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Ki-67 is an Independent Predictor of Metastasis and Cause-Specific Mortality for Prostate Cancer Patients Treated on Radiation Therapy Oncology Group (RTOG) 94-08

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Abstract

Purpose—The association of Ki-67 staining index (Ki67-SI) with overall survival (OS), disease-specific mortality (DSM), distant metastasis (DM), and biochemical failure (BF) was examined in men with favorable-to-intermediate risk prostate cancer receiving radiotherapy (RT) alone or with short-term androgen deprivation (ADT) in Radiation Therapy Oncology Group (RTOG) 94-08.

Methods and Materials—468 patients (23.6%) on RTOG 94-08 had sufficient tissue for Ki67-SI analysis. Median follow-up was 7.9 years. Ki67-SI was determined by immunohistochemistry and quantified manually and by image analysis. Correlative analysis versus clinical outcome was performed using the third quartile (Q3) cut-point. A proportional hazards multivariable analysis (MVA) dichotomized covariates in accordance with trial stratification and randomization criteria.

Results—In MVAs adjusted for all treatment covariates, high Ki67-SI (Q3) was correlated with increased DSM (HR 2.48, $p=0.03$), DM (HR 3.5, $p=0.002$) and BF (HR 3.55, $p<0.0001$). MVA revealed similar Ki67-associated hazard ratios in each separate treatment arm for DSM, DM and BF, these only reached significance for DM in the RT alone arm and for BF in both arms. Ki67-SI was not a significant predictor of intraprostatic recurrence assessed by rebiopsy at 2 years post-

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treatment. Patients with a high or low Ki67-SI appeared to experience similar relative benefit from the addition of ADT to radiation.

Conclusions—High Ki67-SI independently predicts for increased disease specific mortality, distant metastasis and protocol biochemical failure in primarily intermediate risk prostate cancer patients treated with radiation therapy with or without androgen deprivation therapy on RTOG 9408, but does not predict for local recurrence nor for increased relative benefit from ADT. This and prior studies lend support for use of Ki67-SI as a stratification factor in future trials.

Keywords

Prostate carcinoma; Ki-67 Antigen/Analysis; Prognosis; Metastasis; Biomarkers

INTRODUCTION

Radiation therapy (RT) and radical prostatectomy (RP) are standard of care treatments for localized prostate cancer. Treatment failures, however, are still frequent, even among populations defined by clinical prognostic criteria as favorable or intermediate risk. There is increasing evidence that molecular alterations that predispose to metastasis or radiation resistance may contribute to such treatment failures.¹ A better ability to prognosticate outcome, particularly if combined with a better understanding of the molecular pathways of treatment response and metastatic potential, could enhance the ability to individually tailor and optimize therapy.

In 1994, the Radiation Therapy Oncology Group (RTOG) opened a large randomized trial, RTOG 94-08, to study whether combining short term androgen deprivation (ADT) with RT improved outcomes in men with localized prostate cancer.² Enrolled patients had a PSA level of 20 ng/ml or less and T2b or less disease. While a series of correlative studies in other RTOG high-risk prostate cancer trials have demonstrated associations between several molecular biomarkers and clinical outcomes,³⁻⁵ few such studies focused on lower risk patients such as those in RTOG 94-08. Therefore, a correlative study was performed using archived biopsy specimens from RTOG 94-08 that included Ki-67, previously identified as a promising biomarker in prostate cancer patients.⁵⁻¹¹ Ki-67 antigen is a nuclear protein complex, detectable by MIB-1 antibodies, that is present during all active phases of the cell cycle (G1, S, G2 and M-phase) but not the G0 phase, making it a marker of cellular proliferative activity.¹²

PATIENTS AND METHODS

Patient Characteristics

There were 1,979 eligible patients in RTOG protocol 94-08, with 992 in the RT alone arm and 987 in the RT+ADT arm. Tissue was available for Ki67-SI analysis in 468 patients (23.6%), with 253 in the RT alone arm and 215 in the RT+ADT arm.

Initial PSAs and T-categories in RTOG 94-08 were distributed equally between the two treatment arms: 100 (10%) and 109 (11%) patients had an initial PSA of less than 4 ng/ml, while 892 (90%) and 878 (89%) patients had initial PSAs of 4-20 ng/ml in the RT alone and RT+ADT arm, respectively. About 50% of patients had T1 and T2 tumors in each treatment arm. The median follow-ups for surviving patients in the RT alone arm and the RT+ADT arms were 9.2 years and 9.1 years, respectively.² According to National Comprehensive Cancer Network (NCCN) risk stratification, 33.2%, 56.6% and 10.2% of patients with Ki-67 scoring were in the low, intermediate and high-risk categories, respectively.

Treatment Characteristics

For those patients in the RT+ADT arm, ADT was begun 2 months before RT and was continued during RT, for a 4-month total of ADT. Total androgen deprivation was accomplished with flutamide at 250 mg/d plus an LHRH agonist. The prescription RT dose for both arms was 46.8 Gy (1.8 Gy/day four to five times a week for 26 fractions) to the prostate and regional lymphatics, followed by 19.8 Gy (1.8 Gy/day $\times$ 11 fx) for a total of 66.6 Gy to the prostate.

Ki-67 Staining

The staining of Ki-67 using MIB-1 monoclonal antibody (DAKO Corp, Carpinteria, CA) has been described previously.⁵ Negative staining controls were used and normal tonsil sections served as positive controls.

Two investigators independently reviewed the slides without knowledge of patient outcome, both manually under a light microscope and with an image analysis system (ACIS, DAKO Corp, Carpinteria, CA). For manual analysis, the Ki67-SI was defined as the percentage of tumor cells that displayed nuclear MIB-1 staining. Whenever possible, 2,000 tumor cells were counted using an eyepiece grid. For ACIS analysis, the percentages of cells with nuclear staining were quantified by setting a color threshold for brown (positive nuclei) and blue (negative nuclei) staining which was set for every slide analyzed. Slides were scanned at 10 $\times$ magnification and, when amount of tissue permitted, 15 areas of interest equivalent to a 40 $\times$ field in the tissue specimen were quantified. The computer software derived a final sample mean percent index. The two independently determined scores for each case were averaged if discrepant by less than 40% relative to each other. For ACIS analysis, 18% of cases had greater discrepancies than this and their scoring was discussed by the two reviewers with original scores either kept and averaged or new scores assigned based upon the re-review. For manual analysis, because only 3.6% of cases had >40% discrepancy, original scores were averaged without re-review.

Endpoints

The failure event for overall survival (OS) was defined as death due to any cause. A failure event in assessing disease-specific mortality (DSM) was considered death due to: prostate cancer, complications of treatment, other causes with active malignancy (clinical or biochemical), or unknown causes with previously documented relapse (clinical or biochemical). Distant metastasis (DM) was defined as radiographic or clinical evidence of hematogenous spread. Of note, routine-imaging studies to screen for distant metastases at follow-up were not specified by the study and were ordered when deemed clinically indicated by treating physicians. Two different definitions of biochemical failure (BF) were used: one modified from the American Society of Therapeutic Radiology and Oncology consensus definition (protocol BF) and the other according to the Phoenix Consensus Conference definition (PBF). Protocol BF was defined as at least two consecutive rises above the nadir, failure of PSA to reach 4 ng/ml or less at 18 months post RT, or any rise great enough to provoke salvage therapy. PBF was defined as an increase in the PSA level of >2 ng/ml above the nadir, or any rise great enough to provoke salvage therapy. All time events were measured from the date of randomization to the date of their occurrence or last follow-up.

Statistics

All pretreatment characteristics were stratified. Age was dichotomized by the median age in the entire cohort. PSA (<4 ng/mL vs. >4 ng/mL) and T category (T1 vs. T2) were dichotomized in accordance with RTOG 94-08's stratification and randomization criteria

and assigned treatment arm. Gleason score was stratified into three groups: 2 to 6, 7, and 8 to 10. For Overall Survival, the Kaplan-Meier¹³ method was used to estimate yearly survival rates; additionally, Cox regression¹⁴ was used for the univariate and multivariate analyses. For all other time-to-event endpoints including PSA Failure, Distant Metastasis, and Disease-Specific Mortality, the cumulative incidence¹⁵ approach was used to estimate yearly incidence rates; Fine-Gray¹⁶ was used for univariate and multivariable analyses. Ki67-SI was dichotomized for the primary analyses as described in the Results section. All analyses were done on the RT cohort alone, and then repeated on the RT+ADT cohort alone.

RESULTS

Defined as positive nuclear staining (Figure e1), median Ki67-SI was 3.85%, with a range of 0 to 34.85% using the manual scoring method and 2.65%, with a range of 0 to 33.95% when using ACIS. While the differences in means between the two different techniques are relatively small, there was a definite shift of the distribution toward higher scoring indices with the manual method (Figure e2). Correlations with outcomes were stronger for the manual method than for the image-analysis determination; therefore, only manual Ki67-SI results are reported here.

Analyses were performed to determine if the Ki67-SI test cohort (n=468) was representative of the 1,511 cases for which Ki67-SI was not available. Table 1 reveals no statistically significant difference in pretreatment characteristics between the test cohorts in both assigned treatment arms and the remaining cases in the parent cohort. Univariate comparisons of those with and without Ki67-SI data in terms of the end points of OS, DSM, DM, BF and PBF revealed no significant differences between the groups for DSM, DM and BF, while differences reaching statistical significance were seen for OS and PBF (Table e1), meaning that the outcome results for these latter two endpoints may not be generalizable to the entire population enrolled on RTOG 9408.

Three different Ki67-SI quartile-based cut points were initially tested with univariate analysis: the first (<Q1), the median and the third quartile (Q3), representing labeling index cut-points of 2.0%, 3.85% and 6.2%, respectively. When no significant relationships between the 2.0% and 3.85% cut points and the clinical end points were found, further analyses focused only on the 6.2% cut point. Pretreatment characteristics and assigned treatment were distributed similarly between those who had a Ki67-SI <6.2 or ≥6.2% with the exception of T-category, where there was a greater percentage of T2 versus T1 cases when the Ki67-SI was ≥6.2% versus <6.2% (65.3% vs. 48.0%; $p=.001$) (Table e2).

Univariate outcome results for the combined treatment arms are displayed in Table 2. Ki67-SI ≥6.2% was significantly associated with increased rates of DSM ($p=.0064$), DM ($p=.0002$), and BF ($p=.0001$). No significant relationship was found between a ≥6.2% labeling index and OS or PBF, potentially a reflection of the unequal distribution of these endpoints seen between the Ki67-determined and non-determined cases (Table e1) or, in the case of OS, to competing mortality risk. Of the two definitions of PSA failure used, the protocol definition of BF is not a contemporarily conventional one and is considerably more restrictive than the Phoenix definition, which produced a higher failure rate more in the expected range for this cohort of patients. Strong correlations were found, however, between high Ki67-SI and higher rates of failure in the cumulative incidence curves for DSM and DM (Figure 1 A1, B1).

Multivariable analysis validated a Ki67-SI dichotomized at the ≥6.2% cut point as an independent significant correlate of the clinically important end points of DM ($p=.002$) and DSM ($p=.03$), as shown in Table 3. However, none of the more conventional clinical factors

maintained significant association with DM or DSM when the analysis included Ki67-SI, although clinical T-category and a Gleason score of 8-10 did approach significance in their association with DSM. Of note, Ki-67 remained a significant predictor in spite of a significant correlation observed between high Ki67-SI and higher T stage on univariate analysis (Table e2). Although a significant correlation was also seen with protocol BF ($p=.0001$), there was none when the PBF method was examined.

Additional analysis was performed to determine the extent to which these associations of clinical outcomes with Ki67-SI persisted when the individual treatment arms (RT alone vs. RT+ADT) were analyzed separately. Sub-group univariate analysis demonstrated that the 6.2% Ki67-SI cut point was significantly associated with DM in both the RT alone ($p=.004$) and RT+ADT ($p=.03$) arms of the study but was predictive of DSM only in the RT alone arm ($p=.03$), as shown in Table 4. Cumulative incidence curves illustrate the strength of this correlation between high Ki67-SI and DSM and DM (Figure 1 A2, B2). As shown in Table 5, when analyzed by assigned treatment arm, MVA also revealed similar Ki67-associated hazard ratios in each separate treatment arm for DSM, DM and BF, although, likely as a consequence of reduced events, these hazard ratios reached significance only for DM ($p=0.02$) in the RT alone arm and for BF in both the RT alone ($p<0.0001$) and RT +ADT ($p=0.05$) arms. Protocol BF, but not PBF, was significantly correlated with the Ki67-SI in both univariate and multivariable analyses of both arms (Tables 4 & 5).

The potential clinical benefit of adding ADT as a function of Ki67-SI status was also explored. Patients were separated into 6.2% and <6.2% Ki67-SI groups and the hazard ratios for the two treatment arms ($\pm$ ADT) for DSM, DM, BF and PBF were calculated. Comparison of these hazard ratios for the high and low Ki67-SI groups, however, revealed no significant differences (Table e3), suggesting that Ki-67 status does not further inform regarding the potential clinical benefit of adding ADT to radiation.

The relationship between Ki-67 status and tumor local control was also explored. Approximately 40% of patients in RTOG 9408 had re-biopsies obtained 2 years after treatment, with about 20% and 39% of biopsies found positive in the RT+ADT and RT alone arms, respectively. The Ki-67 status of the original diagnostic specimen was determined in a total of 210 of these rebiopsied patients, taken from both treatment arms. A correlative analysis of the positive rebiopsy rates for Ki67-SI 6.2% vs. <6.2%, however, showed a minor trend but no significant difference for the combined arms (Table e4). An analysis was also performed in each treatment arm separately, but again, in neither the RT +ADT nor the RT alone arm did Ki67-SI significantly correlate with the re-biopsy positivity rate. Thus, Ki-67 status, while strongly correlated with the development of metastatic disease, did not appear to inform regarding intraprostatic recurrence/persistence of disease as determined by prostate re-biopsy positivity at 2 years post-treatment.

DISCUSSION

Since dysregulation of proliferation is an essential element of malignant progression, biomarkers of proliferation and cell cycle have been of particular interest in the search for predictors of outcome both in prostate and other cancers. The most often studied proliferation marker, Ki-67, has demonstrated prognostic value for neuroendocrine tumors¹⁷ and gastrointestinal stromal tumors.¹⁸ For prostate cancer also, there is a growing body of evidence that supports an independent prognostic role for Ki67-SI in identifying a tumor subset most likely to progress after various types of treatment. In a number of contemporary prostate cancer studies utilizing various management approaches, the Ki67-SI has been found to be independently prognostic for outcome in patients with clinically localized prostate cancer treated with radiotherapy,^{8,19} or radical prostatectomy,^{6,7} as well as in

patients who were primarily expectantly managed,^{10,11} implying that Ki-67 is a prognosticator rather than a predictor of outcome after any specific treatment.

However, large studies in low-to-intermediate risk prostate cancer patients treated with radiation with or without ADT have been unavailable. Ours is the largest multi-institutional study reported of Ki67-SI in primarily low-to-intermediate risk patients in which adequate diagnostic biopsy material from 468 patients was available for Ki-67 immunohistochemical analysis. Derived from quadrant analysis, a 6.2% labeling index cut point was strongly and independently predictive of disease-specific mortality in the radiation-only arm and independently predictive of distant metastases in both the radiation and the radiation plus ADT arms.

Two prior RTOG clinical correlation biomarker studies have also linked high Ki67-SI with poor clinical outcomes in patients treated with radiation alone or with combined androgen deprivation and radiation, but these trials involved higher risk patients than in the present study. The first, a study of high-risk patients, was carried out on 108 biopsy specimens from patients enrolled on RTOG 86-10.⁵ A Ki-67 scoring index of 3.5% was found to be strongly predictive of cause-specific mortality and distant metastases both in univariate and multivariate analysis. A higher cut-point of 7.1%, similar to the 6.2% cut-point used in the present study also strongly correlated with clinical outcome. The second study was performed on diagnostic biopsy specimens from 537 intermediate-to-high-risk patients enrolled on RTOG 92-02.⁹ The Ki67-SI index was investigated as a continuous and dichotomous variable versus the clinical endpoints of local failure, biochemical control, disease-specific mortality and overall survival. In multivariate analyses that included PSA, Gleason score and T-stage, the Ki67-SI cut-point of 7.1% was associated with DM ($p=0.0008$) and DSM ($p=0.0174$). In fact, the Ki67-SI was the most significant determinant of metastasis and disease specific mortality. Thus, a Ki67-SI threshold in the 6-7% range has consistently been shown to independently distinguish unfavorable from favorable prognosis patients and our study indicates that this relationship extends to early-to-intermediate risk patients, not only the higher risk patients previously reported on in prior studies.

The ability to predict patterns of recurrence, including tumor local recurrence, is particularly important when assessing approaches to optimize treatment. There has, however, been limited information about the relationship between Ki67-SI and local control after radiation therapy. Pollack et al.⁹ investigated such a linkage in RTOG 92-02 and found no significant correlation between a Ki67-SI cut-point of 7.1% and higher rates of local failure after radiation. However, local failure in that trial was assessed only by digital rectal exam, a method likely to underestimate the presence of locally recurrent or persistent disease. In the present study, however, about 40% of enrolled patients had re-biopsies at 2 years, offering a potentially more accurate assessment of local control. Our study, the largest published that compares post-treatment re-biopsy positivity with Ki-67 status, found no significant correlation, further defining Ki-67's role as an independent marker of increased metastatic but not local failure risk.

This study noted a stronger correlation between clinical outcomes and manually determined rather than ACIS-determined Ki67-SI. Intriguingly, a similar trend was found during the Ki67-SI correlative analysis of RTOG protocol 92-02.⁹ While the basis for this observation is unclear, one possibility is that manual scoring could tend to oversample higher labeled regions of the specimen, resulting in the observed shift toward higher staining indices (Figure e2), whereas the image analysis method may produce a more specimen-averaged score. It may then be that the higher labeled elements of the tumor carry the most prognostic impact. Additionally, there were far fewer significant scoring discrepancies between the two independent scorers for manual than for ACIS-determined Ki67-SI, suggesting that the

ACIS method may be less reproducible and therefore less able to predict outcomes. These findings, together with numerous others in which manual scoring of Ki-67 has produced strong correlations with outcome, suggest that simpler, universally available manual scoring for Ki-67 may be sufficient, if not preferable.

Prostate cancer, given its long natural history, is well suited for retrospective biomarker correlative analyses, but potential limitations must be recognized and considered. A foremost potential concern in the present study would be whether the 468 patients with available specimens accurately represented the total enrolled population of RTOG 9408 (n=1,979). Fortunately, although there were differences for OS and PBF, no significant differences in preclinical characteristics (Table 1) or in the key clinical outcomes of DSM, DM and protocol BF (Table e1) were seen between the patients with or without tissue available for analysis, lending increased confidence in the validity of this study's conclusions.

CONCLUSION

High Ki67-SI strongly and independently predicted for disease specific mortality, distant metastasis and protocol biochemical failure in prostate cancer patients treated on RTOG 9408, a study with a majority of intermediate risk prostate cancer patients that were treated with radiation therapy with or without androgen deprivation therapy. Ki67-SI did not, however, significantly predict for biopsy-assessed local recurrence nor for increased relative benefit from ADT. Our results, together with those for two prior RTOG trials whose enrolled patients had higher risk disease, suggest that Ki-67 is an independent predictor of disease specific mortality and distant metastasis across a spectrum of intermediate to high-risk patients. This and prior Ki-67 studies support a potential role for Ki67-SI in identifying metastatic potential in clinical trials focused on improving systemic therapy. These studies also suggest consideration of Ki67 as a stratification factor in future prostate cancer trials.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1. Cumulative incidence disease specific mortality (A) and distant metastasis (B) curves for entire cohort (A1, B1) and by treatment arm (A2, B2) with Ki67-SI dichotomized at the 6.2% cut point. Patients at risk at 0, 2, 4, 6, 8, 10, and 12 years are shown.

Table 1

Pretreatment Characteristics of Patients With Ki-67 Determined vs. Missing (n = 1979)

	Determined Ki-67 (n=468)	Missing Ki-67 (n=1511)	p-value
Age			0.9582
< 70	200 (42.7%)	649 (43.0%)	
70	268 (57.3%)	862 (57.0%)	
Gleason			0.1302
2-6	270 (57.7%)	945 (62.5%)	
7	136 (29.1%)	402 (26.6%)	
8-10	46 (9.8%)	134 (8.9%)	
Unknown	16 (3.4%)	30 (2.0%)	
PSA			0.7377
< 4	47 (10.0%)	162 (10.7%)	
4	421 (90.0%)	1349 (89.3%)	
T Stage			0.6377
T1	223 (47.6%)	741 (49.0%)	
T2	245 (52.4%)	770 (51.0%)	
Assigned Treatment			0.0579
ADT+RT	215 (45.9%)	772 (51.1%)	
RT Alone	253 (54.1%)	739 (48.9%)	

Table 2
Univariate Analyses of Ki67-SI at 6.2% Cut Point in the Complete Test Cohort (n = 468)

Endpoint	Ki-67	n	Failures	HR* (95% CI)	p-value
OS	< 6.20	350	140	1.18(0.85-1.63)	0.32
	6.20	118	49		
DSM	< 6.20	350	14	2.91(1.35-6.26)	0.0064 [‡]
	6.20	118	12		
DM	< 6.20	350	16	3.62(1.85-7.08)	0.0002 [‡]
	6.20	118	18		
BF	< 6.20	350	28	3.29(1.95-5.56)	<0.0001 [‡]
	6.20	118	27		
PBF	< 6.20	350	118	1.24(0.89-1.73)	0.20
	6.20	118	47		

* Fine and Gray's regression model was used for DSM, DM, BF, and PBF.
* Cox-regression model was used for OS.
[‡] Indicates statistical significance at a significance level of 0.05

Table 3

Multivariate Analyses of Dichotomized Ki-67 Using the 6.2% Cut Point (both arms; n = 468)

Endpoint	Variable	Group	HR*	95% CI	p-value
OS	Age, years	70 vs. < 70	1.89	(1.38-2.59)	< 0.0001 [‡]
	Gleason score	7 vs. 2-6	0.81	(0.57-1.13)	0.20
		8-10 vs. 2-6	1.20	(0.75-1.91)	0.45
	PSA	4 vs. < 4	1.26	(0.74-2.15)	0.39
	Clinical stage	T2 vs. T1	1.26	(0.94-1.70)	0.13
	Treatment arm	RT vs. RT/STAD	0.88	(0.66-1.18)	0.38
	Ki67-SI	6.2 vs. < 6.2	1.12	(0.79-1.56)	0.53
DSM	Age, years	70 vs. < 70	1.48	(0.62-3.48)	0.37
	Gleason score	7 vs. 2-6	0.87	(0.34-2.22)	0.76
		8-10 vs. 2-6	2.45	(0.88-6.81)	0.08
	PSA	4 vs. < 4	1.40	(0.30-6.55)	0.67
	Clinical stage	T2 vs. T1	2.34	(0.93-5.89)	0.07
	Treatment arm	RT vs. RT/STAD	1.64	(0.73-3.68)	0.23
	Ki67-SI	6.2 vs. < 6.2	2.48	(1.08-5.72)	0.03 [‡]
DM	Age, years	70 vs. < 70	1.29	(0.64-2.58)	0.47
	Gleason score	7 vs. 2-6	0.87	(0.38-1.20)	0.74
		8-10 vs. 2-6	1.87	(0.74-4.71)	0.19
	PSA	4 vs. < 4	1.17	(0.34-4.03)	0.81
	Clinical stage	T2 vs. T1	0.86	(0.40-1.83)	0.69
	Treatment arm	RT vs. RT/STAD	1.30	(0.65-2.61)	0.46
	Ki67-SI	6.2 vs. < 6.2	3.50	(1.62-7.59)	0.002 [‡]
BF	Age, years	70 vs. < 70	1.11	(0.64-1.90)	0.71
	Gleason score	7 vs. 2-6	1.47	(0.83-2.58)	0.19
		8-10 vs. 2-6	1.17	(0.45-3.03)	0.74
	PSA	4 vs. < 4	6.43	(0.84-49.5)	0.074
	Clinical stage	T2 vs. T1	1.04	(0.58-1.86)	0.90

Endpoint	Variable	Group	HR [*]	95% CI	p-value
	Treatment arm	RT vs. RT/STAD	2.96	(1.59-5.51)	0.0006 [‡]
	Ki67-SI	6.2 vs. < 6.2	3.55	(2.03-6.20)	<0.0001 [‡]
PBF	Age, years	70 vs. < 70	0.79	(0.58-1.08)	0.15
	Gleason score	7 vs. 2-6	1.01	(0.71-1.44)	0.96
		8-10 vs. 2-6	1.42	(0.88-2.30)	0.15
	PSA	4 vs. < 4	1.38	(0.74-2.56)	0.31
	Clinical stage	T2 vs. T1	1.24	(0.89-1.71)	0.20
	Treatment arm	RT vs. RT/STAD	1.89	(1.35-2.63)	0.0002 [‡]
	Ki67-SI	6.2 vs. < 6.2	1.23	(0.87-1.74)	0.25

* Fine and Gray's regression model was used for DSM, DM, BF, and PBF.

* Cox-regression model was used for OS.

[‡] Indicates statistical significance at a significance level of 0.05

Table 4

Univariate Hazard Ratios for Ki67-SI 6.2 vs. < 6.2 % per Treatment Arm

Endpoint	Arm 1: RT + ADT (n =215)		Arm 2: RT (n =253)	
	HR* (95% CI)	p-value	HR* (95% CI)	p-value
OS	1.17(0.73-1.86)	0.52	1.06(0.67-1.67)	0.81
DSM	2.62(0.70-9.71)	0.15	2.80(1.10-7.17)	0.03 [‡]
DM	3.24(1.14-9.17)	0.03 [‡]	3.64(1.53-8.67)	0.004 [‡]
BF	2.91(0.99-8.53)	0.05 [‡]	3.30(1.81-6.01)	<0.0001 [‡]
PBF	1.29(0.72-2.29)	0.39	1.21(0.81-1.80)	0.36

* Fine and Gray's regression model was used for DSM, DM, BF, and PBF.

* Cox-regression model was used for OS.

[‡] Indicates statistical significance at a significance level of 0.05

Table 5

Multivariate Analyses of Ki67-SI at 6.2% Cut Point in Each Treatment Arm

Endpoint	Arm 1: RT + ADT (n = 215)		Arm 2: RT (n = 253)	
	HR* (95% CI)	p-value	HR* (95% CI)	p-value
OS	0.99(0.60-1.63)	0.97	1.10(0.689-1.77)	0.69
DSM	2.47(1.49-12.47)	0.27	2.23(0.75-6.65)	0.15
DM	3.07(0.78-12.07)	0.11	3.21(1.25-8.29)	0.02 [‡]
BF	3.07(0.99-9.44)	0.05 [‡]	3.51(1.88-6.58)	<0.0001 [‡]
PBF	1.07(0.57-2.02)	0.83	1.25(0.82-1.92)	0.30

* Fine and Gray's regression model was used for DSM, DM and BF. The other covariates were age, Gleason score, initial PSA and stage, as in Table 3.

[‡] Indicates statistical significance at a significance level of 0.05