Predictive Biomarkers and Personalized Medicine

**TMPRSS2-ERG Status Is Not Prognostic Following Prostate Cancer Radiotherapy: Implications for Fusion Status and DSB Repair**

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**Abstract**

**Background:** Preclinical data suggest that TMPRSS2-ERG gene fusions, present in about 50% of prostate cancers, may be a surrogate for DNA repair status and therefore a biomarker for DNA-damaging agents. To test this hypothesis, we examined whether TMPRSS2-ERG status was associated with biochemical failure after clinical induction of DNA damage following image-guided radiotherapy (IGRT).

**Methods:** Pretreatment biopsies from two cohorts of patients with intermediate-risk prostate cancer [T1/T2, Gleason score (GS) < 8, prostate-specific antigen (PSA) < 20 ng/mL; >7 years follow-up] were analyzed: (i) 126 patients [comparative genomic hybridization (CGH) cohort] with DNA samples assayed by array CGH (aCGH) for the TMPRSS2-ERG fusion; and (ii) 118 patients [immunohistochemical (IHC) cohort] whose biopsy samples were scored within a defined tissue microarray (TMA) immunostained for ERG overexpression (known surrogate for TMPRSS2-ERG fusion). Patients were treated with IGRT with a median dose of 76 Gy. The potential role of TMPRSS2-ERG status as a prognostic factor for biochemical relapse-free rate (bRFR; nadir + 2 ng/mL) was evaluated in the context of clinical prognostic factors in multivariable analyses using a Cox proportional hazards model.

**Results:** TMPRSS2-ERG fusion by aCGH was identified in 27 (21%) of the cases in the CGH cohort, and ERG overexpression was found in 59 (50%) patients in the IHC cohort. In both cohorts, TMPRSS2-ERG status was not associated with bRFR on univariate or multivariate analysis.

**Conclusions:** In two similarly treated IGRT cohorts, TMPRSS2-ERG status was not prognostic for bRFR, in disagreement with the hypothesis that these prostate cancers have DNA repair defects that render them clinically more radiosensitive. TMPRSS2-ERG is therefore unlikely to be a predictive factor for IGRT response. Clin Cancer Res; 19(18); 5202–9. ©2013 AACR.
Translational Relevance

Improved patient stratification using novel genetic prognosticators or response predictors could help individualize prostate cancer therapies. Preclinical studies have shown that TMPRSS2-ERG gene fusion, leading to ERG overexpression, may be a biomarker of DNA double-strand break (DSB) repair capacity with potential implications for sensitivity to radiotherapy or DNA-damaging modifying agents (e.g., PARP inhibitors). Using two different techniques [array comparative genomic hybridization (aCGH) and immunohistochemistry (IHC)], we did not observe that TMPRSS2-ERG status (as assayed in pretreatment biopsies of patients with intermediate-risk prostate cancer) is prognostic for biochemical outcome after image-guided radiotherapy. These clinical results suggest that the presence of a TMPRSS2-ERG fusion is not, de facto, associated with a clinical DSB repair defect that leads to prostate cancer cell radiosensitivity. If the presence of a fusion, or ERG overexpression, is associated with differential prognosis or treatment response, this would have major implications for its clinical use in a cancer that is diagnosed in more than 250,000 men in North America each year (9).

Intermediate-risk prostate cancer is defined by National Comprehensive Cancer Network (NCCN) as T1/T2–N0–M0 with a Gleason score (GS) 7 and prostate-specific antigen (PSA) < 20 ng/ml or GS < 7 and PSA 10 to 20 ng/ml (10). Clinical outcomes are highly heterogeneous within this risk category, with up to 30% to 40% of patients failing therapy independent of treatment modality (11, 12). Therefore, identification of additional prognostic factors that could stratify these patients into more precise prognostic or predictive subgroups based on individual tumor genetics would be extremely valuable.

Studies addressing the relationship between TMPRSS2-ERG gene fusions and prostate cancer aggressiveness or clinical outcome have provided conflicting results (13–18). However, in the largest cohort tested to date, ERG overexpression (determined by IHC) was not prognostic for biochemical recurrence following radical prostatectomy (19). This lack of prognostic significance in surgery patients was confirmed by a recent meta-analysis using biochemical recurrence and disease-specific mortality as endpoints (20). However, the role of TMPRSS2-ERG as a response modifier in patients receiving modern era radiotherapy has not yet been evaluated.

Precision radiotherapy delivered with image-guided radiotherapy (IGRT) is an important modality for prostate cancer treatment. Recent preclinical data suggest that TMPRSS2-ERG status may relate to DNA repair and radiotherapy-induced DNA damage. Using FISH, androgen signaling was found to induce proximity of the TMPRSS2 and ERG genomic loci (both located on chromosome 21q22.2), particularly following induction of DNA double-strand breaks (DSB) by irradiation or inhibition of topoisomerase II beta (TOP2B; refs. 21, 22). Other data support fusion status associated with altered sensitivity to DNA-damaging agents (23). Stable overexpression of TMPRSS2-ERG fusion product in prostate cancer cells can alter radiosensitivity, and TMPRSS2-ERG fusion status can render tumor cells sensitive to PARP1 inhibition in vitro and in vivo (24). In the latter study, the TMPRSS2-ERG fusion products interacted in a DNA-independent manner with PARP1 and the catalytic subunit of DNA protein kinase, a DSB repair protein. The authors concluded that overexpression of the TMPRSS2-ERG fusion induces DNA damage, which is potentiated by PARP inhibition (PARP1) and leads to cell death. This was similar to the cell death observed in PARP1-treated cells defective in the homologous recombination (HR) pathway of DSB repair.

Taken together, these preclinical data suggest that the TMPRSS2-ERG status of primary prostate cancer may reflect relative a priori DNA repair capacity and thus could alter the therapeutic response to DNA-damaging agents, including precision radiotherapy. If true, prostate cancer gene fusion status could be predictive for treatment outcome. We therefore tested the ability of TMPRSS2-ERG status to predict outcome in patients with intermediate-risk prostate cancer following clinically induced DSBs using IGRT.

Materials and Methods

Patient cohorts and treatment delivery

We investigated TMPRSS2-ERG status in pretreatment biopsies of patients with intermediate-risk prostate cancer using 2 different methods in 2 different cohorts: (i) TMPRSS2-ERG gene fusion assessed at the DNA level using array comparative genomic hybridization (aCGH); or (ii) ERG protein overexpression assayed by IHC. Both cohorts included patients who completed curative radical radiotherapy for histologically confirmed adenocarcinoma of the prostate as part of prospective clinical studies approved by the University Health Network Research Ethics Board and registered (NCT00160979; ISRCTN64733264) in accordance with the criteria outlined by the International Committee of Medical Journal Editors. This work followed the REMARK recommendations for tumor marker prognostic studies (ref. 25; Supplementary Table S1). The aCGH cohort consisted of 126 evaluable patients; further details on the assay technique and background tumor genetics for this cohort have been described previously (26). Clinical characteristics for both aCGH and IHC cohorts are presented in Table 1. To create the IHC cohort, formalin-fixed, paraffin-embedded (FFPE) pretreatment biopsies from 173 patients were used to construct a biopsy tissue microarray (TMA). Post-array, the cohort was reduced to 118 evaluable patients after a quality assurance protocol, which removed patients if malignant cores could not be scored within the histologic section; if the NCCN criteria of intermediate-risk disease were not met (27); or if they lacked follow-up data (see Fig. 1B).
with an assumed BED formula at 2 Gy daily fractions with a α/β ratio of 1.5 for tumor response.

As defined by Phoenix criteria (PSA nadir + 2 ng/mL); except an additional 5 patients in the aCGH cohort who were pre-emptively treated with salvage ADT due to increasing PSA posttreatment.
in prostatic adenocarcinoma cells relative to endothelial cells nuclei (which served as a positive control; see Fig. 1B). Checkers with faint or negative endothelial cell staining were excluded from analysis. ERG expression was then dichotomized for positive and negative expression. We considered a case positive for ERG expression if any of the replicate checkers from that case showed any positive ERG staining.

**Statistical analysis**

The primary outcome was biochemical relapse-free rate (bRFR) defined according to Phoenix criteria (PSA nadir + 2 ng/mL; ref. 34) or institution of salvage ADT (patients treated with ADT by their attending physician due to serial and increasing PSA values, post-IGRT). Time to biochemical failure was measured from the start of treatment until the date of biochemical failure or date of last PSA measurement. Five-year biochemical relapse-free rates were calculated using the Kaplan–Meier method. The associations between either TMPRSS2-ERG fusion or ERG overexpression and clinical factors were examined, using the Fisher’s exact test for GS and T category, and the Mann–Whitney test for pretreatment PSA. The log-rank test was used to compare relapse rates between patients with and without TMPRSS2-ERG fusion or ERG overexpression. The effects of TMPRSS2-ERG fusion and ERG overexpression on bRFR were also tested adjusting for pretreatment PSA, T category, and GS using Cox proportional hazards regression models. The proportional hazards assumption was checked using Schoenfeld residuals and found to be satisfied for all variables, with the exception of ADT in the aCGH cohort. A time-varying coefficient was added to the Cox model to account for this model violation. All statistical analyses were done using the R statistical environment (v2.12.1). HRs, 95% confidence intervals (CI) and P values using the Wald test were generated using the survival package version (v2.36-5). A 2-sided P < 0.05 was used to assess statistical significance.

**Results**

We designed this study to test whether IGRT patients had a differential prognosis based on fusion status. If true, fusion status would become a novel predictive factor for outcome in patients receiving radiotherapy (but not surgery). The clinical characteristics of both aCGH and IHC
cohorts are presented in Table 1. These cohorts were comprised of intermediate-risk patients mostly with T2 disease, GS 7, and PSA < 10 ng/mL. The mean radiation dose was 76 Gy. In the aCGH cohort, 27 of 126 biopsies (21%) were found to be TMPRSS2-ERG fusion–positive. In the IHC cohort, a positive ERG immunohistochemical staining was observed in 59 (50%) of the cases. We next tested whether fusion status was associated with more aggressive clinical states in our IGRT cohort. ERG overexpression was associated with T-category (T2 vs. T1; P = 0.02) but not with Gleason score (7 vs. 6; P = 1.00) or pretreatment PSA (continuous, P = 0.28). TMPRSS2-ERG fusion (aCGH cohort) was not correlated to any of these clinical variables (Supplementary Table S2A–S2F).

We then tested fusion status as a prognostic factor for biochemical failure following IGRT. At a median follow-up of 7.8 years (range, 0.8–12.2), 55 patients (44%) in the aCGH cohort experienced biochemical relapse (see Table 1). Of these, 20 had biopsy-proven local failure in which 5 were fusion-positive and 15 fusion-negative (a similar proportion to the entire cohort and arguing against fusion status associated with increased radiosresponse). For the IHC cohort, at a median follow-up of 7.2 years (range, 0.33–12.2), 31 (26%) patients presented biochemical failure. Of the 31 patients with biochemical failure in this cohort, 8 had biopsy-proven local failure (3 were ERG-positive and 5 ERG-negative); again showing no trend for ERG overexpression to be associated with increased radiosresponse.

The prognostic significance of pretreatment PSA, T-category, and GS for bRFR for both cohorts is shown in Table 2

### Table 2. Multivariate analysis of clinical prognostic factors for bRFR in the aCGH and IHC cohorts

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<th>HR (95% CI)</th>
<th>P</th>
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<td><strong>Clinical model, aCGH cohort</strong></td>
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<td></td>
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<tr>
<td>T category: 2 vs. 1</td>
<td>1.02 (0.56–1.85)</td>
<td>0.96</td>
</tr>
<tr>
<td>PSA (continuous)</td>
<td>1.13 (1.05–1.05)</td>
<td>0.001</td>
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<tr>
<td>GS 7 vs. 6</td>
<td>0.93 (0.49–1.77)</td>
<td>0.83</td>
</tr>
<tr>
<td>ADT</td>
<td>0.16 (0.03–0.87)</td>
<td>0.03</td>
</tr>
<tr>
<td>ADT with time</td>
<td>1.03 (1.01–1.05)</td>
<td>0.02</td>
</tr>
<tr>
<td><strong>Clinical model, IHC cohort</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fusion positive</td>
<td>0.79 (0.40–1.55)</td>
<td>0.49</td>
</tr>
<tr>
<td>T category: 2 vs. 1</td>
<td>2.16 (0.90–0.90)</td>
<td>0.09</td>
</tr>
<tr>
<td>PSA (continuous)</td>
<td>1.06 (0.97–1.15)</td>
<td>0.19</td>
</tr>
<tr>
<td>GS 7 vs. 6</td>
<td>1.32 (0.52–3.34)</td>
<td>0.56</td>
</tr>
<tr>
<td>ADT</td>
<td>0.92 (0.42–2.05)</td>
<td>0.84</td>
</tr>
<tr>
<td>ERG positive</td>
<td>0.89 (0.42–1.88)</td>
<td>0.76</td>
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In concert with other publications showing the potential predictive value of TMPRSS2-ERG status on ADT response (18, 35, 36), ERG overexpression was reported to be a factor in the relative response to salvage ADT following surgery (19). However, in our subgroup of patients treated with ADT, neither TMPRSS2-ERG fusion nor ERG overexpression predicted outcome. In addition, the analysis of those patients treated without ADT also showed no predictive value of TMPRSS2-ERG status. (See HR values associated with Kaplan–Meir plots in Supplementary Figs. S4 and S5.)

Given our goal to analyze IGRT patient outcome on the basis of aCGH or ERG overexpression as a prognostic versus predictive factor, we additionally determined whether fusion status was prognostic in a radical prostatectomy cohort in a similar low- to intermediate-risk cohort using a published dataset (37). Details for this surgical cohort were previously described (26). In this cohort, neither TMPRSS2-ERG fusion (by aCGH) nor ERG overexpression (based on mRNA abundance) was prognostic in 131 men with a median follow-up of 4.6 years (see Supplementary Figs. S6 and S7). Therefore, our studies suggest that TMPRSS2-ERG status is not prognostic in intermediate-risk patients treated with IGR or radical prostatectomy.

**Discussion**

To our knowledge, this is the first study to address the role of TMPRSS2-ERG status in pre-treatment biopsies of patients with prostate cancer treated with radical radiotherapy, one of the main treatment options for this disease. This is a prerequisite to using this information to personalize treatment at the time of diagnosis. Our clinical data shows that TMPRSS2-ERG status, assayed using ERG overexpression or by aCGH, is not a determinant of recurrence following precision local therapies.

There are 2 main pathways of DSB repair: (i) nonhomologous end-joining (NHEJ) in which a defect in this pathway leads to profound radiosensitivity and (ii) homologous recombination (HR) in which less profound, but still appreciable, radiosensitivity is observed (38). As such, if the fusion was associated with a defect in NHEJ or homologous recombination, we would have observed a profound and durable PSA response in fusion-positive prostate cancer relative to fusion-negative prostate cancer. Given that TMPRSS2-ERG status is not predictive for radiotherapy response, our clinical study does not support the preclinical hypothesis that fusion-positive, localized prostate cancer is functionally deficient in DSB repair to the extent that is clinically relevant for an IGRT treatment effect (21, 23, 24).
In addition, although limited data were available for the CGH and IHC cohorts, there was no evidence that post-radiation biopsy positivity was less in fusion-positive patients compared with fusion-negative patients. Finally, unlike the prognostic role of MYC amplification and/or loss of PTEN or NXX3.1 alleles (26, 39), fusion positivity does not lead to rapid early failure post-IGRT (suggestive of an association with occult metastases and relatively aggressive disease at the time of local treatment).

Our study has a number of limitations. Given the multifocality and molecular heterogeneity of prostate cancer, one possible weakness of our analysis is that aCGH data were based on only one biopsy to an index lesion (26) and 78% of the cases from IHC cohort had only one checker assayed. Although there is evidence showing that the dominant lesion is the most common location for recurrence post-treatment (40) and one core per tumor can be sufficient (19, 41), we cannot rule out that we have undercalled fusion status in this aCGH cohort (42). However, our additional and complementary analysis of 118 intermediate-risk patients in the IHC cohort showed ERG overexpression in 50% of those tumors. This is in the range of previous series assessing ERG expression by IHC, RT-PCR, or FISH (8, 43–45). Furthermore, we have shown that when more than one checker per patient was available, there was little intrapatient heterogeneity for ERG status (Fig. 2). Finally, given the CIs for HR as shown in Table 2 (aCGH cohort with CI: 0.40–1.55 and IHC cohort with CI: 0.42–1.88), it would be very unlikely that the true effect size is less than 0.40–0.42 or greater than 1.55–1.88 in the two cohorts. However, in the latter case, values greater than 1.0 would be associated with increasing risk of failure following IGRT (i.e.,

Figure 3. Univariate Kaplan–Meier plots of bRFR versus TMPRSS2-ERG status in the aCGH cohort (A) and IHC cohort (B).
radioresistant phenotype) which would still argue against the hypothesis that fusion status is a marker of defective DSB repair associated with tumor cell radiosensitivity.

In the future, it would be advantageous to collect post-IGRT biopsies for all patients in order to better define the role of fusion status in terms of local control versus systemic relapse. Karnes and colleagues have proposed that ERG-positive patients present a better response to androgen deprivation (36) and, recently, TMPRSS2-ERG status has been shown to be a predictive biomarker for androgen therapy in the form of abiraterone (46). As such, our results could differ in patients receiving combined modality therapy as the primary treatment (e.g., high-risk or locally advanced prostate cancer) in which fusion status could be studied in the context of the need for salvage ADT (including enzalutamide or abiraterone) or systemic chemotherapy. These concepts could be investigated in tissues prospectively collected in randomized clinical trials.

Recent evidence suggests that gene rearrangements involving TMPRSS2 and the ETS transcription factor EVI1 drive a distinct transcriptional program compared with TMPRSS2-ERG. In the context of PTEN deletion, these tumors seem to have more aggressive disease and poorer outcome (47). Furthermore, a quantitative assessment of EVI1 overexpression (47) and ERG overexpression (ref. 18; rather than positive or negative) has been reported to be prognostic across risk groups. Future studies using pretreatment biopsies could test these endpoints in TMAs where prostate cancer cellularity is increased to the extent that quantitative immunohistochemistry is possible (e.g., possibly high-risk prostate cancers). In our intermediate-risk series, selected samples had fewer than 50 cells and therefore quantitative scoring of expression was not deemed feasible.

Molecular prognostic and prediction is an important requirement in novel approaches to personalized cancer medicine. Only large prospective IGRT and dose-escalated cohorts, which also document the presence or absence of TMPRSS2-ERG gene fusion, will define its complete role in prostate cancer treatment. Additional clinical studies are required to understand the potential complex relation-

ship between TMPRSS2-ERG gene fusion, functional DNA repair, androgen receptor (AR) expression (48) and clinical outcome following treatment with agents that modify the DNA damage response, including PARP inhibitors.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): A. Dal Pra, F. Warde, F. Saad, P.C. Bourtos, R.G. Bristow

Study supervision: A. Dal Pra, A. Joshua, R.G. Bristow

Review and editing of manuscript: A. Dal Pra, J. Srigley, R.G. Bristow

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