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Intermediate biomarkers for chemoprevention of prostate cancer

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Use of high-grade prostatic intraepithelial neoplasia (PIN) as an intermediate biomarker for prostate cancer requires additional data concerning its natural biological behaviour. Moreover, it should be recognized that a proportion of PIN lesions may represent intraductal spread of an accompanying prostate cancer rather than a precancerous lesion. The detection rate of isolated PIN in the general population is low, and its clinical significance in the short term may be limited. Additional long-term studies on the significance of isolated PIN detected during population screening are required. Due to inadequate tissue sampling by current biopsy procedures, the presence of an accompanying prostate cancer is difficult to rule out.

Endocrine therapy changes the morphology of PIN, hampering its identification by making it more closely resemble the normal benign glands. In addition, endocrine therapy may lead to molecular changes in PIN, with a potential risk of induction of resistance to endocrine therapy. Prolonged androgen deprivation (for six months) does not generally lead to eradication of PIN. Cessation of endocrine therapy is likely to lead to renewed expansion of PIN, since PIN continues to express androgen receptors and the cell-cycle protein MIB-1 under conditions of low androgen levels.

Recent findings indicate that most high-grade prostate cancers seem to develop from low-grade cancers. The development of a high-grade focus of prostate cancer within a clinically latent low-grade tumour might be a suitable target for future intervention studies, provided that appropriate monitoring for development of high-grade cancer can be achieved in individual patients.

Introduction

Prostate cancer has now become the second most frequent cause of death in the ageing male population of western society (Landis *et al.*, 1999). Its incidence has risen dramatically since the introduction of methods for early detection of prostate cancer (Mettlin *et al.*, 1998). Attempts have been made to identify the mechanisms that underlie the development of prostate cancer, but a major obstacle has been the rarity of this disease in other species, hampering the development of relevant and easily accessible animal models (Riverson & Silverman, 1979). Much research effort has been devoted to the identification of precursor lesions of human prostate cancer. Since the development of prostate cancer is strongly dependent upon the presence of androgens, the effects of androgen deprivation on the potential precursor lesions are of interest.

Prostate cancer has a highly variable biological behaviour. Some prostate cancers are highly aggressive, while others remain indolent for a long period (Chodak *et al.*, 1994). Therefore the ethical acceptability of early detection of prostate cancer by population screening remains a controversial issue, as it is likely that some men diagnosed with prostate cancer will not benefit from treatment. Only randomized studies on prostate screening, measuring both mortality reduction and quality of life, will resolve this issue (Schröder, 1995). Unfortunately, it is not yet possible to predict prostate cancer behaviour preoperatively due to lack of sufficiently reliable serum or tissue markers (Murphy, 1998). Tumour heterogeneity and multifocality of screening-detected prostate cancer has further confused this issue (Hoedemaeker *et al.*, 2000). This chapter discusses current knowledge of the epidemiology and biology of precursor lesions

of prostate cancer and provides some data on early-detected prostate cancers which may serve as a potential target for future chemoprevention studies.

Prostatic intraepithelial neoplasia

Potential precursor lesions of the prostate

In the past, certain lesions have been proposed to be likely precursors of prostate cancer. These include atrophy, atypical adenomatous hyperplasia or adenosis and dysplastic lesions of the prostatic ducts. The latter is now generally referred to as prostatic intraepithelial neoplasia (PIN) (Bostwick, 1995). It has been suggested that such lesions may not account for all prostatic adenocarcinomas and that the human prostate may harbour other hitherto unrecognized premalignant lesions. Some authors have considered atrophy and atypical adenomatous hyperplasia as potential precursor lesions of prostate cancer (Cheng *et al.*, 1998; De Marzo *et al.*, 1999), but this view has not been generally accepted. Evidence for PIN as a premalignant lesion is based on morphological, molecular and epidemiological data. The similarity in morphology of the dysplastic cells of PIN to that of peripheral zone prostatic adenocarcinoma, including nuclear features, and the preferential localization of PIN in the peripheral zone of the prostate, as well as the similarity in molecular changes, strongly support the hypothesis that PIN is a precursor of adenocarcinoma (Myers & Grizzle, 1996). PIN was initially graded in three classes, and subsequently low and high grades were distinguished, but particularly because of great inter-observer variation among pathologists and its lack of clinical relevance, low-grade PIN is now not reported to clinicians (Epstein *et al.*, 1995). Therefore, in the subsequent discussion, high-grade PIN is referred to as PIN.

The histopathology of PIN

Microscopically, at least five architectural patterns of PIN can be distinguished, based on the arrangement of the dysplastic cells within the pre-existing duct or gland. The most common variants of PIN are the tufted and micropapillary patterns. Less common are the flat PIN and the cribriform PIN (Bostwick *et al.*, 1993). In addition, occasional PIN lesions may contain a lumen filled with necrotic debris, resembling comedocarcinoma of the breast. In particular, cribriform PIN and comedocarcinoma-like PIN are associated with concurrent prostatic adenocarcinoma. It has been hypothesized that these two lesions may actually represent intraductal spread of the associated carcinoma rather than precursor lesions (Cohen *et al.*, 2000). Likewise, it cannot be entirely excluded that a proportion of the other, more common, variants of PIN also represent spread of adenocarcinoma within pre-existing ducts or glands rather than a precursor lesion. The similarity of molecular changes in PIN and associated cancer could also be explained in this way. Thus, it is conceivable that morphologically similar PIN lesions represent the extremes of the spectrum from precancerous lesion to spread of an overt cancer.

Prostate-specific antigen and PIN

Since an elevated prostate-specific antigen (PSA) level is now a standard criterion to determine if a man should undergo additional diagnostic procedures for prostate cancer, it has been suggested that a PSA window might be defined to detect specifically men with isolated PIN lesions. Some studies have reported that men with PIN have a PSA level intermediate between those with cancer and those with benign tissue (Brawer & Lange, 1989). More recent studies have not confirmed this and it is now accepted that no PSA window can be defined for isolated PIN (Bostwick, 1999). Immunohistochemical staining of PIN lesions reveals a lower level of PSA expression than in benign prostatic glands. Furthermore, PSA is secreted in the lumina of PIN-containing glands and drained via the prostatic ducts, largely preventing its leakage into the blood circulation. The latter would offer an explanation why PIN is not associated with raised serum PSA levels.

Epidemiology of PIN lesions

Autopsy studies

In 80–90% of radical prostatectomy specimens, PIN can be observed in association with prostate cancer (Kovi *et al.*, 1988; Qian *et al.*, 1997). This high percentage supports the presumed relationship between PIN and prostate cancer. A few (forensic) autopsy studies have compared the frequency of PIN with that of adenocarcinoma. One report suggested that PIN precedes prostate cancer by almost a decade (Bostwick, 1992), confirming an earlier autopsy study reported by Kovi *et al.*

(1988) that showed that the median age of men with atypical acinar hyperplasia (including low- and high-grade PIN) was 56.2 years, while that of men with cancer and PIN was 63.8 years, a lag time of 7.6 years. Sakr *et al.* (1995), however, suggested a much shorter interval between the presence of PIN and adenocarcinoma. In their autopsy series, they noted PIN in 26% and latent cancer in 31% of the prostates of American Caucasian men aged between 30 and 40 years. They also reported that the extent of PIN in prostates of African Americans was much greater than in Caucasians of corresponding ages. This makes it likely that the extent of PIN determines the risk of development of an overt carcinoma.

Isolated PIN in prostatic needle biopsies

Initial studies on prostate needle biopsies of men referred to urological clinics demonstrated a surprisingly high percentage of isolated PIN (without accompanying prostate cancer), with figures reaching 16% (Bostwick *et al.*, 1995). In a retrospective case–control study, repeat biopsies were performed within a period of two years in men with a previous biopsy diagnosis of isolated PIN. This led to the detection of prostate cancer in about 35% of cases, compared with 13% in men without a previous diagnosis of PIN or carcinoma in a previous prostate needle biopsy (Davidson *et al.*, 1995). The outcome of this study was considered to provide evidence for the clinical relevance of isolated PIN in the early detection of prostate cancer.

Later studies on screened populations reported much lower frequencies, in the range 1–2.5% (Hoedemaeker *et al.*, 1999). The differences in reported frequency of PIN can most likely be attributed to differences between the populations selected and in biopsy procedure. In the earlier studies, systematic sextant needle biopsies were not yet a common practice and probably only suspect lesions were biopsied. In these cases, the prostate cancer may have been missed but the frequently occurring adjacent PIN lesion was seen in the biopsy. It was recently suggested that five-region biopsy procedures might increase the yield of isolated PIN in screened men (Rosser *et al.*, 1999). Data on isolated PIN from the Rotterdam section of the European Randomized Screening Program of Prostate Cancer (ERSPC, coordinated by Professor F. Schröder) revealed an incidence of 1% in men aged between 55 and 75 years, using a PSA cut-off value of 3.0 ng/ml for systematic sextant needle biopsies. More importantly, repeat biopsies in these men within six months led to the discovery of prostate cancers in about 10% of the cases (Van der Kwast, unpublished). This is within the background levels of prostate cancers detected in men with a previous benign outcome of their biopsies (Davidson *et al.*, 1995; Epstein *et al.*, 1999), and casts doubt on the value of the current practice of repeat biopsy in this subset of men. In the ERSPC study, the vast majority of isolated PIN lesions were tufted and micropapillary lesions, while cribriform PIN was very rare and comedocarcinoma-like PIN has not yet been found (Van der Kwast, unpublished). Longer follow-up studies of isolated PIN detected in a screened population may give additional insight into the natural behaviour of PIN.

Androgen sensitivity of PIN

Controversies in the literature

Since early studies demonstrated that prostate cancer cannot develop in the absence of adequate testosterone levels, it has been suggested that reduction of androgens may be a useful approach to prevention. Furthermore, it is well known that most (organ-confined or metastasized) prostate cancers initially regress during androgen deprivation. Similarly, several studies have demonstrated that benign prostatic glands show regressive features manifested by apoptosis, vacuolization of cytoplasm and shrinkage of nuclei of the secretory epithelial cells (Armas *et al.*, 1994; Vaillancourt *et al.*, 1996). Some studies on radical prostatectomy specimens from men pretreated for various periods of time for organ-confined prostate cancer (neoadjuvant androgen blockade) have also shown a decline in frequency and extent of PIN (Ferguson *et al.*, 1994). However, published data differ widely, some claiming no decrease and others even a decrease to about 6% of cases (Table 1). Criteria to define PIN in prostates of men pretreated by androgen blockade have not been clearly established, potentially leading to inter-observer variation between pathologists. In particular, one study in which the presence of prominent nucleoli was used as a prerequisite for diagnosis of PIN suggested a strong effect of androgen deprivation on frequency of residual PIN (Vaillancourt *et al.*, 1996).

Table 1. Persistence of PIN during androgen deprivation

Authors	Treatment	% residual PIN
Armas *et al.*, 1994	3 months CAB	77
Montironi *et al.*, 1995	3 months CAB	83
Vaillancourt *et al.*, 1996	3 months CAB	6
Civantos *et al.*, 1995	≥ 3 months CAB	35
Ferguson *et al.*, 1994	(4–53 weeks) Variable	50
Van der Kwast *et al.*, 1999	3 months CAB	72
	6 months CAB	59

CAB, combined androgen blockade

Effects of androgen deprivation on morphology of PIN

It is now well established that in prostatic adenocarcinoma that persists during androgen deprivation therapy mediated by combined androgen blockade, several morphological changes occur, including loss of prominent nucleoli, and it is very likely that this also occurs in PIN lesions. Thus, androgen deprivation could lead to a metamorphosis of dysplastic cells constituting PIN lesions into cells with a histopathologically less apparent phenotype. Since at present only few markers exist that can be employed to distinguish PIN from benign glands, detection of residual PIN in androgen-deprived prostatectomy specimens would depend on histomorphological features of which the definition would be adapted. This is a good illustration of the phenomenon that chemoprevention may lead to morphological changes in such a way that the lesion, though still present, can no longer be identified with certainty. On the other hand, evaluation of the effect on PIN of the mild anti-androgen agent 5α reductase inhibitor (finasteride) did not reveal any changes in PIN lesions (Cote *et al.*, 1998). According to these authors, it is questionable whether finasteride can serve as an effective chemopreventive agent.

We have studied PIN in radical prostatectomy specimens from a series of 40 men with a clinically organ-confined prostate cancer, randomized to three or six months of combined androgen blockade before surgery (Van der Kwast *et al.*, 1999). In radical prostatectomy specimens, foci of PIN were detected in 72% of specimens from men pretreated for their prostate cancer for three months and in 59% of those from men treated for six months. The number of glands involved by PIN decreased from a median number of 19 (± 21 glands) to 7 (± 12) glands with the longer treatment. These differences were, however, not significant. In contrast, the volume of prostate cancer after six months' treatment was significantly reduced by 60% compared with the volume after three months' treatment, while the number of PIN lesions within or adjacent to residual cancer increased (Van der Kwast *et al.*, 1999). The latter observations suggest that prostate cancer may be more susceptible to androgen deprivation than PIN lesions.

Recovery of PIN after cessation of androgen deprivation therapy

Since in residual PIN, nuclear androgen receptors might be detected as well as occasional cells with expression of the cell-cycle molecule MIB-1, the data strongly suggest that PIN may recover and even further expand after cessation of androgen deprivation therapy. Importantly, even a severe regimen of androgen deprivation (so-called combined androgen blockade using flutamide and LHRH agonists) during a period of six months seems not to be sufficient to eradicate all PIN lesions, although a tendency towards further reduction of the extent of PIN was noted after six months of androgen blockade. Nevertheless, prolonged treatment with this combination therapy may lead to a greater reduction of PIN. In a few patients randomized to six months' combined androgen blockade therapy, the therapy was stopped before surgery. In three out of five of these cases, PIN with the classical features, including

prominent nucleoli, was found. This confirms our view that PIN lesions that persist for several months during androgen deprivation can recover rapidly.

Another purpose of chemoprevention of prostate cancer could be to stop progression of PIN to prostatic adenocarcinoma, if eradication of the precursor lesion is not possible. In the latter case, life-long administration of anti-androgens should be envisaged. A study on the prolonged administration of finasteride to men with benign prostatic hyperplasia has suggested that such chemopreventive measures may not be without risk, since in a proportion of men who developed a carcinoma during this treatment, amplification of the androgen receptor gene in the prostate cancer was observed (Koivisto *et al.*, 1999). This androgen receptor amplification was previously shown to mediate resistance to endocrine therapy in metastasized prostate cancers. Androgen receptor gene amplifications have not been documented in cancers not exposed to endocrine therapy (Koivisto *et al.*, 1995).

Prevention of progression of clinically latent low-grade adenocarcinoma to intermediate-grade cancer

Features of early-detected prostate cancers

In radical prostatectomy specimens from men with a prostate cancer detected by first-round screening on the basis of elevated PSA levels, but not clinically, some interesting features can be observed (Hoedemaeker *et al.*, 2000). In about 50% of such specimens, the cancer appears to be multifocal, with a maximum of five different tumours detected in a single prostatectomy specimen. This heterogeneity is also reflected in tumour grade, which may differ considerably. On the other hand, 50% of the detected cancers represent intermediate-grade cancers (Gleason score 7), with variable proportions of high-grade (Gleason grade 4 or 5) tumour. Careful examination of the screen-detected radical prostatectomy specimens obtained in the Rotterdam section of the ERSPC study revealed that about 15% of the early-detected prostate cancers satisfied the criteria of minimal cancer, a category of men who might not benefit from radical prostatectomy or radiotherapy (Hoedemaeker *et al.*, 1997). Tumour volume and grade were well correlated, although a wide scatter of values existed. High-grade tumour was present particularly in the larger tumours. High-grade tumour areas were seen at the centre of tumour areas. This relationship between tumour volume and presence of a high-grade tumour component suggests that most prostate cancers initially are low-grade (Gleason growth pattern 3), while in due course Gleason growth pattern 4 or 5 develops within these low-grade tumour areas. Thus, the development of high-grade cancer within such low-grade tumour areas may represent a potential target for measures aimed at the prevention of tumour progression. PSA velocity could prove a suitable parameter to monitor tumour volume or progression during therapeutic intervention in individual patients with a low-grade latent prostate cancer. This strategy would, however, require a sensitive technique to detect specifically high-grade cancer areas in an otherwise low-grade prostate cancer.

Clinical assessment of prostate cancer grade and stage

Serum PSA level and tumour size do correlate well, but the correlation coefficient is low. Serum PSA levels are influenced by prostatic gland volume, inflammation and obstruction of prostatic glands, in addition to prostate cancer volume. Therefore, in individual patients with prostate cancer, the PSA level cannot be used to predict prostate cancer volume or pathological stage. In larger groups of patients, preoperative parameters such as proportion of involvement of needle biopsies by cancer and tumour grade determined in needle biopsies show a good correlation with tumour volume and grade in prostatectomy specimens. Probably due to sampling problems, these preoperative parameters cannot give a prediction of the biological behaviour of a tumour on an individual basis. Given the multifocality and heterogeneity of early prostate cancer, the problem of adequate sampling seems insurmountable for the application of tissue-based prognostic markers, whether conventional pathological markers or molecular ones. It may be more practical to focus research efforts upon molecules shed by prostate cancer cells into the blood circulation. Although PSA is such a molecule, its lack of specificity for (poorly differentiated) prostate cancer restricts its use as an intermediate end-point marker.

References

Armas, O.A., Aprikian, A.G., Melamed, J., Cordon-Cardo, C., Cohen, D.W., Erlandson, R., Fair, W.R. & Reuter, V.E. (1994) Clinical and pathobiological effects of neoadjuvant total androgen ablation therapy on clinically localized prostatic adenocarcinoma. *Am. J. Surg. Pathol.*, **18**, 979–991

Bostwick, D (1992). Prostatic intraepithelial neoplasia: current concepts. *J. Cell Biochem.*,**16** (S), 10–19

Bostwick, D.G. (1995) High grade prostatic intra-epithelial neoplasia: the most likely precursor of prostate cancer. *Cancer*, **75**, 1823–1836

Bostwick, D.G. (1999) Prostatic intraepithelial neoplasia is a risk factor for cancer. *Semin. Urol. Oncol.*, **17**, 187–198

Bostwick, D.G., Amin, M.B., Dundore, P., Marsh, W. & Schultz, D.S. (1993) Architectural patterns of high-grade prostatic intraepithelial neoplasia. *Human Pathol.*, **24**, 298–310

Bostwick, D.G., Qian, J. & Frankel, K. (1995) The incidence of high grade prostatic intraepithelial neoplasia in needle biopsies. *J. Urol.*, **154**, 1791–1794

Brawer, M.K. & Lange, P.H. (1989) Prostate-specific antigen and premalignant change: implications for early detection. *CA Cancer J. Clin.*, **39**, 361–375

Cheng, L., Shan, A., Cheville, J.C., Qian, J. & Bostwick, D.G. (1998) Atypical adenomatous hyperplasia of the prostate: a premalignant lesion? *Cancer Res.*, **58**, 389–391

Chodak, G.W., Thisted, R.A., Gerber, G.S., Johansson, J.E., Adolfsson, J., Jones, G.W., Chisholm, G.D., Moskovitz, B., Livne, P.M. & Warner, J. (1994) Results of conservative management of clinically localized prostate cancer. *New Engl. J. Med.*, **330**, 242–248

Civantos, F., Marcial, M.A., Banks, E.R., Ho, C.K., Speights, V.O., Drew, P.A., Murphy, W.M. & Soloway, M.S. (1995) Pathology of androgen deprivation therapy in prostate carcinoma. A comparative study of 173 patients. *Cancer*, **75**, 1634–1641

Cohen, R.J., McNeal, J.E. & Baillie, T. (2000) Patterns of differentiation and proliferation in intraductal carcinoma of the prostate: significance for cancer progression *Prostate*, **43**, 11–19

Cote, R.J., Skinner, E.C., Salem, C.E., Mertes, S.J, Stanczyk, F.Z., Henderson, B.E., Pike, M.C. & Ross, R.K. (1998) The effect of finasteride on the prostate gland in men with elevated serum prostate-specific antigen levels. *Br. J. Cancer*, **78**, 413–418

Davidson, D., Bostwick, D.G., Qian, J., Wollan, P.C., Oesterling, J.E., Rudders, R.A., Siroky, M. & Stilmant, M. (1995) Prostatic intraepithelial neoplasia is a risk factor for adenocarcinoma: predictive accuracy in needle biopsies. *J. Urol.*, **154**, 1295–1299

De Marzo, A.M., Marchi, V.L., Epstein, J.I. & Nelson, W.G. (1999) Proliferative inflammatory atrophy of the prostate: implications for prostatic carcinogenesis. *Am. J. Pathol.*, **155**, 1985–1992

Epstein, J.I., Grignon, D.J., Humphrey, P.A., McNeal, J.E., Sesterhenn, I.A., Troncoso, P. & Wheeler, T.M. (1995) Interobserver reproducibility in the diagnosis of prostatic intraepithelial neoplasia. *Am. J. Surg. Pathol.*, **19**, 873–886

Epstein, J.I., Walsh, P.C., Akingba, G. & Carter, H. (1999) The significance of prior benign needle biopsies in men subsequently diagnosed with prostate cancer. *J. Urol.*, **162**, 1649–1652

Ferguson, J., Zincke, H., Ellison, E., Bergstrahl, E. & Bostwick, D.G. (1994) Decrease of prostatic intra-epithelial neoplasia following androgen deprivation therapy in patients with stage T3 carcinoma treated by radical prostatectomy. *Urology*, **44**, 91–95

Hoedemaeker, R.F., Rietbergen, J.B., Kranse, R., Van der Kwast, T.H. & Schroder, F.H. (1997) Comparison of pathologic characteristics of T1c and non-T1c cancers detected in a population-based screening study, the European Randomized Study of Screening for Prostate Cancer. *World J. Urol.*, **15**, 339–345

Hoedemaeker, R.F., Kranse, R., Rietbergen, J.B., Kruger, A.E., Schroder, F.H. & Van der Kwast, T.H. (1999) Evaluation of prostate needle biopsies in a population-based screening study: the impact of borderline lesions. *Cancer*, **85**, 145–152

Hoedemaeker, R.F., Rietbergen, J.B.W., Kranse, R., Schröder, F.H. & Van der Kwast, T.H. (2000) Histopathological prostate cancer characteristics at radical prostatectomy after population-based screening. *J. Urol.*, **164**, 411–415

Koivisto, P., Hyytinen, E., Palmberg, C., Tammela, T., Visakorpi, T., Isola, J. & Kallioniemi, O.P. (1995) Analysis of genetic changes underlying local recurrence of prostate carcinoma during androgen deprivation therapy. *Am. J. Pathol.*, **147**, 1608–1614

Koivisto, P.A., Schleutker J, Helin, H., Ehren-van Eekelen, C., Kallioniemi, O.P. & Trapman, J. (1999) Androgen receptor gene alterations and chromosomal gains and losses in prostate carcinomas appearing during finasteride treatment for benign prostatic hyperplasia. *Clin. Cancer Res.*, **5**, 3578–3582

Kovi, J., Mostofi, F.K., Heshmat, M.Y. & Enterline, J.P. (1988) Large acinar atypical hyperplasia and carcinoma of the prostate. *Cancer*, **61**, 551–561

Landis, S.H., Murray, T., Bolden, S. & Wingo, P.A. (1999) Cancer statistics, 1999. *CA Cancer J. Clin.*, **49**, 8–31

Mettlin, C.J., Murphy, G.P., Rosenthal, D.S. & Menck, H.R. (1998) The National Cancer Data Base report on prostate carcinoma after the peak in incidence rates in the U.S. The American College of Surgeons Commission on Cancer and the American Cancer Society. *Cancer*, **83**, 1679–1684

Montironi, R., Magi-Galuzzi, C. & Fabris G. (1995) Apoptotic bodies in prostatic intraepithelial neoplasia and prostatic adenocarcinoma following total androgen ablation. *Pathol. Res. Pract.*, **191**, 873–880

Murphy, W.M. (1998) Prognostic factors in the pathological assessment of prostate cancer [editorial]. *Human Pathol.*, 29, 427–430

Myers, R.B. & Grizzle, W.E. (1996) Biomarker expression in prostatic intra-epithelial neoplasia. *Eur. Urol.*, **30**, 153–166

Qian, J., Wollan, P. & Bostwick, D.G. (1997) The extent and multicentricity of high-grade prostatic intraepithelial neoplasia in clinically localized prostatic adenocarcinoma. *Human Pathol.*, **28**, 143–148

Riverson, A. & Silverman, J. (1979) The prostatic carcinoma in laboratory animals: a bibliographic survey from 1900 to 1977. *Invest. Urol.*, **16**, 468–472

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Rosser, C.J., Broberg, J., Case, D., Eskew, L.A. & McCullough, D. (1999) Detection of high-grade prostatic intraepithelial neoplasia with the five-region biopsy technique. *Urology*, **54**, 853–856

Sakr, W.A., Grignon, D.J., Haas, G.P., Schomer, K.L., Heilbrun, L.K., Cassin, B.J., Powell, J., Montie, J.A., Pontes, J.E. & Crissman, J.D. (1995) Epidemiology of high grade prostatic intraepithelial neoplasia. *Pathol. Res. Pract.*, **191**, 838–841

Schröder, F.H. (1995) Screening, early detection, and treatment of prostate cancer: a European view. *Urology*, **46** (Suppl A), 62–70

Vaillancourt, L., Têtu, B., Fradet, Y., Dupont, A., Gomez, J., Cusan, L., Suburu, E.R., Diamond, P., Candas, B. & Labrie, F. (1996) Effect of neoadjuvant endocrine therapy (combined androgen blockade) on normal prostate and prostatic carcinoma: a randomized study. *Am. J. Surg. Pathol.*, **20**, 86–93

Van der Kwast, T.H., Labrie, F. & Têtu, B. (1999) Persistence of high-grade prostatic intra-epithelial neoplasia under combined androgen blockade therapy. *Human Pathol.*, **30**, 1503–1507