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AR-V7 and AR-FL expression is associated with clinical outcome: a translational study in patients with castrate resistant prostate cancer

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Abstract

Objectives: To investigate if full-length androgen receptor (AR-FL) is associated with resistance to AR-directed therapy independently and/or combined with AR splice variant 7 (AR-V7).

Patients and methods: Plasma samples were prospectively collected from 73 patients with CRPC before first or second-line AR-directed therapy. mRNA was isolated from exosomes and AR-FL and AR-V7 were analyzed by ddPCR.

Results: AR-FL was detected in all patients while 22% were AR-V7+ at baseline. AR-FL expression was significantly higher in AR-V7+ vs AR-V7- patients ($p<0.0001$). Stratifying patients by tertiles for AR-FL expression, PFS was 22 vs 18 vs 4 months for lower vs intermediate vs higher tertile, respectively ($p=0.0003$). Median PFS and OS were significantly longer in AR-V7- vs AR-V7+ patients (20 vs 4 months, $p<0.0001$; not reached vs 9 months, $p<0.0001$, respectively).

Conclusions: Resistance to AR-directed therapy is associated with the presence of AR-V7; however, AR-FL expression may help better refine response and survival of patients to AR-directed therapy. Both biomarkers, if validated in prospective trials, may be used to select the best treatment strategy.

Key words: AR-V7; AR-FL; CRPC; AR-directed therapy; predictive biomarkers

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Introduction

The androgen receptor (AR) is the major driver of prostate cancer growth and progression, and dysregulation of the pathway (i.e. amplification, point mutations and splice variants of the AR, as well as intracrine synthesis of androgens) may lead to a persistent AR signal transduction [1, 2]. Thus, AR targeting remains at the epicenter of drug development in prostate cancer. Abiraterone and enzalutamide have become the cornerstone of current treatment of castration resistance prostate cancer (CRPC), with abiraterone showing significant overall survival benefit in the hormone naïve setting as well. More recently, apalutamide was also approved by FDA for use in non-metastatic CRPC. Unfortunately, not all patients respond to hormonal therapy, and this represents a clear clinical need for the identification of predictive biomarkers of poor response to hormonal therapy.

In the last years, evidence accumulated suggesting that AR splice variant 7 (AR-V7) may be a biomarker of resistance to hormonal therapy and studies reporting its detection on tumor tissue, circulating tumor cells, extracellular vesicles and peripheral blood have been published [3-7]. Recently, the AR-full length (AR-FL) has also been suggested as a predictive biomarker of response, since the overexpression of the AR-FL was shown to convert prostate cancer growth from a castration-sensitive to a castration-resistant phenotype [8-10].

The present study was aimed at investigating whether the expression of AR-FL in exosomal-RNA is also a predictive biomarker of resistance to hormonal therapy on, in addition to AR-V7.

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Patients and methods

Patient selection

Metastatic patients with CRPC treated with anti-androgen therapy (enzalutamide or abiraterone) as per approved label, were enrolled in the present retrospective translational study. Inclusion criteria allowed the enrolment of patients with: histologically confirmed prostate adenocarcinoma, progressive disease despite castration levels of serum testosterone (<500 ng/l) while on stable androgen-deprivation therapy with documented metastases, confirmed by computed tomography or/and technetium-99 bone scans. Patients must have had at least three increasing serum PSA values taken at least 2 weeks before the last value of at least 2.0 ng/ml, consistent with the Prostate Cancer Working Group-2 guidelines. Prior taxane-based chemotherapy was permitted. Prior AR-directed therapies (abiraterone before enzalutamide or enzalutamide before abiraterone) were exclusion criteria. The study was approved by the Ethics Committee of Pisa University Hospital and conducted in accordance with the principles of the Declaration of Helsinki. All patients gave their signed informed consent before blood collection and data analysis.

Plasma collection, extracellular vesicle isolation, RNA extraction and AR analysis

Six ml of blood were collected in EDTA tubes before the start of abiraterone or enzalutamide (baseline) and was centrifuged at 1900 g for 10 min at 4°C within 2 h after drawing. Plasma was stored at -80 °C until analysis. Plasma samples were then centrifuged again at 1900 g for 15 min to remove cellular debris. Exosome isolation from plasma and RNA extraction was performed using the exoRNeasy kit (Qiagen, Valencia, CA) as previously described [5].

The analysis of AR-FL and AR-V7 in RNA was performed by ddPCR using the One-Step RT-ddPCR kit, as previously published [5]. The ddPCR QuantaSoft software determined the

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absolute target concentration as copies/ml in the samples. AR-FL and AR-V7 were considered positive if at least 3 droplets were amplified.

Statistical analysis

Categorical variables, such as ECOG performance status, tumor stage and Gleason score at diagnosis, type of first line and second line treatments, presence of bone, lymph node and visceral metastases, were described by absolute and relative frequencies, whereas quantitative factors as time from diagnosis to start chemotherapy and hormonal therapy and baseline total PSA level by median and range.

To evaluate the normality of the quantitative data distributions, the Kolmogorov-Smirnov test was performed. Changes in AR-FL and AR-V7 were analyzed with Mann-Whitney test (two tailed). Radiological progression free survival (rPFS) curves were created by the Kaplan-Meier method; log-rank test was used to evaluate differences between curves and hazard ratio was calculated to compare cumulative risks. For AR-FL analysis, patients were grouped by tertiles, to ensure approximately 15 events per group. Differences were considered significant at $p<0.05$.

All statistical analyses, descriptive and inferential, were performed with SPSS version 24 (SPSS Inc. SPSS® Chicago, IL, USA).

Results

AR status analysis

Detailed clinical characteristics of patients are reported in table 1. A total of 73 patients affected by CRPC were prospectively enrolled in this study. Overall, AR-FL was detected in all patients (range: 90 – 21,500 copies/ml, median: 700 copies/ml); 16 patients (22%) were AR-V7+ (range: 80 – 700 copies/ml, median: 310 copies/ml) before the start of abiraterone or

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enzalutamide. Considering the cohort of patients receiving abiraterone or enzalutamide as first line, AR-FL range was 90 – 8700 copies/ml (median: 570 copies/ml) and AR-V7 range was 80 – 500 copies/ml (median: 175 copies/ml). In the cohort of patients receiving abiraterone or enzalutamide as second line, AR-FL range was 100 – 21,500 copies/ml (median: 700 copies/ml) and AR-V7 range was 90 – 700 copies/ml (median: 360 copies/ml). Comparing the exosomal expression of AR-FL in the overall population stratified as AR-V7+ vs AR-V7-, there was a significantly higher level of AR-FL in patients AR-V7+ vs AR-V7- (6,700 vs 460 copies/ml, $p<0.0001$; Fig. 1A). The analysis of the copies/ml of AR-FL in patients AR-V7+ vs AR-V7- divided as per line of therapy (first and second line) demonstrated that the amount of AR-FL was significant in patients treated both as first or second line (first line 1,000 vs 460 copies/ml, $p=0.007$, Fig. 1B; second line 8,700 vs 380 copies/ml, $p=0.0003$, Fig. 1C). Furthermore, there was a significant increase in AR-FL levels in AR-V7+ patients treated as second line (8,700 vs 1,000 copies/ml, $p<0.0001$; Fig. 1D). The scatter plot depicting the correlation between AR-FL vs AR-V7 expression ($r=0.581$; $p<0.0001$) is shown in Fig. 2. A comparative analysis of AR-FL levels in AR-V7- patients was made, showing no differences between first and second line of treatment ($p=0.686$; Fig. 1 Suppl.). In the univariate model, the tumor volume considered as number and localization of metastasis was not correlated with the amount of AR-V7+ or AR-FL ($p>0.05$).

Clinical outcomes according to the AR status

Both the PFS (median 20 vs 4 mo, $p<0.0001$; Fig. 3A) and the OS (median not reached vs 9 mo, $p<0.0001$; Fig. 3B) were longer in AR-V7- vs AR-V7+ patients. Stratifying patients based on their AR-directed therapy, 46 patients received abiraterone and 27 enzalutamide. In both the abiraterone and the enzalutamide groups, the PFS and the OS were longer in AR-V7- vs AR-V7+ patients. In detail, for the abiraterone group the median PFS was 22 vs 3.5

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mo (p<0.0001; Fig. 2A Suppl.) and the median OS was not reached vs 9 mo (p<0.0001; Fig. 2B Suppl.); in the enzalutamide group the median PFS was 16 vs 4 mo (p<0.005; Fig. 2C Suppl.) and the median OS was not reached vs 6.5 mo (p<0.005; Fig. 2D Suppl.). The overall proportion of patients who achieved a PSA response (PSA RR) during abiraterone or enzalutamide was 61.6%. In AR-V7+ patients, the PSA RR was 25%, while in the AR-V7- population it reached 70%. To evaluate the role of AR-FL, independently of AR-V7 status, patients were divided by tertiles based on their AR-FL expression.

PFS was 22 months in patients within the lower tertile (≤400 copies/ml), 18 months in patients with intermediate AR-FL expression (401-899 copies/ml) and 4 months in patients with the higher AR-FL expression (≥900 copies/ml, p=0.014, Fig. 4A). With respect to OS, subjects with AR-FL ≤400 and 401-899 copies/ml had an OS of not reached vs 13 months for patients in the lower tertile (≥900 copies/ml) (p<0.0001; Fig. 4B). In the abiraterone group the median PFS was 22 months in patients within the lower tertile, 20 months in patients with intermediate AR-FL expression and 7 months in patients with the higher AR-FL expression (p=0.023, Fig. 3A Suppl) and the median OS was not reached in patients within the lower tertile, 27 months in patients with intermediate AR-FL expression and 13 months in patients with the higher AR-FL expression (p=0.058, Fig. 3B Suppl). Similarly, in the enzalutamide group the median PFS was 16 months in patients within the lower tertile, 15 months in patients with intermediate AR-FL expression and 4 months in patients with the higher AR-FL expression (p=0.007, Fig. 3C Suppl) and the median OS was not reached in patients within the lower and the intermediate AR-FL expression vs 5 months in patients with the higher AR-FL expression (p=0.003, Fig. 3D Suppl). The PSA RR in patients with AR-FL ≤400, 401-899 and ≥900 copies/ml were 76%, 67%, and 32%, respectively.

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Figure 5 reports the distribution of AR-V7+ and AR-V7- patients in the different groups stratified on the basis of AR-FL. Seven patients carrying a high AR-FL expression (≥900 copies/ml) without AR-V7, had a PFS comparable with patients AR-V7-, being >10 months in all patients but one. On the contrary, 4 out of 16 AR-V7+ positive patients had AR-FL expression <900 copies/ml and their PFS was predicted by their AR-V7 status, being <10 months. In figure 5 are also reported PFS outcomes according to AR status, highlighting the role of the presence of AR-V7. Univariate Cox proportional hazard ratio was used to assess the effect of AR-V7 and AR-FL status on the prediction of time-to-event outcomes. In the univariate model, known risk factors for progression such as Gleason score (≤7 vs >7), age, presence of metastasis, metastasis localization, LDH were analysed. None of these variables were correlated with worse PFS.

Considering that the assessment of the AR-V7 in patients who received the AR-directed therapy as second line was made at the time of progression of the disease after taxanes-based treatment, the PFS of taxanes was calculated stratifying patients based on their AR status, concluding that PFS in patients treated with taxanes was not influenced by AR-V7 (median 10 vs 8 mo in AR-V7- vs AR-V7+, p=0.9) or AR-FL status (median 11 vs 10 vs 8 mo in AR-FL tertiles, p=0.8).

Discussion

The present study shows that the expression AR-FL and AR-V7 is associated with response to abiraterone or enzalutamide in patients affected by CRPC. Since AR signaling is a major driver in CRPC, several studies evaluated if AR expression could be a predictive biomarker of resistance to hormonal therapy [11-13]. Published works conducted with IHC or FISH indicated that AR is expressed in both hormone-naïve and refractory tumors [14-16].

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However, data are controversial about the level of expression of AR [17], since it has been found to be highly heterogeneous, ranging from low to high [12]. Palmberg and colleagues demonstrated that patients with CRPC with AR amplification have a good response to hormonal therapy after first-line androgen deprivation therapy [18]. On the contrary, liquid biopsy data showed that AR amplification is associated with resistance to enzalutamide and abiraterone [19, 20]. The heterogeneous expression of AR has been also confirmed by our results, which also provided evidence that high AR-FL expression was only seen in AR-V7+ patients. Similar results were found in other studies, which demonstrated that AR amplification in bone metastases by CRPC is associated with increased expression of AR, with AR-V7 presence and poor survival [21, 22]. Hörnberg et al, demonstrated that AR-V7 transcript levels seem to be associated with high nuclear AR immunostaining scores, cell cycle dysregulation, high c-MYC and CDK1 activity, and poor prognosis in patients with CRPC [1].

Our results suggest that AR-FL plays an important role in hormonal resistance and may help stratify patients likely to respond to abiraterone/enzalutamide. Interestingly, based on the different expression levels of the AR-FL, it seems possible to identify responders vs unresponsive patients, belonging to lower vs higher tertiles, respectively, but it is also possible to select an intermediate population of subjects who may benefit from AR-directed therapy if AR-V7+ and chemotherapy-unfit. In fact, in these group of patients the hormonal treatment represents a viable option, since they may have a moderate-long response (PFS: 18 months). This hypothesis is also demonstrated by the OS curve, since the intermediate tertile overlaps with the lower one (see proposed flow-chart; Fig. 6).

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On the other hand, since the majority of patients with high AR-FL expression were AR-V7+, AR-FL over-expression may lead to hormone resistance because of the presence of the constitutively active AR-V7. The analysis of few discordant data of our population (patients with AR-FL ≥900 copies/ml without AR-V7 and patients AR-V7+ with AR-FL <900 copies/ml), demonstrated that AR-V7 has the higher impact on response to therapy.

Although the detection of the AR-V7 variant is easier to translate into the clinical management of patients, since only its presence or absence should be demonstrated as opposed to the need of identification of a cut-off value for AR-FL, this study suggests that resistance to AR-directed therapy is better predicted by the availability of both AR-FL and AR-V7 status; therefore, if validated in prospective trials, both biomarkers may support a rational clinical decision.

Competing interest: MDR received honoraria for participation in advisory boards and speakers' bureau of Sanofi and Astellas Pharma. RD received an unrestricted research grant from Sanofi. The other Authors declare no competing interests.

Authors' contributions: MDR, SC, AS, ER, FP, GG; LG, AF, RM, CP, EE, RvS, GJ, RD made substantial contributions to conception and design. MDR, SC, ER, GG, RvS, GJ, RD made substantial contributions to the laboratory analysis and interpretation of data; AS, FP, LG, AF, CP, EE, RD made substantial contributions to the acquisition of clinical data and their interpretation. MDR, SC, AS, ER, FP, GG, LG, AF, RM, CP, EE, RvS, GJ, RD have been involved in drafting the manuscript. RD, RvS, GJ, EE, CP, AF have been involved in revising the paper critically for important intellectual content. RM, SC were responsible for the statistical analysis. All the authors gave their final approval of the version to be published. Each author participated sufficiently in the work to take public responsibility for appropriate

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portions of the content. All authors agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. RD is responsible for the financial support for the project leading to this publication.

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Figure legends

Figure 1. Box-plot graph according to AR-FL and AR-V7 status in all prostate cancer patients (A), in patients treated with first-line (B) and second-line hormonal therapy (C), and in first- vs second-line hormonal therapy (D). Data are shown as median values (lines), 25th percentile to the 75th percentile (boxes) and ranges (whiskers).

Figure 2. Scatter plot depicting the linear correlation between AR-FL and AR-V7 expression (copies/ml).

Figure 3. Progression-free survival (PFS) of AR-V7+ versus AR-V7- patients (A); overall survival of AR-V7+ versus AR-V7- patients (B).

Figure 4. Progression-free survival (A) and overall survival (B) on the basis of expression levels of AR-FL divided by tertiles (≤400; 401-899; ≥900).

Figure 5. Distribution of AR-V7+ and AR-V7- patients in three groups stratified on the basis of AR-FL expression levels and PFS outcomes according to AR status.

Figure 6. Proposed flow-chart of clinical decision making based on AR analysis. Highlighted in red the two groups of patients for whom the knowledge of AR-FL expression allows a better definition of therapeutic approach in AR-V7- patients. Legend: Abi/Enza: abiraterone/enzalutamide.

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Supplementary Figure 1. Box-plot graph according to AR-FL in AR-V7- in patients treated with first- vs second-line hormonal therapy. Data are shown as median values (lines), 25th percentile to the 75th percentile (boxes) and ranges (whiskers).

Supplementary Figure 2. Progression-free survival (PFS) and overall survival (OS) of AR-V7+ versus AR-V7- patients in abiraterone (A and B, respectively) and enzalutamide (C and D, respectively) groups.

Supplementary Figure 3. Progression-free survival (PFS) and overall survival (OS) on the basis of expression levels of AR-FL divided by tertiles (≤400; 401-899; ≥900) in abiraterone (A and B, respectively) and enzalutamide groups (C and D, respectively).

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Table 1. Clinical characteristics of patients.

Baseline characteristics	**Patients treated with hormonal therapy as first-line treatment (n=46)**	**Patients treated with hormonal therapy as second-line treatment (n= 27)**
ECOG performance status, number (%) 0 1 2	 30 (65%) 16 (35%) 0	 20 (74%) 6 (22%) 1 (4%)
Time from diagnosis to start of abiraterone or enzalutamide, median (range), months	44 (11-262)	0
Time from diagnosis to start of first-line chemotherapy, median (range), months	0	45 (5-156)
Time from diagnosis to start of second-line hormonal therapy, median (range), months	0	73 (4-225)
Tumour stage at diagnosis, number (%) T1/2 N0 M0 T3/4 N0 M0 any T N1 M0 any T any N M1 Unknown	 6 (13%) 6 (13%) 10 (21.8%) 10 (21.8%) 14 (30.4%)	 3 (11.1%) 7 (25.9%) 4 (14.8%) 11 (40.8%) 2 (7.4%)
Gleason score at diagnosis, number (%) ≤ 7 ≥ 8 Unknown	 20 (43.5%) 22 (47.8%) 4 (8.7%)	 14 (52%) 10 (38%) 2 (10%)
Type of primary treatment Surgery Radiotherapy None	 25 (54.3%) 5 (10.9%) 16 (34.8%)	 15 (55.6%) 0 12 (44.4%)
First-line treatment Abiraterone Enzalutamide Docetaxel	 30 (65%) 16 (35%) 0	 0 0 27 (100%)
Second-line treatment Abiraterone Enzalutamide	 0 0	 16 (60%) 11 (40%)
Presence of bone metastases No Yes <6 6-20	 12 (26.1%) 34 (73.9%) 18 (53%) 16 (47%)	 3 (11.1%) 24 (88.9%) 16 (6.7) 8 (33.3)
Presence of lymph node metastases No Yes <3 3-6 7-10 >10	 18 (39.1%) 28 (60.9%) 9 (32.1%) 11 (39.3%) 4 (14.3) 4 (14.3)	 5 (18.5%) 22 (81.5%) 8 (36.4%) 10 (45.4%) 2 (9%) 2 (9%)

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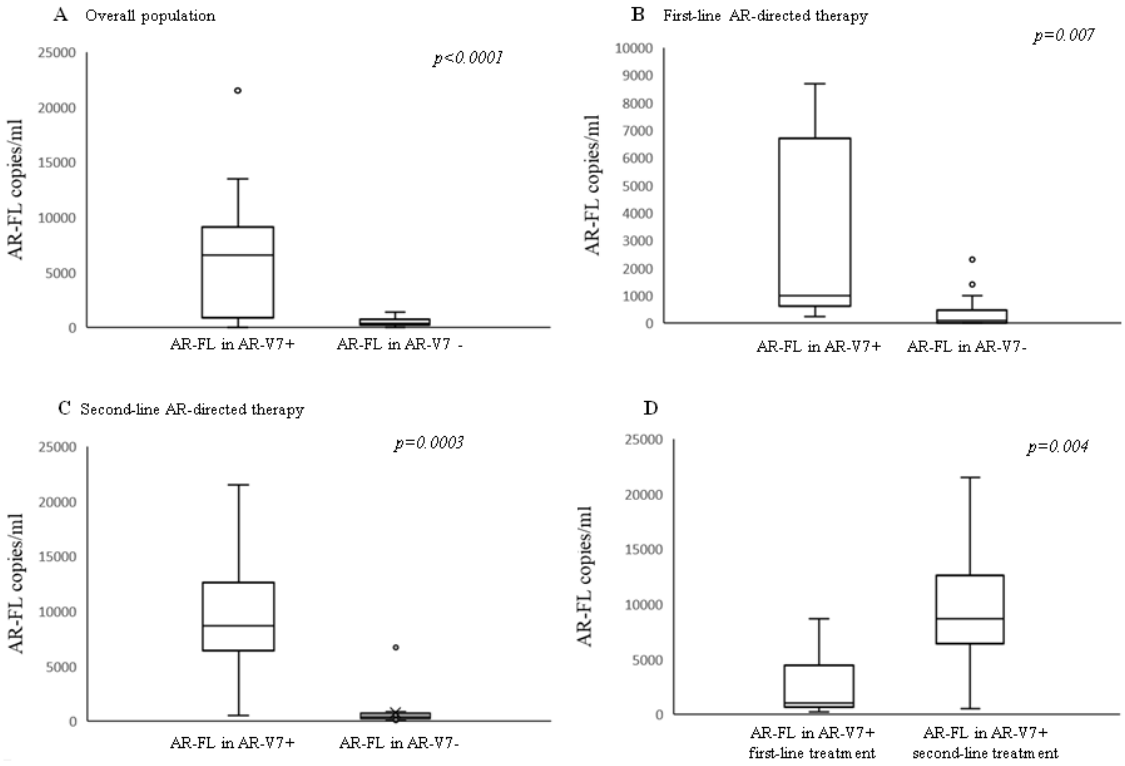
Presence of visceral metastases No Yes Lung Liver Lung and Liver	 44 (95.7%) 2 (4.3%) 1 (50%) 0 (0%) 1 (50%)	 23 (85.2%) 4 (14.8%) 1 (25%) 2 (50%) 1 (25%)
Baseline total PSA level (ng/mL), median (range)	8.7 (0.63-106)	26 (1.33-569.30)



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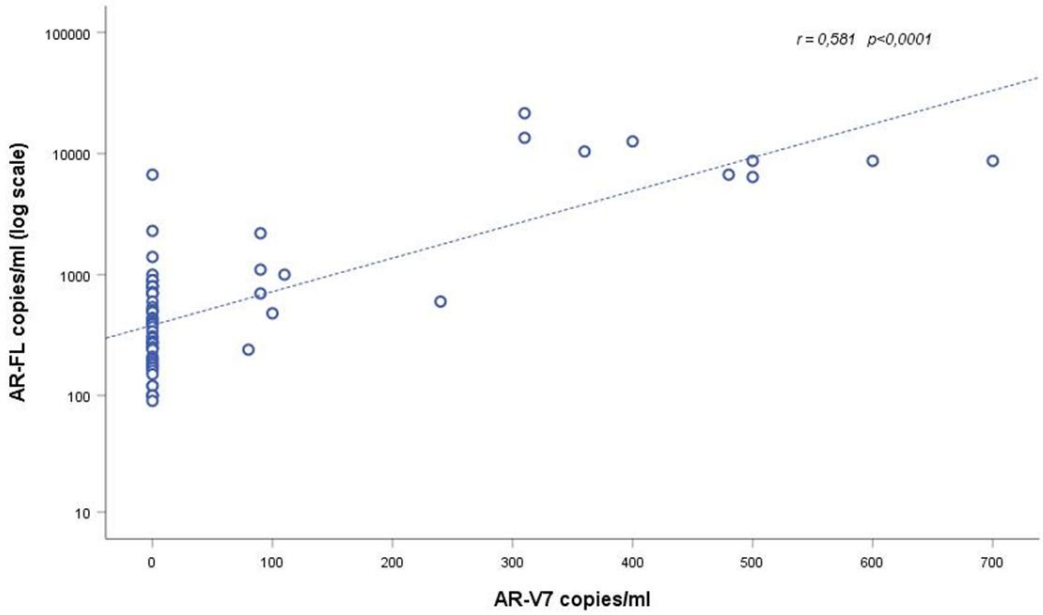

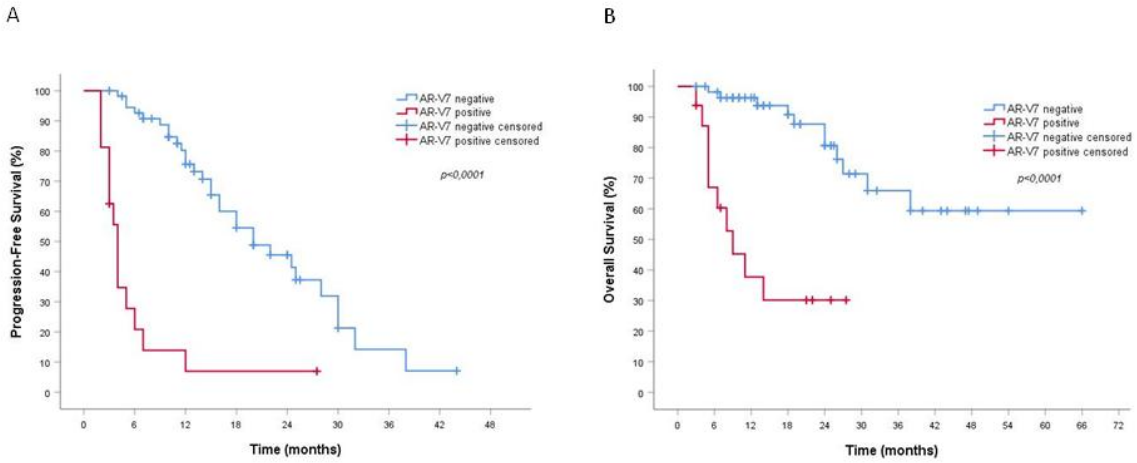

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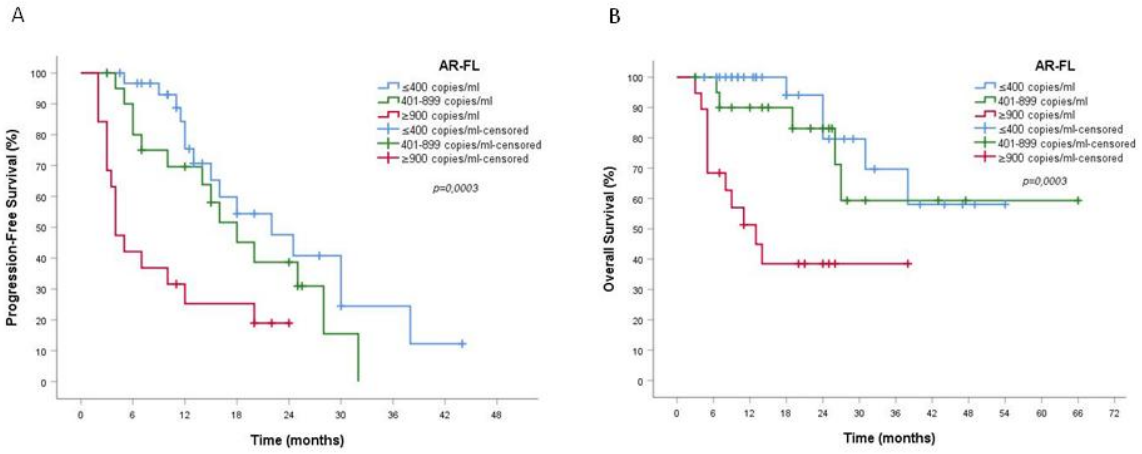

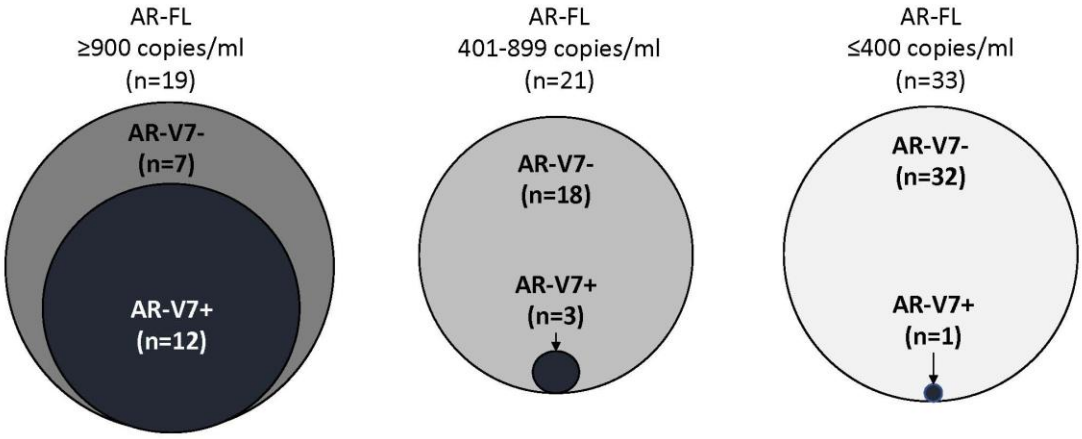

AR *status*	AR-FL high (≥900 copies/ml)	AR-FL intermediate (401-899 copies/ml)	AR-FL low (≤400 copies/ml)
AR-V7+	PFS = 3 months	PFS = 4 months	PFS = 18 months
AR-V7-	PFS = 20 months	PFS = 20 months	PFS = 25 months

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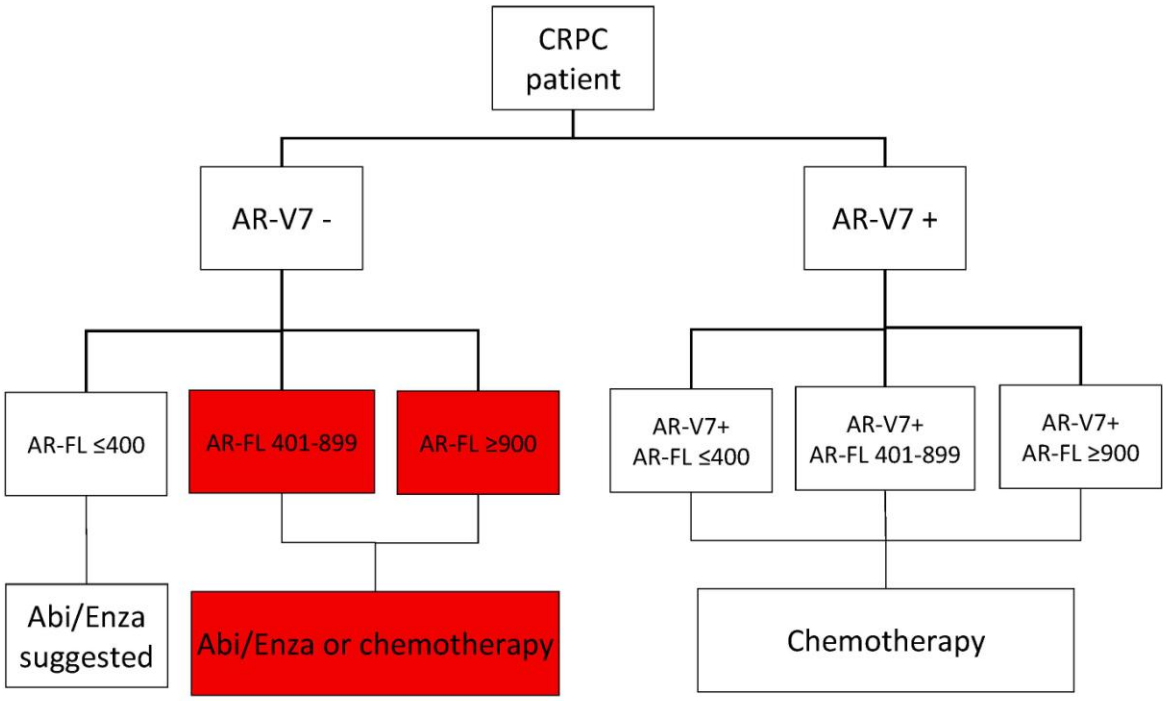

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