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Brief Correspondence

TMPRSS2-ERG in Blood and Docetaxel Resistance in Metastatic Castration-resistant Prostate Cancer

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Abstract

TMPRSS2-ERG rearrangement is a genetic alteration exclusive to prostate cancer, associated with taxane resistance in preclinical models. Its detection in blood samples of metastatic resistant prostate cancer (mCRPC) patients may indicate the presence of circulating tumour cells with this genetic alteration and may predict taxane resistance. To test this hypothesis, we evaluated *TMPRSS2-ERG* expression using quantitative reverse transcription polymerase chain reaction in peripheral blood mononuclear cells and tumour tissue from mCPRC patients treated with taxanes. We examined peripheral blood mononuclear cells from 24 healthy controls, 50 patients treated with docetaxel, and 22 with cabazitaxel. *TMPRSS2-ERG* was detected in 0%, 16%, and 22.7% of them, respectively. In docetaxel-treated patients *TMPRSS2-ERG* detection correlated with lower prostatic-specific antigen (PSA) response rate (12.5% vs 68.3%, $p = 0.005$), PSA-progression-free survival (PFS; 3.1 mo vs 7.5 mo, $p < 0.001$), clinical/radiological-PFS (3.1 mo vs 8.2 mo, $p < 0.001$), and it was independently associated with PSA-PFS (hazard ratio 3.7; $p = 0.009$) and clinical/radiological-PFS (hazard ratio 6.3; $p < 0.001$). Moreover, *TMPRSS2-ERG* also predicted low PSA-PFS to cabazitaxel. At progression, a switch from negative to positive *TMPRSS2-ERG* was observed in 41% of patients with undetected *TMPRSS2-ERG* at the baseline sample. Tissue *TMPRSS2-ERG* expression correlated with lower PSA-PFS ($p = 0.02$) to docetaxel. Our findings support the potential role of *TMPRSS2-ERG* detection as a biomarker to tailor treatment strategies.

Patient summary: Taxanes are the most active chemotherapy agents in metastatic resistant prostate cancer. However, not all patients respond to this therapy. In the present study we show that the detection of *TMPRSS2-ERG* in blood from metastatic resistant prostate cancer patients predicts resistance to docetaxel and it may be useful to select treatment and to avoid possible toxicities in refractory patients.

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Recent advances have seen the introduction of new agents for metastatic castration resistant prostate cancer (mCRPC). Whilst taxanes are the most active chemotherapeutic agents in mCRPC [1,2], cabazitaxel is approved for docetaxel-resistant tumours [3]. As there are no clinical biomarkers to predict taxane response, many nonresponding patients receive these treatments without benefit. We investigated whether the presence of the *TMPRSS2-ERG* fusion could be used to guide taxane use. This gene fusion appears specific to prostate cancer and leads to a high expression of a truncated functional ERG protein [4], which may alter taxane response. Galletti et al [5] demonstrated that ERG binds to soluble tubulin and decreases the taxane–tubulin interaction, leading to impaired taxane sensitivity. Furthermore, *TMPRSS2-ERG* induces epithelial-to-mesenchymal transition, which is also associated with taxane resistance in CRPC [6].

To test our hypothesis, we collected peripheral blood from 50 men receiving docetaxel, 22 receiving cabazitaxel (Supplementary Table 1 and 2), and 24 healthy volunteers included as negative controls. Healthy volunteers were nononcologic patients admitted for different surgical treatments. This cohort is part of a larger prospective collection (Supplementary Fig. 1). Sequential blood samples were available from 27 (36.5%; interval from initial sample collection 11.6 mo $\pm$ 8.4 mo) and matching prostate cancer formalin-fixed paraffin-embedded tissue from 33 (44.6%) patients (Supplementary Fig. 1). We studied the peripheral blood mononuclear cells (PBMC) fraction from the blood, as circulating tumour cells (CTCs) may miss epithelial markers used for its isolation due to an epithelial-to-mesenchymal transition phenomenon [7]. We measured *TMPRSS2-ERG* mRNA expression by quantitative reverse-transcription polymerase chain reaction in each sample using Taqman assay technology. Specifically, commercial primers for *TMPRSS2-ERG* detected the most commonly reported fusion transcript *TMPRSS2* exon 1 fused to *ERG* exon 4. Patients were categorized as *TMPRSS2-ERG* positive when both *TMPRSS2-ERG* and housekeeping genes were detectable (quantification cycle $<$ 35) in duplicate samples. They were considered *TMPRSS2-ERG* negative when housekeeping genes but not *TMPRSS2-ERG* were detectable (quantification cycle $\geq$ 35 or undetermined). Additionally, the expression of prostate specific genes (*AR*, *C1ORF116*, and *KLK3*) was analyzed in PBMC as indirect markers of CTCs. Full study methods are described in the Supplementary material. The hospital's Institutional Ethics Committee approved the study (approval number: 2012_7532). All participants provided written informed consent.

No *TMPRSS2-ERG* was detected in the 24 healthy volunteers. To confirm the prostatic origin of *TMPRSS2-ERG* detection in PBMCs we tested 45 mCRPC patients with known CTC count from a previous study [8]. *TMPRSS2-ERG* was only detected in patients with $\geq$5 CTCs (seven of 22; 32%; $p = 0.004$). Moreover, in the present study, we found higher *KLK3* levels in *TMPRSS2-ERG*-positive samples than in negative ones. No *TMPRSS2-ERG* was detected in *KLK3* negative samples (Supplementary Fig. 2). Our results support the prostate cancer specificity of *TMPRSS2-ERG* and enable its detection over a background of native mRNA from other mononuclear cells apart from CTCs.

Among 50 docetaxel-treated patients, eight (16%) were *TMPRSS2-ERG*-positive and 42 negative. *TMPRSS2-ERG*-positive patients had a lower prostatic-specific antigen (PSA) response rate than negative (12.5% vs 68.3%, $p = 0.005$, respectively) and a lower PSA reduction (Fig. 1A and 1B). *TMPRSS2-ERG* significantly correlated with lower PSA-progression free survival (PFS), clinical/radiological (C/R)-PFS, and overall survival (Fig. 1C–E). Moreover, *TMPRSS2-ERG* was independently associated with PSA-PFS (hazard ratio 3.7; 95% confidence interval 1.4–10; $p = 0.01$) and C/R-PFS (hazard ratio 6.3; 95% confidence interval 2.3–17.3; $p < 0.001$; Supplementary Table 3). We observed that *TMPRSS2-ERG*-positive patients had more adverse prognostic factors compared with *TMPRSS2-ERG*-negative (Supplementary Table 1). However, only *TMPRSS2-ERG* was an independent prognostic factor for PSA-PFS and C/R-PFS in the multivariate analysis. An internal validation using 100 bootstrap samples and a Brier score was performed, obtaining 0.1476 and 0.1572 scores for PSA-PFS and C/R-PFS, respectively. This result supports a good accuracy of the model as a predictor of docetaxel outcome. A larger multicentre independent prospective validation study is ongoing.

We also analyzed if the detection of *KLK3*, as an indirect marker of CTCs, may affect clinical outcome. *TMPRSS2-ERG*-negative/*KLK3*-positive and *TMPRSS2-ERG*-negative/*KLK3*-negative patients had a better PSA response, PSA-PFS, and C/R-PFS than *TMPRSS2-ERG*-positive/*KLK3*-positive patients (Supplementary Fig. 3; Supplementary Table 4).

Among the 22 cabazitaxel-treated patients, five (22.7%) were *TMPRSS2-ERG*-positive. No differences in PSA response rate or best PSA response according to *TMPRSS2-ERG* detection were observed. However, *TMPRSS2-ERG*-positive patients had poorer PSA-PFS and a trend to a poorer C/R-PFS and overall survival compared with negative patients (Fig. 2A–C; Supplementary Fig. 4A and 4B). A larger number of patients are required to define the association between *TMPRSS2-ERG* and cabazitaxel resistance.

Sixty-eight sequential PBMC samples were collected from 27 (36.5%) of 74 patients that underwent systemic treatment, 19 (63.3%) of them with docetaxel therapy. Interestingly, seven (41.2%) of 17 negative baseline samples switched to a positive expression at disease progression (Fig. 2D). This may suggest a possible clonal selection of refractory *TMPRSS2-ERG*-positive tumour cells and/or an increase of *TMPRSS2-ERG*-positive CTC amount.

TMPRSS2-ERG expression in tumour tissue was detected in 16 (48.5%) of the 33 patients with available tumour tissue samples, a percentage similar to previously described in the literature [5]. Among the 33 patients where both tumour tissue and PBMC samples were available, we analysed the concordance in *TMPRSS2-ERG* expression between samples types. We considered concordant results when at least one positive PBMC and a positive tumour tissue samples were observed ($n = 6$), or when both were negative ($n = 17$). These were observed in 23 (69.7%) out of 33 patients. In 10 patients (30.3%) with positive *TMPRSS2-ERG* tumours,

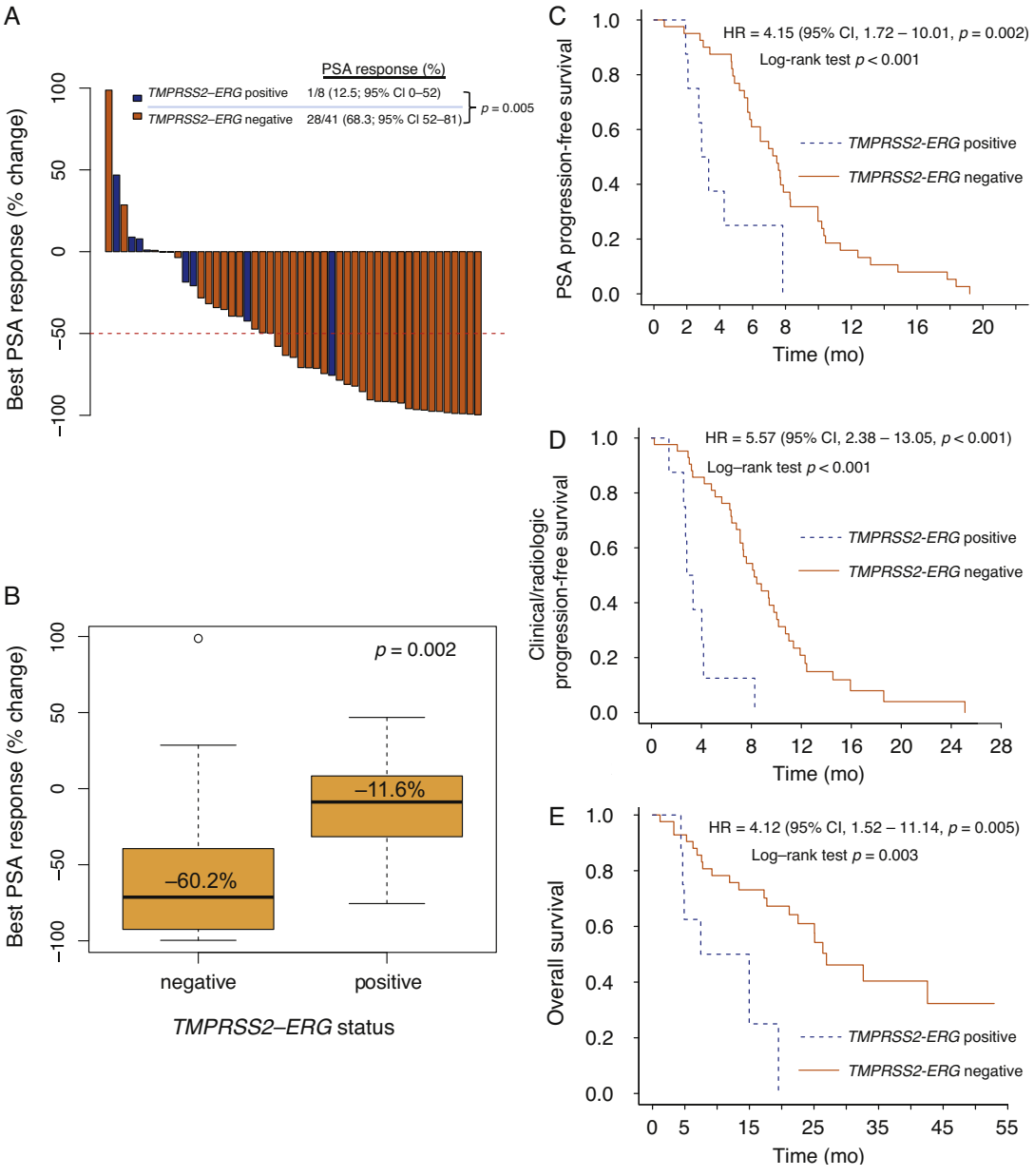

Fig. 1 – Docetaxel outcome according to *TMPRSS2-ERG* status in peripheral blood mononuclear cells samples. (A) Waterfall plot representing individual prostate-specific antigen (PSA) response. Red line shows a ≥50% reduction in PSA level from baseline, indicating a PSA response (Fisher's exact test). (B) Boxplot representing percentage of change in best PSA response (Wilcoxon rank sum test). (C) Kaplan-Meier curves for PSA progression-free survival (PSA-PFS); median PSA-PFS was 3.12 mo (95% confidence interval [CI], 2.07 to not reached) in *TMPRSS2-ERG* positive patients and 7.47 mo (95% CI, 5.83–8.27) in *TMPRSS2-ERG* negative patients. (D) Kaplan-Meier curves for clinical/radiologic PFS (C/R-PFS); median C/R-PFS was 3.08 mo (95% CI, 2.57–4.17) in *TMPRSS2-ERG* positive patients and 8.23 mo (95% CI, 7.1–10.03) in *TMPRSS2-ERG* negative patients. (E) Kaplan-Meier curves for overall survival; median overall survival was 11.2 mo (95% CI, 4.67 to not reached) in *TMPRSS2-ERG* positive patients and 26.9 mo (95% CI, 21.17 to not reached) in *TMPRSS2-ERG* negative patients.
HR = hazard ratio.

PBMCs were negative. No positive PBMCs were observed among patients with negative tumours (Fig. 2D). The lack of detection of *TMPRSS2-ERG* in PBMC from patients with *TMPRSS2-ERG* positive tumours could be explained by the limitations of quantitative reverse-transcription polymerase chain reaction sensitivity, the absence of CTCs, and/or tumour heterogeneity. Indeed, the presence of *TMPRSS2-ERG* rearrangement may be heterogeneous within the same primary tumour, although its expression is consistent within the different metastatic sites from the same patient [9].

Among 25 docetaxel-treated patients with available tissue samples, 14 (56%) were *TMPRSS2-ERG*-positive, finding that is correlated with a shorter PSA-PFS but no statistical differences were found in PSA response rate or C/R-PFS (Fig. 2E–G). If the clinical utility of *TMPRSS2-ERG* detection in blood or CTCs as a biomarker of taxane resistance is superior to its determination in primary tumours has to be determined.

In conclusion, the present study suggests that the detection of *TMPRSS2-ERG* mRNA in blood may be a

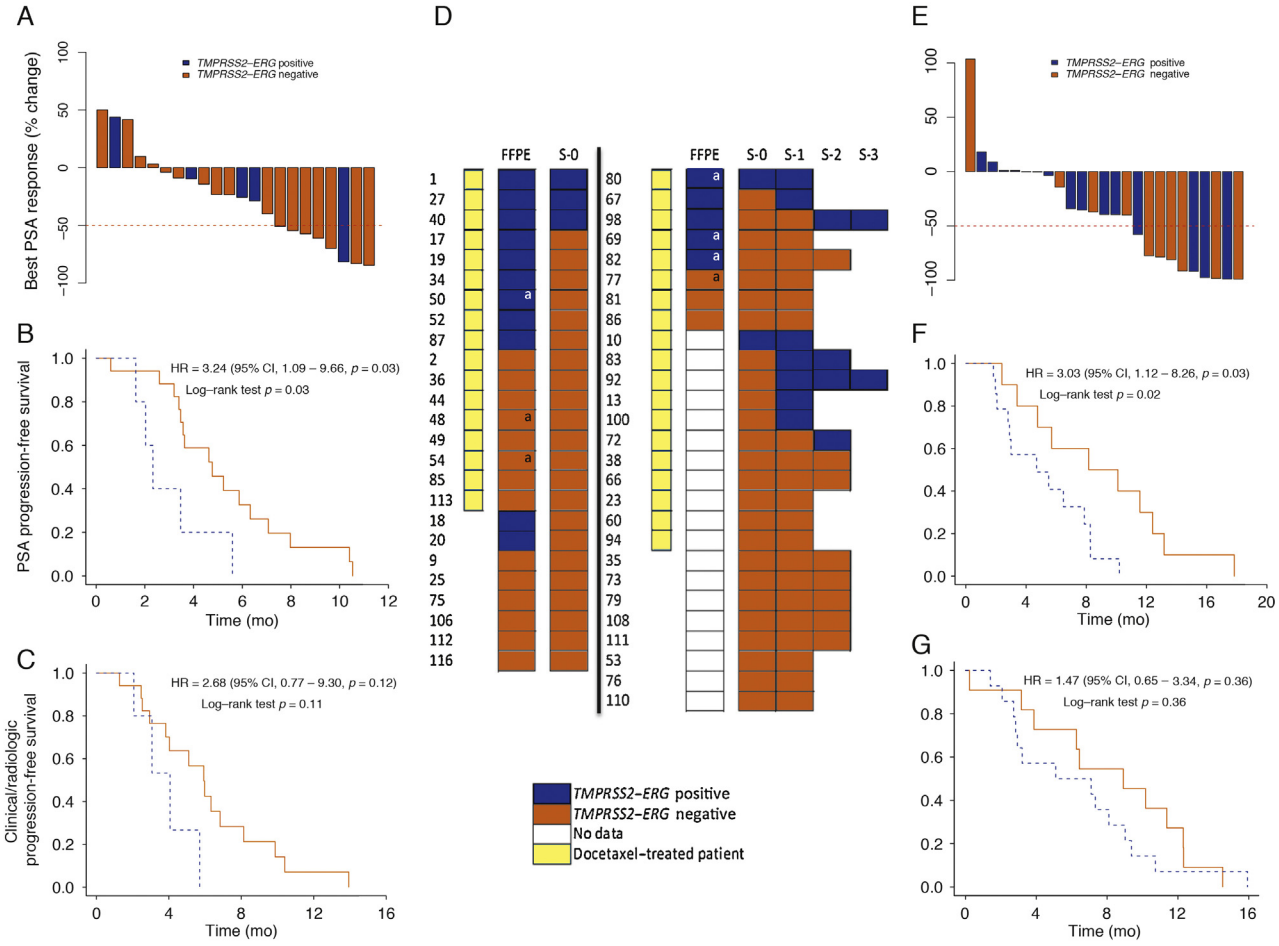

Fig. 2 – Cabazitaxel and docetaxel outcome according to *TMPRSS2-ERG* status in peripheral blood mononuclear cells (PBMCs) samples and tissue. (A) Waterfall plot representing individual prostate-specific antigen (PSA) response of cabazitaxel-treated patients according to *TMPRSS2-ERG* status; red line shows ≥50% reduction in PSA level from baseline, indicating a PSA response. (B) Kaplan-Meier curves for PSA-progression-free survival (PSA-PFS) in cabazitaxel-treated patients; median PSA-PFS was 2.3 mo (95% confidence interval [CI], 1.6 to not reached) in *TMPRSS2-ERG*-positive patients and 4.8 mo (95% CI, 3.5–6.3) in *TMPRSS2-ERG*-negative patients. (C) Kaplan-Meier curves for clinical/radiologic-PFS in cabazitaxel-treated patients; median clinical/radiologic-PFS was 4.1 mo (95% CI, 2.1 to not reached) in *TMPRSS2-ERG*-positive patients and 5.9 mo (95% CI, 3.8–8.1) in *TMPRSS2-ERG*-negative patients. (D) *TMPRSS2-ERG* expression in tissue and sequential PBMC samples. Concordance between *TMPRSS2-ERG* expression in formalin-fixed paraffin-embedded (FFPE) samples and PBMC baseline sample (S-0) is shown on the left and right panel. Right panel includes sequential PBMC samples (S-1, S-2, and S-3). Docetaxel-treated patients are represented with a blue box. (E) Waterfall plot representing individual PSA response according to *TMPRSS2-ERG* expression on tissue. (F) Kaplan-Meier plot representing PSA-PFS in docetaxel-treated patients according to *TMPRSS2-ERG* expression on tissue; median PSA-PFS was 4.7 mo (95% CI, 2.9–8.9) in *TMPRSS2-ERG*-positive patients and 10.1 mo (95% CI, 4.8–13.2) in *TMPRSS2-ERG*-negative patients. (G) Kaplan-Meier plot representing C/R-PFS in docetaxel-treated patients according to *TMPRSS2-ERG* expression on tissue; median C/R-PFS was 6.1 mo (95% CI, 2.9–8.1) in *TMPRSS2-ERG*-positive patients and 8 mo (95% CI, 3.9–12.3) in *TMPRSS2-ERG*-negative patients. HR = hazard ratio.
[a] Samples collected on castration resistance status.

potential biomarker of docetaxel resistance in mCRPC patients. The specificity of this biomarker in prostate cancer and the noninvasive sample collection would facilitate its clinical application.

Author contributions: Begoña Mellado had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: Reig, Marín-Aguilera, Mellado.
Acquisition of data: Reig, Marín-Aguilera, Carrera, Jiménez, García-Recio, Gaba, Pereira, Fernández.
Analysis and interpretation of data: Reig, Marín-Aguilera, Paré, Mellado.
Drafting of the manuscript: Reig, Marín-Aguilera, Mellado.
Critical revision of the manuscript for important intellectual content: Reig, Marín-Aguilera, Prat, Mellado.
Statistical analysis: Reig, Paré, Marín-Aguilera.
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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.eururo.2016.02.034.

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