A Tissue Biomarker–Based Model That Identifies Patients with a High Risk of Distant Metastasis and Differential Survival by Length of Androgen Deprivation Therapy in RTOG Protocol 92-02

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Abstract

Purpose: To examine the relationship between the expression of 7 promising apoptotic/cell proliferation proteins (Ki-67, p53, MDM2, bcl-2, bax, p16, and Cox-2) and risk of distant metastasis.

Experimental Design: RTOG 92-02 compared external beam radiotherapy (EBRT) to approximately 70 Gy + short-term androgen deprivation therapy (STADT) with EBRT + long-term ADT (LTADT). Immunohistochemical analysis was available for ≥4 biomarkers in 616 of 1,521 assessable cases. Biomarkers were evaluated individually and jointly via multivariable modeling of distant metastasis using competing risks hazards regression, adjusting for age, prostate-specific antigen, Gleason score, T stage, and treatment.

Results: Modeling identified four biomarkers (Ki-67, MDM2, p16 and Cox-2) that were jointly associated with distant metastasis. The c-index was 0.77 for the full model and 0.70 for the model without the biomarkers; a relative improvement of about 10% (likelihood ratio P < 0.001). Subdivision of the patients into quartiles based on predicted distant metastasis risk identified a high-risk group with 10-year distant metastasis risk of 52.5% after EBRT + STADT and 31% with EBRT + LTADT; associated 10-year prostate cancer–specific mortality (PCSM) risks were 45.9% and 14.5% with STADT and LTADT.

Conclusion: Four biomarkers were found to contribute significantly to a model that predicted distant metastasis and identified a subgroup of patients at a particularly high risk of both distant metastasis and PCSM when EBRT + STADT was used. LTADT resulted in significant reductions in distant metastasis and improvements in PCSM, and there was a suggestion of greater importance in the very high risk subgroup.

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Introduction

The role of androgen deprivation therapy (ADT) as an adjuvant to external beam radiation therapy (EBRT) for patients with prostate cancer has been a focus of study for decades, particularly for patients at a higher risk of distant metastases (1, 2). RTOG 92-02 (1, 3) was launched to compare standard dose (~70 Gy) EBRT + short-term ADT (STADT) with EBRT+long-term ADT (LTADT). Androgen deprivation therapy consisted of flutamide and goserelin, beginning 2 months before EBRT and continuing until EBRT completion (STADT + EBRT arm) versus goserelin continued for an additional 2 years after EBRT (LTADT + EBRT arm). A significant benefit was observed in biochemical failure, disease-free survival, freedom from distant metastasis and disease-specific survival for patients randomized to LTADT + EBRT. These results, and those of EORTC 22961, (2) support the use of LTADT in men with high-risk prostate cancer. However, LTADT is associated with significant long-term side effects. Better selection of patients for LTADT, as well as for those in need of more aggressive strategies beyond this standard, are needed. Tumor tissue biomarkers have the potential to improve patient selection.

We have identified multiple tumor tissue biomarkers that are significantly associated with outcomes of patients after EBRT + ADT, including Ki-67, p53, MDM2, bcl-2 and bax, p16, and Cox-2. For the most part, these...
Translational Relevance

The association between seven apoptotic/cell proliferation proteins and risk of distant metastasis is examined in immunohistochemical analysis of tissue from RTOG 92–02 patients. The trial compared external beam radiotherapy (EBRT) to approximately 70 Gy + short-term androgen deprivation therapy (STADT) with EBRT + long-term ADT (LTADT). Modeling identified four biomarkers (Ki-67, MDM2, p16, and Cox-2) that were jointly associated with distant metastasis. The model predicted for distant metastasis and identified a subgroup of patients at a particularly high risk of both distant metastasis and prostate cancer–specific mortality (PCSM) when EBRT + STADT were used. LTADT resulted in significant gains in distant metastasis and PCSM, and there was a suggestion of greater importance in a very high risk subgroup. Our findings suggest that the addition of biomarkers to classical clinical–pathologic factors identifies a group at very high risk of distant metastasis after EBRT + ADT that will benefit significantly from LTADT both from the perspective of reduced distant metastasis and PCSM.

Biomarker data analysis

The techniques for biomarker staining and analysis of Ki-67 (4), p53 (5), MDM2 (4), bcl-2 and bax (6), p16 (7), and Cox-2 (8) have been described in detail previously. Briefly, archival paraffin-embedded diagnostic prostate biopsy tumor tissues were cut onto poly-l-lysine slides, deparaffinized in xylene, rehydrated, and washed before antigen retrieval using a pressure cooker. Primary antibodies were then added at titrated dilutions to optimize staining. The primary antibodies used were MB-1 for Ki-67 (No. M7240, 1:100 dilution; Dako Corp.), p53 (No. M7001, clone DO7, 1:100 dilution; Dako Corp.), MDM2 (No. M7146, clone SPM14, 1:100 dilution; Dako Corp.), Bcl-2 (clone 124, 1:100 dilution; Dako Corp.), Bax (clone 2D2, 1:200 dilution; Zymed Laboratories, Inc.), p16 (No. SC-1661, 1:100 dilution; Santa Cruz Biotechnology Inc.), and Cox-2 (No. 804-112-C050, 1:200 dilution: Alexis Biochemicals). Antibody binding was detected by the labeled streptavidin biotin method (Dako LSAB 2 Kit; Dako Corp.) for MDM2, Ki-67, Bcl-2, Bax, p16, and Cox-2, and by the ABC method, using 3-amino-9-ethylcarbazole as the chromogen for p53. Bcl-2 and bax (6), p53 (5), and Ki-67 (4) were based on manual scoring, whereas p16 (7), MDM2 (4), and Cox-2 (8) were based on semiautomated image analysis.

Statistical analysis

Disease endpoints have been described previously (15–17). Distant metastasis was defined as clinical evidence of distant disease by radiographic or pathologic criteria. Prostate cancer–specific mortality (PCSM) was defined as death certified as due to prostate cancer, death due to treatment complications, death from unknown causes with active malignancy (clinical disease relapse), or from another cancer with documented bone metastases attributed to prostate cancer before the appearance of the second independent cancer.

The study aim was to investigate whether a set of molecular features will add to the prognostic classification in a model with recognized prognostic stratification features, seeking a parsimonious model containing molecular characteristics that enhance prediction over known patient and disease characteristics associated with risk of distant metastasis, which include age at diagnosis, initial PSA (iPSA), Gleason score (GLSC), and T stage. Treatment group was also included in the model, and potential interaction effects between treatment and biomarkers were formally tested.

From among 1,521 eligible trial participants (763 STADT, 758 LTADT), there were 616 (41%) patients for whom data was available for at least 4 of the 7 biomarkers. The prognostic significance of these biomarkers was evaluated in a model for cumulative incidence of distant metastasis based on the subdistribution hazards regression approach that accounts for the influence of competing risks (9). Covariates for biomarkers were considered on a continuous linear scale for scaled values [(value – mean)/standard deviation], unless model diagnostics for evaluating functional form suggested otherwise. Each was examined singly in the presence of clinical covariates, and those found to show an
association with distant metastasis were examined jointly. Variables that had previously been omitted were examined again for possible inclusion. Likelihood ratio tests and Akaike information criterion (AIC) were used to contrast models (10). In the resulting model, four biomarkers (Ki-67, MDM2, Cox-2, and p16) remained important predictors in multivariable analyses, with 372 patients having complete data on all 4 of these biomarkers.

The resulting final model was used to explore risk prediction in several ways. First, the c-index for the competing risks regression model was used to evaluate the contribution of biomarkers to prediction after inclusion of clinical characteristics (10, 11). Second, the model was used to generate outcomes for hypothetical patients according to covariates; for example, by fixing clinical characteristics at common representative values and varying biomarker values. Finally, as an empirical check on risk prediction, the “risk scores” that each patient had based on their covariate values and the model coefficients were used to partition patients into quartiles of potential failure risk. Nonparametric cumulative incidence estimates (12) were then generated for patients in each quartile to determine if the model appears to segregate risk.

Model robustness was evaluated by several approaches. To evaluate the influence of individual cases on the model estimates, 1,000 random samples (with replacement, e.g., bootstrap) of size \( n = 75\% \) of the total sample were drawn and model parameter estimates with the larger bootstrap variance estimates were examined to determine whether any of the covariates became superfluous in the model. Second, it is recognized that the c-index estimate on the same data following model fit is overly optimistic for future prediction. A Monte–Carlo analysis was performed, taking 1,000 random subsamples (without replacement) of 50% of the final model cohort, followed by c-index computation for the counterpart of the cohort that was not used to estimate the model. The mean c-index for the validation subcohort is reported.

The median follow-up was 11.6 years (25th percentile = 10.5 years, 75th percentile = 12.3 years). SAS (SAS Institute), Stata (StataCorp), and R (R Foundation for Statistical Computing) software were used for statistical analyses.

Results

Study cohort and biomarker characteristics

Information on the patients in the biomarker cohort relative to all trial participants is shown in Table 1. There are no material differences in patient or disease characteristics between those with and without the marker data; although, there were fewer patients with Gleason score \(< 7\) in the final marker set.

Descriptive statistics for individual biomarkers are shown in Table 2. Associations between marker value distributions indicated that Ki-67 has low correlation with other markers. Other pairwise correlations (e.g., between Cox-2 and MDM2) are larger; although, in no case did the correlation exceed 0.35. The overall associations were moderately small. Relationships between biomarkers and other characteristics were also investigated (not shown). Biomarker values did not differ by age or T stage. Higher values of Ki-67, Cox-2, and MDM2 were more frequent among patients with high GLSC. Patients with low p16 tended to have low iPSA values; but otherwise, iPSA was not related to these markers.

Multivariable model

The influence of the four biomarkers that had statistically significant associations with risk of distant metastasis after inclusion of other patient/disease characteristics and treatment arm are shown in Table 3. In this model, stage and iPSA were retained, even though they no longer reached conventional statistical significance, because these factors are important prognostic covariates in the cohort as a whole and are well-recognized clinical prognostic factors in other studies. For the model in Table 3, the c-index, (13) which provides a measure of model discrimination according to probability of failure, was 0.770 [95% confidence interval (CI), 0.718–0.822]]. This value represents a relative improvement of about 10% over a model on the same cohort that included only the patient/disease characteristics (c-index = 0.702, 95% CI, 0.658–0.759]). The likelihood ratio test contrasting models with and without the four biomarker covariates indicated a statistically significant contribution of the latter to the model (\( X^2 = 22.2, 4 \text{ df}; P < 0.001 \)), and the AIC was smaller (825.0 with biomarker covariates vs. 861.3 without). There was no evidence of statistically significant differential effects (e.g., interactions) between biomarkers and treatment (STADT vs. LTADT) in this final model cohort and thus only main effects were included. However, when examining markers individually, there was some indication that Ki-67 and p16 effects are quantitatively larger in patients receiving LTADT, while MDM2 may exert greater prognostic influence among patients receiving STADT.

Because the markers shown all exert significant influence on the risk of distant metastasis in the presence of clinical characteristics, patients with specific combinations of biomarker values will have greater or lesser predicted risk for distant metastasis for given values of other characteristics. To explore this, the model was used to generate predicted cumulative risks of distant metastasis based on covariate profiles. Using the contribution of biomarkers to distant metastasis risk suggested by the model in Table 3, predicted risk over time was calculated, contrasting different values for the biomarkers while fixing other covariates at a common value. For a hypothetical 65-year-old patient with iPSA of 35, GLSC below 8, and T2 disease, the cumulative probability of distant metastasis was contrasted between a favorable biomarker profile (first quartile values for Ki-67 and Cox-2, third quartile value for p16, and MDM2 <184 intensity units) and an unfavorable profile (third quartile value for Ki-67 and Cox-2, first quartile value for p16, and MDM2 \( \geq 184 \)). For patients receiving STADT, the 10-year predicted risk increased from 6.7% for those with a favorable marker profile, to 33.2% for those with an unfavorable profile; whereas, for patients receiving LTADT, the 10-year predicted risk increased from 2.7% to 14.8% (Fig. 1).
Model evaluation

The statistical model in Table 3 indicates that numerous covariates, including tumor biomarkers, will differentiate risk for distant metastasis. To evaluate robustness of the above model in absence of an independent validation cohort, several steps were taken. First, bootstrap subsampling of 75% samples and reestimating the coefficients confirmed that the biomarker covariates selected remained

### Table 1. Distribution of patient and disease characteristics

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Patients not in final marker set cohort ($n = 1,149$)</th>
<th>Final marker set cohort ($n = 372$)</th>
<th>All patients ($n = 1,521$)</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment group</td>
<td>Frequency (%)</td>
<td>Frequency (%)</td>
<td>Frequency (%)</td>
<td>Frequency (%)</td>
</tr>
<tr>
<td>LTAD + radiotherapy</td>
<td>562 (48.9%)</td>
<td>196 (52.7%)</td>
<td>758 (49.8%)</td>
<td></td>
</tr>
<tr>
<td>STAD + radiotherapy</td>
<td>587 (51.1%)</td>
<td>176 (47.3%)</td>
<td>763 (50.2%)</td>
<td>0.21</td>
</tr>
<tr>
<td>Gleason score</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;7</td>
<td>464 (40.4%)</td>
<td>119 (32.0%)</td>
<td>583 (38.3%)</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>356 (31.0%)</td>
<td>121 (32.5%)</td>
<td>477 (31.4%)</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>137 (11.9%)</td>
<td>60 (16.1%)</td>
<td>197 (13.0%)</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>100 (8.7%)</td>
<td>33 (8.9%)</td>
<td>133 (8.7%)</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>21 (1.8%)</td>
<td>10 (2.7%)</td>
<td>31 (2.0%)</td>
<td></td>
</tr>
<tr>
<td>Unknown</td>
<td>71 (6.2%)</td>
<td>29 (7.8%)</td>
<td>100 (6.6%)</td>
<td>0.05</td>
</tr>
<tr>
<td>Clinical stage (T stage)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T2</td>
<td>523 (45.5%)</td>
<td>169 (45.4%)</td>
<td>692 (45.5%)</td>
<td></td>
</tr>
<tr>
<td>T3</td>
<td>583 (50.7%)</td>
<td>187 (50.3%)</td>
<td>770 (50.6%)</td>
<td></td>
</tr>
<tr>
<td>T4</td>
<td>43 (3.7%)</td>
<td>16 (4.3%)</td>
<td>59 (3.9%)</td>
<td>0.89</td>
</tr>
<tr>
<td>KPS</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>70</td>
<td>8 (0.7%)</td>
<td>5 (1.3%)</td>
<td>13 (0.9%)</td>
<td></td>
</tr>
<tr>
<td>80</td>
<td>73 (6.4%)</td>
<td>29 (7.8%)</td>
<td>102 (6.7%)</td>
<td></td>
</tr>
<tr>
<td>90</td>
<td>555 (48.3%)</td>
<td>179 (48.1%)</td>
<td>734 (48.3%)</td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>513 (44.7%)</td>
<td>159 (42.7%)</td>
<td>672 (44.2%)</td>
<td>0.48</td>
</tr>
<tr>
<td>Age at diagnosis</td>
<td>69.7 (6.5)</td>
<td>69.3 (6.6)</td>
<td>69.6 (6.5)</td>
<td>0.30</td>
</tr>
<tr>
<td>Initial PSA at diagnosis</td>
<td>29.9 (30.3)</td>
<td>33.2 (32.1)</td>
<td>30.7 (30.8)</td>
<td>0.07</td>
</tr>
<tr>
<td>Distant metastasis event status</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Distant metastasis event-free</td>
<td>484 (42.1%)</td>
<td>159 (42.7%)</td>
<td>643 (42.3%)</td>
<td></td>
</tr>
<tr>
<td>Distant metastasis</td>
<td>213 (18.5%)</td>
<td>78 (21.0%)</td>
<td>291 (19.1%)</td>
<td></td>
</tr>
<tr>
<td>Competing death</td>
<td>452 (39.3%)</td>
<td>135 (36.3%)</td>
<td>587 (38.6%)</td>
<td>0.46</td>
</tr>
<tr>
<td>PCSM event status</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alive</td>
<td>524 (45.6%)</td>
<td>171 (46.0%)</td>
<td>695 (45.7%)</td>
<td></td>
</tr>
<tr>
<td>Death (prostate cancer)</td>
<td>166 (14.5%)</td>
<td>62 (16.7%)</td>
<td>228 (15.0%)</td>
<td></td>
</tr>
<tr>
<td>Death (other cause)</td>
<td>459 (40.0%)</td>
<td>139 (37.4%)</td>
<td>598 (39.3%)</td>
<td>0.50</td>
</tr>
</tbody>
</table>

Abbreviation: KPS, Karnofsky performance status.

### Table 2. Descriptive summary of tumor biomarkers in analysis cohort ($N = 372$)

<table>
<thead>
<tr>
<th>Marker</th>
<th>Mean (SD)</th>
<th>Min</th>
<th>Q1</th>
<th>Median</th>
<th>Q3</th>
<th>Max</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ki-67</td>
<td>10.6 (8.2)</td>
<td>0.0</td>
<td>5.0</td>
<td>8.5</td>
<td>13.9</td>
<td>51.8</td>
</tr>
<tr>
<td>MDM2</td>
<td>155.2 (53.8)</td>
<td>0.0</td>
<td>148.0</td>
<td>169.0</td>
<td>186.0</td>
<td>223.0</td>
</tr>
<tr>
<td>Cox-2</td>
<td>132.6 (20.5)</td>
<td>75.0</td>
<td>118.5</td>
<td>132.0</td>
<td>146.0</td>
<td>214.0</td>
</tr>
<tr>
<td>p16</td>
<td>71.2 (24.0)</td>
<td>0.0</td>
<td>60.9</td>
<td>79.7</td>
<td>88.5</td>
<td>100.0</td>
</tr>
</tbody>
</table>

<p>| Spearman rank correlation among markers |</p>
<table>
<thead>
<tr>
<th>Ki-67</th>
<th>MDM2</th>
<th>Cox-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.063</td>
<td>0.350</td>
<td>0.138</td>
</tr>
</tbody>
</table>

Abbreviations: Max, maximum; Min, minimum; Q1, first quartile; Q3, third quartile.
**Table 3. Multivariable model for distant metastasis including clinical and biomarker covariates (N = 372)**

<table>
<thead>
<tr>
<th>Subdistribution HR (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ki-67(^a)</td>
<td>1.53 (1.28–1.82)</td>
</tr>
<tr>
<td>Cox-2(^a)</td>
<td>1.37 (1.01–1.84)</td>
</tr>
<tr>
<td>p16(^a)</td>
<td>0.74 (0.60–0.92)</td>
</tr>
<tr>
<td>MDM2 (≥184 vs. &lt;184)</td>
<td>1.73 (1.04–2.84)</td>
</tr>
<tr>
<td>Treatment (LTADT vs. STADT)</td>
<td>2.53 (1.58–4.04)</td>
</tr>
<tr>
<td>Gleason score (≥8 vs. &lt;8)</td>
<td>3.11 (1.99–4.85)</td>
</tr>
<tr>
<td>Stage (T3/4 vs. T2)</td>
<td>1.48 (0.93–2.36)</td>
</tr>
<tr>
<td>Age (per year)</td>
<td>0.96 (0.93–1.00)</td>
</tr>
<tr>
<td>Initial PSA (per 10 units)</td>
<td>1.003 (0.98–1.09)</td>
</tr>
</tbody>
</table>

\(^{a}\) Continuous scale, standardized.

Statistically significant predictors of distant metastasis risk. Second, prediction computations based on estimation of the model (1,000 random samples) on samples of 50% of the cohort and the c-index then computed on the complement of the cohort resulted in average c-index of 0.737 for the proposed model and 0.675 for the model with clinical–pathologic covariates only. While the predictive ability in novel cases diminished as expected, the value still indicated clinical utility and the relative gain over the model omitting biomarkers was the same.

To demonstrate risk classification based on the model empirically, we calculated each patient’s “risk score” as determined by the cumulative sum of covariate values multiplied by the associated coefficients in the model. Patients were then grouped by quartile of scores (overall and then separately within each treatment group because treatment itself is strongly associated with outcome) and nonparametric (i.e., not model-predicted as in Fig. 1) cumulative incidence of distant metastasis calculated. When subdivided by length of ADT, a gradient of failure risk is clearly observed (Fig. 2). For patients receiving STADT, among those in the highest risk quartile, 10-year cumulative incidence of distant metastasis was 52.5%, compared with 9.4% for patients in the first risk score quartile (Fig. 2, top). For patients receiving LTADT, those in the highest quartile had a 31.0% 10-year cumulative incidence, compared with no failures (0%) in the first quartile (Fig. 2, bottom). These findings were then related to the risk of dying from prostate cancer. Those who received STADT (Fig. 3, top) and were in risk group 4 had a 45.9% cumulative probability of PCSM at 10 years, while those in risk group 3 and below had a 14.5% cumulative probability of PCSM. When LTADT was used (Fig. 3, middle) there was a significant delay in distant metastasis in all risk groups, with separation of risk group 4 from the others not suggested until 12 years. The timing of PCSM after distant metastasis was further investigated in group 4 by calculating the cumulative incidence of PCSM from the initial distant metastasis event (Fig. 3, bottom). Those treated with LTADT had a longer time to PCSM that is most obvious at 3 to 5 years after distant metastasis (absolute difference in PCSM of around 20%); however, this difference was not statistically significant (P = 0.18).

**Discussion**

Our main objective was to determine whether the addition of multiple biomarkers to standard clinical and pathologic covariates would significantly enhance the prediction of prostate cancer patient outcome based on distant metastasis after EBRT + ADT such that patients at the favorable and high-risk ends of the spectrum would be better identified. Multiple models have been described, most of them using classical clinical–pathologic factors (including PSA) and biochemical failure as the endpoints; few models have used distant metastasis as the endpoint (14, 15). We chose distant metastasis because there were a greater number of events, and hence greater power for modeling, than PCSM, and it is a robust predictor of death due to prostate cancer. To our knowledge, none of the prior models have systematically explored tissue biomarkers using the platform or analytic approach described herein. The platform was that of a randomized trial in which the effects of the length of ADT were investigated in an otherwise homogeneously treated group. The recovery of tissue for biomarker studies was rather typical of such studies and there were no substantive differences between the study cohort and the parent protocol cohort (Table 1).

For this investigation, we chose to model on the cumulative incidence scale using the subdistribution hazards competing risks model (9). Covariate effects were similar when we used the more familiar cause-specific hazard regression model (16), a result not uncommonly seen if a relatively large proportion of patients are event-free (e.g., censored; ref. 17). However, the competing risks regression model is likely more appropriate for covariate effect estimation and risk prediction when failures for the event of interest are less frequent than competing events (17), as is the case here (Table 1). This competing risks model has been...
advocated in similar settings modeling recurrence after prostate cancer using clinical characteristics (14, 18).

We investigated the potential of seven promising biomarkers to contribute significantly to clinical–pathologic factors in estimating distant metastasis risk. Of these, four biomarkers, Ki-67, MDM2, p16, and Cox-2 were selected by multivariable modeling that included relevant clinical prognostic variables. All four of these markers had been identified previously as being associated with distant metastasis (4, 7, 8). Ki-67 is a proliferation marker and, notably, is the most consistent prognostic tissue biomarker for prostate cancer outcome after radiotherapy (4) or surgery (19) described thus far. Although p53 is also associated with prostate cancer outcome independently of clinical–pathologic factors in our prior experience (5), in the model described herein (Table 3), MDM2, which is a key regulator of p53, was more significant. The loss of p16, a CDK inhibitor, results in increased phosphorylation of retinoblastoma protein (pRB), dissociation of pRB from E2F1, and greater free E2F1 to promote proliferation from G1–S phase. The inter-relationship between the p19ARF–MDM–p53 and p16–pRB–E2F1 signaling pathways (20) and the balance between proliferation and apoptosis in response to genotoxic stress provide a rationale for why these biomarkers contribute to the model described herein. Cox-2 overexpression has cell-cycle–modulatory effects, as well as a number of other actions likely mediated mainly through prostaglandin E2, such as increased proliferation, invasion,
and angiogenesis, with blunted apoptosis and reduced radiation response (21).

Our selection of biomarkers was based on a systematic approach to explore the relationships of biomarkers associated with cell cycle, cell death, and angiogenesis pathways. Other potential markers not included, but which have shown promise include markers of hypoxia (e.g., HIF1α) or other markers of angiogenesis (e.g., VEGF; ref. 22). We have never examined HIF1α and further biomarker studies in the remaining tissue from the RTOG 92-02 cohort is complicated by the number of missing cases. Of note, VEGF was examined in tissue from RTOG 86-10 and was not predictive of outcome (23).

The biomarkers tested added significantly to the distant metastasis model described here. When patients were divided into quartiles based on model distant metastasis risk, there was a subgroup identified with a very high rate of distant metastasis (highest distant metastasis quartile, Figure 2. Observed cumulative incidence of distant metastasis by model predicted risk. Patients were grouped by quartiles of predicted risk from the model in Table 3, and nonparametric cumulative incidence then computed by quartile group. Top, patients undergoing EBRT + STADT; bottom, patients undergoing EBRT + LTADT.)
Cumulative incidence of prostate cancer–specific mortality by predicted risk: STADT

Risk quartile 1
Risk quartile 2
Risk quartile 3
Risk quartile 4

Patients at risk
Risk quartile 1: 44 40 34 30 22 19 2
Risk quartile 2: 44 42 39 37 33 29 8
Risk quartile 3: 44 42 39 34 31 19 3
Risk quartile 4: 44 39 30 25 17 13 4

Cumulative incidence of prostate cancer–specific mortality by predicted risk: LTADT

Risk quartile 1
Risk quartile 2
Risk quartile 3
Risk quartile 4

Patients at risk
Risk quartile 1: 49 45 43 38 33 28 6
Risk quartile 2: 49 48 46 37 30 25 9
Risk quartile 3: 49 48 45 34 26 23 7
Risk quartile 4: 49 45 42 38 33 25 5

Figure 3. Observed cumulative incidence of death due to prostate cancer by model predicted risk based on distant metastasis. Patients were grouped by quartiles of predicted distant metastasis risk from the model in Table 3, and nonparametric cumulative incidence for PCSM computed. Top, patients by distant metastasis model quartile group after EBRT + STADT with cumulative incidence calculated from enrollment in study; middle, patients by distant metastasis model quartile group after EBRT + LTADT with cumulative incidence calculated from enrollment in the study (bottom, patients). Patients in the highest risk distant metastasis model quartile subdivided by STADT versus LTADT with cumulative incidence calculated from the first distant metastasis event.
group 4) randomized to the STADT arm (>50% at 10 years); this high risk group also had a >45% risk of death due to prostate cancer at 10 years. While group 4 also had a high rate of distant metastasis at 10 years in patients randomized to LTADT (>30%), there was less concordance in the translation to PCSM (<20% at 10 years) and the fourth quartile group had a 10-year PCSM rate that was similar to that of quartile groups 3 and 2 (Fig. 3). To better characterize this unexpected result, we examined the time to PCSM from metastasis with the hypothesis that after LTADT the time from distant metastasis to PCSM was longer than after STADT. There may be conditions in which the rate of growth, i.e., biology of prostate cancer changes based on the timing (24), type (25), or duration of ADT (suggested in present analysis). While the pattern seen (Fig. 3, bottom) is intriguing because patients exposed to LTADT would be expected to respond for a shorter duration upon reinstitution of ADT (salvage ADT) for distant metastasis and the opposite is suggested, the differences were not significant. Larger numbers of patients are needed to understand the mechanisms involved.

In summary, our findings suggest that the addition of biomarkers to classical clinical–pathologic factors identifies a group of patients with prostate cancer at very high risk of distant metastasis after EBRT + ADT. The results indicate LTADT is particularly important in this group both from the perspective of reduced distant metastasis and an even more pronounced reduction in PCSM. The integration of biomarkers into risk classification schemes among patients with clinical characteristics indicating a specific risk (e.g., high risk) may allow reclassification into other risk groups (e.g., intermediate or even low risk) who can then be selected for clinical trials or treatment accordingly. Validation of the biomarker-based model in an independent dataset is needed before broader application of this approach.

Disclosure of Potential Conflicts of Interest

Q. Wu is a consultant/advisory board member for BioStat Solution. H. Sandler is a consultant/advisory board member for Bayer, Eviti, and Medivation. F.Y. Feng is a consultant/advisory board member for GenomicHealth, Myriad, and Nanostring. No potential conflicts of interest were disclosed by the other authors.

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