Prostate Cancer DNA Ploidy and Response to Salvage Hormone Therapy After Radiotherapy With or Without Short-Term Total Androgen Blockade: An Analysis of RTOG 8610

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Purpose: **DNA ploidy has consistently been found to be a correlate of prostate cancer patient outcome. However, a minority of studies have used pretreatment diagnostic material and have involved radiotherapy (RT)-treated patients. In this retrospective study, the predictive value of DNA ploidy was evaluated in patients entered into Radiation Therapy Oncology Group protocol 8610. The protocol treatment randomization was RT alone versus RT plus short-course (~4 months) neoadjuvant and concurrent total androgen blockade (RT+TAB).**

Patients and Methods: **The study population consisted of 149 patients, of whom 74 received RT alone and 75 received RT+TAB. DNA content was determined by image analysis of Feulgen stained tissue sections; 94 patients were diploid and 55 patients were nondiploid. Kaplan-Meier univariate survival, the cumulative incidence method, and Cox proportional hazards multivariate analyses were used to evaluate the relationship of DNA ploidy to distant metastasis and overall survival.**

Results: **DNA nondiploidy was not associated with any of the other prognostic factors in univariate analyses. In Kaplan-Meier analyses, 5-year overall survival was 70% for those with diploid tumors and 42% for nondiploid tumors. Cox proportional hazards regression revealed that nondiploidy was independently associated with reduced overall survival. No correlation was observed between DNA ploidy and distant metastasis. The diminished survival in the absence of an increase in distant metastasis was related to a reduction in the effect of salvage androgen ablation; patients treated initially with RT+TAB and who had nondiploid tumors had reduced survival after salvage androgen ablation.**

Conclusions: **Nondiploidy was associated with shorter survival, which seemed to be related to reduced response to salvage hormone therapy for those previously exposed to short-term TAB.**

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DNA PLOIDY has been investigated as a potential prognostic factor for prostate cancer for many years, and in the vast majority of reports, it has been found to be predictive of patient outcome.[1-20] However, most of these studies were done using tissue from prostatectomy specimens. Far fewer have examined DNA ploidy as a pretreatment correlate of patient outcome using diagnostic material. Moreover, there are few reports wherein the predictive value of DNA ploidy was investigated in prostate cancer patients treated with definitive radiotherapy (RT). Conclusions about the association of DNA ploidy with outcome after RT are unclear because the findings have been somewhat divergent.[21-26] In this analysis, DNA ploidy was characterized in the diagnostic material from patients participating in Radiation Therapy Oncology Group (RTOG) protocol 8610.[27]

RTOG protocol 8610 was a phase III randomized clinical trial designed to assess the effect of RT plus short-term neoadjuvant and concurrent total androgen blockade (RT+TAB) as compared with RT alone. The patients enrolled had locally advanced disease; tumors had a palpable surface area of 25 cm^2 or greater. In addition, nearly one third of the patients had Gleason score 8 to 10 disease and 8% had documented lymph node involvement.[27] The purpose of this analysis was to assess the prognostic significance of DNA ploidy, as determined by image analysis, for prostate cancer patients with high-risk features and to determine whether the addition of androgen ablation to RT affected the prognostic value of these measurements. There were 456 evaluable patients entered into the trial, and of these, 149 (33%) had tissue available for DNA ploidy analysis. These patients are the subjects of this report.

PATIENTS AND METHODS

Study Population Characteristics

The study population included patients entered into RTOG protocol 8610, entitled "A phase III trial of Zoladex and flutamide used as cytoreductive agents in locally advanced carcinoma of the prostate treated with definitive radiotherapy."[27] This phase III randomized clinical trial for locally advanced prostate cancer was closed in 1991 and accrued a total of 471 patients, 456 of whom were evaluable. TAB with flutamide and goserelin acetate (Zoladex) was given for a total of 4 months, starting 2 months before radiotherapy and continuing during RT.

Tissue blocks were obtained from 261 (57%) of the 456 evaluable patients. After hematoxylin- and eosin-stained samples were sectioned and reviewed,

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Table 1. Pretreatment Characteristics

Factor	Diploid No.	Diploid %	Nondiploid No.	Nondiploid %	P*
Total	94		55		
Age, years					
Median	71		70		NS
Range	51-88		49-82		
Performance status (KPS)					
90-100	90	96	53	96	NS
70-80	4	4	2	4	
Gleason score					
2-6	29	31	9	17	.057
7-10	65	69	45	83	
Clinical stage					
T2	23	24	15	27	.71
T3	71	76	40	73	
Clinical nodal status					
NX	91	97	51	93	.30
N+	3	3	4	7	
Assigned treatment					
RT alone	49	52	25	45	.43
RT+TAB	45	48	30	55	
p53 status					
Negative	59	63	36	65	.60
Positive (abnormal)	10	11	8	15	
Unknown†	25	26	11	20	

Abbreviations: KPS, Karnofsky performance status; RT, radiotherapy; TAB, total androgen blockade; NS, not significant.

*χ^2 statistics.

†Those with unknown p53 were not included in the analysis.

Table 2. Potential Pretreatment Predictors of 5-Year* Distant Metastasis, Any Failure, and Overall Survival (n = 456†)

Predictor	5-Year, % DM	P	AF	P	OS	P
Gleason score						
2-6	21		69		81	
7-10	44	.001	85	.001	64	.001
Assigned treatment						
RT alone	41		87		69	
RT+TAB	31	.02	73	.001	71	.08
p53 status						
Negative	38		89		66	
Positive (abnormal)	61	.012	95	.05	39	.06
DNA ploidy						
Diploid	36		90		70	
Nondiploid	45	.49	87	.36	42	.031
Age, years						
< 75	NA		NA		70	
≥ 75					69	.053

Abbreviations: DM, distant metastasis; AF, any failure; OS, overall survival; NA, not analyzed; RT, radiotherapy; TAB, total androgen blockade.

*Kaplan-Meier analysis and log-rank test for AF (including death) and OS. Cumulative incidence and Gray's test for DM.

†There are 429 patients with Gleason score, 149 with DNA-ploidy, and 129 with p53 status.

sufficient tumor for DNA ploidy analysis was present in 149 patient samples. The diagnostic material, which consisted of 113 samples from needle biopsies and 36 samples from transurethral resectates, was requested from participating institutions (> 100), reviewed centrally by the study pathologist (D.J. Grignon) in 98% of cases, and graded according to Gleason.[28] A global Gleason score was assigned. The distribution of patients by Gleason score for the study group was 22 in Gleason score 2 to 5, 16 in Gleason score 6, 60 in Gleason score 7, and 50 in Gleason score 8 to 10; one patient case was not graded. The distribution of patients by clinical T category was 38 in T2 and 111 in T3. At the time the trial was initiated, pretreatment prostate-specific antigen (PSA) was not routinely used in the clinic. Pretreatment serum PSA values were available for only 19 (15%) patients of the study cohort and, as a consequence, are not included in the statistical analyses. A prior immunohistochemical analysis of p53 status was done in 129 patients[29] who participated in RTOG protocol 8610. In that report, abnormal p53 expression (p53-positive by immunohistochemistry) was found to be significantly correlated with reduced survival. For this reason, p53 status is included in the analysis here. p53 status and DNA ploidy were determined in 113 patients.

DNA Content Measurements by Image Analysis

For inclusion in the study, the stained section had to contain identifiable carcinoma. Sections were evaluated without knowledge of patient outcome. Sections cut 6 μm thick on poly-L slides from paraffin-embedded formalin-fixed tissues were deparaffinized in xylene and rehydrated in a series of ethanol washes (100%, 95%) to a final distilled water step. Slides were then placed in 5 N HCl for 60 minutes, stained with Schiff's reagent for 60 minutes, rinsed in a sodium metabisulfite rinse (10% $Na_2S_2O_5$ in 1 N HC1), dehydrated in reagent alcohol, and then cleared in xylene. Coverslips were added to slides using synthetic mounting media.

Measurements were obtained at ×200 magnification using 560-nm monochromatic light. DNA quantification was performed using the Image Measure software program (Phoenix Technology, Inc, Seattle, WA), with a PCVision Plus digitizing frame-grabber board (Imaging Technology, Inc, Woburn, MA) and a Logitech mouse (Fremont, CA). Both a Pulnix TM-745 camera (Sunnyvale, CA) mounted on an Olympus BH-2 microscope (Lake Success, NY) and a Sony monitor (San Jose, CA) were used to scan the specimens.

For each sample, tumor cell and control cell nuclei were taken from the same slide. Each area of interest in the tissue was designated on the slide using a marking pen. For each slide, a black level and incident light level were set for calibration. Each designated area was scanned from left to right covering each field only once. Nuclei were chosen because they appeared not to be overlapped by other nuclei. For each sample, 100 control nuclei (endogenous fibroblasts) and 200 tumor nuclei were measured. The DNA content was plotted as Feulgen-stained DNA versus cell number and displayed in histograms. The DNA content mean, SD, and coefficient of variation (CV) were calculated for the control cells (2C control). The mean was used to calculate the DNA index (DI), which was the ratio of the mean nuclear cell DNA content of tumor population divided by the mean of the 2C control population.

Tumor nuclei populations were considered diploid if the main peak DI was 0.80 to 1.20 with less than 35% of other DNA measurements outside of 2C + 2SD (on the basis of the control nuclei population). Populations were considered aneuploid if the main peak DI was less than 0.80 (hypodiploid) or greater than 1.20 (hyperdiploid) and were not considered tetraploid. Tetraploid populations had a DNA index of 1.80 to 2.20. Patients who had multiple peaks were considered aneuploid if more than 35% of the tumor nuclei population formed peaks in the range greater than 2C ± 2SD.

Definition of End Points

The end points used in the analysis were distant metastasis, any failure, and overall survival. The parameters of distant metastasis and overall survival are as described in the initial report.[27] PSA, or biochemical, failure was included in the definition of any failure. The original treatment protocol was designed before the increasing PSA profile was established as an end point. Consequently, a PSA of more than 1.5 ng/mL 1 year after randomization was used as an approximation of biochemical failure. Five patients died before 1 year, and 11 other patients did not have posttreatment PSA data for determining biochemical failure; these patients were excluded from the analysis of this end point. Local and regional failures were also included in the definition of any failure. Local failure was defined as an increase in tumor size of more than 50% for patients in whom complete tumor regression did not occur or as recurrence of a palpable nodule when there was complete regression or a positive biopsy of the prostate after 2 or more years of follow-up. Regional metastasis included clinical or radiologic evidence of

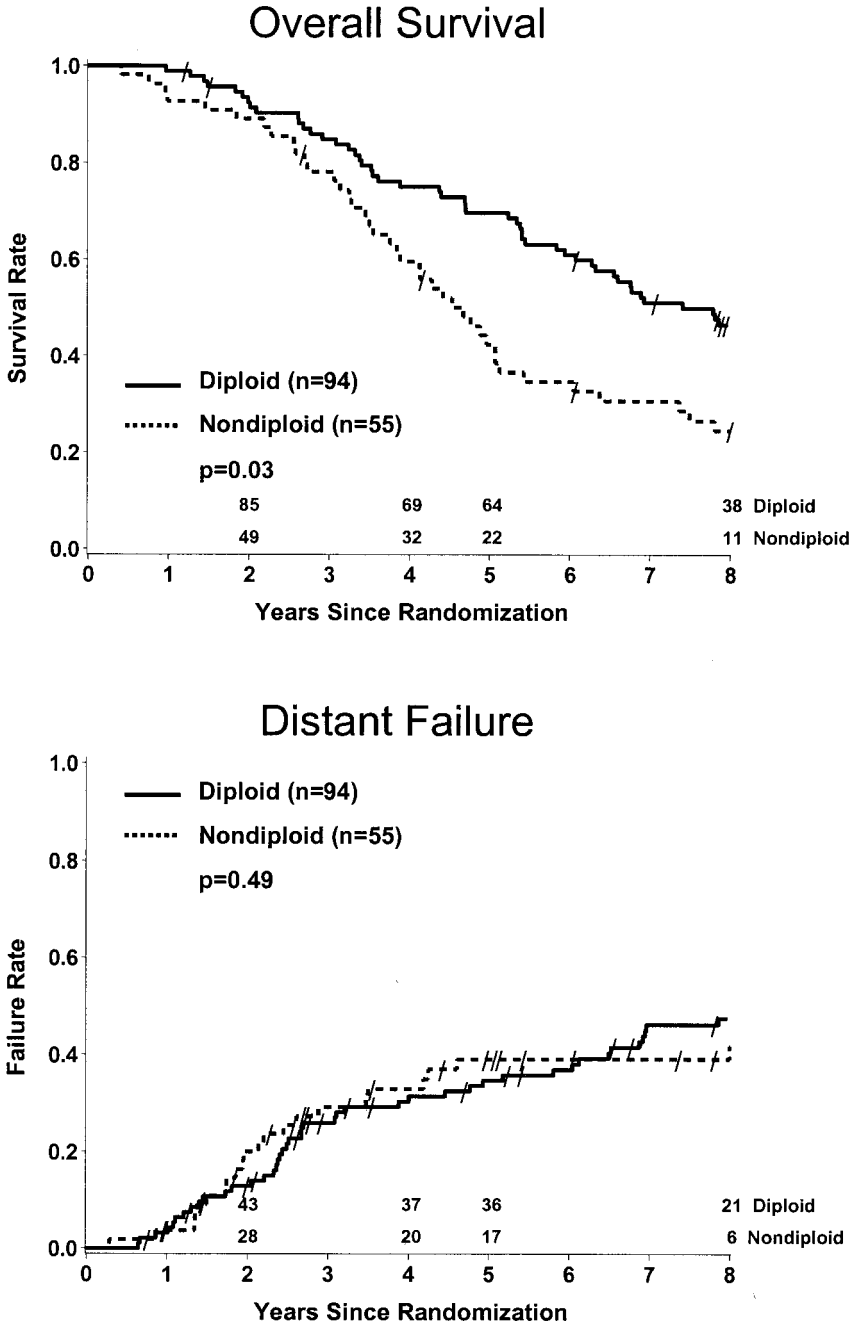

Fig 1. Kaplan-Meier survival analysis of overall survival (upper) and distant failure (lower) for patients with diploid (——) and nondiploid tumors (- - - -). The tic marks represent the times at which patients were censored, and the numbers of patients at risk are displayed above the x-axis.

disease in the pelvis other than in the prostate. Distant metastasis was defined as clinical or radiologic evidence of disease outside the pelvis. Any failure was defined as first reported failure, local failure (n = 9), regional failure (n = 0), distant failure (n = 11), local plus distant failure (n = 11), biochemical failure (n = 112), or death (n = 0). All end points, with the exception of biochemical failure, were measured from the date of randomization to the first reported failure date or last follow-up date in the absence of failure. The biochemical failure end point started 1 year after randomization.

Statistical Analysis

The published analysis of the evaluable patients on the trial was performed on 456 patients. The current analysis was done on 149 patients, with the potential for an additional 6 years of follow-up, as compared with the initial treatment report.[27] As of June 30, 2000, the median follow-up of the alive patients in the study cohort was 9 years (range, 1.2 to 11.8 years).

The distributions of patient characteristics and treatment assignments were compared by the Pearson χ^2 test with the Yates correction factor. Overall survival and any failure estimates were derived using the Kaplan-Meier method.[30] Gelman et al[31] and Gaynor et al[32] indicated in their respective papers that the Kaplan-Meier method generally overestimates distant metastasis. The cumulative incidence approach was used instead to estimate distant metastasis because it specifically adjusts for competing risk such as dying without recurrence of prostate cancer.[33] Univariate comparisons of overall survival and any failure were calculated with the log-rank test.[34] Univariate comparisons of local failure, distant failure, and biochemical failure were accomplished using Gray's test.[35]

Multivariate Cox proportional hazard models were applied to each of the three end points. The initial multivariate analyses were restricted to only patients who had DNA ploidy determination. The analyses determined whether DNA content was of prognostic value after adjusting for treatment

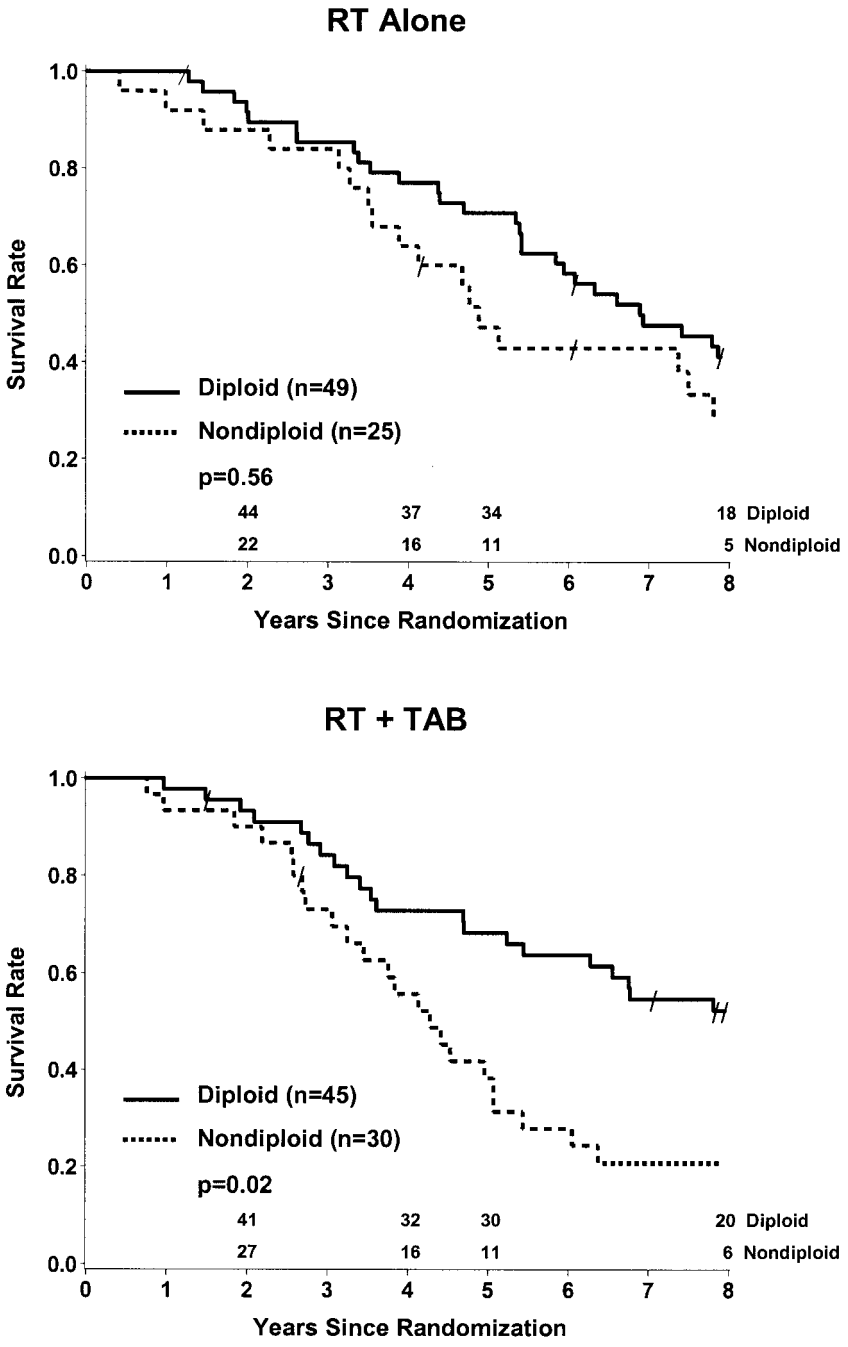

Fig 2. Kaplan-Meier analysis of overall survival for patients randomly assigned to RT alone (upper) or RT+TAB (lower) when subdivided by DNA ploidy status of diploid (solid line) and nondiploid (- - - -). The tic marks represent the times at which patients were censored, and the numbers of patients at risk are displayed above the x-axis.

assignment and Gleason score as fixed covariates.[36] All factors were considered as dichotomous variables and coded as follows: treatment assignment (0 for RT alone *v* 1 for RT + hormones), grouped Gleason sums (0 for sums 2 to 6 *v* 1 for sums 7 to 10), p53 (0 for negative *v* 1 for positive), and DNA content (0 for diploid *v* 1 for nondiploid). The fitted parameter from the Cox model was used to estimate the relative risk associated with each prognostic variable and the corresponding 95% confidence interval. A ratio of 1 would indicate no difference between the two subgroups. The larger the difference from 1, the greater the difference in the failure rates between the two subgroups. The treatment effect was modeled in such a way that a value less than 1 favored the addition of hormones. DNA ploidy was modeled in a way that a value greater than 1 indicates a greater risk of failure for DNA nondiploidy. All of the statistical comparisons were made with two-tailed tests.

Another multivariate analysis adjusted for two additional factors: p53 and missing tumor determinations. Of the 456 evaluable patients, 149 (33%) had ploidy determinations, and 129 (28%) had p53 determinations. In only 113 (25%) patients were both ploidy and p53 determinations available. There are potential problems caused by the missing values. Selection bias may occur, wherein the patients in whom the assays were done do not constitute a random sample from the whole study. Consequently, the study cohort may have a better or worse outcome than the parent cohort. Moreover, when patients with missing values are excluded in the analysis, the number of patients to be analyzed may be relatively small, compromising the statistical power needed to detect clinically meaningful differences.

To adjust for the problem of missing values in the second multivariate analysis, two variables instead of one were used to evaluate each marker. For DNA ploidy, patients were divided into three categories: determination not done, diploid, and nondiploid. For p53, patients were divided into three categories: determination not done, negative, and positive. The first variable for DNA ploidy would then be 0 for diploid/not done versus 1 for nondiploid, and the second variable would be 0 for nondiploid/not done versus 1 for

Table 3. Cox Proportional Hazards Regression Analyses (n = 149)*

Parameter	DM			AF			OS		
	RR	95% CI	P	RR	95% CI	P	RR	95% CI	P
Gleason score									
2-6 *v* 7-10	2.64	1.3 to 5.2	.005	1.7	1.14 to 2.5	.008	1.26	0.79 to 2.02	.31
Assigned treatment									
RT alone *v* RT+TAB	0.68	0.42 to 1.11	.12	0.52	0.37 to 0.73	< .001	1.04	0.7 to 1.5	.83
DNA ploidy									
Diploid *v* nondiploid	0.87	0.5 to 1.46	.5	0.77	0.54 to 1.1	.15	1.5	1.0 to 2.2	.05
Age, years									
< 75 *v* ≥ 75		NA			NA		1.08	0.69 to 1.69	.71

Abbreviations: RR, relative risk; CI, confidence interval; DM, distant metastasis; AF, any failure (including death); OS, overall survival; NA, not analyzed; RT, radiotherapy; TAB, total androgen blockade.

*The analysis was performed on 149 patients, in whom all factors were present. p53 status was not included.

diploid. The estimated relative risk of DNA ploidy was figuratively obtained by subtracting out the two variables. The 27 patients without centrally reviewed Gleason scores were excluded, leaving 429 patients for the analysis.

RESULTS

On the basis of DNA content measurement, 94 patients were classified as diploid, nine patients were classified as tetraploid, and 46 patients were classified as aneuploid. Because the number of tetraploid patients was small and not amenable to separate analysis, the nondiploid patients (tetraploid plus aneuploid; n = 55) were pooled, as has been described previously.[22] Table 1 shows the distribution of patients by pretreatment characteristics and DNA ploidy. There were no statistically significant differences in the distribution of patients between the diploid and nondiploid groups, although a borderline significant relationship was seen with Gleason score. Of those that were nondiploid, 83% had a Gleason score of 7 to 10, as opposed to 69% for those that were diploid ($P = .057$). In a prior analysis of this patient cohort,[29] abnormal p53 expression was reported to be a significant correlate of decreased overall survival and so is included here. There was no association between the distribution of patients by p53 status and DNA ploidy (correlation coefficient = 0.05).

Five-year Kaplan-Meier estimates of overall survival and any failure rates for all patients with the listed variable are shown in Table 2. Five-year estimates of distant failure rates derived using the cumulative incidence method are also shown in Table 2. All factors listed in the table, including DNA ploidy, affected overall survival (assigned treatment and p53 status were borderline). Estimated 5-year overall survival was only 42% when nondiploidy was present, versus 70% in diploid patients (Fig 1). Figure 2 shows the breakdown of overall survival rates by protocol treatment assignment and DNA ploidy. Reduced survival rates were observed with nondiploidy in both treatment groups, but the difference between diploid and nondiploid only reached significance for the patients treated with RT+TAB. The patients in RTOG protocol 8610 had advanced disease, and this is reflected in the high failure of any type and low overall survival rates. Although the main end point of the study was overall survival, the other end points shed light into how survival was affected.

Gleason score, assigned treatment on protocol, and p53 status were associated with all of the end points shown in Table 2. These associations demonstrate a logical relationship between progression, distant metastasis, and overall survival. Such a pattern, however, was not discerned for DNA ploidy in univariate or

Table 4. Characteristics of Patients Who were Entered in RTOG 8610 by Presence or Absence of Ploidy Data

Factor	Without Ploidy Determinations		With Ploidy Determinations		P*
	No. of Patients	%	No. of Patients	%	
Total	307	67	149	33	
Age, years					
Median	70		71		NS
Range	49 to 88		49 to 88		
Performance status (KPS)					
90-100	286	93	143	96	.23
70-80	21	7	6	4	
Gleason score					
2-6	91	30	38	26	.149
7-10	190	62	110	74	
Clinical stage					
T2	99	32	38	26	.14
T3	208	68	111	74	
Clinical nodal status					
NX	291	95	142	95	NS
N+	16	5	7	5	
Assigned treatment					
RT alone	156	51	74	49	NS
RT+TAB	151	49	75	51	
p53 status					
Negative	11	4	95	64	.13
Positive (abnormal)	5	2	18	12	
Unknown†	291	94	36	14	

Abbreviations: RTOG, Radiation Therapy Oncology Group; KPS, Karnofsky performance status; RT, radiotherapy; TAB, total androgen blockade; NS, not significant.

*χ^2 statistics.

†Those with unknown p53 were not included in this analysis.

Table 5. Univariate Analysis of Outcome by Whether DNA Ploidy Determination Was or Was Not Done

Endpoint	Ploidy Determination	Risk Ratio	P
Overall survival	0*: No 1†: Yes	1.5	.0009
Any failure	0: No 1: Yes	1.4	.0014
Distant metastasis	0: No 1: Yes	1.2	.192

*0, Parent cohort, n = 429.

†1, Study cohort, n = 149.

Table 6. Adjusted Cox Proportional Hazards Regression Analyses (n = 429)

Parameter	DM			AF			OS		
	RR	95% CI	*P*	RR	95% CI	*P*	RR	95% CI	*P*
Gleason score									
2-6 *v* 7-10	2.28	1.56 to 3.3	< .0001	1.7	1.35 to 2.14	< .0001	1.47	1.1 to 1.9	.009
Assigned treatment									
RT alone *v* RT+TAB	0.76	0.56 to 1.0	.07	0.52	0.43 to 0.65	< .0001	0.83	0.65 to 1.0	.17
p53 status									
Negative *v* Positive	1.90	1.07 to 3.4	.028	1.55	0.96 to 2.49	.069	2.05	1.18 to 3.54	.01
DNA ploidy									
Diploid *v* nondiploid	0.83	0.49 to 1.4	.50	0.77	0.54 to 1.09	.14	1.55	1.03 to 2.34	.03
Age, years									
< 75 *v* ≥ 75		NA			NA		1.41	1.07 to 1.8	.012

Abbreviations: RR, relative risk; CI, confidence interval; DM, distant metastasis; AF, any failure (including death); OS, overall survival; NA, not analyzed; RT, radiotherapy; TAB, total androgen blockade.

Cox proportional hazards regression analyses. Survival was worse when DNA nondiploidy was found, whereas any failure and distant metastasis rates were not related to ploidy status.

The initial multivariate analyses were performed using the 149 patients with a DNA ploidy determination. DNA ploidy was associated with overall survival after controlling for assigned treatment and Gleason score (Table 3). When this subset was compared with the parent cohort, the differences in baseline characteristics were marginal (Table 4), whereas the differences in patient outcome were highly significant (Table 5). For example, patients with a DNA ploidy determination had an increased risk of death and any failure. In a previously reported analysis, p53 status was found to be a significant prognostic variable for survival but was only available for 113 patients with a DNA ploidy determination. As a consequence, a multivariate analysis that adjusted for this population selection effect was done using 429 patients (see Methods). Table 6 shows that after adjusting for population effects and p53 status, in addition to Gleason score and assigned treatment, DNA ploidy was an independent prognostic factor for overall survival.

DNA ploidy was related to overall survival in multivariate analysis, yet no association with distant metastasis was observed. From these data, it is not intuitive how DNA ploidy affected survival. The lack of a significant correlation between nondiploidy and distant metastasis, although reduced survival was evidenced, led us to examine survival after the institution of salvage hormone therapy. Figure 3 shows that overall survival at 5 years after salvage hormone therapy was significantly lower in the presence of nondiploidy (45% *v* 23%; $P = .018$). Because the difference in overall survival could be related to an imbalance of intercurrent deaths, disease-specific survival results were compared. Even though there were fewer patients available for the analysis of disease-specific survival (n = 40), and therefore power was reduced, a borderline significant trend favoring the diploid population was observed (63% *v* 25% at 3 years; $P = .06$). The disease-specific survival results mirror those of overall survival.

Figure 4 indicates that the reduced survival of nondiploidy patients after salvage hormone therapy was the result of salvage hormone therapy resistance of these patients when they were

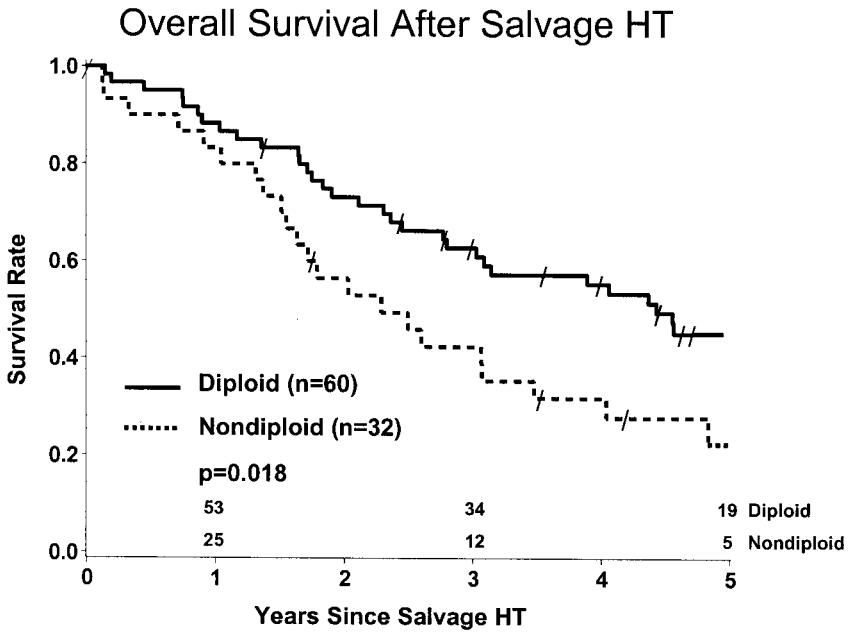

Fig 3. Kaplan-Meier analysis of overall survival after salvage hormone therapy was started for patients with diploid (——) and nondiploid tumors (- - - -). The tic marks represent the times at which patients were censored, and the numbers of patients at risk are displayed above the x-axis.

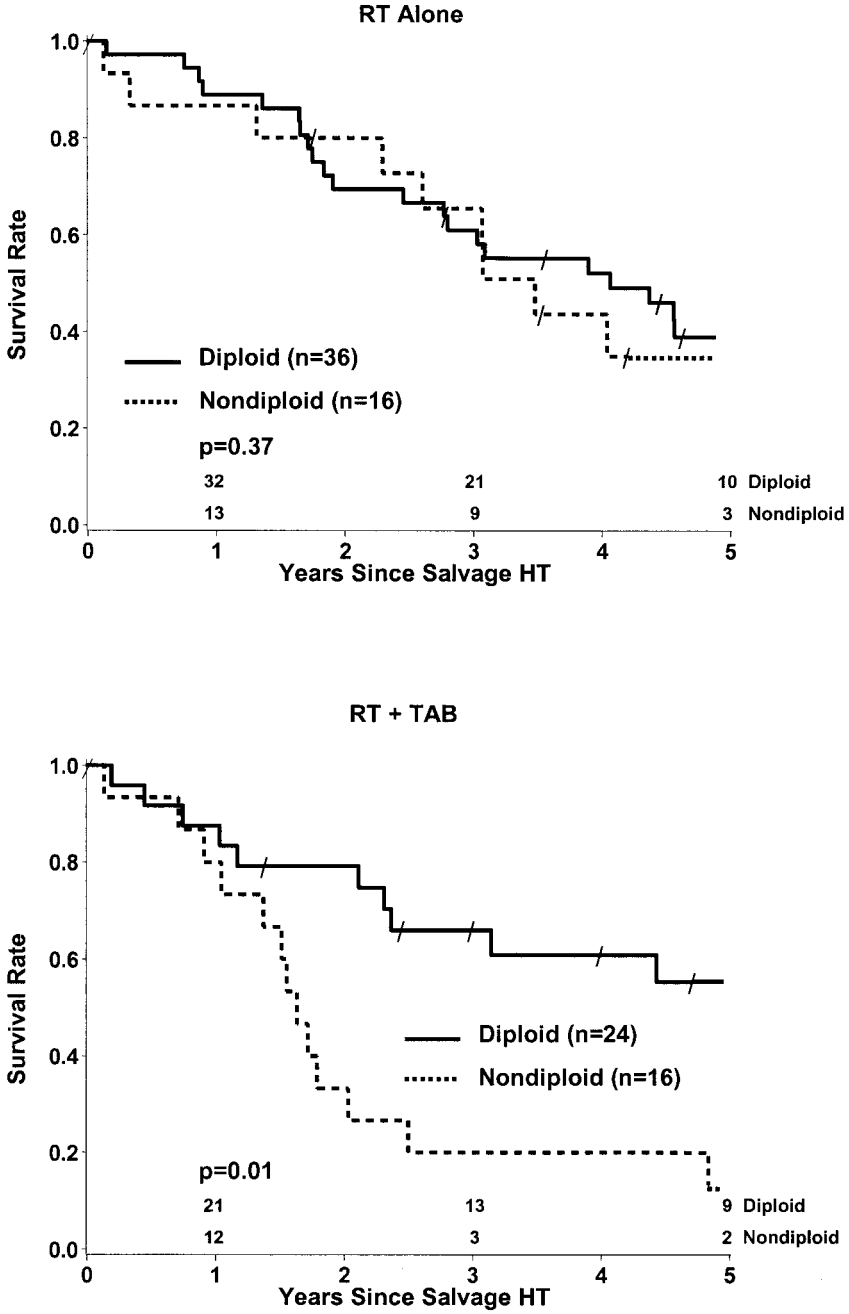

Fig 4. Kaplan-Meier analysis of overall survival after salvage hormone therapy (HT) by the treatment randomization of RT alone (upper) and RT+TAB (lower) when subdivided by DNA ploidy status of diploid (——) and nondiploid (- - - -). The tic marks represent the times at which patients were censored, and the numbers of patients at risk are displayed above the x-axis.

assigned randomly to RT+TAB. This apparent resistance might be explained by an unequal distribution of patients with distant metastasis at the time salvage hormone therapy was initiated. Figure 5 shows that the same pattern was observed for RT+TAB-treated patients who did not have evidence of distant metastasis at the start of salvage hormone therapy. Overall survival at 5 years for patients who had distant metastasis at the time of salvage hormone therapy was 15% (n = 14) for patients randomly assigned to RT alone and 11% (n = 10) for those randomly assigned to RT+TAB. Subdivision by DNA ploidy had no effect on these relationships.

Figure 6 is an analysis of time to distant metastasis after salvage hormone therapy for patients who were free of distant metastasis at that time. The results indicate that it is not the absolute rate of distant metastasis that translated into reduced survival, but probably more rapid progression to death once distant metastasis was discernible. Prior treatment with TAB in nondiploid patients may promote resistance to salvage hormone therapy, thereby shortening survival.

Other potential DNA ploidy-associated differences in treatment outcome based on the protocol treatment assignments of RT alone versus RT+TAB are explored in Table 7. In general, failure rates were more significantly reduced by RT+TAB compared with RT alone for those with diploid tumors than for those with nondiploid tumors. These results combined with the findings that survival after salvage hormone therapy is shortened indicate that short-term adjuvant TAB is not advisable in the presence of nondiploidy. Such subgroup analyses are fraught

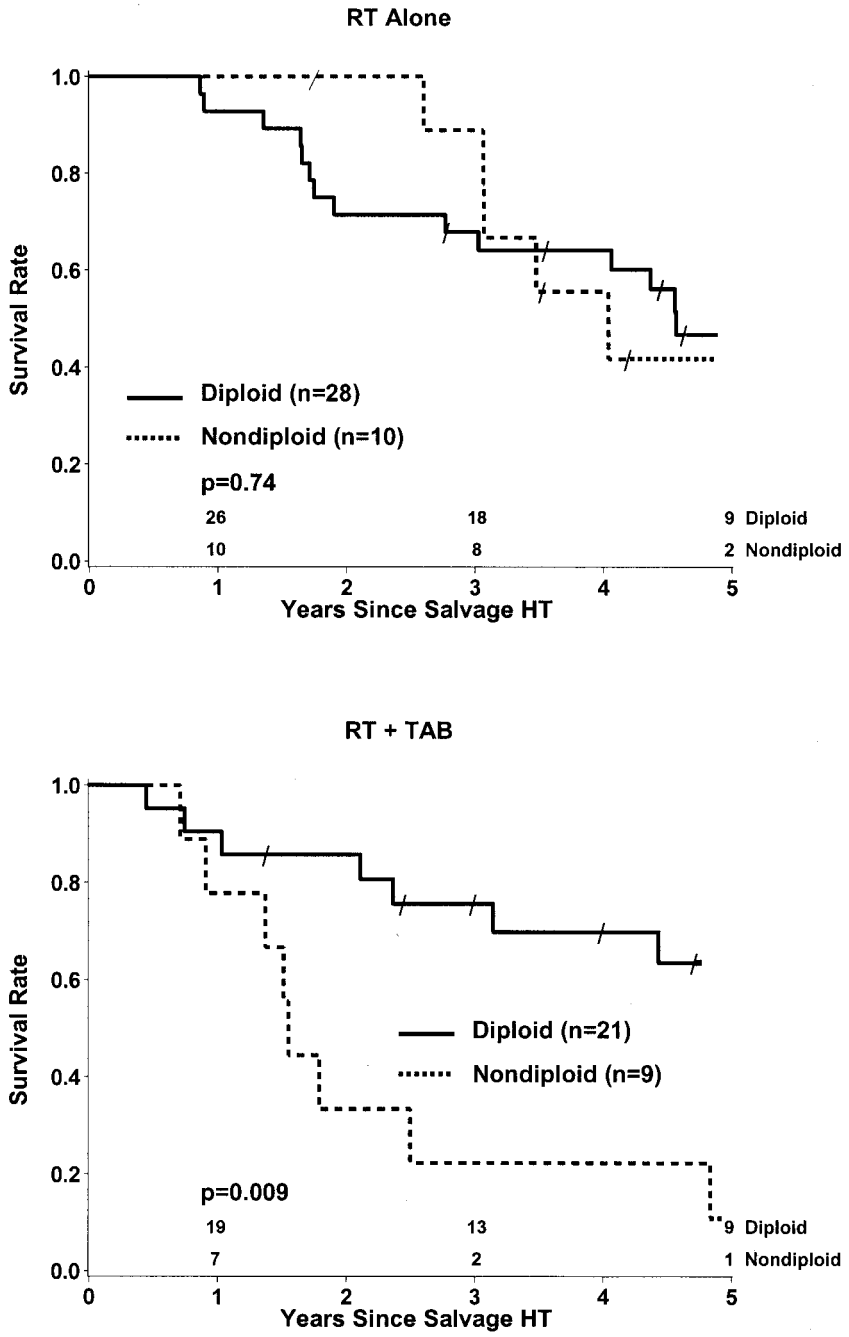

Fig 5. Kaplan-Meier analysis of overall survival after salvage hormone therapy (HT) when distant metastasis (DM) is absent. The patients are categorized by the treatment randomization of RT alone (upper) and RT+TAB (lower) as subdivided by DNA ploidy status of diploid (- - - -) and nondiploid (——). The tic marks represent the times at which patients were censored, and the numbers of patients at risk are displayed above the x-axis.

with potential errors related to the influence of unevenly distributed prognostic factors. Nonetheless, the results support consideration of DNA ploidy analysis in future trials of short-term RT+TAB.

DISCUSSION

Pretreatment prognostic factors have proven valuable in determining prostate cancer patient treatment strategies, especially in defining patients who should receive androgen ablation in combination with RT. The core factors used in clinical practice are serum PSA, Gleason score, and clinical stage.[37] Apart from these, and possibly the proportion of cancer in the biopsy specimens,[38] the most widely investigated and promising marker of disease progression and reduced survival is DNA nondiploidy.[37] However, DNA ploidy analysis has not established a foothold in clinical practice. One explanation is that the majority of prior studies have not analyzed diagnostic material.

For patients managed by RT, only a handful of reports have examined the association of DNA ploidy with outcome.[21-26] Although the results have not been entirely consistent, nondiploidy has been related to poor patient outcome in the majority of studies. These reports have involved relatively small numbers of patients, and additional characterizations of DNA ploidy as a prognostic factor are needed. To our knowledge, no reports have described the predictive value of DNA ploidy for patients managed by RT+TAB.

The data presented revealed a number of relationships between DNA ploidy and patient outcome. For the multivariate

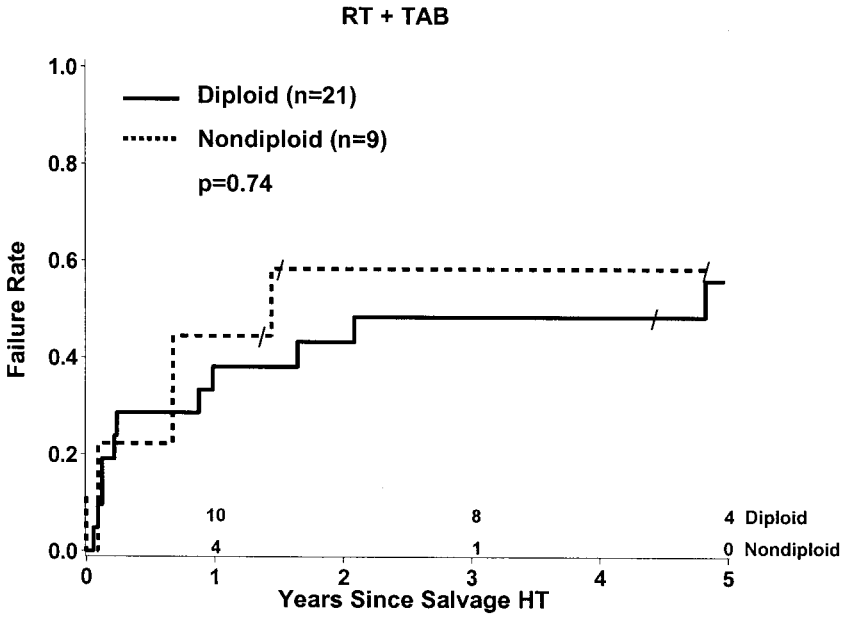

Fig 6. Kaplan-Meier analysis of the relationship of DNA ploidy to distant failure after salvage hormone therapy (HT) when distant metastasis (DM) was absent. This analysis was restricted to patients randomly assigned to RT+TAB. The tic marks represent the times at which patients were censored, and the numbers of patients at risk are displayed above the x-axis.

analyses using the 149 patients with DNA ploidy determinations (Table 3) or the adjusted analyses using the 429 patients available from the entire cohort (Table 6), nondiploidy was associated with reduced overall survival without any increase in distant metastasis. In other studies, nondiploidy consistently has been a robust correlate of clinical disease progression (local, regional, and distant). Few examples exist in which distant metastasis has been analyzed separately,[5,22] but there is every indication that nondiploidy is related to more rapid progression to distant metastasis and that survival is, therefore, reduced.

The prostate cancer patient population studied in RTOG protocol 8610 was clearly locally advanced and not typical of those treated with RT today. Overall, biochemical failure was 78% at 5 years, indicating that the treatments used were inadequate. This biochemical failure rate translated into a 36% distant metastasis rate. The rates of distant metastasis in the presence of diploidy and nondiploidy were 35% and 39%, respectively, which were not different statistically in univariate or multivariate analyses. Because the number of patients exhibiting distant failures was 46 of 94 and 22 of 55 for the diploid and nondiploid cases, respectively, there were sufficient events to measure the effect of DNA ploidy. In multivariate analysis, p53 status and Gleason score were independent correlates of distant metastasis. DNA nondiploidy, in this locally advanced high-risk cohort, was not a predictor of distant metastasis.

Table 7. Relationship of DNA Ploidy Stratified by Study Randomization to Patient Outcome

	5-Year, %					
	Diploid			Nondiploid		
Endpoint	RT Alone (n = 49)	RT+TAB (n = 45)	P*	RT Alone (n = 25)	RT+TAB (n = 30)	P*
DM	45	28	.1	40	38	.54
AF	96	84	.001	92	83	.21
OS	71	68	.53	47	38	.29

Abbreviations: RT, radiotherapy; TAB, total androgen blockade; DM, distant metastasis; AF, any failure; OS, overall survival.

*Log-rank test for AF and OS; Gray's test DM.

DNA nondiploidy has been correlated with reduced prostate cancer patient survival in numerous reports.[37] This correlation was also observed in the data presented here, despite the lack of a relationship between nondiploidy and distant metastasis. The results in Fig 3 indicate that DNA ploidy was associated with survival because the nondiploid cases progressed to death more rapidly after the initiation of salvage hormone therapy. On further examination, the nondiploid patients who were initially treated with RT+TAB were dramatically more resistant to androgen ablation salvage (Fig 4). Short-term adjuvant TAB, combined with RT, could have predisposed patients to resistance to salvage hormone therapy. Although others have shown that response to androgen ablation is less pronounced when DNA nondiploidy is identified,[39-43] this is the first description that adjuvant androgen ablation in such cases may predispose patients to resistance to androgen ablation salvage.

The data presented, however, are not conclusive for a number of reasons. First, there was no evidence of an increased rate of distant metastasis in those with nondiploidy. The compromised survival rate of nondiploid patients treated with RT+TAB after salvage hormone therapy seemed to be independent of distant metastasis rates (Fig 6). Second, Shipley et al[44] recently presented an analysis of this type for the entire group of patients in RTOG protocol 8610, and they did not find a statistically significant difference in survival after salvage hormone therapy based on assigned treatment (RT alone *v* RT+TAB). Likewise, for the DNA-ploidy cohort studied here, there was no difference in survival after salvage hormone therapy based on assigned treatment (data not shown). However, as shown in Fig 4, survival was reduced for patients who had nondiploidy and who were assigned to RT+TAB. Therefore, patients with diploidy who were assigned to RT+TAB should have had a better survival than those assigned to RT alone. A nonsignificant trend in this direction was observed wherein the survival of diploid patients

treated with RT+TAB after salvage hormone therapy was higher than for those treated with RT alone (55% *v* 39%; $P = .54$). This may have contributed to the difference in survival between diploidy and nondiploidy for those treated with RT+TAB. Third, although the cause of death is not always clear for prostate cancer patients receiving salvage therapy, there was a slightly greater proportion of intercurrent deaths in those with nondiploidy (46%) compared with those with diploidy (39%). Despite these potentially complicating factors, the overriding concern raised by the findings is that short-term neoadjuvant or adjuvant TAB may promote resistance to salvage hormone therapy and more rapid progression to death once distant metastasis has occurred. This hypothesis needs to be confirmed in another group of patients.

In conclusion, DNA ploidy shows promise in predicting the outcome of prostate cancer patients. Few reports exist on the relationship of DNA ploidy to outcome after RT, although there is ample evidence that nondiploidy is an independent adverse factor that should be considered in treatment planning. Pretreatment diagnostic material provides a reasonable representation of prostate DNA ploidy status,[12,45,46] which may be measured on thin sections using image analysis. The image analysis technique for quantifying DNA content used in this report is the preferred method for the analysis of DNA ploidy status in diagnostic needle biopsy specimens because it requires less tissue than flow cytometry and allows for the histologic separation of normal epithelial and stromal cells from diploid tumor cells (a problem that plagues flow cytometric analyses). The data presented show that locally advanced patients with nondiploidy have reduced survival. The most striking association was that RT plus short-term neoadjuvant and concurrent TAB may predispose patients with nondiploidy to reduced survival after salvage hormone therapy. As with all retrospective tumor marker studies, this is a subset analysis, and although we attempted to correct for selection bias, it is possible that the findings are not representative. One should consider, however, that these data are consistent with prior reports that affirm the independent merit of DNA ploidy. DNA ploidy will likely become an important factor for the stratification of patients in future trials.

REFERENCES

1. Fordham MVP, Burdget AH, Matthews J, et al: Prostatic carcinoma cell DNA content measured by flow cytometry and its relation to clinical outcome. Br J Surg 73:400-403, 1986
2. Lee SE, Currin SM, Paulson DF, et al: Flow cytometric determination of ploidy in prostatic adenocarcinoma: A comparison with seminal vesicle involvement and histopathological grading as a predictor of clinical recurrence. J Urol 140:769-774, 1988
3. Winkler HZ, Rainwater LM, Myers RP, et al: Stage D1 prostatic adenocarcinoma: Significance of nuclear DNA ploidy patterns studied by flow cytometry. Mayo Clin Proc 63:103-112, 1988
4. Nativ O, Winkler HZ, Raz Y, et al: Stage C prostatic adenocarcinoma: Flow cytometric nuclear DNA ploidy analysis. Mayo Clin Proc 64:911-919, 1989
5. Adolfsson J, Rönström L, Hedlund P-O, et al: The prognostic value of modal deoxyribonucleic acid in low grade, low state untreated prostate cancer. J Urol 144:1404-1407, 1990
6. Haugen OA, Mjølnerød O: DNA-ploidy as prognostic factor in prostatic carcinoma. Int J Cancer 45:224-228, 1990
7. Montgomery BT, Nativ O, Blute ML, et al: Stage B prostate adenocarcinoma: Flow cytometric nuclear DNA ploidy analysis. Arch Surg 125:327-331, 1990
8. Visakorpi T, Kallioniemi O-P, Paronen IYI, et al: Flow cytometric analysis of DNA ploidy and S-phase fraction from prostatic carcinomas: Implications for prognosis and response to endocrine therapy. Br J Cancer 64:578-582, 1991
9. Wirth MP, Müller HA, Mansek A, et al: Value of nuclear DNA ploidy patterns in patients with prostate cancer after radical prostatectomy. Eur Urol 20:248-252, 1991
10. Tinari N, Natoli C, Angelucci D, et al: DNA and S-Phase fraction analysis by flow cytometry in prostate cancer: Clinicopathologic implications. Cancer 71:1289-1296, 1993
11. Blatstein LM, Ginsberg PC, Daskal I, et al: Flow cytometric determination of ploidy in prostatic adenocarcinoma and its relation to clinical outcome. J Am Osteopath Assoc 93:463-472, 1993
12. Ross JS, Figge H, Bui HX, et al: Prediction of pathologic stage and postprostatectomy disease recurrence by DNA ploidy analysis of initial needle biopsy specimens of prostate cancer. Cancer 74:2811-2818, 1994
13. Vesalainen S, Nordling S, Lipponen P, et al: Progression and survival in prostatic adenocarcinoma: A comparison of clinical stage, Gleason grade, S-phase fraction and DNA ploidy. Br J Cancer 70:309-314, 1994
14. Carmichael MJ, Veltri RW, Partin AW, et al: Deoxyribonucleic acid ploidy analysis as a predictor of recurrence following radical prostatectomy for Stage T2 disease. J Urol 153:1015-1019, 1995
15. Hawkins CA, Bergstralh EJ, Lieber MM, et al: Influence of DNA ploidy and adjuvant treatment on progression and survival in patients with pathologic stage T3 (PT3) prostate cancer after radical prostatectomy. Urology 46:356-364, 1995
16. Di Silverio F, D'Eramo G, Buscarini M, et al: DNA ploidy, Gleason score, pathological stage and serum PSA levels as predictors of disease-free survival in C-D1 prostatic cancer patients submitted to radical prostatectomy. Eur Urol 30:316-321, 1996
17. Ahlgren G, Lindholm K, Falkmer U, et al: A DNA cytometric proliferation index improves the value of the DNA ploidy pattern as a prognosticating tool in patients with carcinoma of the prostate. Urology 50:379-384, 1997
18. Amling CL, Lerner SE, Martin SK, et al: Deoxyribonucleic acid ploidy and serum prostate specific antigen predict outcome following salvage prostatectomy for radiation refractory prostate cancer. J Urol 161:857-862, 1999
19. Diaz JI, Mora LB, Austin PF, et al: Predictability of PSA failure in prostate cancer by computerized cytometric assessment of tumoral cell proliferation. Urology 53:931-938, 1999
20. Cheng L, Darson MF, Slezak J, et al: Correlation of margin status and extraprostatic extension with progression of prostate carcinoma. Cancer 86:1775-1782, 1999
21. Song J, Cheng WS, Cupps RE, et al: Nuclear deoxyribonucleic acid content measured by static cytometry: Important prognostic association for patients with clinically localized prostate carcinoma treated by external beam radiotherapy. J Urol 147:794-797, 1992
22. Gauwitz MD, Pollack A, El-Naggar AK, et al: The prognostic significance of DNA ploidy in clinically localized prostate cancer treated with radiation therapy. Int J Radiat Oncol Biol Phys 28:821-828, 1994
23. Centeno BA, Zietman AL, Shipley WU, et al: Flow cytometric analysis of DNA ploidy, percent S-phase fraction, and total proliferative fraction as prognostic indicators of local control and survival following radiation therapy for prostate carcinoma. Int J Radiat Oncol Biol Phys 30:309-315, 1994
24. Pollack A, Zagars GK, El-Naggar A, et al: Near-diploidy: A new prognostic factor for clinically localized prostate cancer treated with external beam radiotherapy. Cancer 73:1896-1903, 1994
25. Pollack A, Zagars GK, El-Naggar A, et al: Relationship of tumor DNA-ploidy to serum prostate-specific antigen doubling time after radiotherapy for prostate cancer. Urology 44:711-718, 1994
26. Khoo VS, Pollack A, Cowen D, et al: Relationship of Ki-67 labeling index to DNA-ploidy, S-phase fraction, and outcome in prostate cancer treated with radiotherapy. Prostate 41:166-172, 1999

27. Pilepich MD, Krall JM, Al-Sarraf M, et al: Androgen deprivation with radiation therapy compared with radiation therapy alone for locally advanced prostatic carcinoma: A randomized comparative trial of the Radiation Therapy Oncology Group. Urology 45:616-623, 1995

28. Gleason DF: Classification of prostatic carcinomas. Cancer Chemother Rep 50:125-128, 1996

29. Grignon D, Caplan R, Sarkar FH, et al: P53 status and prognosis of locally advanced prostatic adenocarcinoma: A study based on RTOG 8610. J Natl Cancer Inst 89:158-165, 1997

30. Kaplan EL, Meier P: Nonparametric estimation from incomplete observations. J Am Stat Assoc 53:457-481, 1958

31. Gelman R, Gelber R, Henderson IC, et al: Improved methodology for analyzing local and distant recurrence. J Clin Oncol 8:548-555, 1990

32. Gaynor JJ, Feuer EJ, Tan CC, et al: On the use of cause-specific failure and conditional failure probabilities: Example from clinical oncology data. J Am Stat Assoc 88:400-490, 1993

33. Kalbfleisch JD, Prentice RL. The Statistical Analysis of Failure Time Data. New York, Wiley, 1980

34. Mantel N: Evaluation of survival data and two new rank order statistics arising in its consideration. Cancer Chemother Rep 5:163-170, 1966

35. Gray RL: A class of K-sample tests for comparing the cumulative incidence of a competing risk. Ann Stat 16:1141-1154, 1988

36. Cox DR: Regression models and life tables. J R Stat Soc Series B 34:187-229, 1972

37. Pollack A, Zagars GK: Pre-treatment prognostic factors for prostate cancer patients treated with external beam radiotherapy, in Greco C, Zelefsky MJ (eds.): Radiotherapy of Prostate Cancer, Harwood Academic, Singapore, 2000, pp. 113-128,

38. Iczkowski KA, Bostwick DG: Prostate biopsy interpretation. Current concepts, 1999. Urol Clin North Am 26:435-452, 1999

39. Forsslund G, Zetterberg A: Ploidy level determinations in high grade and low grade malignant variants of prostatic carcinoma. Cancer Res 50:4281-4285, 1990

40. Stege R, Tribukait B, Lundh B, et al: Quantitative estimation of tissue prostate specific antigen, deoxyribonucleic acid ploidy and cytological grade in fine needle aspiration biopsies for prognosis of hormonally treated prostatic carcinoma. J Urol 148:833-837, 1992

41. Forsslund G, Nilsson B, Zetterberg A: Near tetraploid prostate carcinoma. Cancer 78:1748-1755, 1996

42. Pollack A, Troncoso P, Zagars GK, et al: The significance of DNA-ploidy and S-phase fraction in node-positive (Stage D1) prostate cancer treated with androgen ablation. Prostate 31:21-28, 1997

43. Seay TM, Blute ML, Zincke H: Long term outcome in patients with pTxN+ adenocarcinoma of the prostate treated with radical prostatectomy and early androgen ablation. J Urol 159:357-364, 1998

44. Shipley WU, Lu JD, Pilepich MV, et al: Effect of a short course of neoadjuvant hormonal therapy on the response to subsequent androgen suppression in prostate cancer patients with relapse after radiotherapy: A secondary analysis of the randomized protocol RTOG 86-10. Int J Radiat Oncol Biol Phys 54:1302-1310, 2002

45. Brinker DA, Ross JS, Tran T-A, et al: Can ploidy of prostate carcinoma diagnosed on needle biopsy predict radical prostatectomy stage and grade? J Urol 162:2036-2039, 1999

46. Ahlgren G, Falmer U, Gadaleanu V, et al: Evaluation of DNA ploidy combined with a proliferation index of imprints from core needle biopsies in prostate cancer. Eur Urol 36:314-319, 1999