Published OnlineFirst August 5, 2013; DOI: 10.1158/1078-0432.CCR-13-1049

Clinical Cancer Research

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

Alan Dal Pra, Emilie Lalonde, Jenna Sykes, et al.

Clin Cancer Res 2013;19:5202-5209. Published OnlineFirst August 5, 2013.

Updated version	Access the most recent version of this article at: doi:10.1158/1078-0432.CCR-13-1049
Supplementary Material	Access the most recent supplemental material at: http://clincancerres.aacrjournals.org/content/suppl/2013/08/05/1078-0432.CCR-13-1049.DC1.html

Cited Articles	This article cites by 47 articles, 18 of which you can access for free at: http://clincancerres.aacrjournals.org/content/19/18/5202.full.html#ref-list-1
Citing articles	This article has been cited by 2 HighWire-hosted articles. Access the articles at: http://clincancerres.aacrjournals.org/content/19/18/5202.full.html#related-urls

E-mail alerts	Sign up to receive free email-alerts related to this article or journal.
Reprints and Subscriptions	To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.
Permissions	To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.

Downloaded from clincancerres.aacrjournals.org on August 6, 2014. © 2013 American Association for Cancer Research.

Published OnlineFirst August 5, 2013; DOI: 10.1158/1078-0432.CCR-13-1049

Clinical Cancer Research

Predictive Biomarkers and Personalized Medicine

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

Alan Dal Pra[1,2], Emilie Lalonde[1,3], Jenna Sykes[1], Fiona Warde[1,2], Adrian Ishkanian[1,2], Alice Meng[1], Chad Maloff[4], John Srigley[6], Anthony M. Joshua[1], Gyorgy Petrovics[7], Theodorus van der Kwast[1], Andrew Evans[1], Michael Milosevic[1,2], Fred Saad[8], Colin Collins[5], Jeremy Squire[9], Wan Lam[4], Tarek A. Bismar[10], Paul C. Boutros[1,3], and Robert G. Bristow[1,2]

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 multivariate 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.*

Authors' Affiliations: [1]Departments of Radiation Oncology, Medical Biophysics, Medical Oncology, Laboratory Medicine and Pathology and Biostatistics, University of Toronto; [2]Radiation Medicine Program, Princess Margaret Hospital, University Health Network; [3]Informatics and Bio-Computing Platform, Ontario Institute for Cancer Research, Toronto, Ontario; [4]Department of Cancer Genetics and Developmental Biology, British Columbia Cancer Research Centre; [5]Laboratory for Advanced Genome Analysis - Vancouver Prostate Centre, Vancouver, British Columbia; [6]Department of Pathology and Molecular Medicine, McMaster University, Hamilton, Ontario, Canada; [7]Center for Prostate Disease Research, Department of Surgery, Uniformed Services University of the Health Sciences, Rockville, Maryland; [8]Division of Urology, University of Montreal Health Center, Montreal, Quebec; [9]Department of Pathology and Molecular Medicine, Queen's University, Kingston, Ontario; and [10]Department of Pathology and Laboratory Medicine and Oncology, University of Calgary, Calgary, Alberta, Canada

Note: Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

Corresponding Author: Robert G. Bristow, Radiation Medicine Program, Princess Margaret Hospital, 610 University Avenue, Toronto, Ontario M5G 2M9, Canada. Phone: 416-946-2936; Fax: 416-946-4586; E-mail: rob.bristow@rmp.uhn.on.ca

doi: 10.1158/1078-0432.CCR-13-1049

©2013 American Association for Cancer Research.

Introduction

Chromosomal rearrangements have a critical role in oncogenic events in prostate cancer. Tomlins and colleagues reported a recurrent gene rearrangement involving the 5′-untranslated region of the androgen-regulated *TMPRSS2* (transmembrane protease serine 2) gene with *ETS* (erythroblast transformation specific) gene family members, including *ERG* [v-ets erythroblastosis virus E26 oncogene homolog (avian), chromosome 21q22.3] or *ETV1* (ets variant 1, chromosome 7p21.3; ref. 1). *ETS* family members are involved in multiple signaling pathways associated with cancer formation and progression (2–4). About 50% of clinically localized prostate cancers harbor *TMPRSS2-ERG* gene fusions, leading to ERG overexpression (5). Newer immunohistochemical (IHC) approaches using ERG-specific antibodies have shown that ERG protein overexpression *in situ* is a sensitive and specific surrogate for the presence of *TMPRSS2-ERG* gene fusion detected by FISH or quantitative reverse transcriptase PCR (qRT-PCR; refs. 1,

AACR American Association for Cancer Research

Downloaded from clincancerres.aacrjournals.org on August 6, 2014. © 2013 American Association for Cancer Research.

Published OnlineFirst August 5, 2013; DOI: 10.1158/1078-0432.CCR-13-1049

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 tumor cell radiosensitivity.

6–8). 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 aggression 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 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 (PARPi) and leads to cell death. This was similar to the cell death observed in PARPi-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).

Downloaded from clincancerres.aacrjournals.org on August 6, 2014. © 2013 American Association for Cancer Research.

Published OnlineFirst August 5, 2013; DOI: 10.1158/1078-0432.CCR-13-1049

Table 1. Clinical characteristics of aCGH and IHC treatment cohorts

	IHC cohort (n = 118) n (%)	aCGH cohort (n = 126) n (%)
T score		
T1	43 (36%)	45 (36%)
T2	75 (63%)	81 (64%)
Gleason score		
6	29 (24%)	31 (25%)
7	89 (74%)	95 (75%)
Pretreatment PSA		
≤10	79 (66%)	88 (70%)
>10	39 (33%)	38 (30%)
Median (range)	7.7 (1.3–19.6)	7.8 (0.9–19)
ADT	35 (29%)	33 (26%)
RT dose		
60 Gy/20 fr	7 (6%)	12 (10%)
66 Gy/22 fr	4 (3%)	3 (2%)
75.6 Gy/42 fr	27 (23%)	33 (26%)
78 Gy/39 fr	4 (3%)	3 (2%)
79.8 Gy/42 fr	76 (63%)	75 (60%)
Mean equivalent dose[a]	76.4 Gy	76 Gy
Biochemical failures[b]	31 (26%)	55 (44%)
Deaths	12 (10%)	7 (5%)
Median FU, y	7.2	7.8
Range	(0.33–12.2)	(0.8–12.2)

Abbreviation: FU, follow-up; RT, radiotherapy.
[a]Mean equivalent dose was calculated using BED formula at 2 Gy daily fractions with an α/β ratio of 1.5 for tumor response.
[b]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.

For both cohorts, patients underwent transrectal ultrasound (TRUS)-guided insertion of 3 intraprostatic gold fiducial markers for radiotherapy planning and IGRT. Research biopsies (2 for formalin fixation and 1 fresh frozen in liquid N_2) were taken during fiducial marker insertion. Staging computed tomography (CT) and bone scans were not routinely conducted. The clinical target volume (CTV) encompassed the prostate gland alone. The planning target volume (PTV) was defined by a 10-mm margin around the CTV, except posteriorly where the margin was 7 mm. All patients were treated with 6-field conformal or intensity-modulated radiotherapy (IMRT) with image guidance. The radiotherapy dose was variable within the 2 cohorts, so doses were converted to biologically effective doses (BED) with an assumed α/β of 1.5 (28). Dose details are presented in Table 1 for both cohorts. Neoadjuvant and concurrent hormonal therapy [androgen deprivation therapy (ADT)] was used in 33 patients (26%) in the aCGH cohort and in 35 patients (29%) in the IHC cohort. This ADT consisted of bicalutamide 150 mg daily for 3 months of neoadjuvant treatment followed by a further 2 months as concurrent treatment with radiotherapy (ISRCTN64733264; ref. 29). Patients were followed at 6 monthly intervals after completing treatment with clinical examination and PSA testing. Additional tests and the management of patients with recurrent disease were at the discretion of the treating physician. The median follow-up of surviving patients was 7.8 and 7.2 years following the start of radiotherapy for the aCGH and IHC cohorts, respectively.

aCGH analysis

The biopsy preparation, DNA extraction, aCGH procedure, and copy number detection were previously described (26, 30). For each patient, the presence of a *TMPRSS2-ERG* gene fusion was defined as an observation of a 21q22.2-3 genomic deletion. More specifically, a deletion must overlap with the region contained by the 5′ and 3′ ends of *ERG* and *TMPRSS2*, respectively (Supplementary Fig. S1).

TMA and IHC

The biopsy TMA was constructed from pretreatment prostate biopsies using a "checkerboard" technique as previously described (31). Benign and malignant prostate tissues within each core were denoted for dissection based on hematoxylin and eosin (H&E)-stained sections by an experienced genitourinary pathologist (T. van der Kwast). On the basis of pathologic markings, 4-mm-long "checkers" were cut along the length of the biopsies and flipped 90° and placed within a TMA template (see Supplementary Fig. S2). Although 173 patients had diagnostic biopsy blocks available, a pathologic re-assessment was completed in which each checker was confirmed between contiguous slices for the presence or absence of malignancy. After this quality assurance step, and after removing patients lacking follow-up data or who did not present with intermediate-risk disease, a total of 118 patients remained for comparison to clinical parameters and outcome (Fig. 1A). An assessment of the intrapatient heterogeneity of number of checkers is shown in Fig. 2 as evaluated using the Kappa and Fleiss Kappa approaches (32, 33). This analysis showed that for patients with more than one checker, there was significant agreement between ERG staining results.

Immunostaining of the TMAs for ERG was conducted as follows: deparaffinized 4-μm sections were dehydrated, blocked in 0.6% hydrogen peroxide in methanol for 20 minutes, and processed for antigen retrieval in EDTA (pH 9.0) for 30 minutes in a microwave, followed by 30 minutes of cooling in EDTA buffer. Sections were then blocked in 1% horse serum followed by an overnight incubation with the ERG–MAb mouse monoclonal antibody (Biocare Medical clone 9Fy), diluted 1:300 at room temperature. The immunostaining was developed using the Polymer-HRP IHC Kit (Biogenex) according to manufacturer's instructions. Next, sections were counterstained in hematoxylin for 1 minute, dehydrated, cleared, and mounted. Immunostained TMA checkers were evaluated for ERG staining based on the presence or absence of positive nuclear immunoreactivity

Downloaded from clincancerres.aacrjournals.org on August 6, 2014. © 2013 American Association for Cancer Research.

Published OnlineFirst August 5, 2013; DOI: 10.1158/1078-0432.CCR-13-1049

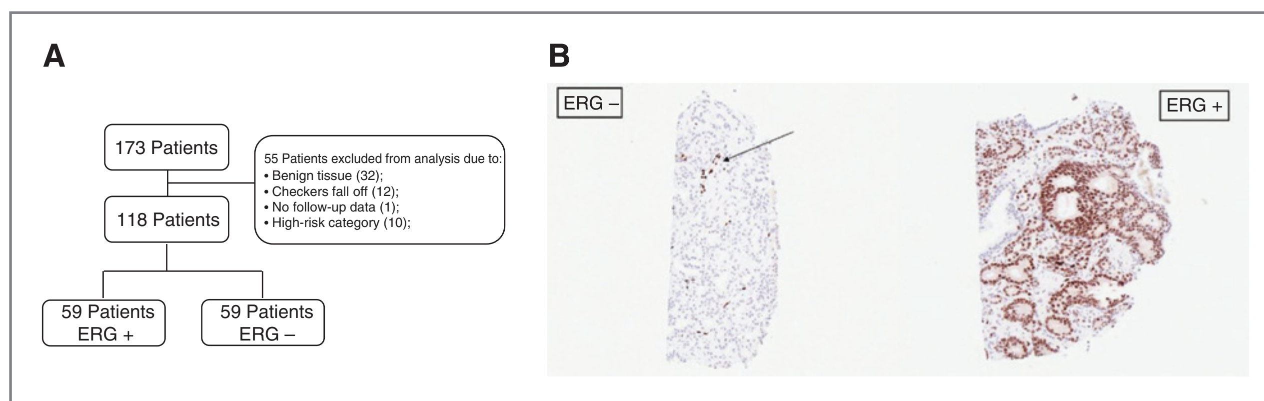
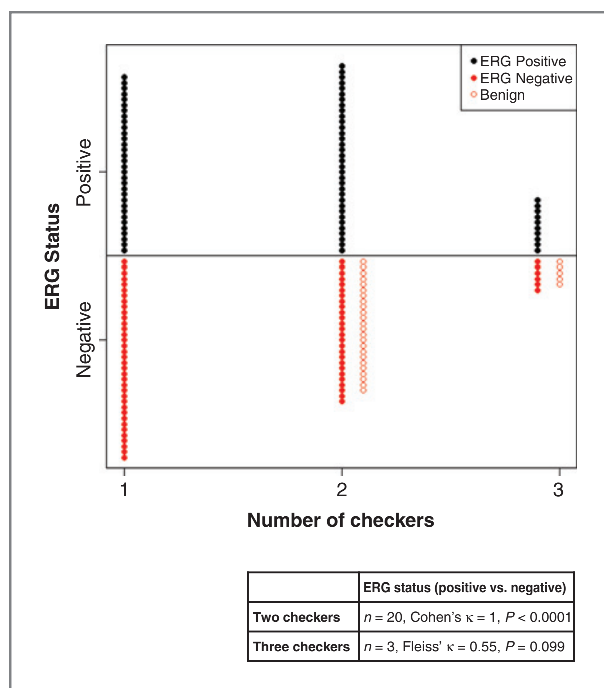


Figure 1. A, study flowchart. After exclusion of 55 patients, a total of 118 patients were available for analysis. B, representative images of ERG immunohistochemistry in an ERG-negative checker in a GS 7 prostate adenocarcinoma (left) showing positive endothelial cells as control (arrow); and an ERG-positive checker in a GS 7 (right).

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.



	ERG status (positive vs. negative)
Two checkers	$n = 20$, Cohen's $\kappa = 1$, $P < 0.0001$
Three checkers	$n = 3$, Fleiss' $\kappa = 0.55$, $P = 0.099$

Figure 2. Frequency histogram showing the distribution of the 118 patients according to ERG staining (positive vs. negative), presence of malignant tissue in the checker (no benign tissue was ERG positive), and number of checkers per patient (ranging from 1 to 3). The table depicts the analysis of intrapatient heterogeneity of checkers. Eighty percent (95 of 118) of the patients had information on a single biopsy checker. There were 20 patients who had ERG scored on 2 checkers, and 3 patients who had ERG scored on 3 checkers. Cohen's Kappa (32) was used to assess patients with 2 checkers and Fleiss' Kappa (33), 3 checkers. Patients with 2 checkers were in perfect agreement (Cohen's $\kappa = 1$, $P < 0.0001$), whereas for the 3 patients with 3 checkers, the checkers were in moderate agreement ($\kappa = 0.55$, $P = 0.099$).

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

Downloaded from clincancerres.aacrjournals.org on August 6, 2014. © 2013 American Association for Cancer Research.

Published OnlineFirst August 5, 2013; DOI: 10.1158/1078-0432.CCR-13-1049

cohorts are presented in Table 1. These cohorts were comprised by 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 radioresponse). 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 radioresponse.

The prognostic significance of pretreatment PSA, T-category, and GS for bRFR for both cohorts is shown in Table 2 and Supplementary Fig. S3A–S3F. Only pretreatment PSA in the aCGH cohort was prognostic for bRFR. We then added data pertaining to fusion status into the model. *TMPRSS2-ERG* status, whether assayed by aCGH or IHC, was not prognostic for bRFR following radiotherapy in either univariate or multivariate analyses (see Fig. 3A and B, respectively). The univariate HRs associated with *TMPRSS2-ERG* in the aCGH and IHC cohorts were 0.78 (95% CI, 0.41–1.49; $P = 0.46$) and 0.99 (95% CI, 0.48–2.02; $P = 0.97$), respectively.

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

	HR (95% CI)	*P*
Clinical model, aCGH cohort		
T category: 2 vs. 1	1.02 (0.56–1.85)	0.96
PSA (continuous)	1.13 (1.05–1.05)	0.001
GS 7 vs. 6	0.93 (0.49–1.77)	0.83
ADT	0.16 (0.03–0.87)	0.03
ADT with time	1.03 (1.01–1.05)	0.02
Clinical model, IHC cohort		
Fusion positive	0.79 (0.40–1.55)	0.49
T category: 2 vs. 1	2.16 (0.90–0.90)	0.09
PSA (continuous)	1.06 (0.97–1.15)	0.19
GS 7 vs. 6	1.32 (0.52–3.34)	0.56
ADT	0.92 (0.42–2.05)	0.84
ERG positive	0.89 (0.42–1.88)	0.76

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) were 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 IGRT 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 prognostic factor for biochemical recurrence after IGRT. This was also true for a small subgroup of patients treated with neoadjuvant and concurrent high-dose (150 mg/d) bicalutamide. Given recent data in surgical cohorts, this suggests that *TMPRSS2-ERG* status 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).

Downloaded from clincancerres.aacrjournals.org on August 6, 2014. © 2013 American Association for Cancer Research.

Published OnlineFirst August 5, 2013; DOI: 10.1158/1078-0432.CCR-13-1049

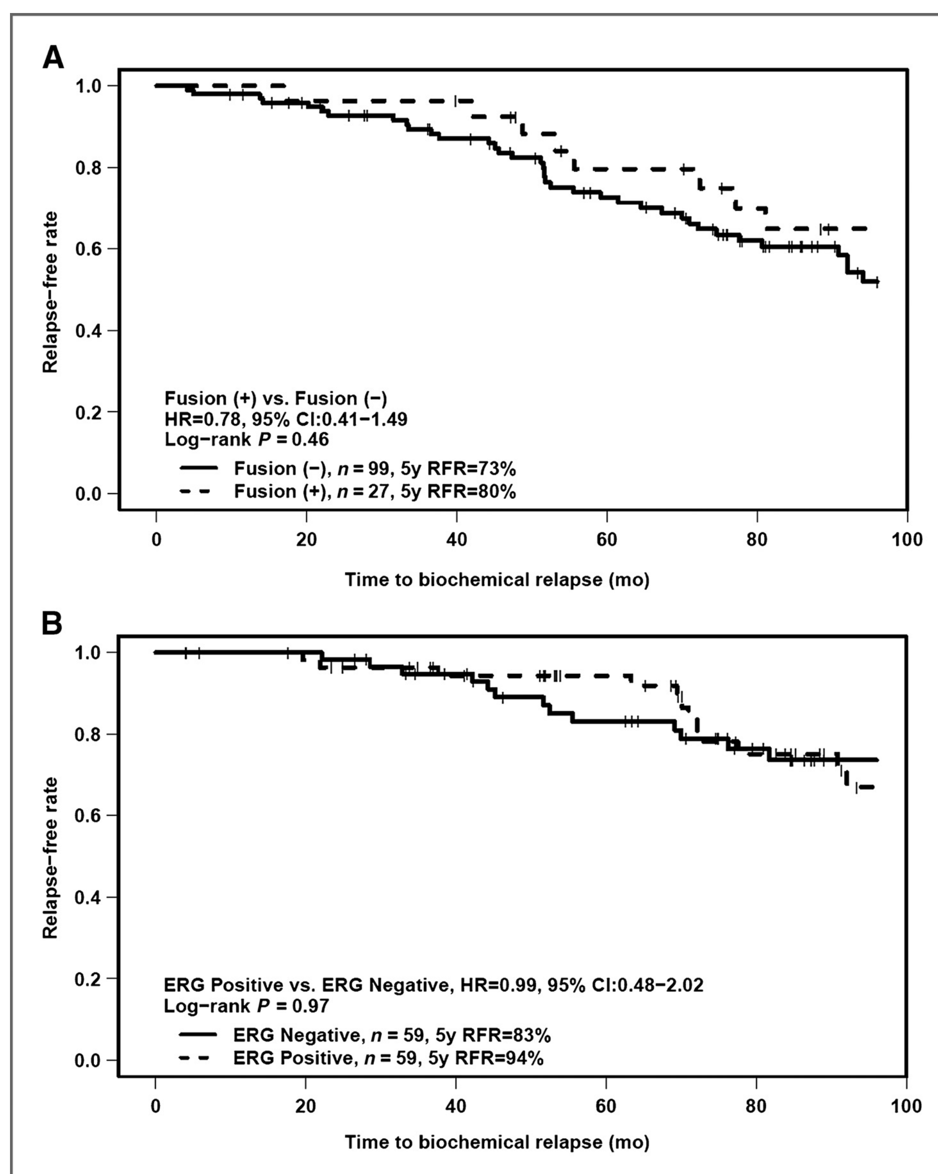


Figure 3. Univariate Kaplan–Meier plots of bRFR versus *TMPRSS2-ERG* status in the aCGH cohort (A) and IHC cohort (B).

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 *NKX3.1* 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.,

Downloaded from clincancerres.aacrjournals.org on August 6, 2014. © 2013 American Association for Cancer Research.

Published OnlineFirst August 5, 2013; DOI: 10.1158/1078-0432.CCR-13-1049

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 *ETV1* 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 ETV1 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 relationship 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.

Authors' Contributions

Conception and design: A. Dal Pra, A.S. Ishkanian, A. Joshua, G. Petrovics, A. Evans, M. Milosevic, R.G. Bristow
Development of methodology: A. Dal Pra, E. Lalonde, F. Warde, A.S. Ishkanian, A.X. Meng, C.A. Malloff, A. Joshua, G. Petrovics, A. Evans, M. Milosevic, P.C. Boutros, R.G. Bristow
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): A. Dal Pra, F. Warde, A.S. Ishkanian, C.A. Malloff, J. Srigley, T. van der Kwast, A. Evans, M. Milosevic, W.L. Lam, R.G. Bristow
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): A. Dal Pra, E. Lalonde, J. Sykes, F. Warde, C.A. Malloff, A. Evans, F. Saad, C.C. Collins, J.A. Squire, W.L. Lam, T.A. Bismar, P.C. Boutros, R.G. Bristow
Writing, review, and/or revision of the manuscript: A. Dal Pra, E. Lalonde, J. Sykes, F. Warde, A. Joshua, T. van der Kwast, A. Evans, M. Milosevic, F. Saad, C.C. Collins, W.L. Lam, T.A. Bismar, P.C. Boutros, R.G. Bristow
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): A. Dal Pra, F. Warde, F. Saad, P.C. Boutros, 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

Grant Support

This study is supported by funding and grants from the Ontario Institute for Cancer Research (to P.C. Boutros and R.G. Bristow), the Canadian Foundation for Innovation grant to the STTARR Innovation Facility (to R. G. Bristow), Prostate Cancer Canada (with funds from the Movember Foundation) to the Canadian Prostate Cancer Genome Network (CPC-GENE; to T. van der Kwast, C.C. Collins, P.C. Boutros, R.G. Bristow), and from the Terry Fox Research Institute to the Canadian Prostate Cancer Biomarker Network (CPCBN; to J. Sykes, T. van der Kwast, F. Saad, T.A. Bismar, R.G. Bristow). This research was also funded in part by the Ontario Ministry of Health and Long Term Care. The views expressed do not necessarily reflect those of the Ontario Ministry of Health and Long Term Care. R.G. Bristow is a Canadian Cancer Society Research Scientist. A.D. Pra received a Canadian Urologic Oncology Group (CUOG) Research Award and E. Lalonde was awarded a CIHR Doctoral Fellowship.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received April 16, 2013; revised July 8, 2013; accepted July 9, 2013; published OnlineFirst August 15, 2013.

References

1. Tomlins SA, Rhodes DR, Perner S, Dhanasekaran SM, Mehra R, Sun XW, et al. Recurrent fusion of TMPRSS2 and ETS transcription factor genes in prostate cancer. Science 2005;310:644–8.
2. Zong Y, Xin L, Goldstein AS, Lawson DA, Teitell MA, Witte ON. ETS family transcription factors collaborate with alternative signaling pathways to induce carcinoma from adult murine prostate cells. Proc Natl Acad Sci U S A 2009;106:12465–70.
3. Gupta S, Iljin K, Sara H, Mpindi JP, Mirtti T, Vainio P, et al. FZD4 as a mediator of ERG oncogene-induced WNT signaling and epithelial-to-mesenchymal transition in human prostate cancer cells. Cancer Res 2010;70:6735–45.
4. Sun C, Dobi A, Mohamed A, Li H, Thangapazham RL, Furusato B, 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.
5. Rubin MA, Maher CA, Chinnaiyan AM. Common gene rearrangements in prostate cancer. J Clin Oncol 2011;29:3659–68.
6. Yoshimoto M, Joshua AM, Cunha IW, Coudry RA, Fonseca FP, Ludkovski O, et al. Absence of TMPRSS2:ERG fusions and PTEN losses in prostate cancer is associated with a favorable outcome. Mod Pathol 2008;21:1451–60.
7. Falzarano SM, Zhou M, Carver P, Tsuzuki T, Simmerman K, He H, et al. ERG gene rearrangement status in prostate cancer detected by immunohistochemistry. Virchows Arch 2011;459:441–7.
8. Rosen P, Sesterhenn IA, Brassell SA, McLeod DG, Srivastava S, Dobi A. Clinical potential of the ERG oncoprotein in prostate cancer. Nat Rev Urol 2012;9:131–7.
9. Siegel R, Naishadham D, Jemal A. Cancer statistics, 2012. CA Cancer J Clin 2012;62:10–29.
10. Mohler JL, Armstrong AJ, Bahnson RR, Boston B, Busby JE, D'Amico AV, et al. Prostate cancer, Version 3.2012: featured

Downloaded from clincancerres.aacrjournals.org on August 6, 2014. © 2013 American Association for Cancer Research.

Published OnlineFirst August 5, 2013; DOI: 10.1158/1078-0432.CCR-13-1049

updates to the NCCN guidelines. J Natl Compr Canc Netw 2012; 10:1081–7.
11. Nichol AM, Warde P, Bristow RG. Optimal treatment of intermediate-risk prostate carcinoma with radiotherapy: clinical and translational issues. Cancer 2005;104:891–905.
12. Grimm P, Billiet I, Bostwick D, Dicker AP, Frank S, Immerzeel J, et al. Comparative analysis of prostate-specific antigen free survival outcomes for patients with low, intermediate and high risk prostate cancer treatment by radical therapy. Results from the Prostate Cancer Results Study Group. BJU Int 2012;109 Suppl 1:22–9.
13. Demichelis F, Fall K, Perner S, Andren O, Schmidt F, Setlur SR, et al. TMPRSS2:ERG gene fusion associated with lethal prostate cancer in a watchful waiting cohort. Oncogene 2007;26:4596–9.
14. Nam RK, Sugar L, Yang W, Srivastava S, Klotz LH, Yang LY, et al. Expression of the TMPRSS2:ERG fusion gene predicts cancer recurrence after surgery for localised prostate cancer. Br J Cancer 2007; 97:1690–5.
15. Attard G, Clark J, Ambroisine L, Mills IG, Fisher G, Flohr P, et al. Heterogeneity and clinical significance of ETV1 translocations in human prostate cancer. Br J Cancer 2008;99:314–20.
16. Cheville JC, Karnes RJ, Therneau TM, Kosari F, Munz JM, Tillmans L, et al. Gene panel model predictive of outcome in men at high-risk of systemic progression and death from prostate cancer after radical retropubic prostatectomy. J Clin Oncol 2008;26: 3930–6.
17. Gopalan A, Leversha MA, Satagopan JM, Zhou Q, Al-Ahmadie HA, Fine SW, et al. TMPRSS2-ERG gene fusion is not associated with outcome in patients treated by prostatectomy. Cancer Res 2009;69: 1400–6.
18. Bismar TA, Dolph M, Teng LH, Liu S, Donnelly B. ERG protein expression reflects hormonal treatment response and is associated with Gleason score and prostate cancer specific mortality. Eur J Cancer 2012;48:538–46.
19. Minner S, Enodien M, Sirma H, Luebke AM, Krohn A, Mayer PS, et al. ERG status is unrelated to PSA recurrence in radically operated prostate cancer in the absence of antihormonal therapy. Clin Cancer Res 2011;17:5878–88.
20. Pettersson A, Graff RE, Bauer SR, Pitt MJ, Lis RT, Stack EC, et al. The TMPRSS2:ERG rearrangement, ERG expression, and prostate cancer outcomes: a cohort study and meta-analysis. Cancer Epidemiol Biomarkers Prev 2012;21:1497–509.
21. Mani RS, Tomlins SA, Callahan K, Ghosh A, Nyati MK, Varambally S, et al. Induced chromosomal proximity and gene fusions in prostate cancer. Science 2009;326:1230.
22. Haffner MC, Aryee MJ, Toubaji A, Esopi DM, Albadine R, Gurel B, et al. Androgen-induced TOP2B-mediated double-strand breaks and prostate cancer gene rearrangements. Nat Genet 2010;42: 668–75.
23. Swanson TA, Krueger SA, Galoforo S, Thibodeau BJ, Martinez AA, Wilson GD, et al. TMPRSS2/ERG fusion gene expression alters chemo- and radio-responsiveness in cell culture models of androgen independent prostate cancer. Prostate. 2011 Mar 10. [Epub ahead of print].
24. Brenner JC, Ateeq B, Li Y, Yocum AK, Cao Q, Asangani IA, et al. Mechanistic rationale for inhibition of poly(ADP-ribose) polymerase in ETS gene fusion-positive prostate cancer. Cancer Cell 2011;19: 664–78.
25. McShane LM, Altman DG, Sauerbrei W, Taube SE, Gion M, Clark GM, et al. Reporting recommendations for tumor marker prognostic studies (REMARK). J Natl Cancer Inst 2005;97:1180–4.
26. Locke JA, Zafarana G, Ishkanian AS, Milosevic M, Thoms J, Have CL, et al. NKX3.1 haploinsufficiency is prognostic for prostate cancer relapse following surgery or image-guided radiotherapy. Clin Cancer Res 2012;18:308–16.
27. Mohler J, Bahnson RR, Boston B, Busby JE, D'Amico A, Eastham JA, et al. NCCN clinical practice guidelines in oncology: prostate cancer. J Natl Compr Canc Netw 2010;8:162–200.
28. Barendsen GW. Dose fractionation, dose rate and iso-effect relationships for normal tissue responses. Int J Radiat Oncol Biol Phys 1982;8: 1981–97.
29. Mok G, Glicksman R, Sykes J, Bayley A, Chung P, Bristow R, et al. Short term hormone therapy and dose escalated radiation for localized prostate cancer: a randomized phase III study. Radiol Oncol 2012; 105:273–382.
30. Ishkanian AS, Zafarana G, Thoms J, Bristow RG. Array CGH as a potential predictor of radiocurability in intermediate risk prostate cancer. Acta Oncol 2010;49:888–94.
31. Jhavar S, Corbishley CM, Dearnaley D, Fisher C, Falconer A, Parker C, et al. Construction of tissue microarrays from prostate needle biopsy specimens. Br J Cancer 2005;93:478–82.
32. Fleiss J. Measuring Nominal Scale Agreement among many raters. Psychol Bull 1971;76:378–82.
33. Cohen J. A coefficient of agreement for nominal scales. Educ Psychol Meas 1960;20:37–46.
34. Roach M III, Hanks G, Thames H Jr, Schellhammer P, Shipley WU, Sokol GH, et al. Defining biochemical failure following radiotherapy with or without hormonal therapy in men with clinically localized prostate cancer: recommendations of the RTOG-ASTRO Phoenix Consensus Conference. Int J Radiat Oncol Biol Phys 2006;65:965–74.
35. Attard G, Swennenhuis JF, Olmos D, Reid AH, Vickers E, A'Hern R, 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.
36. Karnes RJ, Cheville JC, Ida CM, Sebo TJ, Nair AA, Tang H, et al. The ability of biomarkers to predict systemic progression in men with high-risk prostate cancer treated surgically is dependent on ERG status. Cancer Res 2010;70:8994–9002.
37. Taylor BS, Schultz N, Hieronymus H, Gopalan A, Xiao Y, Carver BS, et al. Integrative genomic profiling of human prostate cancer. Cancer Cell 2010;18:11–22.
38. Thoms J, Bristow RG. DNA repair targeting and radiotherapy: a focus on the therapeutic ratio. Semin Radiat Oncol 2010;20:217–22.
39. Zafarana G, Ishkanian AS, Malloff CA, Locke JA, Sykes J, Thoms J, et al. Copy number alterations of c-MYC and PTEN are prognostic factors for relapse after prostate cancer radiotherapy. Cancer 2012; 118:4053–62.
40. Freedland SJ, Pantuck AJ, Paik SH, Zisman A, Graeber TG, Eisenberg D, et al. Heterogeneity of molecular targets on clonal cancer lines derived from a novel hormone-refractory prostate cancer tumor system. Prostate 2003;55:299–307.
41. Kollermann J, Schlomm T, Bang H, Schwall GP, von Eichel-Streiber C, Simon R, et al. Expression and prognostic relevance of annexin A3 in prostate cancer. Eur Urol 2008;54:1314–23.
42. Minner S, Gartner M, Freudenthaler F, Bauer M, Kluth M, Salomon G, et al. Marked heterogeneity of ERG expression in large primary prostate cancers. Mod Pathol 2013;26:106–16.
43. Yoshimoto M, Joshua AM, Chilton-Macneill S, Bayani J, Selvarajah S, Evans AJ, et al. Three-color FISH analysis of TMPRSS2/ERG fusions in prostate cancer indicates that genomic microdeletion of chromosome 21 is associated with rearrangement. Neoplasia 2006;8:465–9.
44. Kumar-Sinha C, Tomlins SA, Chinnaiyan AM. Recurrent gene fusions in prostate cancer. Nat Rev Cancer 2008;8:497–511.
45. Park K, Tomlins SA, Mudaliar KM, Chiu YL, Esgueva R, Mehra R, et al. Antibody-based detection of ERG rearrangement-positive prostate cancer. Neoplasia 2010;12:590–8.
46. Attard G, Reid AH, A'Hern R, Parker C, Oommen NB, Folkerd E, et al. Selective inhibition of CYP17 with abiraterone acetate is highly active in the treatment of castration-resistant prostate cancer. J Clin Oncol 2009;27:3742–8.
47. Baena E, Shao Z, Linn DE, Glass K, Hamblen MJ, Fujiwara Y, et al. ETV1 directs androgen metabolism and confers aggressive prostate cancer in targeted mice and patients. Genes Dev 2013;27:683–98.
48. Schiewer MJ, Goodwin JF, Han S, Brenner JC, Augello MA, Dean JL, et al. Dual roles of PARP-1 promote cancer growth and progression. Cancer Discov 2012;2:1134–49.

Downloaded from clincancerres.aacrjournals.org on August 6, 2014. © 2013 American Association for Cancer Research.