

Tumor Hypoxia Predicts Biochemical Failure following Radiotherapy for Clinically Localized Prostate Cancer

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Abstract

Purpose: Tumor hypoxia is an important determinant of outcome in many human malignancies and is associated with treatment resistance and metastases. The aim of this study was to determine the effect of hypoxia in patients with prostate cancer treated with radiotherapy.

Experimental Design: Tumor hypoxia was measured in 247 patients with clinically localized prostate cancer before radiotherapy, with or without hormonal therapy. The median pO₂ was 6.8 mm Hg and the median hypoxic percentage less than 10 mm Hg (HP₁₀) was 63%. The median follow-up was 6.6 years.

Results: The 5-year biochemical relapse-free rate (bRFR) was 78%. Prostate-specific antigen and Gleason score were both associated with biochemical relapse and formed a baseline clinical model. The effect of hypoxia was found to vary with the duration of patient follow-up. HP₁₀, when added to the clinical model, was an independent predictor of early bRFR ($P = 0.019$). The relationship between hypoxia and early bRFR was more pronounced when the analysis was restricted to 142 patients with bulk tumor at the site of the oxygen measurements ($P = 0.004$). Hypoxia was the only factor predictive of local recurrence in 70 patients who had biopsies conducted during follow-up ($P = 0.043$), again with the effect being greatest early after completing treatment.

Conclusions: This is the largest clinical study of prostate cancer hypoxia with direct measurement of tumor oxygen levels. It shows that hypoxia is associated with early biochemical relapse after radiotherapy and also with local recurrence in the prostate gland. *Clin Cancer Res*; 18(7): 2108–14. ©2012 AACR.

Introduction

Prostate cancer is the most common malignancy among North American men, and imposes a huge social and economic burden. Most patients have low- or intermediate-risk disease at diagnosis that is clinically confined to the prostate gland (1). Depending on comorbidities and individual preference, they are candidates for potentially curative treatment with either radical prostatectomy or radiotherapy. Despite technical advances in both of these treatments over the past decades, approximately 25% of these patients will develop progressive disease locally in the prostate gland, in regional lymph nodes or at distant sites (2). This underscores the importance of better understand-

ing the biologic factors that are responsible for malignant progression, the development of metastases and the failure of currently available treatments, as prerequisites to developing new therapeutic strategies and improving clinical outcomes.

Hypoxia is a feature of many human tumors and has been implicated as an important modulator of clinical behavior and treatment response, mediated via genomic and molecular changes that promote both local aggressiveness and metastasis formation (3, 4). We and others have previously reported that many prostate cancers are hypoxic, and that the distribution of oxygen values is similar to other human tumors (5–8). However, little is known about the impact of hypoxia on prostate cancer progression over time or long-term clinical outcome following radical prostatectomy or radiotherapy. Upregulation of hypoxia-inducible factor (HIF) signaling was recently reported to be an independent predictor of biochemical failure in prostate cancer patients treated with either surgery or radiotherapy (9). While this implicates hypoxia as a biologically and clinically relevant determinant of treatment outcome in this disease, HIF also may be upregulated by hypoxia-independent factors including altered expression of oncogenes and tumor suppressors, oxygen-free radicals, androgens, and other growth factors (10–13). The complex interactions involving hypoxia, androgens, HIF signaling, and tumor angiogenesis are

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Translational Relevance

There is an important need to better understand the biologic determinant of clinical outcome in patients with intermediate-risk prostate cancer. This study shows that prostate cancer is hypoxic and, for the first time, shows that hypoxia measured before treatment is associated with early biochemical recurrence after dose-escalated conformal or intensity-modulated radiotherapy. This pattern of recurrence is compatible with the presence of hypoxia-driven occult metastatic disease, as has been reported in experimental studies and clinically in other tumor sites. Overall, the results provide new insight about the biologic behavior of intermediate-risk prostate cancer after radiotherapy and have important implications for combining radiotherapy with hypoxia-targeted therapeutics, hormonal therapy, or other systemic anticancer treatments.

highlighted by studies that have shown hormonal treatment to reduce hypoxia in prostate cancer (14, 15).

This study is the largest to date of direct oxygen measurements before treatment in clinically localized prostate cancer. It shows that hypoxia is associated with early biochemical relapse after radiotherapy and also with local recurrence in the prostate gland.

Materials and Methods

Patient population

A total of 279 patients with histologically confirmed adenocarcinoma of the prostate were accrued between 1999 and 2006 to a prospective clinical study, which was approved by the University Health Network Research Ethics Board and registered (NCT00160979) in accordance with the criteria outlined by the International Committee of Medical Journal Editors. Informed consent was obtained in all cases. Thirty-two patients were excluded from the analysis, 11 because of technical problems with the oxygen measurements, 7 because the oxygen measurements were made after the start of hormonal treatment, 8 because adjuvant hormonal treatment was used after radiotherapy, 5 because radiotherapy was not completed as planned, and 1 because the pathologic diagnosis was changed to small-cell carcinoma on subsequent review. The clinical characteristics of the 247 eligible patients are summarized in Table 1.

Measurement of prostate oxygen levels

Prostate cancer hypoxia was measured before any treatment using an ultrasound-guided transrectal needle-electrode technique, as previously described (8). Between 40 and 80, individual oxygen readings were obtained along 2 to 4 linear measurement tracks 1.5 to 2 cm in length through regions of the prostate gland likely to contain tumor based on information from previous diagnostic biopsies, digital

rectal examination, and real-time Doppler ultrasound during the procedure. Needle biopsies were then obtained along the measurement tracks for correlative molecular studies. Patients were awake throughout and local anesthetic was not used. We previously reported that tumor was present along 70% of the measurement tracks with this technique, that average oxygen levels were similar regardless of whether or not tumor was present in the postmeasurement biopsies, and that both malignant and nonmalignant regions of the gland contained hypoxic foci (8). Therefore, all oxygen measurements (excluding nonphysiologic values <-3 or >100 mm Hg) along all tracks were included in the analyses.

Treatment of prostate cancer

Immediately following the oxygen measurements, as part of the same procedure, 3 inert gold fiducial markers were inserted into the prostate gland transrectally under ultrasound guidance for radiotherapy planning and day-to-day target verification during treatment. 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. As summarized in Table 1, one patient ($<1\%$) received a dose of 70 Gy in

Table 1. Clinical characteristics

Variable	Category	Distribution
Age, y	Median (range)	71 (55–83)
cT-category ^a , <i>n</i> (%)	T1	104 (42)
	T2	142 (57)
	T3	1 (<1)
Gleason score, <i>n</i> (%)	6	71 (29)
	7	163 (66)
	8, 9	13 (5)
PSA, <i>n</i> (%)	Median (range)	7.8 (0.9–33) ng/mL
	≤ 10 ng/mL	164 (66%)
	10.1–20 ng/mL	76 (31%)
	>20 ng/mL	7 (3%)
Proportion-positive biopsies ^b	Median (range)	0.43 (0–1.0)
Hormonal treatment ^c , <i>n</i> (%)	Yes	52 (21)
	No	195 (79)
Radiotherapy dose, <i>n</i> (%)	70 Gy ^d	1 (<1)
	75.6 Gy ^d	71 (29)
	78–79.8 Gy ^d	148 (60)
	60–66 Gy ^e	27 (11)

^aUICC-TNM classification, 6th edition, 2002.

^bThe proportion of diagnostic biopsy cores that contained cancer.

^cNeoadjuvant and concurrent hormonal treatment.

^d1.8 to 2 Gy per fraction.

^e3 Gy per fraction.

2 Gy daily fractions, 72 patients (29%) received 75.6 Gy in 1.8 Gy daily fractions, and 148 patients (60%) received 78 to 79.8 Gy in 1.8 to 2 Gy daily fractions. Twenty-seven patients (11%) participated in a study of hypofractionated radiotherapy and received 60 to 66 Gy in 3 Gy daily fractions. Neoadjuvant and concurrent hormonal therapy was used in 52 patients (21%). None of the patients received adjuvant hormonal treatment.

Patients were followed at 6 monthly intervals after completing treatment with clinical examination and prostate-specific antigen (PSA). 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 6.6 years from the date of the oxygen measurements.

Sample size and data analysis

A variety of oxygen parameters have been used in previous electrode studies of tumor hypoxia, including the median pO_2 and the percentage of the individual oxygen measurements in each tumor less than 5 mm Hg or less than 10 mm Hg (16). Figure 1 shows the distribution of these parameters for the 247 patients with prostate cancer in this study. The percentage of oxygen measurements less than 10 mm Hg (HP_{10}) was selected as the independent variable for this analysis because it offered the greatest potential to discriminate among patients with relatively well-oxygenated tumors (small hypoxic percentages) and was approxi-

mately normally distributed. The median HP_{10} was 63% with a range of 0% to 100%. There were no correlations between HP_{10} and clinical prognostic factors, including T-category, Gleason score, or pretreatment PSA.

The primary endpoint of the study was biochemical failure using the Phoenix definition of a 2 ng/mL PSA increase above the nadir (17). Patients with a rising PSA who received salvage treatment for recurrent prostate cancer before meeting the Phoenix definition were also included as failures for the purpose of this analysis. Secondary endpoints were biochemical failure in patients with bulk disease at the site of the oxygen measurements and local recurrence in the prostate gland. Patients with bulk disease were identified by an independent observer blinded to the oxygen status using a definition of sufficient tumor in the core biopsies (obtained at the time of the measurements from the same region of the gland) to permit manual microdissection for molecular profiling (18). Local recurrence was determined from transrectal ultrasound or MRI-guided prostate biopsies obtained either routinely 3 years after completing treatment, as a requirement of separate phase I/II radiation dose-escalation protocols in which some of the patients also participated, or in response to a rising PSA. In general, sextant biopsies were obtained as well as biopsies targeted to areas of suspected recurrence based on imaging characteristics. The biopsies were reported by experienced genitourinary pathologists knowledgeable in the diagnosis of tumor recurrence following radiotherapy.

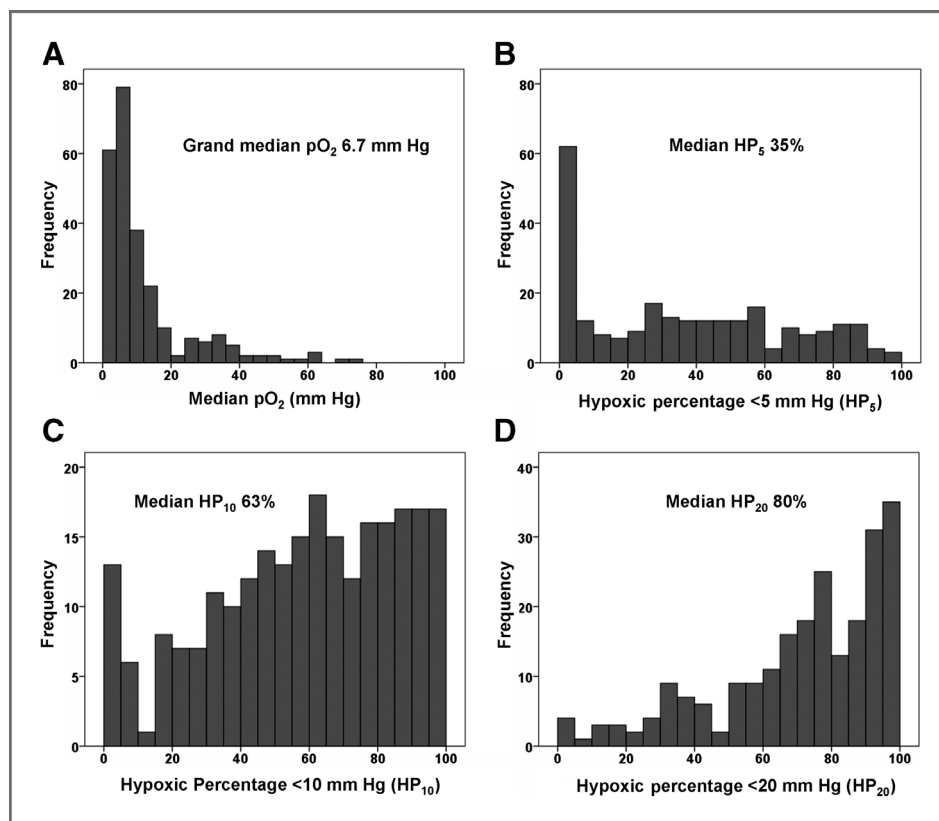


Figure 1. Distribution of oxygen parameters in 247 patients with prostate cancer: median pO_2 (A) and the percentage of oxygen readings in individual tumors less than 5 mm Hg (B), 10 mm Hg (C), and 20 mm Hg (D).

The main objective of the study was to determine whether prostate hypoxia influenced disease recurrence after radiotherapy, independent of standard clinical prognostic factors. A clinical multivariate Cox model was first derived with forward stepwise selection of covariates (age, clinical T-category, Gleason score, pretreatment PSA, proportion of diagnostic biopsy cores positive for cancer, radiotherapy dose, and the use of hormonal therapy) and biochemical failure as the endpoint. HP₁₀ was then added to this clinical model to determine its independent predictive effect. The α level for rejecting the null hypothesis that HP₁₀ has no relationship to patient outcome was set at 0.05. Clinical T-category, Gleason score, the use of hormonal treatment, and radiotherapy dose were modeled as categorical variables and age, pretreatment PSA, the proportion of positive diagnostic biopsy cores, and HP₁₀ as continuous variables. The assumption of proportional hazards was tested for each covariate first by univariate Cox analysis and examination of the Schoenfeld residuals and then using a bivariate Cox model, including both the covariate and its interaction with time (19, 20).

A sample size of 200 patients was estimated initially to detect an HR of 1.015 with hypoxia expressed as a continuous, normally distributed covariate with mean of 50% and SD of 25% (equivalent to an HR of 2.3 for a dichotomous covariate split at the median value) with 80% power and a 2-sided α level of 0.05, assuming an overall 5-year biochemical relapse-free rate (bRFR) of 70% and a median follow-up of 5 years. However, because of fewer events than expected, the sample size estimate subsequently was increased to 280 patients.

Results

Biochemical recurrence was identified in 79 patients, including 74 who met the Phoenix failure definition of a PSA increase ≥ 2 ng/ml above the nadir and 5 with a rising PSA who received salvage treatment before the Phoenix PSA failure threshold was reached. The actuarial bRFR was 91% at 3 years and 78% at 5 years, as shown in Fig. 2.

Univariate analysis of clinical covariates identified significant effects of Gleason score (Gleason 6 vs. 7 vs.

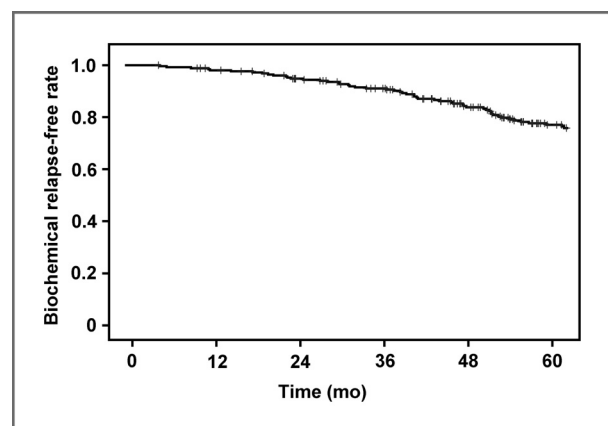


Figure 2. Kaplan-Meier plot of bRFR in 247 patients with prostate cancer.

8, $P = 0.015$; log-rank) and PSA ($P = 0.005$) on bRFR. Multivariate analysis using the Cox proportional hazard model identified Gleason score (6 or 7 vs. 8, $P = 0.005$) and PSA ($P < 0.001$) to be the only significant, independent clinical predictors of outcome. These 2 covariates comprised the baseline clinical model, to which HP₁₀ was added to determine its independent predictive value. There was no relationship between age, clinical T-category, the proportion of positive diagnostic biopsy cores, radiotherapy dose, or the use of hormonal therapy and bRFR by either univariate or multivariate analysis.

All of the clinical covariates satisfied the assumption of proportional hazards. In contrast, HP₁₀ was found to violate this assumption. On univariate analysis, the effect of HP₁₀ on bRFR was maximal early in follow-up (HR = 1.026, $P = 0.01$) and diminished with increasing time ($P \leq 0.001$). This implies that patients with hypoxic tumors were more likely than those with well-oxygenated tumors to develop biochemical failure within the first 48 months of completing treatment but not at longer times. HP₁₀ and an HP₁₀ interaction term with time were added to the clinical multivariate model to determine the independent effect of hypoxia on bRFR. As shown in Table 2 and Fig. 3, hypoxia remained a significant predictor of early biochemical relapse (HR = 1.023, $P = 0.019$) after correcting for Gleason score and PSA.

Table 2. Multivariate predictive models for biochemical and LRFRs

Variable	HR	P
Entire cohort of 247 patients with prostate cancer, bRFR		
Gleason score ^a	2.66	0.015
PSA	1.075	<0.001
HP ₁₀ ^b	1.023	0.019
HP ₁₀ with time ^c	0.9995	0.001
142 patients with bulk^d tumor at the site of the oxygen measurements, bRFR		
Age	1.073	0.021
PSA	1.085	<0.001
HP ₁₀ ^b	1.036	0.004
HP ₁₀ with time ^c	0.9992	<0.001
70 patients with prostate biopsies for local control, LRFR		
HP ₁₀ ^b	1.037	0.043
HP ₁₀ with time ^c	0.9991	0.032

NOTE: No effect of age, clinical T-category, the proportion of positive diagnostic biopsy cores, radiotherapy dose, or the use of hormonal therapy on either bRFR or LRFR.

^aGleason 8 versus 6 or 7.

^bEffect of HP₁₀ on outcome (bRFR or LRFR) at the completion of treatment.

^cHP₁₀ time dependence. Time expressed in months from the date of the oxygen measurements.

^dSee text for definition of bulk tumor.

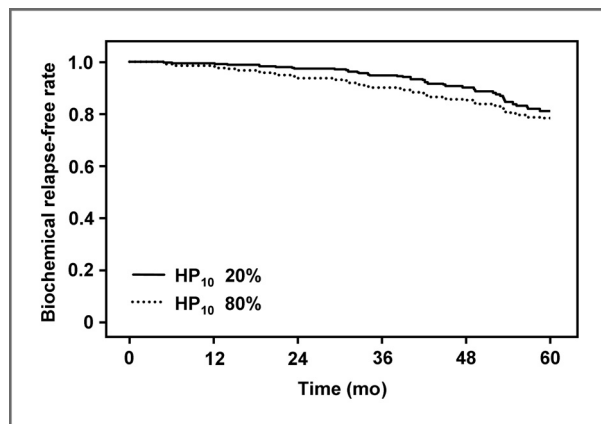


Figure 3. Effect of hypoxia (HP_{10}) on bRFR in the entire cohort of 247 patients. The lines show the predicted bRFR for HP_{10} values of 80% (more hypoxic) and 20% (less hypoxic) and are corrected for the effect of other significant clinical prognostic factors.

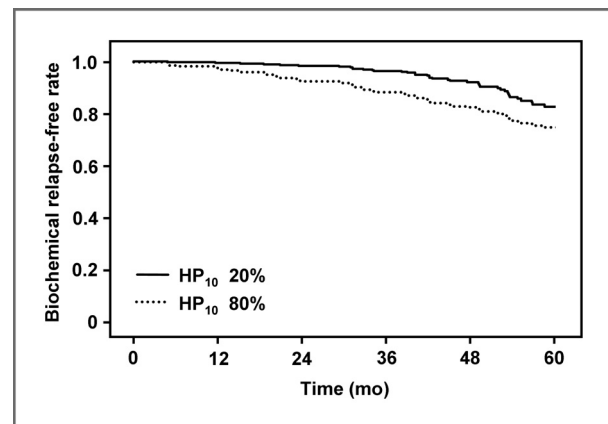


Figure 4. Effect of hypoxia (HP_{10}) on bRFR in the subgroup of 142 patients with bulk tumor at the site of the oxygen measurements. The lines show the predicted bRFR for HP_{10} values of 80% (more hypoxic) and 20% (less hypoxic) and are corrected for the effect of other significant clinical prognostic factors.

Prostate cancer is a multifocal, infiltrative disease particularly early in its natural history (21). Although the oxygen measurements in this study were targeted at tumor, it is likely that some of the readings were obtained from nonmalignant regions of the gland. Therefore, a secondary analysis was conducted to evaluate the effect of hypoxia on bRFR in patients with bulk disease at the site of the oxygen measurements, increasing the likelihood that the measurements were made in tumor. Bulk disease, defined by an independent observer as sufficient tumor in biopsies from the measurement sites to permit manual microdissection, was identified in 142 of 194 patients (tissue was unavailable in 53 patients). Those with bulk disease were more likely to have cT2 tumors than those without (64% vs. 48%), but there were no differences in the distribution of other clinical factors or hypoxia between the groups. HP_{10} remained a significant predictor of early bRFR by univariate analysis ($HR = 1.035$, $P = 0.007$) and also after correcting for the effect of important clinical prognostic factors ($HR = 1.036$, $P = 0.004$), as shown in Table 2 and Fig. 4. Even though this subgroup contained substantially fewer patients than the cohort as a whole (142 vs. 247), there was a trend towards a larger effect of hypoxia on early bRFR.

Transrectal ultrasound or MRI-guided prostate biopsies were obtained during follow-up in 70 patients to assess local tumor control. The median interval from the completion of treatment to biopsy was 34 months, with a range of 20 to 107 months. Only one patient underwent biopsy within the first 24 months of follow-up. Biochemical failure had occurred in 36 of these patients (51%) at the time of biopsy. Tumor was identified in the biopsies of 48 patients (69%), including 31 of 36 with biochemical failure (86%) and 17 of 34 without (50%). Hypoxia was the only factor predictive of local control in this subgroup, as summarized in Table 2. The effect of hypoxia on local relapse-free rate (LRFR) was greatest early in follow-up ($HR = 1.037$, $P = 0.043$) and dimin-

ished with increasing time ($P = 0.032$), similar to the bRFR results.

Discussion

Hypoxia often arises early in solid tumor development because of imbalances between oxygen supply and consumption and leads to a cascade of genetic and molecular signaling events that together influence biologic evolution, clinical behavior, and treatment response (3, 4). This is the largest study of direct oxygen measurements in prostate cancer before treatment. It shows that hypoxia is an independent predictor of biochemical recurrence after radiotherapy alone, or radiotherapy in combination with neoadjuvant and concurrent hormonal therapy. It also provides new information about the hypoxia-related time dependence of biochemical recurrence, which is greatest early after treatment and diminishes with increasing time. This effect of hypoxia was maintained when the analysis was restricted to a subgroup of patients with biopsy-proven bulk disease at the site of the oxygen measurements, consistent with emerging evidence indicating that early biologic and clinical behavior is determined by the dominant focus of disease in the prostate gland and less so by secondary more indolent foci (22, 23). Overall, the results support the hypothesis that hypoxia influences clinical outcome after radiotherapy in men with intermediate-risk prostate cancer, and provide a strong rationale for integrating radiotherapy with new hypoxia-targeted treatment approaches in future clinical trials.

This is the first study to identify a relationship between pretreatment prostate hypoxia and local recurrence after radiotherapy. Some of the patients underwent prostate biopsy during follow-up, either as part of separate dose-escalation studies or at the discretion of individual treating physicians. This subgroup was biased toward patients deemed to be at high risk of recurrence on the basis of

increasing PSA or other clinical indicators, and the results therefore need to be interpreted cautiously. Nevertheless, hypoxia was the only factor predictive of LRFR in these patients. The impact of hypoxia on the risk of metastatic recurrence in lymph nodes or bone, or at other distant sites, was not examined because most patients with rising PSAs began salvage hormonal therapy without first undergoing imaging for metastases.

Only 2 other studies have reported a relationship between prostate cancer hypoxia and outcome after radiotherapy. Movsas and colleagues made direct oxygen measurements in 57 patients with low- or intermediate-risk prostate cancer and found the prostate-to-muscle oxygen ratio to be an important predictor of biochemical recurrence following brachytherapy (24, 25). Vergis and colleagues generated tissue microarrays from pretreatment needle biopsies of 201 men with clinically localized prostate cancer who received neoadjuvant hormonal therapy followed by external beam radiotherapy. They showed that upregulation of HIF and one of its target genes, *VEGF*, were independently associated with biochemical recurrence (9). This group also showed a similar result in 289 patients who underwent radical prostatectomy, using tissue microarrays generated from the surgical specimens (9). Hypoxia is a strong driver of HIF expression, but other hypoxia-independent factors may also be important (10, 11). For example, in prostate cancer, HIF expression has been linked to activation of the phosphoinositide 3-kinase pathway because of changes in the activity of oncogenes or tumor suppressors (13) or an effect of circulating androgens (12). Given this complexity, direct measurements of oxygen levels may not necessarily correlate with HIF-related gene expression (11). In our study, needle biopsies were obtained from the same sites as the oxygen measurements and will allow us to relate prostate hypoxia to the expression of HIF-dependent and -independent biomarkers.

This study identified an effect of prostate hypoxia on early biochemical and local recurrence that was maximal immediately after completing treatment, diminished with increasing follow-up and disappeared after about 4 years, as shown in Figs. 3 and 4. The explanation for this is unclear, but it may reflect an interaction between the effects of hypoxia on local versus metastatic recurrence in the context of modern, high-dose radiotherapy, coupled to the longer natural history of prostate cancer compared with other tumors. Several reports have described a biphasic recurrence pattern in prostate cancer patients who receive radiotherapy, with an early wave in the first 4 years mainly due to unrecognized disseminated disease at diagnosis and a late wave between 8 and 10 years thought to arise from locally persistent tumor leading to secondary metastases (26–28). Primary tumor hypoxia has been shown in laboratory and clinical studies to impair the local response to radiotherapy and increase the development of metastases (3, 4). Overall, the pattern of hypoxia-dependent early biochemical recurrence that we observed is most consistent with a linkage between hypoxia and dominant, biologically aggressive

disease in the prostate gland that may be both resistant to radiotherapy and metastatic at diagnosis. The diminished long-term predictive power of hypoxia may reflect the fact that oxygen dynamics in tumors change over time and in response to treatment (11), so that hypoxia measured at diagnosis may not be relevant to the biologic or clinical behavior several years later. Also, there is evidence to indicate that radiotherapy dose-escalation to the levels used in this study may more effectively suppress late recurrences than early recurrences (28), consistent with the former arising from more indolent, better oxygenated, more radio-sensitive disease at the time of treatment. The median follow-up of patients in this study was only 6.6 years, and a clearer picture of the relationship between pretreatment tumor hypoxia and late disease recurrence may emerge with further surveillance.

In summary, this study provides new evidence that hypoxia in clinically localized prostate cancer influences the outcome of patients after high-dose external beam radiotherapy, with or without neoadjuvant and concurrent hormonal therapy. It raises several intriguing questions about the underlying mechanisms of treatment failure and disease progression that will be addressed with longer follow-up, and in future companion studies using biopsies obtained at the same time and from the same sites as the oxygen measurements. A validation cohort is required to confirm these findings. This highlights an important need for better, more widely available ways of directly measuring hypoxia at diagnosis and repeatedly over time in patients with prostate cancer, perhaps using minimally invasive functional MRI or positron emission tomography imaging techniques. Ultimately, a validated, minimally invasive biomarker will be required to select patients for more intensive or targeted therapies. New treatment approaches for high-risk patients with hypoxic tumors might consist of further radiation dose-escalation, the use of hypoxic cell sensitizers or cytotoxins in combination with radiotherapy (29), or molecular therapeutic strategies that either alter the balance between oxygen supply and consumption or exploit the molecular consequences of hypoxia (3) to enhance radiation response, tumor control, and patient survival.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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Tumor Hypoxia Predicts Biochemical Failure following Radiotherapy for Clinically Localized Prostate Cancer

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