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The influence of treatment sequence in the prognostic value of *TMPRSS2-ERG* as biomarker of taxane resistance in castration-resistant prostate cancer

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Novelty and Impact

TMPRSS2-ERG alteration results in ERG overexpression which impairs taxanes activity. Previously, it has been described that *TMPRSS2-ERG* mRNA detection in blood is associated with shorter docetaxel response in metastatic castration resistant prostate cancer. Here, authors evaluated its role as a prognostic marker in tumour biopsies. They found that administering abiraterone/enzalutamide (A/E) therapy prior to taxanes affects the prognostic capacity of *TMPRSS2-ERG* in tumour, maintaining its prognostic role in those patients who did not receive A/E prior to taxanes.

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ABSTRACT

TMPRSS2-ERG expression in blood has been correlated with low docetaxel benefit in metastatic castration-resistant prostate cancer (mCRPC). This multicentre study aimed to prospectively asses its role as a taxane-resistance biomarker in blood and retrospectively in tumours, exploring also the impact of prior abiraterone/enzalutamide (A/E) in patients and *in vitro*. *TMPRSS2-ERG* was tested by quantitative reverse-transcription PCR. We included 204 patients (137 blood and 124 tumour samples) treated with taxanes. *TMPRSS2-ERG* expression was correlated with prostate-specific antigen (PSA)-progression-free survival (PFS), radiological-PFS (RX-PFS), and overall survival (OS). Independent association with survival was evaluated by multivariate Cox modelling. *In vitro* ERG knockdown and combinatorial and sequential experiments with enzalutamide and docetaxel were performed in VCaP cells. Prior A/E (HR 1.8, 95%CI 1.2-2.8) and blood *TMPRSS2-ERG* detection (HR 2, 95%CI 1.1-3.7) were independently associated to lower PSA-PFS. In patients without prior A/E, blood and tumour *TMPRSS2-ERG* independently predicted lower PSA-PFS (HR 3.3, 95%CI 1.4-7.9 and HR 1.8, 95%CI 1.02-3.3, respectively) to taxanes. When prior A/E was administered, *TMPRSS2-ERG* was not associated with outcome. There was a significant interaction between blood *TMPRSS2-ERG* and prior A/E related to PSA-PFS (P=0.032) and RX-PFS (P=0.009). *In vitro* stable *ERG* inhibition did not sensitize VCaP cells to docetaxel. Concomitant enzalutamide and taxanes were synergistic, but prior

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enzalutamide reduced docetaxel cytotoxicity in VCaP cells. Enzalutamide induced the expression of neuroendocrine markers and reduced that of E-cadherin. We conclude that prior hormone-therapy may influence taxanes response and *TMPRSS2-ERG* prognostic value. Thus, multiple and sequential biomarkers are needed in CRPC follow-up evaluation.

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INTRODUCTION

Taxanes are the most active chemotherapy agents and demonstrated survival benefit in metastatic castration resistant prostate cancer (mCRPC).[1-3] Despite known resistance mechanisms no molecular biomarkers to predict taxane resistance are clinically available.

TMPRSS2-ERG rearrangement is a prostate cancer-specific genetic alteration present in about 50% of prostate cancers. It leads to overexpression of ERG,[4] which binds to soluble tubulin impairing taxane sensitivity in preclinical models.[5] In prior work, we found a correlation with low treatment benefit when *TMPRSS2-ERG* mRNA was detected in peripheral blood mononuclear cells (PBMCs) of patients with mCRPC before docetaxel administration.[6] As *TMPRSS2-ERG* occurs in early prostate cancer and persists in metastatic disease,[7] we hypothesized that *TMPRSS2-ERG* may predict ulterior taxane-resistance when detected in early tumour biopsies of mCRPC patients.

ERG overexpression resulting from *TMPRSS2-ERG* alteration is regulated by the androgen receptor (AR) though direct bound to the ETS domain of the ERG protein. At the same time, ERG overexpression decreases AR transcripts in several cell lines models.[8] This auto-regulatory loop tightly controls levels of both transcription factors and may be affected by hormonal treatments, modifying the role of ERG protein in taxanes effectiveness and the potential role of *TMPRSS2-ERG* as a biomarker of resistance.

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This multicentre study prospectively assessed the role of blood *TMPRSS2-ERG* as a biomarker of taxane resistance and retrospectively studied its predictive value in tumours. Moreover, we explored the impact of prior abiraterone/enzalutamide (A/E) in mCRPC patients and *in vitro.*

MATERIALS AND METHODS

Study design and patients

This is a multicentre prospective study to evaluate the taxanes-response prognostic value of *TMPRSS2-ERG* mRNA in pre-taxanes PBMCs and in a retrospective set of formalin-fixed paraffin-embedded (FFPE) samples. Institutional Ethics Committees approved the study at all participating centres. The inclusion criteria were: 1) patients with mCRPC diagnosis that received systemic docetaxel or cabazitaxel therapy as standard clinical practice;[9] 2) patients with available tumour or with the possibility of extracting PBMCs samples for molecular analysis. Patients were treated with docetaxel (75mg/m^2 iv every 3 weeks) or cabazitaxel (25mg/m^2 iv every 3 weeks, or, 10mg/m^2 iv weekly), both in association with prednisone, 10 mg/day p.o.

The blood series included updated data from 50 patients from a prior publication[6] and 71 new patients treated in the various participating centres.

Primary endpoint was radiological (RX)-progression free survival (PFS). The estimated sample size for the blood study was of at least 60 patients

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(considering a 20% *TMPRSS2-ERG* positivity), to detect a hazard ratio (HR) of 3 (alpha error 0.05 and beta of 0.1, power of 90%) for RX-PFS. For the tissue study, the estimated sample size was 94 to detect HR=3 (alpha error 0.05 and beta 0.1) for RX-PFS. Secondary endpoints included prostatic-specific antigen (PSA)-PFS, and overall survival (OS).

Samples collection and RNA extraction

Before treatment initiation, peripheral blood samples (10ml) were collected in Monovette EDTA–containing Vacutainers (Sarstedt). A prior tube with 5ml of blood was extracted and discarded to avoid epithelial contamination during venepuncture. Samples were kept at 4ºC for up to 2 hours until processing. Blood specimens were layered onto 10mL of Ficoll-Paque (GE Healthcare Life Sciences). After centrifugation, PBMCs were isolated and total RNA was extracted using TRI-Reagent (Thermo Fisher Scientific) according to manufacturer's instructions. RNA was quantified by ND-1000 Spectrophotometer (Nanodrop Technologies).

Regarding FFPE samples, haematoxylin- and eosin-stained sections from tumours and adjacent tissues were prepared to confirm the histological diagnosis. A representative tumour area was selected and, depending on its size, at least 2 and up to 12 sections of 10µm thickness were cut and used for RNA isolation. Total RNA was isolated using the RecoverAll Total Nucleic Acid Isolation Kit (Life Technologies) according to manufacturer's protocol. RNA was quantified by ND-1000 Spectrophotometer (Nanodrop Technologies).

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Patients' follow-up and response evaluation

PSA levels were measured every 3 to 6 weeks, and computed tomography (CT) and/or bone scan were performed every 2 to 4 months or when clinically indicated. PSA response rate was defined as at least 50% decrease in PSA from baseline (maintained >4 weeks) at any time after treatment initiation. PSA-PFS, RX-PFS, and OS were calculated from the date of taxanes initiation to PSA progression, radiologic progression, and death or last follow-up visit, respectively. Treatment response criteria and progressive disease definition followed Prostate Cancer Working Group 2 criteria.[9] Briefly, PSA progression was defined as at least 25% increase above the nadir and confirmed three weeks later. RX progression was defined as an occurrence of two or more new bone lesions on bone scanning or progression by Response Evaluation Criteria in Solid Tumors (v1.1) in CT scanning.[10] Patients were prospectively followed from inclusion until death or last visit.

Quantitative reverse-transcription PCR (qRT-PCR)

Half ug of total RNA was reverse-transcribed using the High Capacity cDNA Archive Kit (Thermo Fisher Scientific) following manufacturer's instructions. *TMPRSS2-ERG* and the housekeeping gene *GUSB* were pre-amplified for 14 cycles, following manufacturer instructions for the TaqMan PreAmp Master Mix Kit (Thermo Fisher Scientific), except that the final volume of the reaction was reduced to 12.5µl. QRT-PCR was performed in a StepOne Plus Real-Time PCR system (Life Technologies) according to manufacturer's recommendations. Data

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were acquired using SDS Software 1.4. Amplification reactions were performed in duplicate. Expression values were based on the quantification cycle (Cq) from target genes relative to the Cq of *GUSB* endogenous gene. Commercial primers and probes were used to amplify *TMPRSS2-ERG* and *GUSB* genes (Hs03063375_ft and Hs99999908_m1, respectively; Thermo Fisher Scientific). We used thresholds of 0.1 and 0.2 for *TMPRSS2-ERG* and *GUSB,* respectively, to record Cq data.

TMPRSS2-ERG categorization

Patients were categorized as *TMPRSS2-ERG* positive when both *TMPRSS2-ERG* and the housekeeping gene *GUSB* were detectable (Cq ≤ 35) in duplicate reactions. Patients were considered *TMPRSS2-ERG* negative when *GUSB* was detectable but not *TMPRSS2-ERG* (undetermined Cq).

***In vitro* experiments**

The human prostate carcinoma cell line VCaP was purchased from ATCC (CRL-2876) and cultured in Dulbecco's Modified Eagle's Medium (ATCC) supplemented with 10% fetal bovine serum (FBS). Taxanes, docetaxel and cabazitaxel, and enzalutamide (Selleckchem) were dissolved at 10 mM and 100nM in DMSO, respectively.

Gene expression studies in cell lines were performed based on RNA extraction using TRI-Reagent (Thermo Fisher Scientific). One million of cells were seeded per well and treated after 24h with the corresponding drugs. QRT-PCR was

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performed as explained above. Commercial primers and probes were used to amplify *TMPRSS2-ERG, ERG, KLK3, MYCN, CHGA, SYP, CDH1* and *GUSB* genes (Hs03063375_ft, Hs01554634_m1, Hs03063374_m1, Hs00232074_m1, Hs00900375_m1, Hs00300531_m1, Hs01023895_m1 and Hs99999908_m1, respectively; Thermo Fisher Scientific). Primers and probe for *ARV7* detection were: forward: 5'-gaaatgttatgaagcagggatgact-3', reverse: 5'-ggtcattttgagatgcttgcaa-3' and probe 5'-tgggagaaaaattccgg-3'. We used the threshold of 0.1 to record the quantification cycle of *ERG*, *CHGA*, *MYCN* and *ARV7* expression, and 0.2 for the rest of the genes. Relative quantification was performed.

Whole-cell extracts were prepared and Western blot performed as described previously.[11] Monoclonal antibodies used were ERG (EPR3864, ab92513 Abcam), SYP (D35E4, Cell Signalling), CDH1 (#4065 Cell Signalling), MYCN (D4B2Y, Cell Signalling), PSA (D6B1, Cell Signalling), ARV7 (AG10008, Precision antibody) and GAPDH (Ref. AM4300, Ambion). Odyssey fluorescence system was used to detect protein signals.

Cytotoxicity of drugs alone or combined was evaluated by using the Cell Titer 96 Aqueous One Solution Cell Proliferation Assay kit (Promega) according to manufacturer's instructions. Briefly, $1x10^4$ cells were seeded per well at 96-wells plated and treated 24 hours after. Cell viability was evaluated after 72h of treatment. For synergy quantification of drug combination experiments Chou-Talalay method was used.[12]

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Sequential experiments were performed by seeding $1x10^4$ cells per well in 96-wells plates separately for 36 and 72 hours treatments. Treatments were performed after 24 h. Single treatments were evaluated at 36 hours plates. At 72 hours plates, sequential treatments were performed by replacing drug treatment after 36h. Cytotoxicity was evaluated by using the Cell Titer 96 Aqueous One Solution Cell Proliferation Assay kit (Promega) according to manufacturer's instructions.

Stable knockdown of *TMPRSS2-ERG* was performed through lentiviral delivery of short hairpin RNA (shRNA) in VCaP cells. Lentiviral particles were generated by co-transfection of the shERG (code TRCN0000429354 from Sigma Aldrich) plus viral packaging vectors (VSVG, REV and RRE) into HEK293T cells using Polyethylenimine (Polysciences Europe). Forty-eight hours post-transfection, cell culture supernatants were collected, filtered (0.45 µm) and were used to infect VCaP cells. Positive selection was performed with puromycin (2ug/ml) for 1 month.

Statistical analysis

Fisher´s exact test was used to compare proportions between groups, including PSA response rate between *TMPRSS2-ERG*-positive and *TMPRSS2-ERG*-negative patients. Wilcoxon-Mann-Whitney test was used to compare continuous variables between groups. PSA-PFS, RX-PFS and OS were evaluated by Kaplan-Meier analysis using log-rank test. Univariate analysis of *TMPRSS2-ERG* status and other clinical variables was performed with Cox

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regression; P<0.1 was required for inclusion in multivariate analysis. Test of interaction was performed by entering into the proportional hazard models selected multiplicative interaction terms between the following binary variables: prior A/E to taxanes and *TMPRSS2-ERG* detection. In *in vitro* experiments data were expressed as mean ± SD and were analysed by the Student *t* test. All tests were 2-sided and P values <0.05 were considered statistically significant. Statistical analysis was done with SPSS statistics v20 and R software.

RESULTS

Patients

We included 204 patients: *TMPRSS2-ERG* was tested in PBMC in 121 and FFPE in 124. Patients' characteristics are shown in Table 1.

PSA-response rate according to *TMPRSS2-ERG* status and taxane treatment in each cohort of patients (blood and tumour analysis) are shown in supporting information Table S1. Multivariate analysis in the whole series showed that, together with lactate dehydrogenase levels and ECOG Performance Status, prior A/E was independently associated to lower PSA-PFS (HR 2.1, 95%CI 1.5-2.9; P<0.001), RX-PFS (HR 1.8, 95%CI 1.2-2.6; P=0.004) and overall survival (OS) (HR 1.6, 95%CI 1.04-2.4; P=0.034) to taxanes (Supporting information Fig. S1).

TMPRSS2-ERG mRNA in peripheral blood

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We collected 137 PBMC samples from 121 patients that were analysed prior to receiving taxanes treatment (from 16 patients who received both docetaxel and cabazitaxel treatments we collected two samples, one prior to each treatment) (Fig. 1). *TMPRSS2-ERG* was detected in 24 (17.5%) samples equivalent to 21 (17.36%) patients. Of note, in 13 of the 16 patients with two samples collected, both samples had the same result for *TMPRSS2-ERG* detection. In 3 of the 16 patients there was a discrepancy, being in all the three cases negative the first sample obtained prior to docetaxel treatment and positive the sample obtained after docetaxel progression and prior to cabazitaxel.

It predicted lower response rate (16.7% vs 49%, P=0.005), PSA-PFS (HR 1.7, 95%CI 1.1-2.7; P=0.026), RX-PFS (HR 1.8, 95%CI 1.1-2.9; P=0.03), and OS (HR 2.2, 95%CI 1.3-3.7; P=0.006) to taxanes (Supporting information Fig. 2A). In the multivariate analysis prior A/E (HR: 1.8; 95%CI: 1.2-2.8; P=0.005) and *TMPRSS2-ERG* (HR 2; 95%CI: 1.1-3.7; P=0.018) were independently associated to lower PSA-PFS (Supporting information Table S2). Stratifying patients according *TMPRSS2-ERG* status, prior A/E was independently associated to lower PSA-PFS only in *TMPRSS2-ERG* negative (HR 2.1; 95%CI: 1.3 – 3.5; P=0.002) (Fig. 2). Moreover, there was a significant interaction between the blood detection of *TMPRSS2-ERG* and prior A/E related to PSA-PFS (P=0.032) and RX-PFS (P=0.009). *TMPRSS2-ERG* also predicted independently lower OS (HR 1.9; 95%CI: 1.1-3.4; P=0.03) (Supporting information Table S2).

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Based on these results, an exploratory analysis of *TMPRSS2-ERG* prognostic value according to prior A/E exposure was performed. We found that patients without A/E prior to taxanes (N=67; 48.9%) and positive blood *TMPRSS2-ERG* (N=14, 20.9%) presented lower PSA response (14.3% vs 61.2%; P=0.002), PSA-PFS, RX-PFS, and OS (Fig. 3) than negatives. *TMPRSS2-ERG* was an independent prognostic factor for PSA-PFS (HR 3.3; 95%CI 1.4-7.9 P=0.009), RX-PFS (HR 6.1; 95%CI 2.6-14.19 P<0.001) and OS (HR 2.6; 95%CI 1.3-5.4 P=0.008) (Supporting information Table S2). However, *TMPRSS2-ERG* did not predict taxane resistance when prior A/E was administered (N=66; 48.2%; *TMPRSS2-ERG* positive in 10 samples, 15.2%) (Fig. 3).

***TMPRSS2-ERG* mRNA in tumour samples**

We also analysed 124 tumour samples from 124 patients (Fig. 1). FFPE corresponded to initial biopsy in 109 (87.9%) and to metastasis in 15 (12.1%) patients. Metastatic tissues belonged to lymph nodes (n=3), bone (n=4), liver (n=4), testicle (n=1), bladder (n=1), brain (n=1) and lung (n=1) tissues. *TMPRSS2-ERG* was detected in 58 (46.8%) samples and, in contrast with blood results there were no significant differences between patients with *TMPRSS2-ERG* positive and negative tumours in their PSA response to taxanes (40.3% vs 49.3%) or clinical outcome (Supporting information Fig. S2B). However, as observed in blood, tumour *TMPRSS2-ERG* was associated to lower PSA-PFS in patients who did not receive prior A/E (6.6 vs 10 months; HR 1.76, 95%CI 1.1-2.8; P=0.018) but not in those with prior A/E (Fig. 4).

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On the other hand, prior A/E was independently associated to lower PSA-PFS (HR 2.02, 95%CI 1.3-3.1; P=0.002) and OS (HR 1.9, 95%CI 1.2-3.3; P=0.01) (Supporting information Table S3). However, as occurred with blood samples, stratifying patients by *TMPRSS2-ERG* status this was only observed in *TMPRSS2-ERG* negative patients (HR 2.4; 95%CI 1.4-4.1, P=0.001), where A/E prior to taxanes was an independent prognostic factor of shorter PSA-PFS to taxanes (Fig. 2). A significant interaction between *TMPRSS2-ERG* status and receiving prior A/E to taxanes was observed related to OS (P=0.037).

***TMPRSS2-ERG* mRNA in blood and tumour correlation**

In 41 patients with both tumour and blood available samples a significant correlation in *TMPRSS2-ERG* detection in both tissues was observed (74.1%, P<0.001) taking into account that in 13 of the 41 patients we obtained two blood samples. *TMPRSS2-ERG* prognostic value in blood and in tumour in this group was significant only in patients who did not received A/E prior taxanes, similar to the overall population (Supporting information Fig. S3).

***In vitro ERG* inhibition**

We studied the effect of stable *ERG* knockdown by short hairpin RNA in the *TMPRSS2-ERG* positive CRPC cell line VCaP (Fig. 5A). *ERG* inhibition did not sensitize tumour cells to docetaxel (Fig. 5B) while increased the expression of the androgen receptor (AR)-related gene *KLK3* (Fig. 5C) and induced lower proliferation (Fig. 5D).

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We also tested the molecular and cytotoxic effect of both AR and ERG inhibition treating VCaP cells with enzalutamide, docetaxel, and cabazitaxel alone, in combination, or in sequenced therapy. As a result, enzalutamide, but not taxanes, reduced androgen-induced expression of ERG at the mRNA and protein levels (Fig. 5E and 5F). Moreover, the combination of enzalutamide plus taxanes had a cytotoxic synergistic effect (Fig. 5G). However, prior treatment with enzalutamide reduced subsequent *in vitro* docetaxel activity (Fig. 5H). On the other hand, prior enzalutamide increased ulterior *in vitro* cabazitaxel cytotoxicity (Fig. 5H). Short-term enzalutamide exposure decreased the expression of AR-regulated genes such as *KLK3* and *ERG* (Fig. 5E), increased the expression of AR variant 7 (*ARV7*), neuroendocrine markers (*CHGA* and *SYP)* and induced *CDH1* down-regulation, which is associated to epithelial-to-mesenchymal transition (EMT) (Fig. 5I and Fig. 5J).

DISCUSSION

This study shows that the detection of blood *TMPRSSR2-ERG* mRNA before the start of therapy is associated with a lower response rate and lower PSA-PFS to taxanes, confirming in a larger series of patients our prior results.[6] However, this was not observed when *TMPRSSR2-ERG* mRNA was tested in tumor samples.[6] Notably, we also found in an exploratory analysis that the administration of A/E prior to taxanes affected the potential value of *TMPRSSR2-ERG* as a biomarker of taxane resistance. Indeed, in patients who

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did not receive prior A/E, both blood and tumour *TMPRSSR2-ERG* were independently associated to shorter PSA-PFS. Conversely, in patients who received prior A/E, neither blood nor tumour *TMPRSSR2-ERG* was correlated with taxane activity. As previously suggested,[13] we observed that taxanes activity was lower in patients who had received prior A/E, and it was independently associated to lower PSA-PFS. However, subgroup analysis showed that A/E prior taxanes was an independent prognostic factor of shorter PFS in *TMPRSSR2-ERG* negative but not in *TMPRSS2-ERG* positive patients. Moreover, a statistically significant interaction between A/E and *TMPRSS2-ERG* detection in blood and PSA-PFS was observed in the multivariate analysis.

As previously reported,[6] blood *TMPRSSR2-ERG* detection was lower than in tumour (17.5% vs 46.8%, respectively). Moreover, in patients with matched blood and tumour samples, *TMPRSSR2-ERG* detection correlated in 74.1% of samples. Distinct detection rates and prognostic value between blood and tumour may be due to the different time in course of disease at sample collection (hormone-sensitive vs CRPC) and, also, different assay methodology and sensitivity (FFPE tumours vs PBMC including circulating tumour cells). In concordance with our results in tumour samples, a recently published retrospective study did not found any correlation between ERG protein detected by immunohistochemistry and clinical outcome or response to docetaxel in mCRPC patients.[14] *PTEN* loss was also evaluated in the same work and it was not associated with docetaxel response either.[14] However, in a biomarker

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retrospective study of two phase III trials, ERG protein was associated with docetaxel benefit in hormone-sensitive prostate cancer (HSPC) patients treated with concomitant androgen suppression and docetaxel.[15]

The cross-talk regulation between AR and the over-expression *ERG* in *TMPRSS2-ERG* tumours may explain that the predictive value of this alteration may be different in HSPC or CRPC, and may be modified by prior androgen inhibition therapy, as suggested in our exploratory analysis.[8,16] It has been reported that *in vitro* over-expression of ERG disrupts AR signalling by decreasing AR transcripts and repressing its transcription factor activity. Concordantly, we show that knockdown of ERG in VCaP cells led to upregulation of *KLK3*, a marker of AR activation.[8] However, the inhibition of ERG did not sensitize tumour cells to docetaxel, but induced lower proliferation,[17] which has been associated to cell cycle arrest and then lower docetaxel activity.[18] Thus, the AR-pathway activation and cell cycle stop may prevent the inhibition of ERG as a single strategy to revert docetaxel resistance in VCaP *TMPRSS2-ERG* positive model.

Moreover, the combination of enzalutamide (inducing AR-inhibition and ERG downregulation) plus taxanes in VCaP cells was synergistic, suggesting that concomitant AR and ERG inhibition may sensitize tumour cells to chemotherapy. This may explain that ERG expression predicted docetaxel benefit in hormone-sensitive prostate cancer patients treated with concomitant androgen suppression plus docetaxel.[15]

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On the other hand, in treatment sequencing experiments, we observed that prior enzalutamide reduced ulterior *in vitro* docetaxel cytotoxicity, as well modified the expression of genes involved in neuroendocrine differentiation or EMT, also involved in chemotherapy resistance.[19,20] The up-regulation of NE markers after androgen deprivation therapy has been previously reported to be almost exclusively associated with *TMPRSS2-ERG* positive tumours.[21] This may explain, at least in part, the more adverse clinical outcome of patients treated with docetaxel after A/E and the lack of association between *TMPRSS2-ERG* and resistance to therapy. In a recently published study by Aggarwal et al., the acquisition of NE phenotype was observed in 17% patients who progressed to abiraterone, and this was associated with adverse clinical outcome, but no data regarding to response to chemotherapy were reported.[22] Other genetic markers such as *RB1* and *TP53* lost have been related to antiandrogen resistance therapy but no conclusive data have been reported in association with taxanes either.[23] The predictive value of NE markers and other common genetic alterations in taxane-resistance have to be further defined.

The complex molecular landscape accompanying ERG overexpression may include AR-independent cell de-differentiation by activating the epigenetic regulator *EZH2* and the oncogene *MYC* signalling.[8,24] Other epigenetic pathways, WNT signalling and cell death programs have been also associated with ERG expression profile.[25] Recently, Blee et al reported that the cooperation of ERG protein with other molecular lesions such as PTEN/TP53 mutations can

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affect the sensitivity to antiandrogen therapies in prostate cancer.[26] We hypothesize that in a biological context comparable to VCaP model in which PTEN is intact and AR signalling is repressed by ERG overexpression (in *TMPRSS2-ERG* positive tumours) but not reactivated by PI3K/AKT pathway, the administration of A/E agents reduces ERG levels resembling the outcome to docetaxel to the group without *TMPRSS2-ERG* alteration. In contrast, at patients without A/E prior taxanes, ERG remains abundant to disrupt the docetaxel-tubulin interaction described by Galletti and impairing taxane sensitivity.[5]

It is also important to point out that AR status may also affect taxanes activity. *ARV7* expression in circulating tumour cells has been related to A/E resistance and taxanes response.[27-29] Similarly, AR gain in circulating DNA has been associated to A/E resistance and a greater taxane benefit.[30] Thus, AR status as predictor of taxane-activity in combination with *TMPRSS2-ERG* should be further investigated. Additionally, estrogen receptor and glucocorticoid receptor interaction with *TMPRSS2-ERG* can be also considered.[31,32]

Altogether, results indicate that prior hormone-therapy may influence tumour *TMPRSS2-ERG* prognostic value of taxanes response. Multiple sequential biomarkers studies are needed to stratify treatment sequence in mCRPC patients.

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REFERENCES

1. Tannock IF, de WR, Berry WR, et al. Docetaxel plus prednisone or mitoxantrone plus prednisone for advanced prostate cancer. *N Engl J Med* 2004; 351:1502-12.

2. Petrylak DP, Tangen CM, Hussain MH, et al. Docetaxel and estramustine compared with mitoxantrone and prednisone for advanced refractory prostate cancer. *N Engl J Med* 2004; 351:1513-20.

3. de Bono JS, Oudard S, Ozguroglu M, et al. Prednisone plus cabazitaxel or mitoxantrone for metastatic castration-resistant prostate cancer progressing after docetaxel treatment: a randomised open-label trial. *Lancet* 2010; 376:1147-54.

4. Tomlins SA, Rhodes DR, Perner S, et al. Recurrent fusion of TMPRSS2 and ETS transcription factor genes in prostate cancer. *Science* 2005; 310:644-8.

5. Galletti G, Matov A, Beltran H, et al. ERG induces taxane resistance in castration-resistant prostate cancer. *Nat Commun* 2014; 5:5548.

6. Reig O, Marin-Aguilera M, Carrera G, et al. TMPRSS2-ERG in Blood and Docetaxel Resistance in Metastatic Castration-resistant Prostate Cancer. *Eur Urol* 2016; 70:709-13.

7. Attard G, Swennenhuis JF, Olmos D, et al. Characterization of ERG, AR and PTEN gene status in circulating tumor cells from patients with castration-resistant prostate cancer. *Cancer Res* 2009; 69:2912-8.

8. Yu J, Yu J, Mani RS, et al. An integrated network of androgen receptor, polycomb, and TMPRSS2-ERG gene fusions in prostate cancer progression. *Cancer Cell* 2010; 17:443-54.

9. Scher HI, Halabi S, Tannock I, et al. Design and end points of clinical trials for patients with progressive prostate cancer and castrate levels of testosterone: recommendations of the Prostate Cancer Clinical Trials Working Group. *J Clin Oncol* 2008; 26:1148-59.

10. Eisenhauer EA, Therasse P, Bogaerts J, et al. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). *Eur J Cancer* 2009; 45:228-47.

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11. Domingo-Domenech J, Mellado B, Ferrer B, et al. Activation of nuclear factor-kappaB in human prostate carcinogenesis and association to biochemical relapse. *Br J Cancer* 2005; 93:1285-94.

12. Chou TC. Drug combination studies and their synergy quantification using the Chou-Talalay method. *Cancer Res* 2010; 70:440-6.

13. Nuhn P, de Bono JS, Fizazi K, et al. Update on Systemic Prostate Cancer Therapies: Management of Metastatic Castration-resistant Prostate Cancer in the Era of Precision Oncology. *Eur Urol* 2018

14. Rescigno P, Lorente D, Dolling D, et al. Docetaxel Treatment in PTEN- and ERG-aberrant Metastatic Prostate Cancers. *Eur Urol Oncol* 2018; 1:71-7.

15. Shanna Rajpar, Alexandra Carmel, Zahira Merabet et al. The benefit of combining docetaxel to androgen deprivation therapy in localized and metastatic castration-sensitive prostate cancer as predicted by ERG status: An analysis of two GETUG phase III trials. J Clin Oncol 35[supp; abstr 5012]. 2017.

16. Chen Y, Sawyers CL. Coordinate transcriptional regulation by ERG and androgen receptor in fusion-positive prostate cancers. *Cancer Cell* 2010; 17:415-6.

17. Wang Z, Wang Y, Zhang J, et al. Significance of the TMPRSS2:ERG gene fusion in prostate cancer. *Mol Med Rep* 2017; 16:5450-8.

18. Hennequin C, Giocanti N, Favaudon V. S-phase specificity of cell killing by docetaxel (Taxotere) in synchronised HeLa cells. *Br J Cancer* 1995; 71:1194-8.

19. Marin-Aguilera M, Codony-Servat J, Reig O, et al. Epithelial-to-mesenchymal transition mediates docetaxel resistance and high risk of relapse in prostate cancer. *Mol Cancer Ther* 2014; 13:1270-84.

20. Davies AH, Beltran H, Zoubeidi A. Cellular plasticity and the neuroendocrine phenotype in prostate cancer. *Nat Rev Urol* 2018

21. Volante M, Tota D, Giorcelli J, et al. Androgen deprivation modulates gene expression profile along prostate cancer progression. *Hum Pathol* 2016; 56:81-8.

22. Aggarwal R, Huang J, Alumkal JJ, et al. Clinical and Genomic Characterization of Treatment-Emergent Small-Cell Neuroendocrine

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Prostate Cancer: A Multi-institutional Prospective Study. *J Clin Oncol* 2018; 36:2492-503.

23. Ku SY, Rosario S, Wang Y, et al. Rb1 and Trp53 cooperate to suppress prostate cancer lineage plasticity, metastasis, and antiandrogen resistance. *Science* 2017; 355:78-83.

24. Sun C, Dobi A, Mohamed A, et al. TMPRSS2-ERG fusion, a common genomic alteration in prostate cancer activates C-MYC and abrogates prostate epithelial differentiation. *Oncogene* 2008; 27:5348-53.

25. Iljin K, Wolf M, Edgren H, et al. TMPRSS2 fusions with oncogenic ETS factors in prostate cancer involve unbalanced genomic rearrangements and are associated with HDAC1 and epigenetic reprogramming. *Cancer Res* 2006; 66:10242-6.

26. Blee AM, He Y, Yang Y, et al. TMPRSS2-ERG Controls Luminal Epithelial Lineage and Antiandrogen Sensitivity in PTEN and TP53-Mutated Prostate Cancer. *Clin Cancer Res* 2018; 24:4551-65.

27. Antonarakis ES, Lu C, Luber B, et al. Androgen Receptor Splice Variant 7 and Efficacy of Taxane Chemotherapy in Patients With Metastatic Castration-Resistant Prostate Cancer. *JAMA Oncol* 2015; 1:582-91.

28. Scher HI, Lu D, Schreiber NA, et al. Association of AR-V7 on Circulating Tumor Cells as a Treatment-Specific Biomarker With Outcomes and Survival in Castration-Resistant Prostate Cancer. *JAMA Oncol* 2016; 2:1441-9.

29. Thadani-Mulero M, Portella L, Sun S, et al. Androgen receptor splice variants determine taxane sensitivity in prostate cancer. *Cancer Res* 2014; 74:2270-82.

30. Conteduca V, Jayaram A, Romero-Laorden N, et al. Plasma Androgen Receptor and Docetaxel for Metastatic Castration-resistant Prostate Cancer. *Eur Urol* 2018

31. Xu Z, Wang Y, Xiao ZG, et al. Nuclear receptor ERRalpha and transcription factor ERG form a reciprocal loop in the regulation of TMPRSS2:ERG fusion gene in prostate cancer. *Oncogene* 2018

32. Narayanan S, Srinivas S, Feldman D. Androgen-glucocorticoid interactions in the era of novel prostate cancer therapy. *Nat Rev Urol* 2016; 13:47-60.

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FIGURE LEGENDS

Figure 1: Scheme of number of patients and samples included in the study. Blue boxes represent patients/samples that are included in both tumour and blood or docetaxel and cabazitaxel categories. A/E: abiraterone/enzalutamide.

Figure 2: Survival and multivariate analysis according to *TMPRSS2-ERG* status and A/E prior to taxanes in blood and tumour samples. Kaplan-Meier curves represent PSA progression-free survival (PSA-PFS). Table shows multivariate Cox model for PSA-PFS for clinically significant variables (P<0.1) in univariate analysis. ECOG, LDH, Hb, PSA, AP and the presence of visceral metastasis were evaluated before starting the treatment with taxanes; *variables considered dichotomous; **variables considered continuous. A/E: new anti-androgen therapy; ECOG: Eastern Cooperative Oncology Group; LDH: lactate dehydrogenase; Hb: haemoglobin concentration; PSA: prostate-specific antigen; AP: alkaline phosphatase; HR: hazard ratio; CI: confidence interval.CI: confidence interval; HR: hazard ratio.

Figure 3: Survival analysis of taxanes-treated patients according to *TMPRSS2-ERG* and A/E prior to taxanes in PBMC. Kaplan-Meier curves represent PSA progression-free survival (PSA-PFS), radiological progression-free survival (RX-PFS) and overall survival (OS). CI: confidence interval; HR: hazard ratio.

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Figure 4: Survival analysis of taxanes-treated patients according to *TMPRSS2-ERG* and A/E prior to taxanes in tumour samples. Kaplan-Meier curves represent PSA progression-free survival (PSA-PFS), radiological progression-free survival (RX-PFS) and overall survival (OS). CI: confidence interval; HR: hazard ratio.

Figure 5: *In vitro* experiments of ERG knockdown in VCaP cells and of taxanes and enzalutamide treatment. A) Western blot of VCaP lysates after shRNA treatment. Lane 1 represents scrambled shRNA treatment (shCT) and lane 2 ERG inhibition (shERG). GAPDH was used as a load control; B) Viability analysis of VCaP cells with shERG and shCT under docetaxel treatment during 72 hours; C) Bars plot and western blot representing *KLK3* expression levels in VCaP cells transfected with shERG and shCT; D) Growth curves of VCaP cells transfected with shERG and shCT. Cells were counted at days 4, 7, 9, 12 and 15 after seeded, using trypan blue and the Neubauer chamber, by triplicate; E) Bar graphs representing *TMPRSS2-ERG*, *ERG* and *KLK3* relative expression in VCaP cells treated with enzalutamide (Ez) 45uM, docetaxel (Dx) 1nM, and cabazitaxel (Cz) 1nM individually and combining Ez with taxanes at the same doses during 48 hours. Each condition was performed by triplicate (*: t-test $P<0.05$); F) Western blot of VCaP cells treated with Ez 45uM, Dx 1nM and Cz 1nM individually and combining Ez with taxanes at the same doses during 48 hours. GAPDH was used as a load control; Bar graph represents the western blot quantification (Odyssey application software v3.0). Experiment performed

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by triplicate (*: t-test $P<0.05$); G) Viability analysis in VCaP cells of both docetaxel and cabazitaxel drugs combined with enzalutamide during 72 hours. Ez, Dx, and Cz were tested at fix doses of 1uM, 1nM and 1nM, respectively, in combination with growing concentrations of Dx, Cz and Ez. Synergy evaluation was calculated by using IC50 doses through Chou-Talalay method being the combination index (CI) between 0.7-0.85, and 0.3-0.7, indicative of moderate synergism and synergism, respectively; H) Bar graphs representing cytotoxicity levels of VCaP cells individually and sequentially treated with Dx 1nM, Cz 1nM and Ez 45uM. ; I) Bar graphs representing *KLK3*, neuroendocrine markers *MYCN*, *CHGA*, and *SYP*, and the epithelial marker *CDH1* relative expression in VCaP cells treated with enzalutamide (Ez) 10uM in a time-course experiment. Each condition was performed by triplicate (*: t-test $P<0.05$); J) Western blot of VCaP cells treated with enzalutamide (Ez) 10uM in a time-course experiment. GAPDH was used as a load control; bar graph represents the image quantification at 72h (Odyssey application software v3.0).

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TABLES

Table 1: Clinical patients' characteristics. P-value is based on Fisher exact test and Wilcoxon Mann-Whitney test for categorical and continuous variables, respectively. N: number of cases; *data at taxanes start time; ECOG: Eastern Cooperative Oncology Group; A/E: Abiraterone/Enzalutamide therapy; NA: not available

	Tumour					Blood			
	Total	*TMPRSS2-ERG* negative	*TMPRSS2-ERG* positive	*TMPRSS2-ERG* NA	P-value	Total	*TMPRSS2-ERG* negative	*TMPRSS2-ERG* positive	P-value
Number of patients	124	-	-	-		121	-	-	
Number of samples, N(%)	124	62 (50%)	58 (46.8%)	4 (3.2%)		137	113 (82.5%)	24 (17.5%)	
Docetaxel-treated patients, N(%)	111 (89.5%)	52 (46.8%)	55 (49.5%)	4 (3.6%)		102 (74.5%)	86 (84.3%)	16 (15.7%)	
Cabazitaxel-treated patients, N(%)	42 (33.9%)	23 (54.8%)	18 (42.8%)	1 (2.4%)		35 (25.5%)	27 (77.1%)	8 (22.9%)	
Age* (years)									
Median (range)	69.5 (41.6-87.1)	69.4 (51.6-87.1)	69.9 (55.8-84.5)	68.8 (54.5-78.8)		69.6 (37.3-85.9)	69.4 (37.3-85.9)	70.2 (52.2-83)	
Stage at diagnosis, N (%)									
<IV	41 (33.1%)	18 (43.9%)	20 (48.8%)	3 (7.3%)	0.551	48 (39.7%)	45 (93.8%)	3 (6.3%)	0.035
IV	67 (54%)	37 (55.2%)	29 (43.3%)	1 (1.5%)		68 (56.2%)	54 (79.4%)	14 (20.6%)	
NA	16 (12.9%)	7 (43.8%)	9 (56.3%)	-		5 (4.1%)	4 (80%)	1 (20%)	
Gleason sum at diagnosis, N (%)									
≤7	40 (32.3%)	15 (37.5%)	24 (60%)	1 (2.5%)	0.122	48 (39.6%)	42 (87.5%)	6 (12.5%)	0.6073
≥8	77 (62.1%)	44 (57.1%)	31 (40.3%)	2 (2.6%)		71 (58.7%)	59 (83.1%)	12 (16.9%)	

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NA	7 (5.6%)	3 (42.9%)	3 (42.9%)	1 (14.3%)		2 (1.65%)	2 (100%)	-	
Presence of bone metastases, N (%)									
Yes	105 (84.7%)	52 (49.5%)	49 (46.7%)	4 (3.8%)	0.967	104 (85.9%)	87 (83.7%)	17 (16.3%)	0.4632
No	10 (8.1%)	5 (50%)	5 (50%)	-		17 (14.1%)	16 (94.1%)	1 (5.9%)	
NA	9 (7.3%)	5 (55.6%)	4 (44.4%)	-		-	-	-	
Presence of visceral metastases* , N (%)									
Yes	42 (33.9%)	20 (47.6%)	21 (50%)	1 (2.4%)	0.622	37 (30.6%)	32 (86.5%)	5 (13.5%)	1
No	73 (58.9%)	36 (49.3%)	34 (46.6%)	3 (4.1%)		84 (69.4%)	71 (84.5%)	13 (15.5%)	
NA	9 (7.3%)	6 (66.7%)	3 (33.3%)	-		-	-	-	
ECOG performance status score*, N (%)									
0	18 (14.5%)	8 (44.4%)	9 (50%)	1 (5.6%)	0.816	26 (21.5%)	24 (92.3%)	2 (7.7%)	0.3526
1 or 2	87 (70.2%)	45 (51.7%)	39 (44.8%)	3 (3.4%)		89 (73.6%)	74 (83.1%)	15 (16.9%)	
NA	19 (15.3%)	9 (47.4%)	10 (52.6%)	-		6 (4.9%)	5 (83.3%)	1 (16.7 %)	
Baseline Prostate-specific antigen (ng/mL)									
Median (range)	68.1 (0.04-2006)	103.2 (0.04-2006)	56 (0.17-1565)		0.237	42.1 (0.04-1284)	33.52 (0.04-1284)	104 (16.8-983)	0.003
Baseline haemoglobin concentration (g/L)									
Median (range)	96 (7-154)	98 (8-151)	87 (7-151)		0.618	123 (9-154)	124 (9-154)	121.5 (9.3-144)	0.642
Baseline alkaline phosphatase (U/L)									
Median (range)	192 (17-4397)	178 (17-1607)	220 (27-4397)		0.309	176 (50-2311)	175 (50-2311)	268 (87-1660)	0.032
Baseline lactate dehydrogenase									

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Median (range)	396 (153-3864)	436 (153-2145)	380.5 (156-3864)		0.123	388.5 (163-1979)	384 (163-1979)	476 (245-967)	0.030
A/E treatment pre-taxanes (%)									
Yes	55 (35.9%)	33 (60%)	19 (34.5%)	3 (5.5%)	0.026	66 (48.2%)	56 (84.8%)	10 (15.2%)	0.5
No	98 (64.1%)	42 (42.9%)	54 (55.1%)	2 (2%)		67 (48.9%)	53 (79.1%)	14 (20.9%)	
NA	-					4 (2.9%)	4 (100%)	-	

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	Tumor		Blood	Total
Nº Patients	83	41	80	204
Nº Samples	124		137	261

	Docetaxel		Cabazitaxel	Docetaxel		Cabazitaxel	
Nº Patients	82	29	13	86	16	19	204
Nº Samples	82	29	13	102		35	261

	A/E	No A/E	A/E	No A/E	A/E	No A/E	A/E	No A/E	
Nº cases of study	31	80	24	18	38	60	28	7	286*

* In 4 cases information about A/E in blood samples is missing

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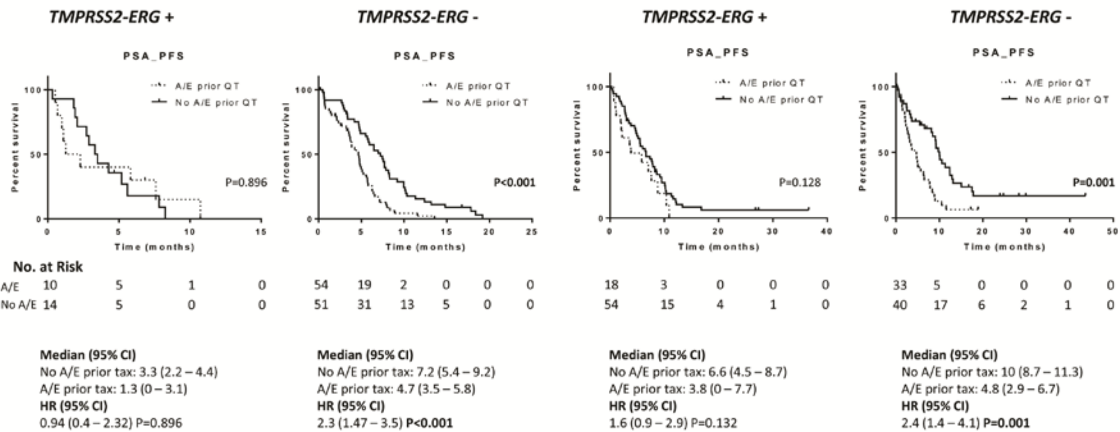

Variable/multivariate	BLOOD						TUMOR					
	TMPRSS2-ERG +			*TMPRSS2-ERG -*			*TMPRSS2-ERG +*			*TMPRSS2-ERG -*		
	HR	95% CI	P-value	HR	95% CI	P-value	HR	95% CI	P-value	HR	95% CI	P-value
A/E prior taxanes*	-	-	-	2.134	1.309 - 3.479	**0.002**	-	-	-	2.315	1.222 - 4.384	**0.010**
Stage at diagnosis*	-	-	-	1.314	0.831 - 2.079	0.243	-	-	-	-	-	-
ECOG*	-	-	-	1.04	0.6 - 1.801	0.889	-	-	-	-	-	-
LDH**	-	-	-	-	-	-	-	-	-	1.003	1.001 - 1.005	**0.010**
Hb**	1.014	0.998 - 1.014	0.081	-	-	-	1.011	1.005 - 1.017	**<0.001**	1.007	1.002 - 1.013	**0.010**
PSA**	-	-	-	1.001	1 - 1.002	0.064	1.003	1.001 - 1.005	**0.011**	-	-	-
AP**	-	-	-	-	-	-	1.000	1.000 - 1.001	0.789	1.000	0.999 - 1.002	0.457
Visceral metastases*	-	-	-	-	-	-	-	-	-	-	-	-

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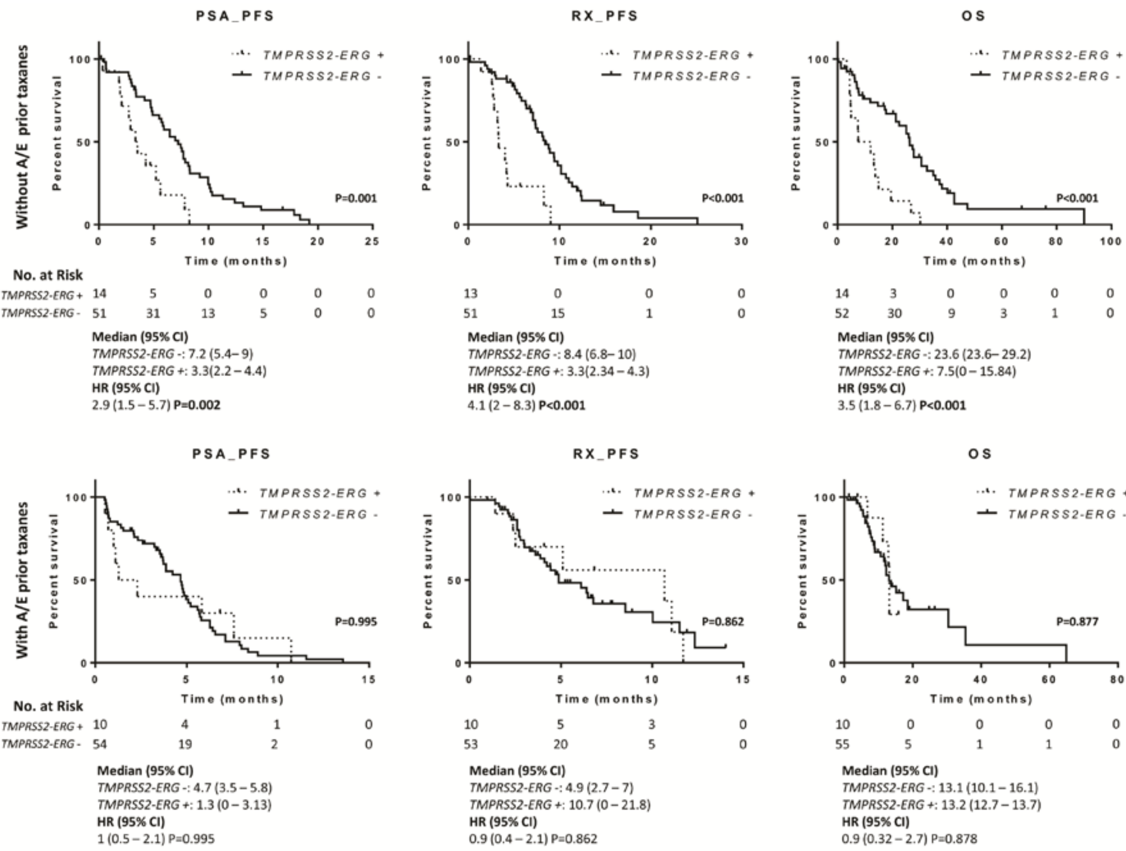

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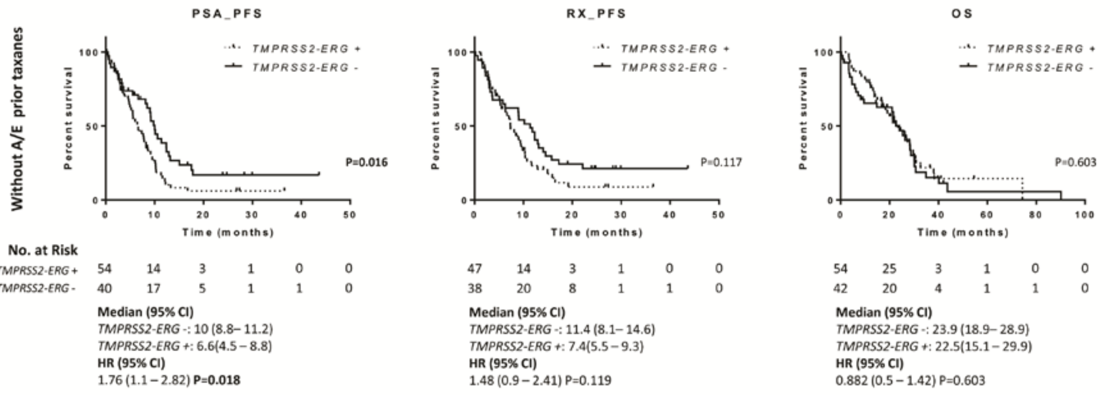

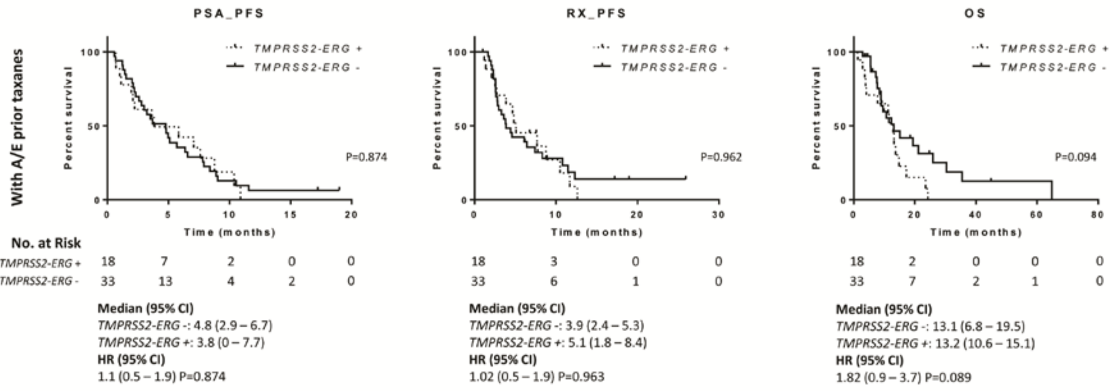

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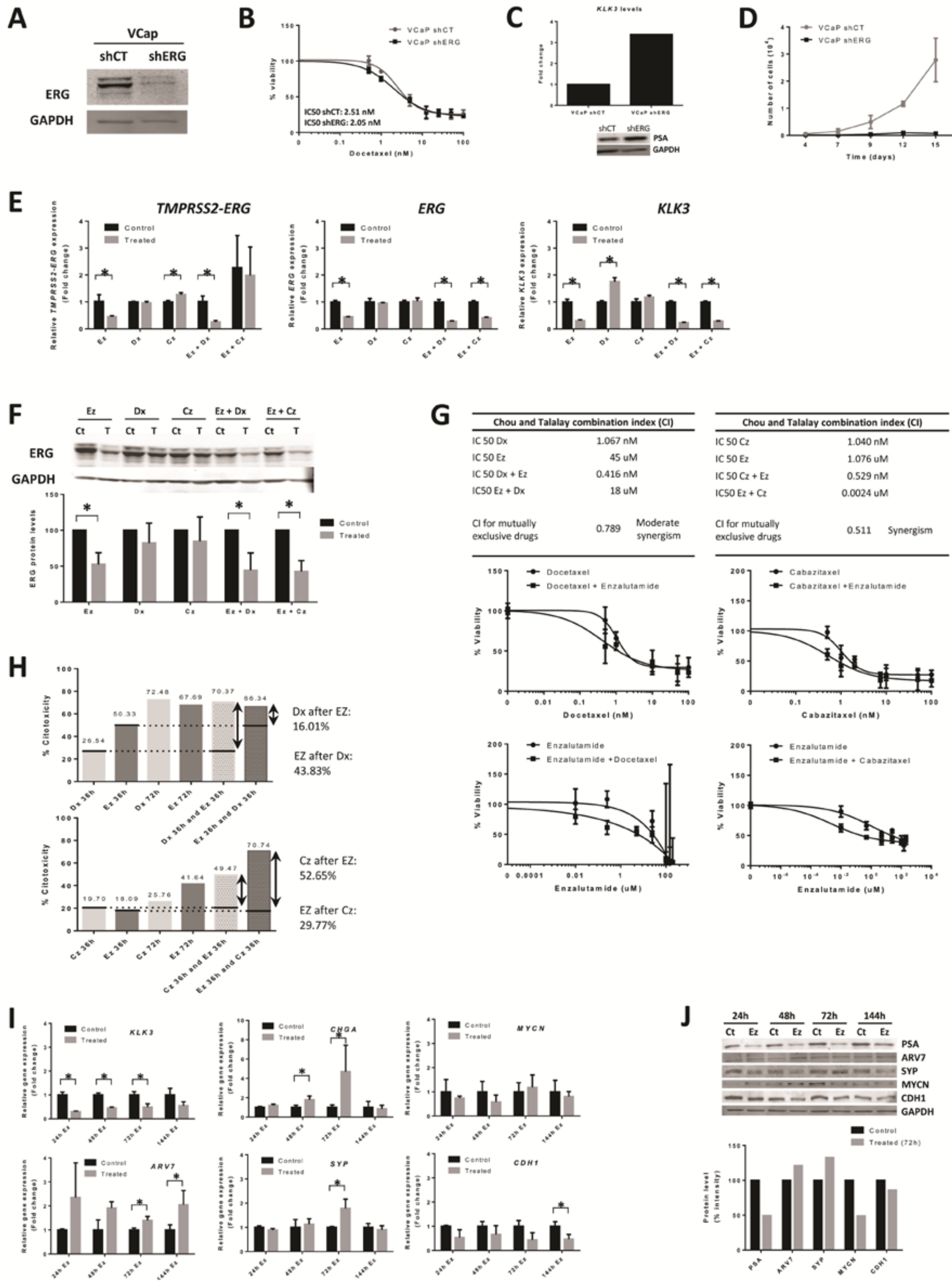

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Despite known resistance mechanisms, no molecular biomarkers to predict taxane resistance are clinically available. TMPRSS2-ERG rearrangement is a prostate cancer-specific genetic alteration known to impair taxane sensitivity in preclinical models. This study shows that blood and tumour TMPRSS2-ERG expression independently predicted lower prostate-specific antigen progression-free survival in metastatic castration-resistant prostate cancer (mCRPC) patients following taxanes. When prior abiraterone/ enzalutamide therapy was administered, TMPRSS2-ERG was not associated with outcome. Prior hormone-therapy may influence taxane response and TMPRSS2-ERG prognostic value, suggesting the need for multiple and sequential biomarkers in mCRPC follow-up evaluation.

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