TMPRSS2:ERG Gene Fusion Predicts Subsequent Detection of Prostate Cancer in Patients With High-Grade Prostatic Intraepithelial Neoplasia

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ABSTRACT

Purpose

High-grade prostatic intraepithelial neoplasia (HGPIN) is considered a precursor lesion of prostate cancer (PCa). The predictive value of *ERG* gene fusion in HGPIN for PCa was interrogated as a post hoc analysis in the context of a randomized clinical trial.

Patients and Methods

The GTx Protocol G300104 randomly assigned 1,590 men with biopsy-diagnosed HGPIN to receive toremifene or placebo for 3 years or until a diagnosis of PCa was made on prostate biopsy. As part of this phase III clinical trial, a central pathologist evaluated biopsies of patients with isolated HGPIN at baseline and 12, 24, and 36 months of follow-up. ERG immunohistochemistry was performed on biopsies from 461 patients and evaluated for protein overexpression.

Results

ERG expression was detected in 11.1% of patients (51 of 461 patients) with isolated HGPIN. In the first year and during the 3-year clinical trial, 14.7% and 36.9% of 461 patients were diagnosed with PCa, respectively. Patients with ERG expression were more likely to develop PCa, with 27 (53%) of 51 ERG-positive and 143 (35%) of 410 ERG-negative patients experiencing progression to PCa ($P = .014$, Fisher's exact test). ERG expression was not associated with age, baseline PSA, Gleason score, or tumor volume.

Conclusion

This study underscores the necessity of more stringent follow-up for men with HGPIN that is also positive for ERG overexpression. Clinicians should consider molecular characterization of HGPIN as a means to improve risk stratification.

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INTRODUCTION

Prostate cancer (PCa) is the second leading cause of cancer deaths among men in the United States, with an expected 30,000 deaths each year.¹ One of the determinants of a patient's risk for having or developing PCa, independent of age, race, and family history, is the presence of the precursor lesion called **high-grade prostatic intraepithelial neoplasia (HGPIN)**.² Historically, more than 40% of patients with HGPIN will be diagnosed with PCa within 3 years of the diagnosis of HGPIN and up to 80% will be diagnosed within 8 years^{3,4}; however, the diagnosis rates of PCa after an initial diagnosis of HGPIN have decreased as more aggressive biopsy templates have reduced the number of cancers missed on initial biopsy.⁵ Recommendations for care of patients with HGPIN include repeat prostate biopsy in 3 to 6 months and continued monitoring of prostate-

specific antigen (PSA) levels and digital rectal exam.⁶ However, considerable heterogeneity exists in both academic and community practices regarding management of HGPIN. Many urologists today will not rebiopsy based solely on the presence of HGPIN as a result of the low likelihood of recurrent disease in the absence of an increasing PSA. In the setting of a large clinical trial evaluating HGPIN progression, we sought to explore whether the presence of a neoplastic specific molecular event in HGPIN would improve our ability to identify men at a significantly higher risk of harboring or developing cancer determined by a repeat needle biopsy.

Approximately 50% of PCa harbors an acquired chromosomal translocation that results in the fusion of the promoter region of the androgen-regulated transmembrane protease serine 2 (*TMPRSS2*) gene to the coding region of members of the erythroblast transformation-specific (*ETS*) family of

transcription factors.^{7,8} The most common PCa gene fusion involves *TMPRSS2* and v-ets erythroblastosis virus E26 oncogene homolog (avian) (*ERG*). This recurrent molecular event is observed in HGPIN and PCa but not benign prostate tissue.⁹ Prior work shows that the gene fusion occurs early in PCa development, with approximately 15% of HGPIN demonstrating *ERG* rearrangement,⁹⁻¹³ suggesting that *TMPRSS2-ERG* gene fusion defines a distinct molecular subclass of PCa.¹⁴ Subsequent studies have demonstrated that *ERG* can also be rearranged and fused with other 5' androgen-regulated partners such as *SLC45A3* or *NDRG1*,¹⁵⁻¹⁷ accounting for approximately an additional 5% of the *ERG*-overexpressing PCa. In this current study, we retrospectively interrogated the presence of *ERG* gene fusion in HGPIN and the association of PCa in the context of a clinical trial.¹⁸

PATIENTS AND METHODS

Patient Selection Criteria

A randomized phase III, double-blinded, placebo-controlled clinical trial was conducted to test the effects of toremifene citrate in preventing PCa.¹⁸ To be eligible for the trial, patients had to be male, over the age of 30 years, have a serum PSA ≤ 10 ng/mL, and a diagnosis of HGPIN from any previous biopsy. They were required to have had a prostate biopsy in the last 6 months with a minimum of 10 cores or have had two prostate biopsies (each with a minimum of six cores) in the 12 months before screening with at least one of the biopsies occurring within 6 months before the screening visit. These biopsies were required to have had no evidence of cancer as confirmed by the central pathologist (D.G.B.). The protocol was approved by the institutional review board of each investigative site, and informed consent was obtained from each patient before initiation of the clinical trial.

Protocol G300104 randomly assigned 1,590 men with HGPIN, and 1,589 patients received either toremifene 20 mg ($n = 787$) orally per day or placebo ($n = 802$) for 3 years or until a diagnosis of PCa was made on prostate needle biopsy (Fig 1). The primary end point of the trial was progression-free PCa survival in the modified intent-to-treat (MITT) population. The MITT population included men who received at least one dose of study drug and underwent at least one on-study prostate biopsy. A minimum of 10 prostate

biopsy cores were obtained from each patient and sent directly to the central pathologist at the 12-, 24-, and 36-month visits, unless they were diagnosed with PCa and discontinued from the trial.

Of the 1,589 patients with HGPIN in the trial, 249 men (34.7%) in the placebo group and 229 men (32.3%) in the toremifene-treated group developed PCa during the 3-year trial period ($P = .39$, log-rank test).¹⁸ The 3-year Kaplan-Meier cancer-free survival estimates were 54.9% (99% CI, 43.3% to 66.5%) and 59.5% (99% CI, 48.1% to 70.9%) in the placebo and toremifene-treated groups, respectively.¹⁸ *ERG* analysis on a subset of these patients was conducted as an exploratory post hoc study.

Six hundred sixty-three biopsy cores from 461 patients acquired at various time points during the trial period, prepared as back-ups and available after the trial, were archived by the central pathologist and used for *ERG* analysis. Biopsy cores from the remaining patients, which were originally processed by local pathologists, were not available for this study.

ERG Immunohistochemistry

Formalin-fixed, paraffin-embedded tissue sections prepared from biopsies were labeled with a unique sample identification number (sample ID) by the central laboratory (Bostwick Laboratories, Glen Allen, VA), and immunohistochemical analyses were carried out at Weill Medical College of Cornell University (New York, NY) using the automated DiscoveryXT staining platform (Ventana Medical Systems [VMSI], Tucson, AZ).¹⁹ Antigen recovery was performed using heat retrieval and CC1 standard, a high pH Tris/borate/EDTA buffer (VMSI, catalog no. 950-124). Slides were incubated with 1:100 of the primary rabbit monoclonal *ERG* antibody (Epitomics, Burlingame, CA) for 1 hour at room temperature. Primary antibody was detected using the ChromoMap DAB detection kit (VMSI, catalog no. 760-159) and UltraMap anti-Rb HRP (VMSI, catalog no. 760-4315). The anti-Rb HRP secondary antibody was applied for 16 minutes at room temperature. Slides were counterstained with Hematoxylin II (VMSI, catalog no. 790-2208) for 8 minutes followed by Bluing Reagent (VMSI, catalog no. 760-2037) for 4 minutes at 37°C.

As previously documented, *ERG* protein expression is a proxy for gene rearrangement with high sensitivity and specificity.¹⁹ Evaluated without knowledge to treatment status (placebo or toremifene) or clinical outcome, *ERG* overexpression in any isolated HGPINs in a given core was considered positive in this study. *ERG* expression in endothelial cells and lymphocytes served as internal control for the assay.

Fig 1. CONSORT diagram illustrating study participants, *ERG* status in high-grade prostatic intraepithelial neoplasia (HGPIN), and prostate cancer incidence. PSA, prostate-specific antigen.

Statistical Analysis

Clinical features including age, PSA levels at baseline, changes in PSA, and cancer volume were compared between patients who did and did not express ERG using the Wilcoxon rank sum test. The distribution of Gleason scores and the raw proportions of patients who developed PCa were compared between the ERG status groups using Fisher's exact test. PCa-free survival was estimated using the Kaplan-Meier method, and the distributions were compared using the log-rank test. The hazard ratio of risk of PCa development was calculated using the Cox

proportional hazards model. For the Cox model, we assumed that ERG status does not change over time, but is the same at baseline as the time point it was assayed.

RESULTS

Among the study cohort of 461 patients, 14.7% and 36.9% were diagnosed with PCa in the first year and during the 3-year clinical trial,

Fig 2. ERG immunohistochemistry on prostate needle biopsies with isolated high-grade prostatic intraepithelial neoplasia (HGPIN). (A to F) Representative photomicrographs of strong and diffuse ERG expression in HGPINs of various morphology ($\times 20$ magnification). (G and H) ERG-negative HGPINs with ERG-positive endothelial cells and lymphocytes ($\times 20$ magnification).

respectively. The overall percentage of patients who expressed ERG (11.1%; 51 of 461 patients) was similar to previously reported values.⁹⁻¹² When ERG protein expression was evaluated, 96% demonstrated strong nuclear staining (Fig 2). It was noted that not all glands involved with HGPIN in a biopsy specimen were ERG positive (Appendix Fig A1, online only). This clonal ERG expression was also seen within a single HGPIN lesion. Some of the biopsies had ERG expression in low-grade prostatic intraepithelial neoplasia, a lesion that does not meet the diagnostic characteristics of HGPIN.²⁰

ERG expression was detected in 51 prostate biopsies, with 53% of patients with ERG-positive HGPIN experiencing progression to PCa during the clinical trial (Table 1). Among these 27 patients who were ERG positive and detected with PCa, 14 had ERG-expressing isolated HGPIN in a core other than one with concomitant PCa. In contrast, only 35% of the patients with ERG-negative biopsies experienced progression to PCa, indicating that patients with ERG expression were more likely to have PCa ($P = .014$, Fisher's exact test).

By Kaplan-Meier estimate, the 3-year PCa-free survival rates were 32.5% (95% CI, 14.5% to 50.5%) and 55.9% (48.8% to 63.2%) among men who were ERG positive and ERG negative, respectively (Fig 3). Log-rank test showed that the PCa-free survival distribution for ERG-positive patients differed significantly from ERG-negative patients ($P = .009$). The hazard ratio for PCa development among ERG-negative patients relative to ERG-positive patients was 0.58 (95% CI, 0.38 to 0.88). This hazard ratio estimate remained significant and stable after adjustment for a number of other baseline covariates (Table 2).

The percentage of men with ERG-positive HGPIN who developed cancer (53%) was similar in men who received toremifene and in men who received placebo during the clinical trial (Fig 1). However, the effects of toremifene on *ERG* gene fusion cannot completely be excluded because of the small sample size.

There was no association between ERG expression in HGPIN and Gleason score, with the majority of patients with PCa (84%) having a Gleason score of 6. When age, PSA at baseline, changes in PSA, and tumor volume were compared according to ERG status among the patients who developed PCa, no difference was observed (Table 3).

DISCUSSION

To our knowledge, the clinical trial reported by Taneja et al¹⁸ is the largest prospective evaluation of men with isolated HGPIN to date and allows insight into the natural history of this lesion with contemporary management. Four hundred seventy-eight (33.5%) of 1,426 patients with HGPIN who had a postbaseline biopsy (MITT) developed PCa,

Fig 3. By Kaplan-Meier estimate, prostate cancer-free survival was significantly worse among patients who expressed ERG than patients who did not.

and 68 (40%) of 170 of these PCas were detected within the first year of the clinical trial. The observed rate of cancer detection has substantial implications for the clinical management of these men with isolated HGPIN, particularly those diagnosed at a young age, in whom prolonged follow-up by serial prostate biopsies will be necessary. However, prostate biopsy is not a completely benign procedure; up to 6.7% of patients undergoing prostate biopsy require hospitalization within 30 days,²¹ with the risk of infectious complications significantly increased in recent years.²² With approximately 115,000 newly diagnosed isolated HGPIN cases each year,²³ improved risk stratification may assist in allocating limited health care resources and minimizing potential morbidity of prostate biopsy. Here, we show that ERG expression in HGPIN is associated with development of invasive PCa. A biomarker such as ERG that predicts increased rates of cancer detection after HGPIN diagnosis can assist in risk stratification, limiting prostate biopsy to appropriately selected patients and maximizing clinical benefit.

Recently, two studies examined ERG in isolated HGPIN to predict PCa detection. Gao et al²⁴ reported *ERG* fusion in 59 (36%) of 162 patients with isolated HGPIN. Ninety-five percent of patients with

Table 1. Frequency of ERG Positivity by Prostate Cancer

Prostate Cancer					
	No		Yes		
ERG Positive	No. of Patients	%	No. of Patients	%	Total No. of Patients
No	267	65	143	35	410
Yes	24	47	27	53	51
Total	291		170		461

Table 2. Hazard Ratio of Risk of Development of Prostate Cancer Among ERG-Negative Patients Relative to ERG-Positive Patients After Adjustment for Various Baseline Characteristics

Factor	Hazard Ratio*	95% CI	P
Age (median, 65 years)	0.580	0.383 to 0.876	< .01
PSA	0.560	0.370 to 0.848	.01
Presence of ASAP	0.582	0.385 to 0.879	.01
PSA velocity	0.581	0.384 to 0.879	.01
Normal DRE	0.528	0.349 to 0.799	.003
Treatment	0.580	0.384 to 0.877	< .01
> 1 HGPIN core	0.550†	0.363 to 0.834	.005

Abbreviations: ASAP, atypical small acinar proliferation; DRE, digital rectal examination; HGPIN, high-grade prostatic intraepithelial neoplasia; PSA, prostate-specific antigen.

*Hazard ratios < 1.0 signify a hazard ratio in favor of ERG-negative patients.

†HGPIN detected in > one core was significant ($P = .045$) after adjustment for ERG status.

Table 3. Clinical Parameters of 170 Men With Cancer Development According to ERG Status in High-Grade Prostatic Intraepithelial Neoplasia

Clinical Parameter	ERG Status	
	Positive (n = 27)	Negative (n = 143)
Age at screening, years		
Mean	62.85	65.15
Median	65	66
Range	47-80	48-81
Prebiopsy prostate-specific antigen, ng/mL		
Mean	4.48	4.89
Median	4.2	4.8
Range	0.2-9.2	0.6-24.7
Cancer volume, mL		
Mean	0.01	0
Median	0	0
Range	0-0.15	0-0.03
Gleason score		
6	24	119
7	3	20
8	0	2
9	0	2

ERG-positive HGPIN were subsequently diagnosed with PCa compared with 5% of patients with ERG-negative HGPIN; however, variability in PSA levels and biopsy patterns between these groups complicates the interpretation of these data. The discrepancy between Gao et al²⁴ and this study may derive from the patient cohorts; our cohort represents a general PSA-screened population in the United States, differing from that of the Chinese cohort of Gao et al²⁴ in ethnicity, environmental factors, screening practices, selection, and study bias. Nevertheless, both studies demonstrate higher incidence of cancer after ERG-positive HGPIN. He et al²⁵ had a cohort of 94 patients with biopsies with isolated HGPIN in which only five patients (5.3%) were ERG positive; no differences were observed in subsequent PCa detection between ERG-positive and ERG-negative patients, but the small study size and few number of ERG-positive patients limit interpretation of these results. From the perspective of a randomized, systemically controlled clinical trial with more than 1,500 patients, our results support that patients with ERG-positive HGPIN are more likely to develop PCa than those with ERG-negative HGPIN.

Most cancers detected in this study were Gleason score 6.¹⁸ Previous studies also have reported that 75% to 95% of patients with HGPIN experienced progression to Gleason score 6 cancers after 3 years.²⁶⁻²⁸ However, frequent biopsy, sampling bias, and short follow-up as part of a clinical trial could have contributed to these results and obscured the prognostic value of ERG. It can be concluded that ERG is a potential predictive marker for PCa detection after HGPIN diagnosis but not for the grade of the cancers.

The current study also underscores the usefulness of ERG as a tissue biomarker to identify clinically significant HGPIN. HGPIN is often confused with benign mimics such as basal cell hyperplasia, benign proliferative epithelium, and reactive changes.²⁹ In addition, we have observed that atypical glands that do not meet the more stringent criteria for HGPIN, which might be considered most consis-

tent with low-grade prostatic intraepithelial neoplasia, may express ERG. As a PCa-specific gene fusion marker, ERG expression in PIN indicates a neoplastic lesion, highly associated with concurrent cancer, and may imply the presence of undersampled cancer.

One limitation of the study is the small number of patients with ERG-positive HGPIN, which and additional studies are required to verify the findings. Studies on HGPIN are often limited in size because of the difficulty in collecting patients and conducting clinical follow-up. As far as we are aware, this is the largest cohort of HGPIN reported to date accompanied by clinical data on systemically controlled patients.

In the era of whole genome sequencing and personalized medicine, molecular testing has become a routine practice in pathology. The presence of hormone receptors or *Her-2/neu* amplification in breast cancers, as well as *EGFR/KRAS* mutations and *EML4-ALK* fusion in lung cancers, not only predicts the behavior of individual tumor, but also dictates the type of therapy and management of each patient. As PCa is molecularly characterized and subclassified, the same approach is required for HGPIN, which may have variable clinical courses and warrant differential management depending on the molecular alterations identified. In this exploratory study, ERG expression in HGPIN suggests a higher risk of PCa development and earlier cancer detection. Further studies will be necessary to confirm these findings in additional cohorts and to determine whether assessment of ERG expression will improve clinical decision making in patients with HGPIN on initial prostate needle biopsies.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Although all authors completed the disclosure declaration, the following author(s) and/or an author's immediate family member(s) indicated a financial or other interest that is relevant to the subject matter under consideration in this article. Certain relationships marked with a "U" are those for which no compensation was received; those relationships marked with a "C" were compensated. For a detailed description of the disclosure categories, or for more information about ASCO's conflict of interest policy, please refer to the Author Disclosure Declaration and the Disclosures of Potential Conflicts of Interest section in Information for Contributors.

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GLOSSARY TERMS

ERG (ETS-related gene): ERG, located on chromosome 21q22, encodes a transforming proto-oncogene that is expressed in hematopoietic progenitor and endothelial cells. Furthermore, ERG is expressed in early thymocytes and becomes downregulated as cells develop toward the T-cell lineage. ERG and other members of the ETS family are downstream effectors of mitogenic signaling transduction pathways and are involved in key steps regulating cell proliferation, differentiation, and apoptosis.

HGPIN (high-grade prostatic intraepithelial neoplasia): The only accepted precursor of prostate cancer characterized by morphologic and molecular abnormalities that are intermediate between benign prostatic epithelium and cancer. It is associated with concurrent or subsequent prostate cancer.

TMPRSS2-ERG (transmembrane protease, serine 2-v-ets erythroblastosis virus E26 oncogene homolog [avian]): The recurrent gene fusion between androgen-regulated TMPRSS2 and ERG is present in more than 50% of prostate-specific antigen-screened prostate cancers. It results in ERG protein overexpression in hormone-naïve prostate cancer.

Appendix

Fig A1. ERG expression defines molecular tumorigenesis in precancerous prostate glands. (A to C) Heterogeneous ERG expression between and within high-grade prostatic intraepithelial neoplasias (HGPINs) implies beginning of malignant molecular alteration and confirms that HGPIN is the precursor lesion of prostate cancer ($\times 20$ magnification). (D to F) Clear demarcation between ERG-positive and ERG-negative cells is noted within the same gland ($\times 40$ magnification). (G and H) Glands that might not meet the histologic criteria for HGPIN demonstrate ERG expression, adjacent to ERG-negative HGPINs ($\times 40$ magnification).