the formation of disrupted cancer genes such as ERG fusion with Tmprss2, NKX3-1, TP53, RB1, CDKN13 and PTEN [17]. Alternatively, the chromotripsis model, based on high genetic instability with numerous chromosomal rearrangements and hypermutational status, has been proposed to explain the catastrophe scenario related to terrible disease evolution [18]. The chromoplexy and chromotripsis phenomena are summarized in Supplemental Table 1 and Figure 1.

Genetic aspects of pathways involved in the progression to CRPC

At the bio-pathological level, progression to CRPC could be divided in two schematic pathways [19]. The more frequent one remains dependent of the AR activity, which binds and drives genes characterized by AR genomic signature with androgens response elements sequences such as PSA or Tmprss2. The second one is independent of AR activity, and is characterized by loss of differentiation towards an epithelial/mesenchymal transition (EMT) or towards hybrids tumors with trans-differentiation harboring neuroendocrine features according to the ontogenic origin of prostatic gland (Supplemental Figures 2A and 2B) [20].

Androgen receptor activity dependent pathway

Historically, the residual concentration of androgens after hormonal castration was seen as an event without any consequence, and resistance was considered to be related to androgen-independent mechanisms. However, the recent discovery of the efficacy of next-generation drugs targeting androgen-dependent AR signaling through inhibition of either AR ligand-binding (ENZ) or androgen synthesis (AA) supports the view of the activity of the AR pathway during progression to the CRPC phenotype. Schematically, two non-exclusive mechanisms

have been described to explain the maintenance of such AR activity with low circulating testosterone levels after castration.

The first one is logically associated to local synthesis of ligand to AR. A number of genes involved in androgen metabolism such as AKR1C3 and SRD5A1 are deregulated and overexpressed in CRPC compared to HNPC, leading to greater in situ concentrations of testosterone [21]. This mechanism is supported by the recent randomized clinical trials comparing AA versus placebo, which have demonstrated that CRPC is still hormone sensitive when using this androgen biosynthesis inhibitor additionally targeting both intra tumoral and extra gonadal biosynthesis of androgens by inhibiting the critical enzyme CYP17A1 [5,6].

The second mechanism involved in the progression to CRPC through the AR pathway is a ligand independent constitutive activation of the AR itself. Beyond the AR gene activating mutations, deregulation of co-activators and repressors or alternative splicing of AR, and other recurrent mutations in multiple histone modifying genes including MLL2 gene have been recently identified to explain over expression of the AR-mediated signaling [2]. Similarly to AA clinical trials, randomized placebo controlled data obtained for ENZ provides clinical support to the implication of such altered pathway [7,8]. In addition to its competitive antagonist effect, ENZ has been demonstrated to display ligand independent anti-tumor activity by inhibiting nuclear translocation of AR and impairing both DNA binding to androgen response elements and recruitment of co-activators [22].

Androgen receptor activity independent pathway

The cell plasticity with dedifferentiation leading to proliferation, apoptosis resistance and EMT is alternatively involved in the progression to CRPC through the AR activity independent pathway, even though some clones might still be dependent on AR signaling [23]. Activation of the EMT program is characterized by the loss of E-cadherin and the gain

of mesenchymal markers such as Vimentin and N-cadherin, which increases the migratory ability of the cells and contribute notably to the interaction of tumors cells with the microenvironment at the bone [24–26]. Such a phenomenon leads to the activation of a set of transcription factors including Twist and Snail, and is orchestrated by epigenetic remodeling involving the Polycomb complex [27].

All these molecular events are driven by key regulators including Myc and EZH2, which are deregulated either directly by genetical events or indirectly by epigenetical upstream events such as ERG overexpression or specific microRNA activation [28,29]. Notably, new insights in such a signalling pathway include regulation of Myc and EZH2 expressions by miR101/miR449 and miR101/miR26, respectively [28,29]. Other microRNAs have been related to CRPC biology, including miR125b/miR146/miR205 for androgen independent growth, miR21/miR221/miR15a for migration or invasion ability and miR20a/miR32/miR23 for apoptosis resistance [30]. Although the better knowledge of the genomic events leading to CRPC progression is under way, limited clinical applications are available at the moment, underlying the usefulness of prognostic and predictive biomarkers in daily practice.

Prognostic biomarkers in CRPC

Prognostic biomarkers in CRPC allow to predict outcomes preceding or following treatment without necessarily providing evidence regarding potential sensitivity to a given agent [31,32]. A synthesized list of pre- and post-treatment prognostic biomarkers of interest in the management of CRPC is provided in Table 1. Many of these clinical or biological characteristics have been used as eligibility criteria for inclusion in randomized clinical trials designed to assess effectiveness of drugs under development in the field of CRPC.

Specifically, the finding of five or more circulating tumor cells (CTCs) prior to the initiation of cytotoxic chemotherapy is associated with inferior OS while the decline of CTCs count below five has been demonstrated to result in increased OS [31,32]. Goldkorn et al have also confirmed the prognostic value of CTCs at baseline and before cycle two in a large DCT-based prospective cohort [33]. Of note is that the conversion from unfavorable to favorable CTCs count in DCT-resistant patients treated with AA or ENZ might prolong life expectancy [34,35]. Furthermore, a biomarker panel containing CTCs number has been shown to meet Prentice criteria for surrogacy of OS at the individual-patient level [36]. In addition, CTC visualization may enable a direct measurement of the underlying tumor biology. However, one drawback of the CTCs detection using the current CellSearch[®] technology is the lack of sensibility within CRPC setting, limiting its clinical interest [32]. As such, other liquid biopsy techniques including circulating cell-free tumor DNA may help to identify genomic alterations and track patient's genomic landscape over time to assess individual prognosis and adapt further treatment strategies. [37,38]

The vast majority of other clinical and biological prognostic biomarkers of CRPC have been validated and included in nomograms, which help physicians to estimate survivals using tumor's and patient's characteristics. Originally, PSA based testing results, performance status (PS) score, and hemoglobin level have been combined with age, albumin, LDH or ALP

levels, Gleason score, pain intensity and metastases characteristics in different prognostic models [31,39,40]. Nevertheless, emergence of new treatment options in CRPC requests constant re appraisal of the clinical interest to use such biomarkers. Based on the prospective data obtained from ALLIANCE and ENTHUSE trials, Halabi et al. have recently developed and validated an updated nomogram for predicting survival in men with metastatic CRPC receiving first line chemotherapy [41]. This model was designed to incorporate well-established biomarkers such as PS score and PSA, albumin, LDH, ALP or hemoglobin levels but also new variables of interest such as metastatic site and opioid analgesic use [42]. Accordingly, the prognostic value of metastatic site has been confirmed by Pond et al, based on the data of the TAX 327 trial [43].

More recently, expression of molecular biomarkers in whole-blood has been found to correlate with CRPC outcomes [44,45]. Ross et al. have assessed a panel of 168 inflammation- and prostate-related genes using PAXgene/TaqMan systems and RT-PCR to detect RNA transcripts and prostate cancer-enhanced RNA messenger, respectively [44]. A 6-gene model separating patients into 2 risk groups according to their signature has been developed to help physician with prognostication of CRPC. Interestingly, this nomogram outperformed those exclusively based on clinicopathologic factors by showing greater accuracy using ROC curve analysis. Danila et al. have selected a different panel of prostate-related genes including KLK3, KLK2, HOXB13, GRL2 and FOXA1 detected by multiplex RT-PCR [45]. After combining the results of gene profile with CTCs counts, risk group were defined to pinpoint patients with poor prognosis. This RT-PCR assay detecting prostate-specific RNA in whole blood associated with CTCs count is currently being prospectively tested as a prognostic factor and response indicator [45]. Loss of PTEN expression has also been reported to be associated with worse survival [46] and analyses of extracellular microRNAs embedded in circulating exomes revealed that plasma exomal miR-1290 and

miR-375 could be promising prognostic biomarkers for CRPC patients [47]. Although none of them has been fully validated as a surrogate for OS, a wide range of prognostic biomarkers as well as nomograms are currently available to assess disease evolution risk in patients diagnosed with CRPC [48]. In addition, different predictive biomarkers seems to be also promising.

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Predictive biomarkers in CRPC

Predictive biomarkers can be clinical (e.g. prior exposure to a specific therapy, response to treatment), hormonal (e.g. serum testosterone levels), or molecular determinants (e.g. AR overexpression, increased biosynthesis of androgens, splice variants and mutations of AR, altered PTEN signaling, translocation allowing ETS transcription to be under control of androgens) that are associated with sensitivity or resistance to a specific treatment [32].

Specifically, considerable interest has been generated with the recent report on the splice variant AR-V7 [49]. Indeed, Antonarakis et al have demonstrated that the expression of such AR-V7 variant was correlated with the response to AA or ENZ [49]. This novel predictive biomarker has emerged after the analysis of CTCs in 62 patients treated prospectively with AA (n = 31) or ENZ (n = 31) for mCPRC. Interestingly, AR-V7 expression was correlated with treatment resistance to AA or ENZ, including shorter biochemical progression-free survival (PFS), radiological PFS and OS, compared to patients without AR-V7 expression.

These response indicator biomarkers include also pharmacodynamics or imaging factors that typically show a change after treatment has occurred, without necessarily indicating that this change is in relation with a patient benefit [50–53]. Specifically, PSA level decline and tumor size changes according to RECIST criteria are two examples of them[31,32,54] but other imaging biomarkers are currently emerging. Indeed, the Prostate Cancer Working Group 2 (PCWG2) criteria and the bone scan index (BSI) developed at the MSKCC are other promising tools [32,55]. This lastly mentioned quantitative measurement of the tumor burden, as a percentage of the skeletal mass, is able to take into account increase in size of bone lesions.¹⁴ A doubling BSI is associated with a 2-fold increase risk of death but this imaging biomarker has to be validated in phase III clinical trials [56]. Investigators of COU-AA-302 trial have also proposed radiographic PFS as a response biomarker in metastatic CRPC considering that it was highly consistent and highly associated with OS [57]. Also, ¹⁸F-

fluorodeoxyglucose (FDG)-PET scan seems to be another imaging technique with better performance to detect early metastases compared to bone scan. Its prognostic significance is under evaluation as well as its value as response indicator. Table 2 summarizes the biomarkers with potential predictive value on response, patients benefit and candidate for surrogate end-points. Despite the emergence of these prognostic and predictive biomarkers in CPRC, a particular difficulty arising with the new agents available is the optimal sequence to administer them.

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Sequential administration of new agents in CRPC

To date, only few clinical trials attempted to assess the impact of different sequences of new agents such as AA, ENZ and CZT by using retrospective post-hoc analyses with inherent bias. Specifically, the administration of AA after resistance to DCT followed by ENZ, was tested in 38 patients included in the phase 3 AFFIRM trial [56]. Because of a PSA decline $\geq 50\%$ in only 3 patients and a median PFS of 2.7 months, this study suggested a potential cross-resistance between AA and ENZ which was confirmed in an other report [58]. The reverse sequence with ENZ after DCT and AA failure showed similar results with low response rates and short PFS [59–64]. The cross-resistance between AA and ENZ could be explained by increased levels of AR Δ LBD observed after the use of both drugs [65,66].

Inversely, the administration of AA following synthetic oestrogen diethylstilboestrol (DES), was effective in a pre- and post-DCT setting [67]. Indeed, the response rates and time to progression were similar in the groups of DES-naïve or pre-treated patients in both settings. Consequently, AA seems to maintain antitumour activity in men with CRPC after DES exposure. In addition, effectiveness of CZT has also been assessed in a sequencing trial including patients previously treated with DCT and next generation endocrine agents such as AA and ENZ [68]. Interestingly, prolonged OS and PFS were observed in this cohort of patients and such results were confirmed in other recent studies [69–71]. Because the AR amino-terminal domain has been previously reported to be critically important for the tubulin binding agent activity, one could hypothesize that the AA and ENZ resistant AR splice variants, lacking only the carboxy-terminal ligand-binding domain terminal, would still be inhibited by CZT [72]. This has been confirmed by recent findings which demonstrated that efficacy of CZT for CRPC was independent of the presence of AR-V7 in circulating tumor cell [73]. However, these findings might not apply to DCT considering that men receiving AA before DCT are more likely to progress on DCT and less likely to achieve a PSA response

that AA-naïve patients [70,74–78]. In addition, large multicenter studies failed to show any significant difference in the clinical outcomes of third line treatment with AA, ENZ or CZT regardless of previous therapy delivered [79], whereas in a less advanced treatment setting, the prospective evidence from the recent TERRAIN trial support the use of ENZ rather than bicalutamide in patients with asymptomatic or mildly symptomatic CRPC [80]. Supplemental Figure 3 shows options of targeting CRPC according to the activation of AR.

It is noteworthy that the recent results of the CHARTED [81] and STAMPEDE [82] trials may also change the treatment landscape and sequencing of CRPC, as the use of DCT has been advocated in the last NCCN guidelines [83] to treat patients with metastatic HNPC, especially those with high-volume disease – despite the inconclusive post-hoc analyses from the GETUG-AFU 15 trial [84]. Nonetheless, DCT used as a second line therapy after AA for CRPC retains reasonable activity [85] (Table 3).

Finally, new drugs are currently emerging, adding even more confusion to the sequential treatment of CRPC [86]. Ongoing preclinical studies found new therapeutic targets such as signal transducer and activator of transcription 3 (STAT3) [87], while novel PI3K/AKT-targeted drugs associated with androgen deprivation therapy have shown promising results in CRPC models [88].

As a consequence, robust data on the best sequencing strategy for CRPC are growingly lacking. The absence of validated surrogate for OS in CRPC makes it difficult to conduct sequencing trials [89]. In addition, no agent has actually been developed with a companion predictive biomarker that may help to choose among the numerous possible sequences based on the six validated therapies for CRPC. Thus, several strategies using either the most active or least toxic agent first could be used but the rational sequencing, which consists in choosing an agent that is not expected to have cross-resistance with previous therapy in preference to one that may, appears to be more reasonable [90]. Because estimated median survival time of

CRPC patients is approximately 40 months from diagnosis, it is likely that many of them will not live long enough to receive all these systemic therapies in sequence, and one additional issue of recent progress will be to identify those that could be used in combination based on the assessment of biomarkers derived from fundamental and clinical research.

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Conclusion

Stratifying patients with CRPC to receive next-generation drugs targeting the AR activity dependent or independent pathways remains an unmet need. Furthermore, designing novel therapies is currently a challenge at the crossroads of several fields including tumor genetics and epigenetics as well as micro-environmental changes. The main reason is simply related to the spatio-temporal evolution of prostate cancer cells towards such highly variable pathways. A comprehensive characterization of all these processes, simultaneously, all along the development of lethal CRPC in a defined patient remains unknown, although next-generation sequencing has dramatically helped to define the landscape of aggressive CRPC variants but also to find prognostic and predictive biomarkers.

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Review criteria

A search of original and review articles focusing on the conservative management of upper tract urothelial carcinoma was performed in MEDLINE and PubMed. The following search terms ("Prostate cancer") AND ("Castration" OR "Castrate") AND ("Resistant" OR "Resistance") were used according a free text protocol that applied only "Humans" and "English language" filters without a time-period restriction. Reference lists of selected articles were cross-searched for additional literature.

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Figure 1: Androgens receptor axis and action sites of news hormonal agents in CRPC

adapted from Kohli et al[12]

Figure 2: Genomic lesions in evolution of prostate cancer adapted from Barbieri et al.[1]

Genes with common genomic lesions {including mutation, rearrangement, or copy number alteration) are shown. Solid arrows designate a temporal relationship between events; presumptive “early” lesions are at the top, with “later” lesions below. Tumors with ETS fusions (ETS+) are shown on the top; ETS- tumors are shown on the bottom.

ADT: androgen deprivation therapy.

Figure 3A: Molecular history of prostate cancer evolution.

The progression to the CRPC stage, is usually describe at the molecular level, step by step, involving mainly chromosomal rearrangements, according the model of the punctuated progression named of chromoplexy. A shortcut to metastatic and resistant stages, related to a high genetic instability with numerous chromosomal rearrangements “chromotripsis” and hypermutational status “kaetagis” can occur.

Figure 3B: Mechanisms of CRPC progression driven by heterogeneous modalities of androgen receptor deregulation.

CRPC related to an uncontrolled Androgen receptor (AR) have been related to heterogeneous mechanisms (residual ligands to AR, promiscuous activation involving AR expression and structure or Co-activators). AR activation pathway (AR+ signature) drives genes regulated by androgen responses elements, such as PSA or AR dependant fusions genes classified as type 1,2 or 5 (such as Tmprss2-ETS).

Supplemental Figure 1: PCa progression and phenomenon of chromoplexy and chromotripsis in prostate cancer evolution, adapted from Bacca et al.[17]

Concept of Genomic Evolution of Prostate Cancer: Three different situations could occur: gradual accumulation in cancer genome of aberrations (upper curve), punctuated progression/chromoplexy (middle), or single catastrophic event/ chromotripsis (lower curve). Larger-scale rearrangements that affect broader swaths of the genome may be more difficult for a cell to survive and may tend to require co-occurring oncogenic lesions to become fixed in a tumor.

Supplemental Figure 2A: Ontogenic origins of prostate cells

Androgen receptor drives luminal differentiation (and PSA expression) phenotype during development of prostatic gland and during progression of CRPC. Cell survival without androgen receptor activation is associated to a phenotype switch to neuroendocrine or stem cell like phenotypes according ontogenic origin of prostatic glands. CRPC is a heterogeneous disease involving accumulation of mutations or epigenic changes in cancerous cells. So, CRPC exhibits various phenotypes which are able to suggest a biological classification.

Supplemental Figure 2B: Bio-pathological classification of CRPC

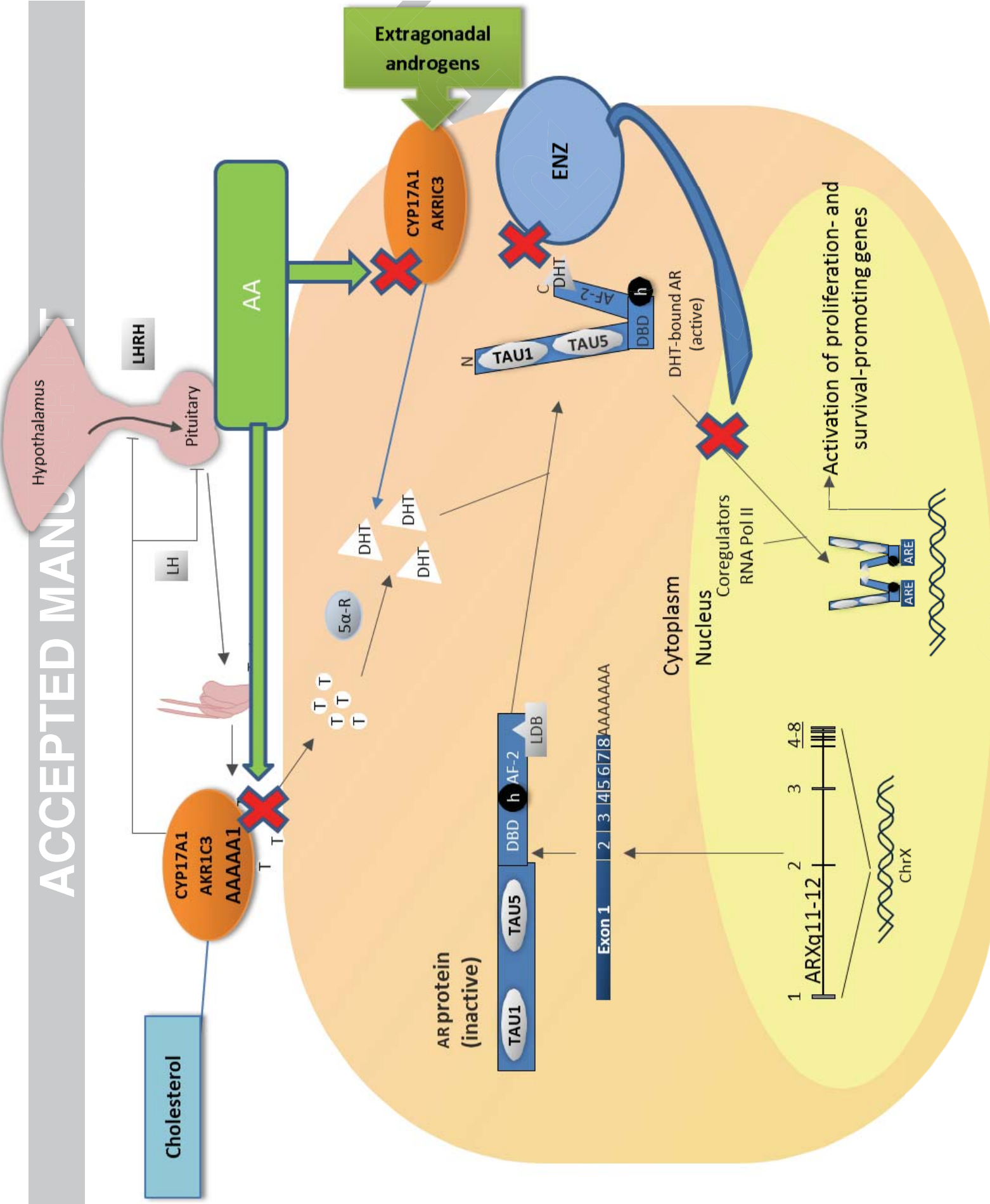
On one hand CRPC could be secretory (expressing luminal markers such as PSA) or neuroendocrine markers such as chromogranin A. On the other hand, CRPC could be non-secretory, expressing stem cells' markers such as CD44/aldehyde dehydrogenase 1A1, like neuroendocrine phenotype they do not express AR and his “reporter” gene PSA. CRPC with high level of PSA expression are determined by an uncontrolled androgen activity which

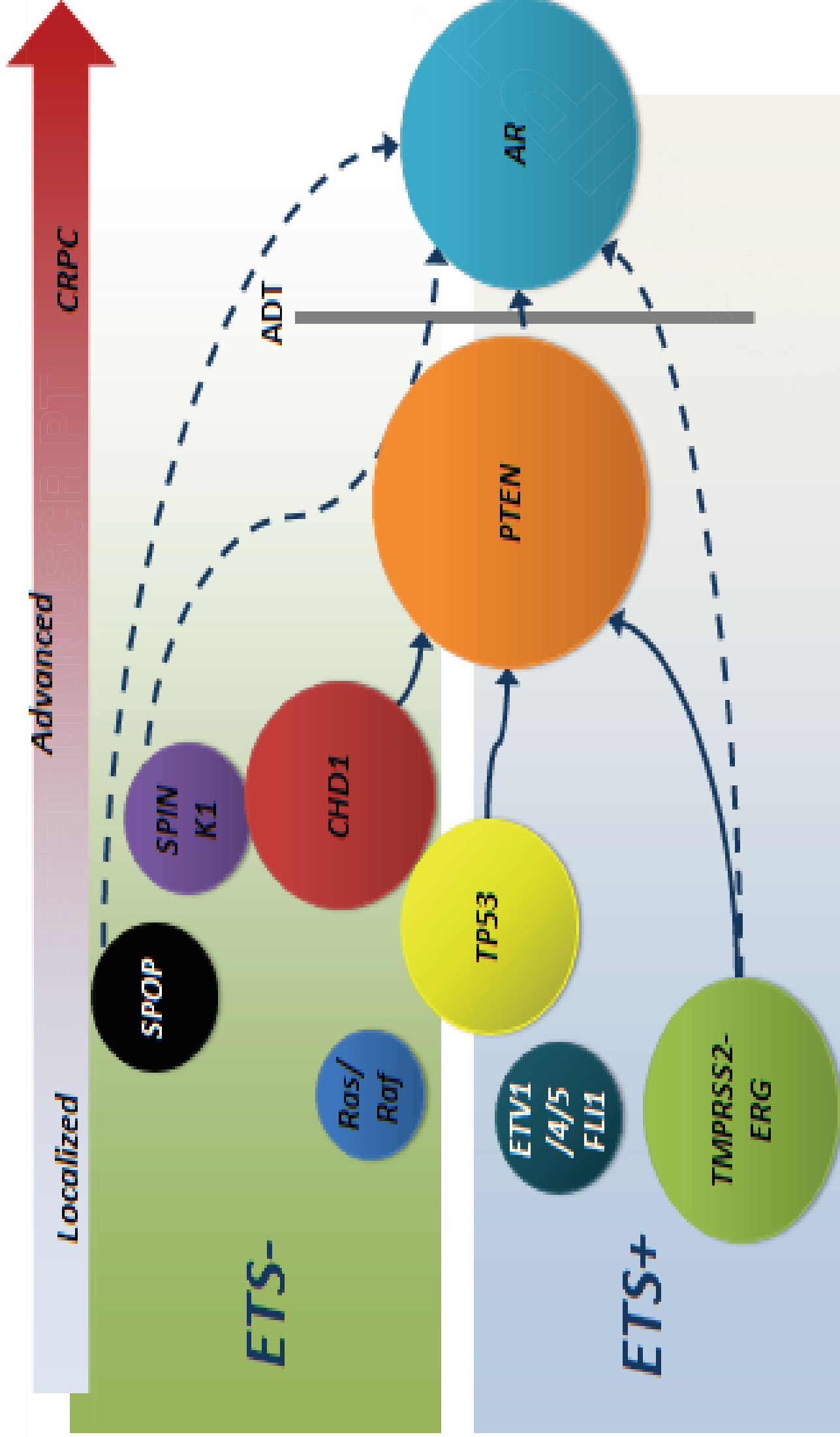
could be driven by residual androgens (potential responder to a depth androgen withdrawal) or AR mutation leading to a constitutive oncogenic activation.

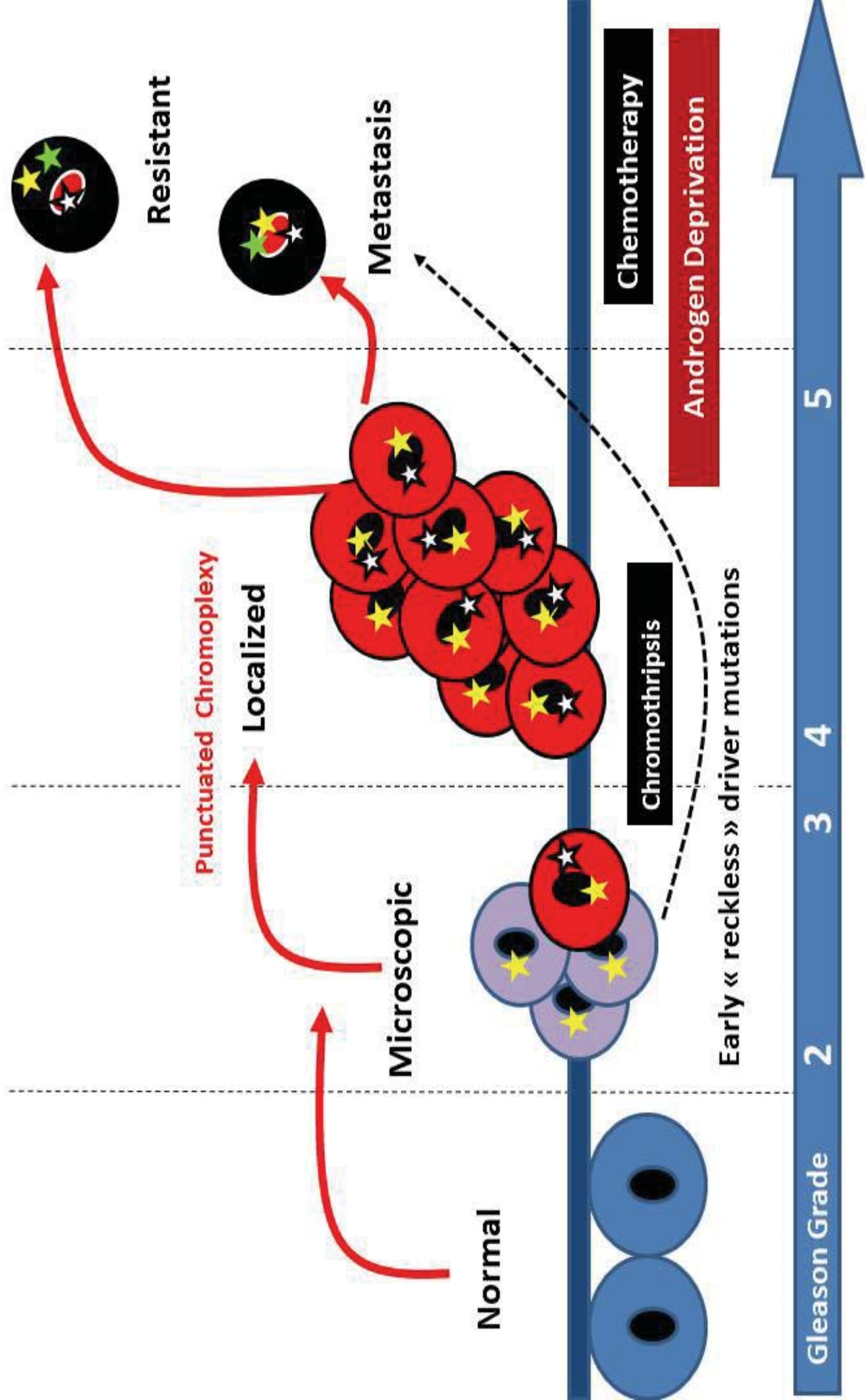
AR: androgen receptor; PSA: Prostate Specific Antigen; NSE: Neuro Specific Enolase; VP16: etoposide

Supplemental Figure 3: Options of targeting CRPC according to the activation of androgen receptors.

If AR pathway is activated, AA or ENZ might be the first option therapy to propose. If the CRPC become independent from the AR signaling, many pathways have to be explored including targeting the immune system, EMT, epigenetics, growth factor receptors or anti-mitotic agents.



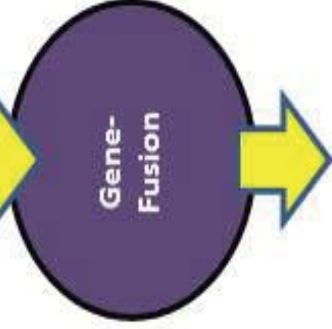
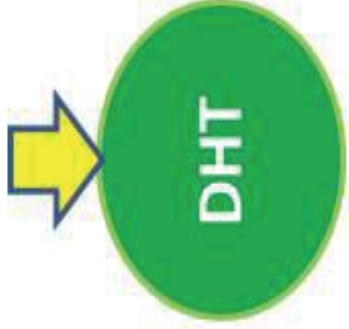




CRPC AR Signature +

| Residual androgens |
|-----------------------|
| Endocrine (Adrenal) |
| Intracrine (Prostate) |

Abiraterone
(Enzatumamide)



Proliferation
Invasion

Apoptosis
Blockade
+

PSA expression

| promiscuous activation |
|-----------------------------|
| Amplification |
| Hypersensitivity (Mutation) |
| Phosphorylation |



Enzatumamide
(Abiraterone)

| Ligand independent activation |
|--|
| AR constitutively active (truncated mutation) |
| EGF, IGF-1, IL6 |

Typology of fusion-genes
AR dependant

Type 1, Type 2, Type 5

Table 1: Pre and post-treatment prognostic biomarkers in CRPC.

| | Pre-treatment prognostic biomarkers | Post-treatment prognostic biomarkers |
|--|--|--|
| Clinical parameters and primary tumor characteristics at baseline | Age Performance Status Pain Gleason score, Gleason sum Number of metastatic sites Bone metastases Liver metastases | Pain improvement Quality of life improvement |
| Biological parameters | Total PSA and PSA kinetics Hemoglobin LDH, ALP Albumin CTCs VEGF, IL6, Chromogranin A CRP Bone turnover markers Urinary N -Telopeptide | PSA decline Change in CTCs count (≥ 5 to <5) Anemia LDH changes ALP changes Induction of immunity to tumor antigens (Sipuleucel-T) |
| Type of progression | PSA only, bone, measurable lesions | Skeletal events, rPFS |

PSA: prostate specific antigen; LDH: Lactate dehydrogenase; ALP: Alkaline phosphatase; CTCs: circulating tumor cells; rPFS: radiologic progression-free survival.

Table 2: Biomarkers with potential predictive value on response, patients benefit and candidate for surrogate end-points.

| Biomarker | Main problematic |
|-------------------------------|--|
| PSA decline | Some new agents demonstrated an OS benefit without any PSA change Threshold of decline unclear (decrease of 30 % or 50 %) |
| Baseline PSA | Low value at baseline is associated with greater OS only in Sipuleucel-T phase III trial |
| Composite score | Association of PSA response over 50%, neutrophil-to lymphocyte ratio ≤ 5 and low value of LDH seems to be predictive of only abiraterone acetate response and OS |
| Pain improvement | Subject to change with analgesic treatment alone Causes of pain independent of tumor progression Reliable validated scales to be validated |
| Quality of life | Definition of clinically significant changes |
| Bone turn-over markers | Normal in patients with visceral-only or node-only disease and in some with bone metastasis Normalization of high baseline ALP value seems to be an independent predictive factor for OS in patients with bone metastases treated with chemotherapy |
| CTCs | Only 50% of patients having detectable CTCs using the FDA approved Cell Search platform Unable to be banked or stored; expensive procedure in specialized labs only |
| Radiographic response | Bone metastases are not considered by RECIST as measurable target lesions Bone scans flare Modest correlation with OS |
| PFS | No correlation between PFS and OS for Sipuleucel-T Anti-angiogenic agents improved PFS without OS increase |

PSA: prostate specific antigen; CTCs: circulating tumor cells; OS: overall survival; LDH: Lactate dehydrogenase; ALP: Alkaline phosphatase; PFS: progression free survival

Table 3: Studies reporting the outcomes of treatment sequencing in castration-resistant prostate cancer

| Study | Study design | Number of patients | PSA response $\geq 50\%$, % | Partial radiographic response, % | Median PFS, months | Median OS, months |
|--|---------------|--------------------|------------------------------|----------------------------------|--------------------|-------------------|
| Abiraterone acetate after docetaxel and enzalutamide | | | | | | |
| Loriot et al., 2013 [56] | Retrospective | 38 | 8 | 8 | 2.7 | 7.2 |
| Noonan et al., 2013 [58] | Retrospective | 30 | 4 | 0 | 3.6 | 11.6 |
| Enzalutamide after docetaxel and abiraterone acetate | | | | | | |
| Badrising et al., 2014 [59] | Retrospective | 61 | 21 | NR | 2.8 | 7.3 |
| Bianchini et al., 2014 [60] | Retrospective | 39 | 13 | 4 | 2.8 | Not reached |
| Brasso et al., 2014 [61] | Retrospective | 137 | 18 | 12 | 3.1 | 8.3 |
| Schmid et al., 2014 [62] | Prospective | 35 | 10 | 4 | 3.1 | 7.5 |
| Schrader et al., 2014 [63] | Retrospective | 35 | 29 | 6 | NR | 7.1 |
| Thomsen et al., 2014 [64] | Retrospective | 24 | 17 | NR | NR | 4.8 |
| Cabazitaxel after docetaxel and abiraterone acetate or enzalutamide | | | | | | |
| Pezaro et al., 2013 [68] | Retrospective | 37 | 41 | 15 | 5.5 | 20.3 |
| Al Nakouzi et al., 2014 [69] | Retrospective | 79 | 35 | NR | 4.4 | 10.9 |
| Houts et al., 2014 [70] | Retrospective | 18 | NR | NR | 4.9 | NR |
| Sella et al., 2014 [71] | Retrospective | 24 | 32 | 15 | 3.2 | 8.2 |
| Docetaxel after abiraterone acetate | | | | | | |
| Aggarwal et al., 2014 [74] | Retrospective | 23 | 48 | NR | NR | 12.4 |
| Azad et al., 2014 [75] | Retrospective | 86 | 35 | NR | 4.0 | 11.7 |
| Mezynski et al., 2014 [76] | Retrospective | 35 | 26 | 17 | NR | 12.5 |
| Houts et al., 2014 [70] | Retrospective | 71 | NR | NR | 7.8 | 10.6 |
| Schweizer et al., 2014 [77] | Retrospective | 24 | 38 | NR | 4.4 | NR |
| Suzman et al., 2014 [78] | Retrospective | 31 | 40 | NR | 4.4 | NR |

Highlights

1. Chromoplexy and chromotripsis models for CRPC genmoic landscape
2. Dependent and independent androgen receptor activity pathways in CRPC development
3. No biomarker validated as a surrogate for overall survival in CRPC patients
4. AR-V7 splice variant to predict resistance to abiraterone and enzalutamide in CRPC.
5. Only rational treatment sequencing can be currently advised for CRPC.

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