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Mechanisms and markers of resistance to androgen signaling inhibitors in patients with metastatic castration-resistant prostate cancer

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Abstract

Next-generation androgen signaling inhibitors such as abiraterone and enzalutamide are widely used for the treatment of metastatic castration-resistant prostate cancer. Unfortunately, baseline and acquired resistance to these treatments is commonly observed. In the last few years, significant effort has been devoted to uncover the molecular mechanisms and predictive markers of resistance. These analyses identified various DNA (single nucleotide variations, amplifications) and RNA variants (e.g., the splice variant AR-V7) of androgen receptor in association with resistance to abiraterone and enzalutamide therapies. Additionally, androgen receptor independent resistance mechanisms were also described. Some of these alterations can be detected in tumor tissues and/or in liquid biopsies of prostate cancer patients and therefore may serve as predictive biomarkers. According to the diversity of potential resistance mechanisms, it appears that a combination of markers representing various resistance mechanisms may provide better performance as single markers. In the present review, we summarize the most important androgen receptor dependent and independent resistance mechanisms and pay attention to methodological details. Recent data has highlighted that some of the resistance mechanisms to next-generation antiandrogen agents are associated with a better response to other therapies, we give an overview on currently ongoing clinical studies evaluating this promising aspect.

Key words: Prostate cancer; Enzalutamide; Resistance

1. Introduction

Prostate cancer (CaP) is a common cause of cancer mortality in men with 350,000 estimated deaths per year [1]. For advanced CaP primary androgen deprivation therapy (ADT) has been the standard of care for over 50 years and ADT alone was used as first-line therapy. Despite its initial efficacy, most patients develop resistance to ADT. Docetaxel (DOC) chemotherapy has been the standard first-line treatment for metastatic castration-resistant prostate cancer (mCRPC) for nearly 20 years. In the last decade, two next-generation androgen receptor (AR) signaling inhibitors, abiraterone (ABI) and enzalutamide (ENZA) have been approved for the treatment of mCRPC. Abiraterone inhibits intratumoral androgen biosynthesis by blocking cytochrome P450 17A1 (CYP17A1) enzyme. Enzalutamide is a specific antagonist of the AR, which can bind to the ligand-binding domain of AR, impede AR nuclear translocation and inhibit AR binding to DNA. In phase III trials, ABI and ENZA have demonstrated improved survival of mCRPC both in the pre- and post-docetaxel setting [2,3,4,5]. Unfortunately, baseline and acquired as well as cross-resistance against ENZA and ABI treatments have been observed. Therefore, it is crucial to understand the mechanisms of resistance and identify predictive biomarkers in order to help clinical decision and select the optimal therapy sequences for the individual patients.

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In this review, we summarize various resistance mechanisms against ABI and ENZA in mCRPC and provided an overview on the potential prognostic markers of mCRPC.

2. Search Strategy

For AR-related resistance mechanisms, a literature search of PubMed database was conducted for articles, published between January 2012 and August 2020. The search was performed using combination the following keywords: castration-resistant prostate cancer, CaP, abiraterone, enzalutamide, resistance. Articles were selected based on title and abstract. Search results were restricted to English language. Review, letters, case-reports, editorial comments and papers with only abstract were excluded. Papers reported treatment response of ABI or ENZA-treated mCRPC patient were included in this review. 29 articles were excluded because of they were non-relevant biomarker studies. Additional references were identified from references of selected articles. Ongoing clinical trials were searched on the clinicalTrials.gov website. Eligible findings that we presumed to be of clinical interest were included in our review article (Fig. 1) [6]. For non-AR-related resistance mechanisms, we performed a subjective selection of published literature.

3. Androgen receptor

AR is a transcription factor and a member of the steroid hormone nuclear receptor superfamily. It is located on the X chromosome at Xq11-12. AR protein consist of four main functional regions; N-terminal transactivation domain, the DNA-binding domain, the small hinge region and the ligand-binding domain (LBD) (Fig. 1). In absence of dihydrotestosterone (DHT), the inactive AR is in the cytoplasm and bound to chaperone proteins. After androgens (DHT or testosterone) bind to AR, the protein undergoes a conformational change and this complex translocates to the nucleus, dimerizes and binds to specific

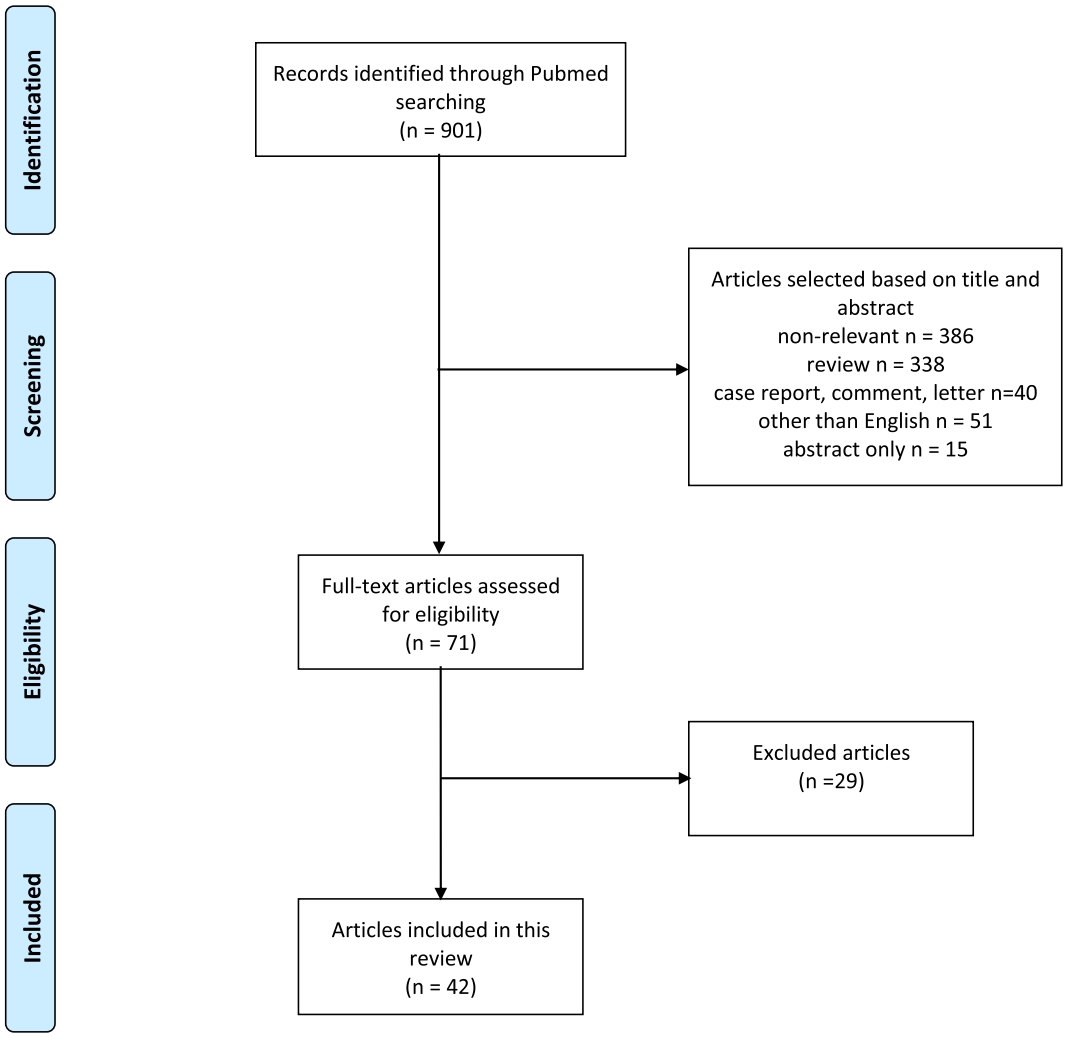

Fig. 1. PRISMA flow diagram of literature search.

DNA motifs, the so-called androgen response elements to regulate transcription of AR target genes [7].

3.1. Androgen receptor amplification and/or overexpression

Overexpression of AR can be a result of AR gene amplification (AR gain) which has been associated with resistance to AR targeting therapies. AR gain was found in 10-15% of plasma samples of treatment naïve compared to 25-50% in the second or later line setting of mCRPC patients [8,9,10,11,12,13]. Several independent studies demonstrated that patients with AR alterations (amplification, mutation) had shorter radiographic, and clinical progression-free survival (PFS) under ABI or ENZA treatment [8,9,10,11]. Additionally, a recent large multicohort study with >500 mCRPC patients who received first-line ABI or ENZA treatment found that patients with plasma AR copy number value of ≥1.92 had significantly shorter OS and PFS and have significantly shorter response to prior primary ADT [13]. However, the correlation between the AR gain and PFS or OS was consequently found, a potential limitation remains to be considered. As AR gain was strongly correlated with higher cfDNA levels and in liquid biopsy tests high cfDNA levels (and also positive results) are often associated with higher tumor load, a confounding effect between these factors may account for the prognostic value of AR gain in the above studies [13]. This raises the question whether AR gain is prognostic or predictive? Against this background the results of a current meta-analysis of more than 1000 patients treated with next-generation AR inhibitors is interesting, revealing that AR gain (as detected by cfDNA analyses) was associated with a worse response to ABI and ENZA treatment and in contrast it was not associated with OS or PFS in patients who received first-line DOC or second-/ third-line cabazitaxel therapy [14]. These data suggest that AR gain may select mCRPC patients for DOC rather than for ABI or ENZA treatment [14], however prospective randomized studies are required and are ongoing (NCT03700099, NCT02922218) to decide whether AR gain can be used for treatment selection.

3.2. Androgen receptor point mutation

Various point mutations in the LBD of AR gene have been implicated in the resistance to ABI and ENZA treatment (figure 2).

In vitro and *ex vivo* data uniformly demonstrated that a F876L somatic mutation in LBD confers resistance to ENZA. This mutation is able to convert ENZA from an antagonist into a partial agonist of AR [9,15,16]. Interestingly, F876L mutation bearing ENZA resistant CaP cells were sensitive to other AR antagonists, such as bicalutamide and to cyclin-dependent kinase (CDK4/6) inhibitors suggesting potential therapeutic options for patients present with F876L mutation [15].

The T878A mutation reduces the ligand-binding specificity of AR, making the receptor sensitive to endogenous molecules such as estrogen or progesterone [17]. Additional *in vitro* analyses revealed that, the presence of T878A mutation was associated with resistance to ABI but not to other anti-AR agents such as bicalutamide, which suggest that ABI may be more effective in combination with anti-AR agents [17], but further clinical studies are required to confirm this hypothesis.

Similar to T878A, H875Y is also a ligand-promiscuity-conferring AR mutation. Annala *et al.* performing whole-exome sequencing on 115 treatment-naive CRPC patients' plasma cfDNA found missense mutations in the LBD of AR in 14 (12%) samples. The most common mutation was H875Y (n=9), which was not associated with shorter PFS [18].

The L702L AR mutation, changes the LBD structure of AR thereby AR can be activated by other steroids such as glucocorticoids and anti-inflammatory drugs. Romanel *et al.* analyzing plasma samples of 97 ABI-treated CRPC patients observed that L702H and T878A AR mutations were significantly associated with worse OS and PFS [19].

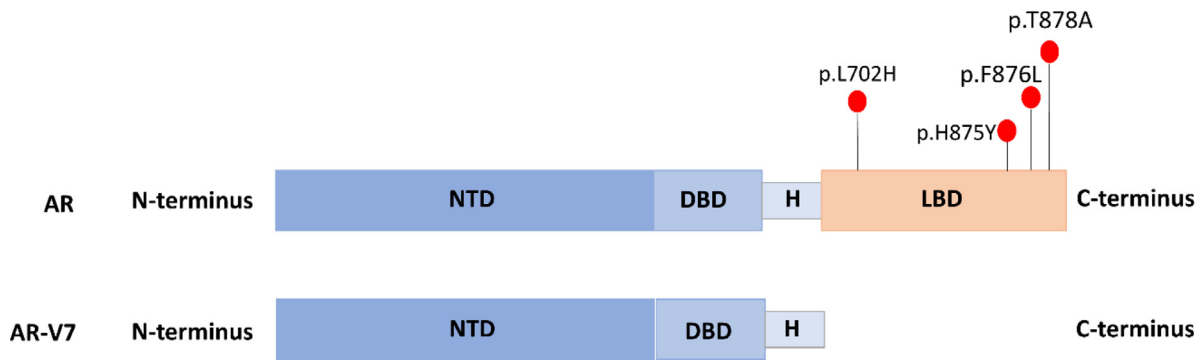

Fig. 2. Structure of AR and its activating point mutations and splice variant AR-V7.AR: androgen receptor, NTD: N-terminal domain, DBD: DNA binding domain, H: hinge region, LBD: Ligand binding domain (Szarvas T, Csizmarik A, Nagy N, Keresztes D, Varadi M, Kuronya Zs, Riesz P, Nyirady P. Molecular underpinnings of systemic treatment resistance in metastatic castration-resistant prostate cancer. Orv Hetil. 2020; 161: 813-820. (https://creativecommons.org/licenses/by-nc/4.0)

In another study, Conteduca *et al*. found AR mutations (T878A, L702H) in 8 of 171 ABI/ENZA-treated mCRPC patients' plasma samples which were associated with shorter OS. Interestingly, these mutations were only detected in docetaxel pre-treated patients [10].

A recent study reported a parallel cfDNA and cfRNA sequencing method which is able to detect AR gain and AR mutations as well as AR mRNA variants from the same plasma sample. Authors found an independent correlation between AR gain and poor PFS and OS in ABI/ENZA-treated patients [20]. In contrast, AR variants were not associated with any of the above end-points [20].

In conclusion, clinically significant AR mutations are detected in 7% to 17% of mCRPC patients however their low occurrence and relative weak prognostic value limits their clinical importance (Table 1).

3.4. Androgen receptor splice variant 7

Resistance against next-generation androgen receptor (AR) inhibitors was found to be associated with the presence of AR splice variants (AR-V). AR-V7 encodes a truncated AR protein, which lacks the LBD but shows constitutive activity independent of androgen stimulus. AR-V7 can be detected in various samples such as tumor tissue, circulating tumor cells (CTC) from whole blood or plasma cfRNA by using, digital droplet PCR, real-time PCR and immunohistochemistry. (table 2)

Antonarakis *et al*. found 29% of mCRPC patients to be present with AR-V7 mRNA in their CTCs These patients achieved significantly lower PSA response rates compared to AR-V7 negative patients (0% vs. 61%). Accordingly, PFS was shorter in patients with AR-V7 positive among both ENZA- (2.1 vs. 6.1 months, respectively) and ABI-treated men (2.3 vs. 6.3 months, respectively) [21]. In a subsequent, prospective study on 202 ENZA- and ABI-treated patients, authors divided patients into three groups based on CTC and AR-V7 detection; CTC negative; CTC positive but AR-V7 negative and CTC/AR-V7 positive patients. The most favorable OS and PFS was found in the CTC negative group, while the worst response to ABI/ENZA treatment was observed in the AR-V7 positive group, suggesting that not only the AR-V7 status but also the presence of detectable CTCs is associated with patients' prognosis [22].

Scher *et al.* used immunohistochemistry for the detection of AR-V7 expression in CTCs from mCRPC patients who received next-generation antiandrogen (ENZA, ABI, apalutamide [APA]) and taxane therapy [23]. Among the 128 patients, receiving next-generation antiandrogen therapy 16 (13%) proved to be AR-V7 positive. AR-V7 positive ENZA, ABI and APA treated patients had worse PSA response rate with shorter PFS and OS. Interestingly, AR-V7 positive patients had superior OS when treated with taxane therapy compared to those who received next-generation antiandrogen treatment [23]. In a subsequent analysis, AR-V7 positive cases were divided based on their subcellular localization into nuclear, cytoplasmic and both nuclear and cytoplasmic (nuclear-agnostic) groups [24]. None of the 16 nuclear-positive positive patients showed a PSA-response to ENZA, ABI or APA treatment, whereas 9 of 32 patients with nuclear-agnostic AR-V7 positivity responded to these treatments underlining the importance of AR-V7 localization. Furthermore, AR-V7 nuclear positive men had a more favorable OS, when treated with taxane in comparison to next-generation antiandrogen therapies [24]. These results could be validated in an independent study [25]. Based on these, not only the presence of AR-V7 in CTCs, but also its subcellular localization may provide useful information for improved therapeutic decision-making.

Although the majority of published literature [26,27] reports a significant association between the presence of AR-V7 and poor response to androgen-targeting therapies, three studies reported less consistent results. In 1 study, surprisingly, 6 of 21 (29%) AR-V7 positive patients responded well to ABI/ENZA treatment, while the other two studies found no correlation between presence of AR-V7 and response to therapies [20,28,29]. Methodological differences may account for these contradictory results.

A prospective biomarker study (PROPHECY) demonstrated a prognostic value for AR-V7 in ABI/ENZA treated patients as well. In this study, AR-V7 protein expression in CTCs has been identified by using two different blood-based assays (AdnaTest and EPIC Sciences). Both methods showed that AR-V7 positive patients had a significantly shorter PFS and OS [30]. In contrast, AR-V7 positivity was not associated with OS and PFS in taxane treated group [30]. Therefore AR-V7 seems to be predictive rather than prognostic for ABI/ENZA treatment.

From a practical point of view, it is important to compare the two commercially available AR-V7 assays; the AdnaTest uses three specific antibodies for CTC separation from whole blood and in a subsequent step AR-V7 can be detected by real-time qPCR. In contrast, the EPIC Sciences test can identify CTCs and AR-V7 in blood sample by using an immunofluorescent technique. Additionally, this assay is able to distinguish between the nuclear and cytoplasmic localization of AR-V7. In a comparative study, the AdnaTest identified 28 AR-V7 positive patients of which 11 patients had >50% PSA declines to ENZA/ABI-treatment. In the same patient cohort, the EPIC Sciences method detected only 11 AR-V7 positive patients, of whom none had a PSA decline of >50% to ABI/ENZA treatment. These results show that methodological differences may significantly affect results of AR-V7 analysis and may account for previous contradictory results.

A further important aspect is that CTCs cannot always found in patients' samples. In the ARMOR3-SV Phase III study, that compared galeterone to ENZA, only AR-V7 positive mCRPC patients were enrolled. The hypothesis was that AR-V7 positive patients will better respond to galeterone compared to ENZA. Overall, 953 patients were

Table 1
AR genetic alterations related ABI/ENZA resistance

Marker	Ref.	Type of study	Treatment	Therapy lines at analysis	Sample	Assay	ΣN	Npos	PSA RR (%)		Univariate analysis			Multivariate analysis		
									Pos.	Neg.	End-point	HR (95%)	P	End-point	HR (95%)	P
AR gain	Azad [8]	Retrospective	ABI, ENZA	mixed	cfDNA	aCGH	48	23	-	-	PFS	5.08 (2.25 - 11.49)	<0.001	PFS	4.05 (1.40 - 11.76)	0.010
AR gain	Conteduca [10]	Prospective	ABI, ENZA	mixed	cfDNA	ddPCR	171	43	-	-	PFS	2.33 (1.61 - 3.36)	<0.001	PFS	2.22 (1.48 - 3.34)	<0.001
					cfDNA						OS	4.07 (2.68 - 6.20)	<0.001	OS	4.26 (2.76 - 6.55)	<0.001
		Prospective	ENZA	1st	cfDNA	ddPCR	94	11	-	-	PSA-PFS	4.33 (1.94 - 9.68)	<0.001	-	-	-
					cfDNA						rPFS	8.06 (3.26 - 19.93)	<0.001	-	-	-
					cfDNA						OS	11.08 (2.16-56.95)	0.004	-	-	-
AR gain	Wyatt [11]	Retrospective	ENZA	mixed	cfDNA	aCGH	65	19	16	48	PFS	2.92 (1.59 - 5.37)	0.001	-	-	-
AR gain	Salvi [12]	Retrospective	ABI	2nd	cfDNA	qPCR	53	16	31	57	PFS	3.73 (1.95−7.13)	<0.0001	PFS	4.06 (1.86−8.86)	0.0004
											OS	4.68 (2.17−10.10)	<0.0001	OS	3.59 (1.38−9.31)	0.0026
AR gain	Jayaram [13]	Prospective	ABI	1st	cfDNA	ddPCR /NGS	133	22	-	-	PFS	1.94 (0.897 - 3.87)	<0.001	PFS	2.60 (2.00 - 3.50)	<0.001
					cfDNA						OS	2.37 (1.07 - 5.25)	<0.001	OS	3.10 (2.20 - 4.30)	<0.001
		Prospective	ABI, ENZA	1st	cfDNA	ddPCR /NGS	73	-	-	-	PFS	2.08 (0.92 - 4.72)	0.010	-	-	-
					cfDNA						OS	3.22 (1.17 - 8.85)	0.010	-	-	-
		Prospective	ENZA	1st	cfDNA	ddPCR	94	-	-	-	PFS	3.90 (1.27 - 12.03)	<0.001	-	-	-
					cfDNA						OS	5.62 (1.42 - 22.17)	<0.001	-	-	-
		Prospective	ABI, ENZA	1st	cfDNA	NGS	201	-	-	-	PFS	2.45 (1.44 - 4.18)	<0.001	-	-	-
					cfDNA						OS	5.40 (2.63 - 10.94)	<0.001	-	-	-
AR gain	Annala [18]	Prospective	ABI, ENZA	1st	cfDNA	NGS	202	67	68	64	TTP	2.05 (1.43 -2.93)	<0.001	TTP	1.21 (0.77 - 1.91)	0.401
AR gain	Fettke [20]	Prospective	ABI, ENZA	mixed	cfDNA	NGS	41	15	33	69	PSA-PFS	2.80 (1.30 - 6.10)	0.010	-	-	-
											rPFS	3.40 (1.40 - 8.20)	0.006	-	-	-
											0S	3.20 (1.20 - 8.50)	0.020	-	-	-
		Prospective	ABI, ENZA, taxane	mixed	cfDNA	NGS	40	18	-	-	0S	7.80 (3.00 - 21.00)	<0.001	-	-	-
AR mut.	Conteduca [10]	Prospective	ABI, ENZA	mixed	cfDNA	ddPCR	171	8	-	-	PFS	2.86 (1.24 - 6.59)	0.014	PFS	2.59 (1.24 - 5.44)	0.012
											OS	4.81 (2.02 - 11.44)	<0.001	OS	3.8 (1.77 - 8.15)	0.001
AR mut.	Wyatt [11]	Retrospective	ENZA	mixed	cfDNA	aCGH	65	14	20	39	PFS	3.94 (1.46 - 10.64)	0.007	-	-	-
AR mut.	Annala [18]	Prospective	ABI, ENZA	1st	cfDNA	NGS	202	14	86	64	TTP	1.02 (0.53 - 1.95)	0.950	TTP	0.82 (0.40 - 1.68)	0.581
AR mut.	Romanel [19]	Prospective	ABI	mixed	cfDNA	NGS	80	16	-	-	OS	7.33 (3.51 - 15.34)	<0.001	OS	6.85 (3.21 - 14.60)	<0.001
											PFS	3.73 (2.17 - 6.41)	<0.001	PFS	3.58 (1.92 - 6.69)	<0.001
AR mut.	Fettke [20]	Prospective	ABI, ENZA	mixed	cfDNA	NGS	41	12	67	52	PSA-PFS	1.00 (0.44 - 2.20)	1.000	-	-	-
											rPFS	0.90 (0.52 - 1.80)	0.080	-	-	-
											0S	1.00 (0.34 - 2.80)	0.900	-	-	-
		Prospective	ABI, ENZA, taxane	mixed	cfDNA	NGS	40	8	-	-	OS	1.60 (0.74 - 3.60)	0.200	-	-	-

AR= androgen receptor; ABI= abiraterone; ENZA= enzalutamide; CRPC= castration-resistant prostate cancer; PFS= progression-free survival; OS= overall survival; rPFS= radiographic progression-free survival; ddPCR= digital droplet PCR; qPCR= quantitative PCR; NGS= next-generation sequencing; aCGH= array comparative genomic hybridization; HR= hazard ratio; mut= mutation; cfDNA= cell-free DNA; TTP= time to progression; RR= response rate

Table 2
Published AR-V7 studies of CaP

Ref.	Type of study	Treatment	Therapy lines at analysis	Sample	Assay	Method	ΣN	CTC + (n)	AR-V7 + (n)	PSA RR (%)		Univariate analysis			Multivariate analysis		
										ARV7+	ARV7-	End-point	HR (95%)	P	End-point	HR (95%)	P
Antonarakis [21]	Prospective	ABI	mixed	CTC	AdnaTest	RT-PCR	36	31	6	0	68	PSA-PFS	16.10 (3.90 - 66.00)	<0.001	PSA-PFS	17.51 (3.53 - 87.03)	<0.001
												PFS	16.50 (3.30 - 82.90)	<0.001	PFS	5.25 (1.09 - 25.21)	0.038
												OS	12.70 (1.30 - 125.30)	0.006	OS	-	-
	Prospective	ENZA	mixed	CTC	AdnaTest	RT-PCR	35	31	12	0	53	PSA-PFS	7.40 (2.70 - 20.60)	<0.001	PSA-PFS	3.40 (1.43 - 8.08)	0.006
												PFS	8.50 (2.80 - 25.50)	<0.001	PFS	3.38 (1.35 - 8.46)	0.009
												OS	6.90 (1.70 - 28.10)	0.002	OS	-	-
Antonarakis [22]	Prospective	ABI, ENZA	mixed	CTC	AdnaTest	RT-PCR	202	149	36	13.9	52.2	-	-	-	PSA-PFS	2.90 (1.83 - 4.61)	<0.001
												-	-	-	PFS	2.49 (1.55 - 3.99)	<0.001
												-	-	-	OS	2.98 (1.66 - 5.32)	<0.001
Scher [23]	Prospective	anti-androgen	mixed	CTC	Epic Sciences	CTC IHC	128	128	16	-	-	OS	10.39 (2.10 - 51.47)	<0.001	-	-	-
		taxane	mixed	CTC		CTC IHC	63	63	18	-	-	OS	3.19 (1.45 - 7.02)	<0.001	-	-	-
Scher [24]	Prospective	anti-androgen	mixed	CTC	Epic Sciences	CTC IHC	128	128	16	-	-	OS	11.45 (5.67 - 23.82)	<0.001	-	-	-
		taxane	mixed				63	63	18	-	-	OS	3.74 (1.95 - 7.20)	<0.001	-	-	-
Scher [25]	Prospective	anti-androgen	mixed	CTC	Epic Sciences	CTC IHC	70	70	14	-	-	OS	1.67 (1.00 - 2.81)	0.050	-	-	-
		taxane	mixed				72	72	22	-	-	OS	0.62 (0.28 - 1.39)	0.250	-	-	-
Del Re M [26]	Prospective	ABI, ENZA	mixed	RNA	-	ddPCR	36	-	14	7	64	-	-	-	-	-	-
Qu [27]	Retrospective	ABI	mixed	RNA	-	ddPCR	81	-	27	-	-	TTF	-	-	TTF	1.31 (0.74 - 2.32)	0.353
												OS	-	-	OS	1.73 (0.83 - 3.60)	0.145
		ENZA	mixed	RNA	-	ddPCR	51	-	17	-	-	TTF	-	-	TTF	2.02 (1.00 - 4.00)	0.048
												OS	-	-	OS	2.08 (0.83 - 5.24)	0.119
Fettke [20]	Prospective	ABI, ENZA	mixed	cfRNA	-	NGS	37	-	6	57	57	PSA-PFS	2.10 (081 - 5.90)	0.100	-	-	-
												rPFS	2.40 (0.82 - 6.90)	0.100	-	-	-
												0S	3.50 (1.10 - 11.00)	0.030	-	-	-
	Prospective	taxane	mixed	cfRNA	-	NGS	22	-	5	50	50	PSA-PFS	2.00 (0.55 - 8.10)	0.300	-	-	-
												rPFS	2.10 (0.54 - 9.80)	0.300	-	-	-
												0S	0.79 (0.09 -6.70)	0.800	-	-	-
	Prospective	ABI, ENZA taxane	mixed	cfRNA	-	NGS	40	-	4	-	-	OS	2.50 (0.83 - 7.70)	0.100	-	-	-
To [29]	Prospective	ABI, ENZA	mixed	RNA	-	qPCR	37	-	7	57	66	-	-	-	-	-	-
Amstrong [30]	Prospective	ABI, ENZA	2nd	CTC	AdnaTest	RT-PCR	118	116	28	11	30	PFS	2.30 (1.50 - 3.50)	-	PFS	1.7 (1.00 - 2.90)	-
												OS	2.80 (1.70 - 4.50)	-	OS	3.30 (1.70 - 6.30)	-
	Prospective	ABI, ENZA	2nd	CTC	Epic Sciences	CTC IHC	118	107	11	0	26	PFS	2.20 (1.20 - 4.30)	-	PFS	2.10 (1.00 - 4.40)	-
												OS	3.10 (1.60 - 5.90)	-	OS	3.00 (1.40 - 6.30)	-
Taplin [31]	Prospective	GAL, ENZA	1st	CTC	AdnaTest	RT-PCR	953	315	73	28	-	-	-	-	-	-	-
Seitz [32]	Prospective	ABI, ENZA	mixed	RNA	-	ddPCR	85	-	15	0	50	-	-	-	PSA-PFS	6.99 (2.36 - 20.7)	<0.001
												-	-	-	PFS	2.33 (1.12 - 4.86)	0.020
Tagawa [33]	Prospective	taxane	1st	CTC	-	ddPCR	63	54	36	58	78	-	-	-	-	-	-
Belderbos [35]	Prospective	ABI, ENZA, taxane	mixed	CTC	-	RT-PCR	94	94	45	-	-	OS	1.33 (0.81 - 2.15)	0.250	-	-	-

mCRPC= metastatic, castration-resistant prostate cancer; RT-PCR= real-time PCR; ddPCR= digital-droplet PCR; CTC= circulating tumor cell; cfRNA= cell-free RNA; IHC= immunohistochemistry; NGS= next-generation sequencing; ABI= abiraterone; ENZA= enzalutamide; GAL − galaterone; PFS= progression-free survival; OS= overall survival; RR= response rate

screened for AR-V7, 73 (8%) patients were AR-V7 positive, 250 (26%) were AR-V7 negative and in 66% of patients ($n = 630$) no CTCs could be detected for AR-V7 analysis. The study was terminated because galeterone did not improve PFS [31].

A further experimental, digital droplet PCR assay is able to detect AR-V7 mRNA in whole blood. The first results are promising; 18% of 85 patients had high AR-V7 expression and these patients had shorter PSA-PFS and OS under ABI or ENZA treatment [32].

Interestingly, AR-V7 was found to be associated with response to taxanes. The TAXYNERGY study demonstrated, that the absence of AR-V7 in CTCs is associated with superior PSA response and PFS in docetaxel and cabazitaxel-treated mCRPC patients [33]. This may be explained by the previous observation that the nuclear translocation of AR proteins - which is a prerequisite for AR transcriptional activity - is microtubule-dependent and as such may be predictive of response to taxane treatment [34]. On the other hand, in an analysis with 124 DOC, ABI or ENZA pretreated patients; in CTC detected AR-V7 positivity (35%) was not prognostic for subsequent cabazitaxel treatment [35].

Overall, AR-V7 has been shown in many independent retrospective and prospective studies to be associated with poor PSA response and shorter PFS and OS in ABI/ENZA treated but not taxane treated patients. Therefore, AR-V7 analysis can be considered for clinical routine for the decision of second- or later-line therapies (ABI/ENZA or taxane) after prior treatment with next-generation androgen targeting therapies (ENZA or ABI).

3.5. Clinical studies for AR-V7 positive mCRPC patients

Several novel AR-V7 targeting agents are being developed to overcome anti-androgen resistance (Table 3). Onvansertib is a polo-like kinase 1 inhibitor that can inhibit the growth of AR-V7 positive CaP cells as well as in preclinical xenograft models. Currently, onvansertib in combination with ABI is being tested in a phase II clinical study (NCT03414034) for the treatment of mCRPC patients who progressed to ABI. [36]. Another potent AR-V7 inhibitor is niclosamide, which was tested in combination with ENZA in a phase I study (NCT02532114) and in combination with ABI in a phase II study (NCT02807805) which showed promising safety and efficacy results [37]. In addition, cabazitaxel is being tested in mCRPC patients with AR-V7 CTC in a phase II study (NCT03050866). A further ongoing study is testing Rad-223 in men with asymptomatic mCRPC, who progressed to ABI or ENZA therapy (NCT03002220) with the aim to confirm the association between Rad-223 activity and AR-V7 status. A currently ongoing phase II study evaluates the efficacy of ipilimumab plus nivolumab in mCRPC with AR-V7 positive CTCs (NCT02601014). Based on first results, ipilimumab plus nivolumab had poor clinical efficacy as only 2 of 15 patients had a PSA response of >50% [38].

Another strategy to inhibit the AR-V7 is the selective targeting the N-terminal domain (NTD) of AR. Currently, EPI-7386, a second generation NTD inhibitor, (NCT04421222). and ZEN-3694, a bromodomain extraterminal inhibitor are being tested in phase I and II studies (NCT02711956) [39].

4. Non-AR related resistance mechanisms

There are several AR independent mechanisms involved in ABI and ENZA resistance, such as neuroendocrine transdifferentiation of CaP, alterations of Wnt- and DNA repair-pathway, *TP53* and *RB1* mutations and glucocorticoid receptor overexpression (table 4).

4.1. Neuroendocrine-transdifferentiation and TP53 or RB1 loss

While pure neuroendocrine or small cell CaPs are rare, most prostatic adenocarcinomas include single neuroendocrine tumor cells in a scattered localization. These cells are less responsive to androgen-targeting therapies, therefore they may overgrow adenocarcinomas under ADT. This phenomenon is called treatment-related or treatment-emergent neuroendocrine transdifferentiation [40]. Neuroendocrine

Table 3
Clinical trials for treatment of CRPC patients with AR-V7 positive tumor

Treatment	Description	Disease	Trial phase	NCT number	Ref.
Onvansertib + ABI	polo-like kinase 1 inhibitor + CYP17A1 inhibitor	mCRPC	2	NCT03414034	Einstein [36]
Niclosamide + ENZA	AR-V7 inhibitor + AR antagonist	mCRPC	1	NCT02532114	
Niclosamide + ABI	AR-V7 inhibitor + CYP17A1 inhibitor	CRPC	2	NCT02807805	Pan [37]
Cabazitaxel	taxane	mCRPC with AR-V7 positive CTCs	2	NCT03050866	
Ipilimumab + Nivolumab	anti-CTLA4 + anti-PD1	mCRPC with AR-V7 positive CTCs	2	NCT02601014	Boudadi [38]
DOC + ENZA	taxane + AR antagonist	mCRPC	2	NCT03700099	
ENZA	AR antagonist	mCRPC	2	NCT02922218	
EPI-7386	AR-LBD inhibitor	mCRPC	1	NCT04421222	
ZEN-3694	AR-LBD inhibitor	mCRPC	1b/2a	NCT02711956	Aggarwal [39]

ABI= abiraterone; ENZA= enzalutamide; AR= androgen receptor; mCRPC= metastatic, castration-resistant prostate cancer; CTC= circulating tumor cell

Table 4
Non-AR related resistance markers

Marker	Ref.	Type of study	Treatment	Therapy lines at analysis	Sample	Assay	ΣN	Npos	PSA RR (%)		Univariate analysis			Multivariate analysis		
									Pos.	Neg.	End-point	HR (95%)	P	End-point	HR (95%)	P
CGA and NSE (>85 and >19ng/ml)	Heck [41]	Prospective	ABI	mixed	serum prot.	KRYPTOR and Cobas e602	45	36	-	-	PFS	3.65 (1.51 - 8.82)	0.004	PFS	2.92 (1.17 - 7.28)	0.022
											OS	7.82 (2.33 - 26.23)	0.001	OS	7.17 (1.62 - 31.70)	0.009
											PSA-PFS	2.92 (1.17 - 7.30)	0.022	PSA-PFS	2.80 (1.12 - 7.05)	0.028
CGA (>100ng/ml) NSE (>18ng/ml)	Fan [42]	Retrospective	ABI	2nd	serum prot.	ELISA	40	38	-	-	rPFS	13.99 (4.27 - 45.80)	<0.001	rPFS	1.02 (1.00 - 1.04)	0.041
											OS	32.70 (4.06 - 263.19)	0.001	OS	13.71 (1.03 - 183.31)	0.048
											PSA-PFS	29.19 (6.22 - 136.88)	<0.001	PSA-PFS	10.15 (1.57 - 65.87)	0.015
CGA (>360ng/ml) and AR gain	Conteduca [43]	Retrospective	ABI,ENZA	mixed	serum prot.	ELISA	197	22	33	74	OS	4.80 (2.90 - 8.60)	0.003	OS	2.62 (1.46 - 4.70)	0.004
											PFS	3.10 (3.10 - 5.30)	0.476	PFS	1.39 (0.80 - 2.42)	0.480
		Prospective	ABI,ENZA	mixed	serum prot.	ELISA	59	2	50	57	OS	4.00 (2.50 - 5.60)	0.289	-	-	-
											PFS	3.60 (2.50 - 4.60)	0.289	PFS	5.92 (0.51 - 68.61)	0.155
CGA (>81.29 ng/ml)	Szarvas [44]	Retrospective	ABI, ENZA	mixed	serum prot.	KRYPTOR	143	105	-	-	OS	2.266 (1.359 - 3.779)	0.002	OS	1.926 (1.009 - 3.677)	0.047
CGA and NSE (>85 and >19ng/ml)							143	11	-	-	OS	4.216 (2.188 - 8.121)	<0.001	OS	2.754 (1.225 - 6.191)	0.014
BRCA2/ATM truncated mut.	Annala [18]	Prospective	ABI, ENZA	1st	cfDNA	NGS	202	14	36.4	67	TTP	2.58 (1.58 - 4.21)	<0.001	TTP	1.44 (0.82 - 2.53)	0.205
TP53 single defect								66	63.5	66.2	TTP	2.70 (1.86 - 3.91)	<0.001	TTP	1.96 (1.23 - 3.11)	0.005
TP53 two or more defect								19	68.8	66.2	TTP	5.65 (3.14 - 10.17)	<0.001	TTP	3.40 (1.70 - 6.80)	<0.001
TP53 mut.	De Laere [46]	Prospective	ABI, ENZA	mixed	cfDNA	NGS	145	36	15.4	46.8	PFS	3.25 (2.14 - 4.92)	<0.001	PFS	1.88 (1.18 - 3.00)	0.008
TP53 mut/ loss	Torquato [47]	Prospective	ABI, ENZA	mixed	cfDNA	NGS	62	23	-	-	OS	3.19 (1.53 - 6.64)	0.002	OS	2.70 (1.27 - 5.72)	0.009
											PFS	1.33 (0.77 - 2.30)	0.314	PFS	1.26 (0.73 - 2.19)	0.406
PI3K pathway mut.							62	15	-	-	OS	2.92 (1.28 - 6.68)	0.011	OS	2.62 (1.12 - 6.1)	0.026
											PFS	1.77 (0.97 - 3.22)	0.064	PFS	1.40 (0.71 - 2.77)	0.327
WNT mut.	Velho [56]	Retrospective	ABI, ENZA	1st	DNA	NGS	137	15	53	75	OS	2.28 (1.15 - 4.53)	0.010	OS	2.27 (1.13 - 4.56)	0.021

CGA= chromogranin A; NSE= neuron-specific enolase; ABI= abiraterone; ENZA= enzalutamide; mCRPC= metastatic castration-resistant prostate cancer; TTP= time to progression; PFS= progression-free survival; Npos= number of marker positive / elevated cases; OS= overall survival; rPFS= radiographic progression-free survival; ddPCR= digital droplet PCR; NGS= next-generation sequencing; mut= mutation; prot= protein; cfDNA= cell-free DNA; RR= response rate

transdifferentiation of CaP is associated with aggressive tumor growth higher occurrence of *TP53, RB1* and Phosphatase and tensin homolog (*PTEN*) loss-of-functions mutations and secretion of neuroendocrine markers, such as chromogranin A (CGA) and neuron-specific enolase (NSE). Several studies showed that elevated serum CGA and NSE levels are promising tools for prediction and monitoring of ABI and ENZA therapies [41,42,43,44]. Heck *et al.* found that elevated CGA and NSE serum levels independently predict worse OS and PSA-PFS in ABI-treated patients [41]. In accordance, our study showed that high baseline CGA levels and its changes during treatment with ABI and ENZA are independently associated with poor OS [44]. Conteduca *et al.* analysed CGA levels in combination with AR alterations in ENZA or ABI-treated patient samples. They found that elevated CGA level and AR gain were independent predictors of poor OS and PFS [43]. Additionally, a current phase II study showed that taxane-carboplatin combination therapy provides a significantly longer PFS compared to cabazitaxel alone for mCRPC patients with aggressive variant CaP (with *TP53, RB1, or PTEN* loss), suggesting different therapeutic sensitivity for neuroendocrine transdifferentiated CaPs [45]. Further correlations were found between *TP53* mutation and resistance to anti-androgen therapy. In a study by Annala *et al.,* 19 of 65 patients had two or more *TP53* alterations and these patients had significantly shorter PFS under ABI/ENZA treatment [18]. Accordingly, De Laere *et al.* revealed that *TP53* gene defects were associated with worse PFS in ABI/ENZA-treated mCRPC patients [46]. Another prospective study showed that ABI/ENZA-treated patients with *TP53* loss or PI3K pathway defect to be associated with a worse OS [49].

RB1 genomic alteration are also associated with ABI/ENZA resistance. Abida *et al.* used whole-exome and transcriptome sequencing on 128 ABI/ENZA-treated patients and revealed that *RB1* mutations were significantly associated with poor OS [48]. In accordance, a current study showed that the combined loss of *TP53* and *RB1* is associated with a devastating clinical outcome, loss of androgen activity and therefore show resistance to androgen-targeting therapies. On the other hand, these subgroup may better respond to PARP and ATR inhibition [49]

4.2. DNA-repair alterations

DNA-repair gene alterations such as defects in Breast cancer 1/2 gene (BRCA2) or ATM genes were found to be associated with ABI/ENZA resistance. Annala *et al.* using whole-exome and deep targeted sequencing of plasma cfDNA of 202 treatment naïve mCRPC patient demonstrated worse outcome under subsequent ABI or ENZA treatment in patients with BRCA2 or ATM alterations [18]. Thus, the presence of DNA repair alterations seems to be a negative predictor of AR-targeting therapies, but on the other hand the same alterations are known to be positive predictors for poly(ADP-ribose) polymerase (PARP) inhibitor and also for platinum-based therapies [50,51,52]. Interestingly, DNA-repair alterations were found to be almost mutually exclusive with *TP53* and *RB1* loss-of-function mutations suggesting that these are two molecularly different CaP subtypes which are however common in their resistance to androgen-targeting therapies [40].

4.3. Wnt-pathway alterations

Furthermore, Wnt pathway alterations are associated with resistance to next-generation antiandrogen therapies. Gene expression profile analysis of ENZA resistant *vs.* sensitive CaP cells showed overexpression of Wnt pathway genes in ENZA resistant cells. In addition, inhibition of Wnt pathway led to decrease viability of ENZA resistant cells by enhancing apoptosis [53]. In a prospective study, Wang *et al.* showed that Wnt/β-catenin pathway activation and increased expression of cell cycle regulator genes have been associated with ABI resistance [54]. Chen *et al.* used whole-genome sequencing and whole-transcriptome RNA sequencing on 101 mCRPC patients' sample to identify key driver gene alterations of ENZA resistance. Based on gene set enrichment analysis, the most upregulated pathway was the Wnt/β-catenin pathway among ENZA resistance patients. Additionally, *CTNNB1* mutations were associated with a poor OS [55]. A further retrospective study found Wnt-pathway activating mutations (*CTNNB1* activating or *APC* or *RNF43* inactivating mutations) in 15 of 124 (11%) of ABI or ENZA treated patients and revealed a significant association between their presence and poor PFS and OS [56].

Currently, several targeted therapies are being tested in clinical trials for men with mCRPC who bear Wnt or PI3K pathway alterations [57]. Based on a current phase III trial (IPATential 150), Ipartesib, a PI3K pathway inhibitor in combination with ABI significantly improved OS and rPFS compared to ABI alone in mCRPC patients with an aggressive form of tumors carrying damaged *PTEN* gene [58]. This finding suggests that CaP patients present with PI3K pathway alterations may better benefit from a combination therapy rather than from ABI monotherapy.

4.4. Glucocorticoid receptor overexpression

In vitro and *ex vivo results* demonstrated that upregulation of glucocorticoid receptor (GR) is also associated with ENZA resistance [59]. Moreover, in preclinical models, Puhr *et al*. reported that GR is upregulated upon long-term ABI or ENZA treatment and GR upregulation can trigger antiandrogen resistance by bypassing AR blockade. Therefore, antiandrogen therapy in combination with GR inhibitor agents might be a potential therapy option for mCRPC patients in order to overcome antiandrogen resistance [60].

5. Limitations

We performed a systemic literature search for AR-associated resistance mechanisms. Majority of publications reports retrospective analyses with possible selection bias and generally low numbers of AR-altered cases, which often limits their statistical power. In addition, most of these papers include patients with mixed lines of therapies, which makes difficult to draw firm conclusions for their application. Therefore, in order to provide an objective overview, we summarized available case numbers and main results as well as data on pretreatments in our tables.

6. Conclusions

In the last few years, several new therapies with various mechanism of action became available for the treatment of mCRPC. Unfortunately, despite the relatively high initial response rates to next-generation antiandrogen therapies, most patients become resistant to these treatments. Resistances mechanisms can be classified in three main groups. One of the driving mechanisms of this resistance is directly associated with the alterations of the AR (Group Nr.1). Various AR point mutations have been described, however because of their low frequency largely limits their clinical potential. AR gain is a promising tool for treatment selection, however ongoing prospective randomized studies need to be completed before its recommendation in the clinical routine. AR-V7 is the most well established marker of this group with available commercial test methods, which have been already underwent prospective multicenter validation. As AR-V7 positive men had superior OS with taxanes compared to ABI/ENZA, AR-V7 can be considered to help guide selection of therapy after progression on ABI or ENZA in mCRPC.

It is important to note that other also non-AR-related mechanisms are significantly associated with ABI- and ENZA-resistance. The most important of them are neuroendocrine transdifferentiation (Group Nr.2) and DNA-repair pathway (BRCA/ATM/MRR) alterations (Group Nr.3). These types are found to be mutually exclusive, suggesting that these are two distinct subsets of CaP. Probably these later mechanisms are responsible for therapy resistance in those cases where no AR-alterations are detectable. Therefore, a panel of predictive markers covering various mechanisms is more likely able to adequately predict response to androgen targeting treatments.

Importantly, different non-AR related ABI/ENZA resistance mechanisms and/or markers are able to predict positive response to other therapies and therefore have significant therapeutic implications for both already approved and experimental drugs. For mCRPC patients with BRCA/ATM and other homologue recombinant repair gene mutations (10%−25% of mCRPC patients) PARP inhibitors have recently been approved and several retrospective studies showed that these patients may show exceptional response to platinum therapy. In addition, mCRPC patients present with mismatch repair gene alterations (occurring in 1-3% of CaP patients) may receive PD-1 inhibitor therapy. Therefore, alterations of the homologue and mismatch repair genes have already significant clinical relevance for the second-line treatment of mCRPC.

In addition, several clinical studies are underway aiming to find effective therapies for ABI/ENZA resistant patients. Neuroendocrine transdifferentiated (high-risk variants) mCRPC patients may benefit from a platinum-based therapy, while ABI/ENZA resistant patients with PI3K pathway alterations (with PTEN loss occurring in 40% of mCRPC) may respond to a PI3K pathway inhibitor treatment. All these developments projects that the therapeutic landscape of mCRPC will continue to evolve towards a molecular background-driven strategy, which requires the involvement molecular analysis in the clinical decision-making.

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