ELSEVIER

ADULT UROLOGY

A DNA CYTOMETRIC PROLIFERATION INDEX IMPROVES THE VALUE OF THE DNA PLOIDY PATTERN AS A PROGNOSTICATING TOOL IN PATIENTS WITH CARCINOMA OF THE PROSTATE

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ABSTRACT

Objectives. A still controversial issue is whether the results of a cytometric assessment of the DNA distribution pattern of the nuclei of the neoplastic parenchymal cells of a prostatic adenocarcinoma has additional prognostic value to that of the stage and grade of the disease. To increase the accuracy of the DNA ploidy assessments, image cytometry (ICM) has been used and combined with the determination of an ICM proliferation index (PI) to increase its value as an additional prognosticating tool.

Methods. We investigated 96 patients, followed up since diagnosis in 1980/1981 until death or, in 11 surviving patients, for an average of 14.5 years. Survival analysis was made by the conventional Kaplan-Meier method. Fine-needle aspiration biopsy was used as the major diagnostic tool. The neoplastic cell nuclei were classified as ICM DNA diploid, tetraploid, or aneuploid by means of the ploidy-establishing peak in the ICM DNA histograms, as well as the fraction of tumor cells in the S-phase. Scattered cells to the right of the ploidy-establishing peak, the S-phase fraction, and those in the G2M area of the ICM DNA histograms were counted as percent of the total number of tumor cells; this percentage was defined as the PI. Arbitrarily, tumors with a PI less than 5% were classified as having a low proliferation rate, those with a PI greater than 10% were considered highly proliferating, and those with a PI between 5% and 10% as carcinomas with an intermediate proliferation potency.

Results. By univariate analyses, clinical stage, cytodiagnostic grade, cytometric DNA ploidy pattern and PI all had significant prognostic value. By multivariate analyses, the PI was found to add prognostic information to that of the ICM DNA ploidy pattern variable, giving it an increase in its statistical *P* value from 0.002 to 0.0005. As a consequence, the combination of these two variables was found to give rise to three new patient groups with regards to their prognosis: DNA group I had tumors with a diploid ICM DNA pattern with a low PI; DNA group II had tumors with a diploid or tetraploid ICM DNA tumor cell nuclei pattern with an intermediate PI; and DNA group III had a diploid or tetraploid ICM DNA pattern with high PI and all tumors with an aneuploid pattern. By multivariate analysis, including tumor grade and clinical stage, these new DNA groups ($P = 0.0004$) and M stage disease ($P = 0.0006$) were the only significant prognostic variables.

Conclusions. A DNA cytometric PI improves the prognosticating value of DNA ploidy. Patients with prostatic adenocarcinomas, classified as DNA group I, have a low risk of death from their neoplastic disease with deferred or hormonal treatment only. UROLOGY **50:** 379–384, 1997.

The cytometrically assessed DNA ploidy pattern of the nuclei of the neoplastic parenchymal cells of a prostatic adenocarcinoma is a well-established prognosticating factor with a significant correlation to the histopathologic differentiation grade of the tumor.[1] Still, the prognostic infor-

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TABLE I. *Type of primary treatment in 96 patients*

	Cytodiagnostic Cell Differentiation				Survivors >10 yr
	Grade 1	Grade 2	Grade 3	Total	
Castration	8	28	10	46	8
Estrogens	2	10	6	18	2
Emcyte	1	4	4	9	2
Radiation	1	0	7	8	4
TURP	0	1	0	1	0
No treatment	3	9	2	14	2
Total	15	52	29	96	18

KEY: TURP = transurethral resection of the prostate.
Data presented are number of patients.

mation obtained from such DNA ploidy assessments varies to a great extent in different studies. As previously reviewed by one of us, these discrepancies obviously depend on differences, even errors, of a methodologic nature, both when flow cytometry (FCM) and image cytometry (ICM) were used.[2] It is known that ICM has a higher sensitivity than FCM for detecting nondiploid cell clones[3] and that there is a lack of ICM studies in prostatic carcinomas, including data on patient survival.[4] Assessments by means of FCM of the S-phase fraction and the proliferation potency of the parenchymal prostatic adenocarcinoma cells have added relevant pieces of prognostic information in some studies[5] but not in others.[6] Against this background, it was thought worthwhile to perform an analogous investigation by means of ICM in which the DNA ploidy of the nuclei of the carcinomatous cells was assessed and, concomitantly, their proliferation capacity. For this purpose, a "proliferation index" (PI) was used that consisted of the percentage of tumor cells in the ICM DNA histograms that were found to be scattered to the right on the abscissa of the ploidy-establishing peak together with those in the G2M area of the histograms.[7]

MATERIAL AND METHODS

PATIENT CHARACTERISTICS

All slides from fine-needle aspiration (FNA) biopsy specimens from 125 consecutively diagnosed patients with prostatic carcinoma at the Malmö General Hospital during 1980 and 1981 were scrutinized by one of us (K.L.). Specimens from 29 patients were excluded because of insufficient material. Thus, FNA specimens from 96 patients were available for ICM DNA analysis. The mean age of the patients was 73 years (range 50 to 90). All case histories were reviewed according to a protocol without any knowledge of the ICM DNA data. Autopsy studies were performed in 41 (48%) of the 85 patients who died. In the remaining nonsurviving patients, the cause of death was evaluated in each individual case according to the information given in the clinical history and/or death certificate. A disseminated disease in progress required the determination of cancer of the prostate as the main cause of the death. All patients were followed up until death or until the end of our scrutiny of the case histories (mean 176 ± 3 months).

Most patients received primary or delayed hormonal treatment. A survey of the various types of treatment received by the 96 patients in relation to the cytodiagnostic grading of the nuclear cell differentiation is shown in Table I. Primary treatment received by 18 patients surviving for more than 120 months is also shown.

CLINICAL STAGING AND CYTODIAGNOSTIC GRADING

At the scrutiny of the slides, the carcinomas were reclassified cytodiagnostically in analogy with the World Health Organization histopathologic grading system into well-differentiated, grade 1 (G1); moderately differentiated, grade 2 (G2); and poorly differentiated, grade 3 (G3) adenocarcinomas.[8] The concordance between the reclassification and the original cytodiagnostic grading was 15 (58%) of 26 in G1, 37 (73%) of 51 in G2, and 15 (79%) of 19 in G3.

For all 96 patients, except for 3 with insufficient clinical data, the neoplastic disease stage was assessed according to the TNM classification and was clinical Stage T2 in 54, Stage T3 in 33, and Stage T4 in 6. In the present clinical analysis, Stages T3 and T4 are regarded as one group. Lymph node status was unknown. At diagnosis, bone scan was performed in 92 of 96 patients, and evidence of metastatic dissemination to the skeleton was obtained in 29 (32%).

DNA CYTOMETRIC TECHNIQUES AND CALCULATION OF PI

All original specimens had been May-Grünwald-Giemsa stained at the time of diagnosis; they were now destained in a solution of methanol and diluted acetic acid. The slides were then refixed in 4% neutral buffered formaldehyde overnight and subsequently Feulgen stained (acid hydrolysis in 5 N HCl for 60 minutes at room temperature, then staining with Schiff's reagent for 2 hours at room temperature.[9] The DNA densitometric assessments were made by means of a commercially available image analysis system.[10] Autopsy specimens from the cortex of the cerebellum supplied those cells that were used as reference cells (both quality control and "external 2c standard" cells) within each separate staining bath. Tumor cells were analyzed randomly all over the specimen. A minimum of 100 neoplastic cells were counted (mean 177 ± 5). Lymphocytes or leukocytes were used as "internal 2c standard" cells. The DNA histogram evaluation and classification was made by means of software according to the criteria described earlier.[7] Tumors were defined as *diploid* if they had a DNA index (DI) between 0.9 and 1.1, as *tetraploid* with a DI between 1.9 and 2.1 and containing more than 15% of the total cell counts.

Tumors showing a histogram with a peak greater than 10% of all counts and a DI outside the diploid and tetraploid ranges

were classified as *aneuploid*. Scattered cells above the ploidy-establishing peak and in the G2M range were considered the PI and were counted in the percent of the total number of tumor cells.[7] Tumors were arbitrarily classified having *low proliferation* if the PI was less than 5%, *intermediate proliferation* if the PI was greater than 5% but less than 10%, and *high proliferation* if the PI was greater than 10%. The mean coefficient of variation value for the G1 peak was 7.3 ± 0.2%.

STATISTICS

Results are presented as mean values ± SEM. The unpaired *t* test was used to calculate differences in continuous variables. Survival analysis was done according to the Kaplan-Meier method. Differences between curves were tested with the log-rank test (Mantel-Cox). Multivariate analysis was done using a Cox proportional hazard model.

RESULTS

DNA PLOIDY VERSUS TUMOR GRADE AND CLINICAL STAGE

A significant correlation was found between the DNA results and the cytodiagnostic grade of the cancer cells ($P < 0.0001$). This correlation was particularly evident in G1 and 3 tumors, most of them being diploid and aneuploid, respectively, whereas G2 tumors had a mixed nuclear DNA ploidy pattern (Table II). There was also a clear-cut correlation between DNA results and clinical stage ($P < 0.005$).

PI VERSUS DNA PLOIDY, TUMOR GRADE, AND CLINICAL STAGE

In DNA diploid carcinomas, the mean PI was found to be 8%; in DNA tetraploid carcinomas, the PI was 6%; and in DNA aneuploid carcinomas, the PI was 11%, which was statistically significantly different from that of the tetraploid tumors ($P = 0.012$) but not the diploid tumors ($P = 0.17$) (Table III).

The PI was found to be increased with increasing grade of malignancy of the carcinomas. Thus, the mean PI was significantly higher in G3 tumors (13%) than G2 tumors (7%, $P < 0.01$) and G1 tumors (3%, $P = 0.03$). Clinical Stage T3 and T4 tumors had a mean PI of 10%, significantly higher than that for T2 tumors (PI 6%, $P = 0.003$).

SURVIVAL: UNIVARIATE ANALYSIS

Tumor grade, stage, DNA ploidy, and PI were all significant prognostic factors in the Kaplan-Meier survival analysis (Fig. 1, a to d). When the carcinomas of diploid, tetraploid, and aneuploid types, respectively, were stratified into the three levels of proliferation described above, statistically significant differences in survival were obtained in the carcinomas of diploid ($P = 0.02$) and tetraploid types ($P = 0.002$), whereas those of aneuploid type had a poor prognosis, irrespective of PI (Fig. 2, a to c). Based on this information, three tentative prognostic groups could be formed. Only 1 (9%) of 11 patients with diploid-type carcinoma and a low PI (DNA group I) died of cancer within the follow-up period, whereas 11 (35%) of 31 with diploid-type tumors and an intermediate PI or tetraploid-type tumors with a low or intermediate PI (DNA group II) died of prostatic cancer. Among those patients with a DNA aneuploid tumor or a diploid or tetraploid type combined with high PI (DNA group III), 36 (67%) of 54 patients died of prostate cancer within 14.5 years (Table III, Fig. 2d).

TABLE II. *Relation between cytodiagnostic grade and image cytometric DNA ploidy pattern*

Cytodiagnostic of Cell Differentiation	ICM DNA Pattern		
	Diploid	Tetraploid	Aneuploid
Grade 1	12	2	1
Grade 2	12	26	14
Grade 3	2	8	19
Total	26	36	34

KEY: ICM = image cytometric.
Data presented are number of patients.

SURVIVAL: MULTIVARIATE ANALYSIS

The PI was tested as a continuous variable in a multivariate analysis and was found to add significant prognostic information to that of the DNA ploidy pattern of the cancer cells ($P = 0.0005$). The three prognostic DNA groups were tested in a Cox proportional hazard model, including clinical stage and cytodiagnostic cell differentiation grade. Classification into these groups was found to be a statistically significant, independent prognosticating factor for risk of dying of prostate cancer ($P = 0.004$). The clinical Stage T disease was also found to be an independent prognosticating variable in this model ($P = 0.002$), whereas the cell differentiation grade did not add prognostic information to the three risk groups and Stage T disease ($P = 0.79$). To further evaluate whether the prognostic information obtained from the three new DNA groups could also be independent of the presence or absence of metastatic dissemination of the carcinoma, a proportional hazard model that included DNA groups I to III and Stage T and M disease was created. The three DNA groups were again found to be a strong prognostic factor ($P = 0.0004$), as was Stage M disease ($P = 0.0006$); however, Stage T disease was no longer considered an independent prognostic variable.

COMMENT

In our series, the mortality rate from prostate cancer was 50%, a figure similar to that of other

TABLE III. *Relation between image cytometric DNA pattern and proliferation index*

ICM DNA Pattern	Proliferation Index			Total
	Low	Intermediate	High	
Diploid	11 (1)	5 (3)	10 (5)	26 (9)
Tetraploid	20 (6)	6 (2)	10 (9)	36 (17)
Aneuploid	12 (8)	8 (4)	14 (10)	34 (22)
Total	43 (15)	19 (9)	34 (24)	96 (48)

KEY: *ICM = image cytometric.*
Data presented are number of patients (number who died of prostate cancer).

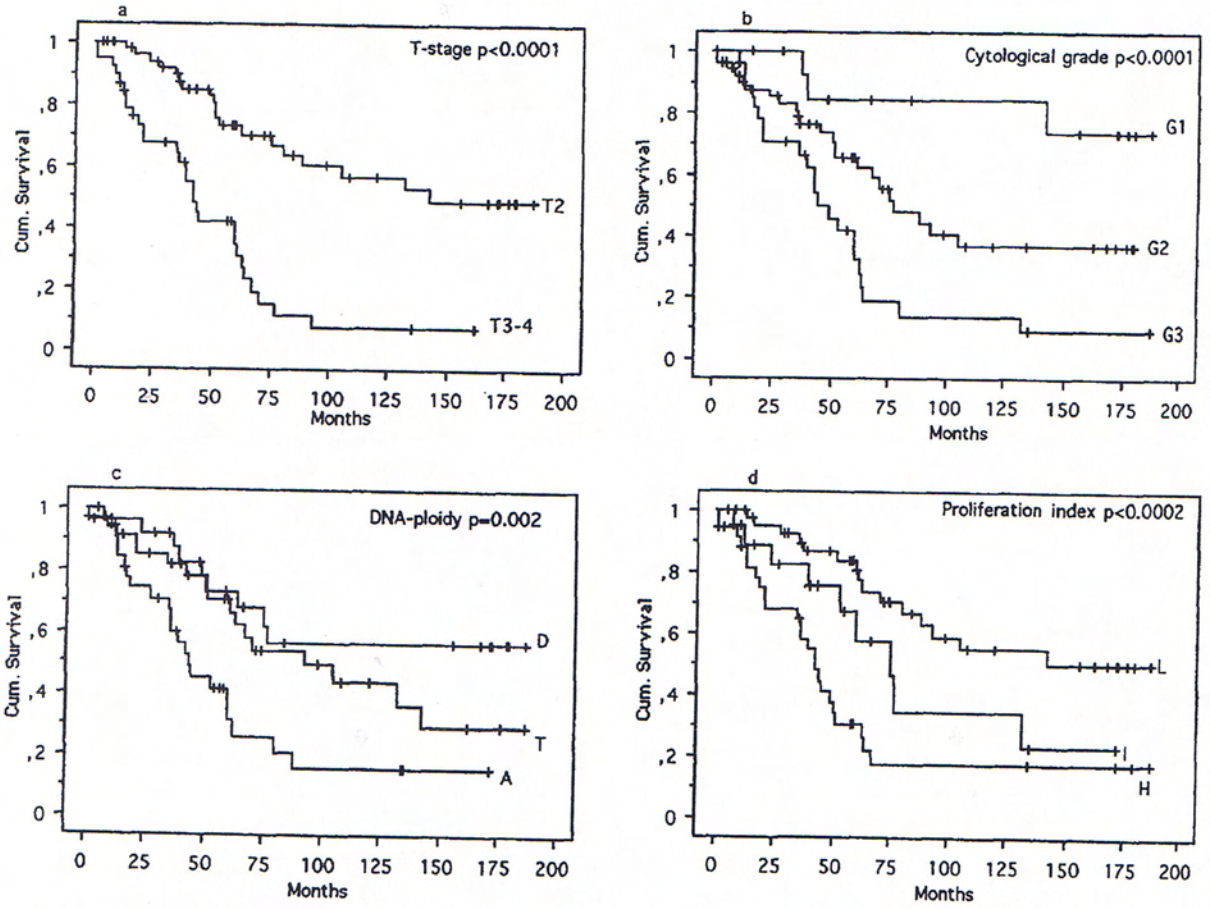

FIGURE 1. *Kaplan-Meier disease-specific survival curves according to clinical Stage T disease (a), cytodiagnostic cell differentiation grade (b), DNA ploidy pattern (c), and proliferation index (PI) (d). The PI is subdivided into low (L, less than 5%), intermediate (I, 5% to 10%) and high (H, greater than 10%). Vertical bars = censored observations. Cum. = cumulative; G = grade; D = diploid; A = aneuploid; T = tetraploid.*

Swedish studies on prostate cancer not treated with radical surgery.[11,12] This finding indicates that this series can be looked on as being representative for patients with prostate cancer in Sweden. Furthermore, the distribution of the patients with regard to clinical stage of the neoplastic disease and to cell differentiation grades in our study resemble that seen during different time periods in Sweden, including 1980 to 1981.[13] The degree of concordance between the primary cytodiagnostic grading of the FNA biopsy specimens and that made at reclassification in the present study is also similar to that of previous investigations of this kind. It indicates the inherent degree of uncertainty in the cytodiagnostic grading system and, again, it supports the representativity of our patients material.

By exclusively identifying tumor cells with ICM, a higher sensitivity and specificity for cells with nondiploid DNA content than that for FCM may be obtained.[3] The prognostic information gained from this improvement in sensitivity is not known.

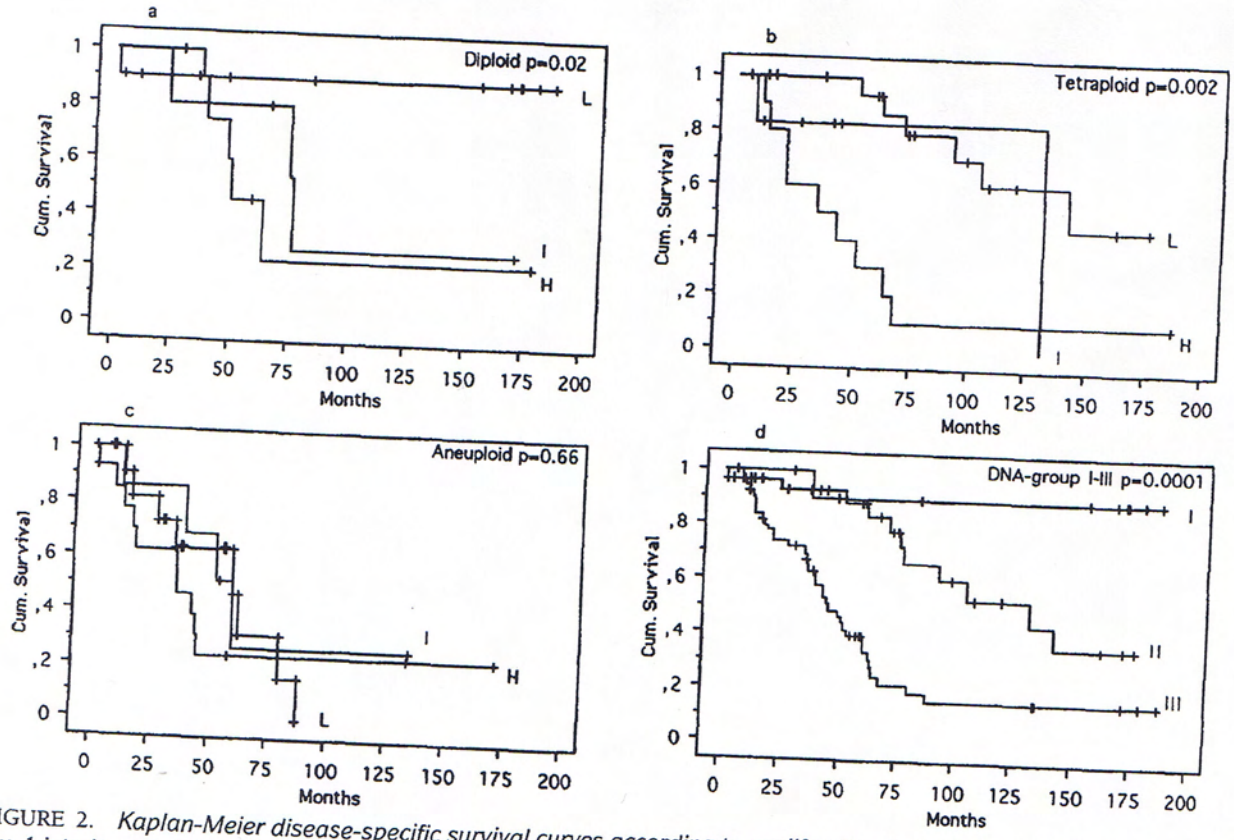

FIGURE 2. *Kaplan-Meier disease-specific survival curves according to proliferation index (PI), subdivided as in Figure 1 into low (L), intermediate (I), and high (H) PI in carcinomas with a nuclear DNA pattern of diploid (a), tetraploid (b), and aneuploid (c) types. The course of the cumulative (Cum.) survival curves for 96 patients classified into new prognostic DNA groups I to III, as described in the text, is also shown (d). Vertical bars = censored observations.*

Hypothetically, small cell populations with aneuploid DNA content outside the ploidy-establishing peak, as well as scattered cells from highly proliferating tumors, can be detected in the DNA histogram, thus improving the prognostic information obtained from the specimen.

There are restrictions in receiving prognostic information from FNA biopsy specimens. First, tumor heterogeneity is a substantial problem because different DNA profiles have been found in up to 40% of prostate cancers using FCM on pieces from whole-mount sections of radical prostatectomy specimens.[14] As described above, in the present study we reviewed in each patient four FNA slides from different parts of the hard, cancer-suspect lesions of the prostate gland felt at rectal palpation, selecting the most representative one for subsequent ICM DNA analysis (ie, the one with ample amount of cancer cells displaying the most poorly differentiated cells). Still, it is obvious that even in FNA biopsy specimens from different parts of the prostate gland, the grade of cell differentiation as well as that of the DNA ploidy pattern may be underestimated compared with whole-mount sections from radical prostatectomy specimens.[15] Secondly, the possibility of DNA progression may lower the value of prognostic information obtained from an FNA biopsy specimen gained from a prostate cancer in an early, still localized stage. In repeated FNA biopsies from patients with low-stage, low-grade disease undergoing surveillance only, an increased incidence of cancer cell nuclei of DNA aneuploid type was found in 24% of the tumors by FCM.[16] A change in FCM DNA ploidy status was more common in the cancer cell nuclei of so-called nondiploid tumors, whereas those with a distinctly DNA diploid type seemed to be more stable. The phenomenon of DNA progression may also be explained by the possibility that small-volume foci of a more poorly differentiated phenotype of the carcinoma with a nondiploid DNA distribution pattern exist that are not identified until repeat biopsies are made. Thus, this phenotype is not discovered until these foci have grown larger with time. This possibility is supported by observations made in radical prostatectomy specimens in which not less than 31% of small-volume (0.1 to 1.0 cm^3) cancers were detected in which the nuclear DNA distribution pattern was of the nondiploid type.[17]

Our observations demonstrate that patients with so-called diploid carcinomas with no, or only few,

scattered cells in their ICM DNA histograms had excellent disease-specific survival. This finding can be interpreted as indicating that so-called chromatin-stable carcinoma cells with an ICM DNA pattern of the diploid type remain stable with time. Patients with such diploid tumors have also been found to have a significantly lower recurrence rate after radical prostatectomy.[18] However, the follow-up times in such studies are rather limited.

In the present study, the results of DNA ploidy assessments, as obtained by the DNA index alone, did not add prognostic information to those of cytodiagnostic grading and clinical staging. However, combining the DNA ploidy results and the PI improved the value of the prognostic information significantly. A similar improvement in prognostic information in diploid tumors by adding the results of S-phase analysis has been described using FCM.[5] As previously stated (see Results), our three new prognostic groups added significant prognostic information in a multivariate analysis of disease-specific survival, independent of cell differentiation grade and clinical stage. Furthermore, the three prognostic groups were strongly significant prognostic factors independent of advanced disease, whereas Stage T disease did not add prognostic information when metastatic disease was included in the model. Our prognosticating DNA/PI groups should now be further tested in a consecutive, prospective series of patients with prostate cancer.

Notwithstanding, by means of ICM assessments of the DNA ploidy of the nuclei of the neoplastic cells in primary adenocarcinomas of the prostate gland, it is possible to select those patients who have a low risk of dying of their neoplastic disease.

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