Prostate Cancer: Screening, Prevention and Therapy – Lessons Learnt from Current Trials

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INTRODUCTION

Introduction from the chairmen of the WHO International Consultation on Prostate cancer

The reports from the two large PSA-based screening studies of prostate cancer reported in 2009, ERCP in Europe and PLCO in USA and Canada, supplemented by a Scandinavian trial, based in Gothenburg, have widened our understanding of the value of systemic screening and, at the same time, thrown light upon the shortcoming, or rather imperfection, inherited in studies based upon PSA values. These data inspired us to undertake a more wide front updating of not only screening bus as well diagnostic procedures and therapy of prostate cancer. What has emerged in modern research with respect to prognostic markers, evaluation of tumor extent and progressive character, treatment modalities, etc? When is active surveillance justified? Feasibility of prophylactic measures? Are some of the drugs in the big flora of substances with a presumed cytostatic property of any value in the treatment of prostate cancer? And – not least – how can the profession collaborate with our patients and their organizations to arrive at optimal solutions in situations, many times of a difficult character – when it comes to the selection of treatment modality.

With all this in view, we decided to invite a faculty of scientists representing a manifold expertise and all of them selected among those world-leading at present, to collaborate with us to focus on these issues in the light of modern research. After a period of preparation we came together for an open conference in Stockholm, entitled "Prostate Cancer: Screening, Prevention and Therapy – Lessons Learnt from Current Trials. WHO International Consultation". This book presents those conclusions we arrived at. In case at least some of these of our conclusions will stand the test of time, our common efforts will be worthwhile. We even hope our reports will open a window to new research. And – like in all our international consultations – the ultimate aim of our efforts is to serve our patients.

We wish to convey our warm thanks to all our Faculty members for your devoted contributions at non-profit conditions and for our stimulating debates. We also wish to convey our sincere thanks to our supporting organizations. Without your financial and idealistic support this endeavor would never had become a reality.

Sten Nilsson
Lennart Andersson
EDITORIAL

Screening for prostate cancer: Defining critical issues

LARS HOLMBERG & OLOF AKRE

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Several facts point towards that prostate cancer in theory is a suitable goal for secondary prevention with screening. It is a common disease in many countries with often a malignant course when detected clinically. Clinical symptoms are unspecific or few and clinical diagnosis often means late diagnosis in a stage where medical interventions cannot today claim to be curative. There is presently no clearly identifiable life style risk factor that could be changed to influence the incidence substantially. There is a long preclinically identifiable phase where the disease is limited and with a low probability of having produced clinically viable micrometastases during that time. For the last 5–10 years there has been increasing evidence that locally radical intervention at a stage where the disease is limited can save lives [1,2].

However, we already knew when the randomised studies to evaluate the efficacy of prostate cancer screening [3,4] started that two major obstacles stand in our way to realise expectations of a successful screening program: First, the current means to detect prostate cancer in a symptomatic stage – PSA testing followed by biopsies – will diagnose a substantial number of men with cancer that would not have surfaced clinically during their lifetime, thus causing overdiagnosis [5,6]. Our hope to find a reliable method to distinguish the patients that benefit from treatment from those that do not remains unfulfilled. Second, a local radical intervention for prostate cancer is still despite many remarkable improvements in surgical technique associated with a clinically significant risk of side-effects that may be unacceptable given the potentially indolent course of disease. These two obstacles combine in an unfortunate way to risk of overtreatment with severe consequences that then threatens the cost-benefit balance certainly for small, but even for modest and clearly clinically relevant eventual mortality reductions by screening.

We now have the first evidence from two large randomised trials in prostate cancer screening, the ERSPC [4] and the PLCO [3] trial. In this section of the proceedings of the WHO consultation on prostate cancer, further evidence than those already published in the New England Journal of Medicine will be presented and discussed. Furthermore, the trialists set the empirical findings in a context of the discussions emerging from the first publications. Given what we know hitherto from the publications, a first priority is to understand the very basic fact: is there a mortality reduction to expect from PSA screening or not? This task is not trivial. Mass screening trials are extremely complex to undertake, analyse and interpret. A multiprofessional, multidisciplinary, careful, realistic and sincere approach free from irrational conflicts of interest – be they commercial, political, academic or any – is needed from all stakeholders. We need to understand that we are only in the beginning of the follow-up and of this discussion. It is sobering to think that the first randomised data in breast screening now came some 40 years ago, and still, though a majority of experts and reviews are very clear about a mortality reduction following mammography screening, new debates pops up from time to time and health care providers many times get confused. The same is true for screening for colorectal cancer, where as in mammography screening the randomised data are so much richer and older than for prostate cancer screening. The medical community should show that we have a collective memory and learning so that we in the prostate cancer debate avoid the sometimes irrational and emotional overtones in
cancer screening history, which have hindered people’s understanding of the screening issues.

A second task is to define the most important and pressing questions that rise from the current problem situation. We suggest that rather than using a number of critical questions only as criticisms and dismissal of trial data, they should be brought forward as important research issues to be solved either with data from the current trials or in other research in existing datasets or in prospective trials. For such an approach to give answers sooner than later, the ERSPC and PLCO trialists need support for their efforts and others have to collaborate constructively in focused efforts. Some such questions are obvious and are already on the research agenda for many groups, i.e. finding a way to distinguish potentially lethal cancer from the very slow growing or improving the screening test itself. However, such a magic bullet may not be found for many years and if screening continues, we need to engage in large studies of immediate clinical problems: Is active surveillance a safe option? Is there a low-toxic intervention that could be offered for men with low volume disease with beneficial prognostic markers? Such studies need to be done in large, collaborative networks. In this section of the proceedings there is an important discussion about the possible contribution from genetic biomarkers to solve these problems.

If the results of these two tasks can be clearly formulated it will go a long way to help us with the ultimate goal: to have science inform men and health care providers what the current state of affairs tells us about prostate cancer screening, what is known, which are the great enigmas and what are the possible clinical implications. We cannot in the long run shy away from trying to solve one of the most important medical and ethical dilemmas today: should screening with PSA for prostate cancer be stopped, should it be encouraged on a large population scale, or should it only be offered after a very careful individual information including an informed consent? This problem solving requires a lot of very good, honest collaborative teamwork.

References

Screening for prostate cancer – The controversy continues, but can it be resolved?

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Abstract

Background. In 2009, the European Randomized Study of Screening for Prostate Cancer (ERSPC) was one of two studies to report interim data on the effect of screening for prostate cancer (PC) on the disease specific mortality. Contradictory results caused considerable discussion and misunderstanding in secondary literature. Methods. This document is based on a non systematic review of recent evidence for and against screening for PC, specifically considering three recently published randomized screening trials [1–3]. Results. The ERSPC data are based on a core age group of 162 387 men, aged 55–69 years, who were identified through population registries in seven European countries. Men were randomized between a screening group that received screening at an average of once every four years and a control group. After a median follow-up of nine years, a reduction in the rate of death from PC by 20% was shown which increased to 31% after adjusting for non-compliance and contamination. Overdetection and subsequent overtreatment (with a number needed to treat (NNT) of 48) are considered to be the major downsides of screening. The recently published 14-year results have shown that these downsides strongly depend on the duration of follow-up. In response to the outcomes of the ERSPC, several points of discussion have been brought up by various authors concerning the usefulness of screening considering benefits, harms and costs, the methodology of the ERSPC and the interpretation of its outcomes. Important issues to address regarding PC screening are addressed. Conclusions. This paper sheds a light on the controversial points of the ERSPC as well as on the priority issues of PC screening. On July 2, 2010 the Swedish section of ERSPC (Göteborg screening trial) published their results with a median follow-up of 14 years. With longer follow-up the data confirm the trend seen in improvement of PC mortality and suggest much more favorable future outcomes also with respect to the NNT to prevent one PC death.

In 2009, two large, randomized, controlled trials [1,2] reported their interim data on prostate cancer specific mortality, addressing the matter of screening for prostate cancer (PC). The two studies have apparently contradictory results, which created considerable discussion and misunderstanding in secondary literature. Recent literature profoundly outlined the differences between the two trials and the underlying causes explaining the contradictory outcomes [4]. However, as one editorial stated [5], the main point regarding present prostate cancer screening studies, is to avoid meaningless controversy over which study is right or wrong, thus hiding the real issues behind a smoke screen.

In this paper we would like to define the results of the ERSPC as well as its supposed controversial points as they were mentioned in various communications, in order to throw light on different sides of the study and to report the priority issues and the way they should be taken on. In addition, the recently published results of the Göteborg screening trial [3], which is part of the ERSPC, will be considered in the context of the ERSPC trial as a whole.

Evidence from the European Randomized Study of Screening for Prostate Cancer

The European Randomized Study of Screening for Prostate Cancer (ERSPC) [1] was initiated in 1991 as a randomized, multicenter trial of screening for PC, with PC mortality being the main endpoint. In eight different countries men, aged 50–74 years, were randomized between either a screening group or control group. The core age group consisted of men aged 55–69 years (N=162 387), who were screened with a four-year (87%) or two-year (13%) interval. Screening
was performed by means of a prostate-specific antigen (PSA) test, with a cut-off value of 3.0 ng/ml as an indication for lateralized sextant biopsy. Nevertheless, slightly differing PSA cut-offs of 2.5–4.0 ng/ml with ancillary tests as digital rectal examination, free to total PSA ratio and transrectal ultrasound have been used by different centers during the study period as was all clearly reported earlier in the original paper (Table I).

After randomization, a total of 72 890 men were assigned to the screening group and 89 353 to the control group, with a mean age of 60.8 years. Of all PSA-tests performed, 20 437 (16.2%) were positive and led to biopsy recommendation, which 17 543 (85.5%) of men complied with. In 24.1% of men biopsied, results turned out positive (positive predictive value or PPV), amounting to 75.9% of false positive results. PC was detected in 5 990 (8.2%) men in the screening group and in 4 307 (4.8%) men in the control group.

After a mean and median follow-up of 8.8 and 9.0 years respectively, 214 PC deaths occurred in the screening group and 326 PC deaths in the control group (Table II). This corresponded to a significant reduction of 20% fewer men dying of PC in the screening group (p \(<0.04\)). The cumulative risk of death from PC over time for both groups is shown in Figure 1, with diverging rates of death from seven to eight years on and a trend suggesting larger effects with longer follow-up.

The intention-to-screen (ITS) analysis showed an absolute risk reduction of seven per 10 000 screened men, with a number of 1 410 men who need to be screened (NNS) and another 48 men who need to be treated (NNT) in order to prevent one PC death. When adjusted for non-compliance, a 27% reduction in PC mortality was seen in men who were actually screened. After adjustment for the differences in stage distribution between the two arms, no difference was seen in treatment, which makes a mortality reduction solely caused by a treatment effect very improbable [6].

One of the major drawbacks of PC screening in general is detecting PC in men who would not have clinical symptoms during their lifetime if it was not for screening, with an over detection rate in the ERSPC screening group that has been estimated to be as high as 50% [7].

A secondary analysis [8] was carried out according to the method described by Cuzick et al. [9] to assess the impact of adjustment for men assigned to the screening arm, who did no undergo screening (non-compliance) and for men assigned to the control arm, who sought opportunistic PSA-based screening (contamination). This analysis showed a further

Table I. Methods used for the ERSPC.

<table>
<thead>
<tr>
<th>Recruitment</th>
<th>Men identified from population registries</th>
</tr>
</thead>
<tbody>
<tr>
<td>Randomization</td>
<td></td>
</tr>
<tr>
<td>- Before consent</td>
<td>Finland, Sweden, Italy</td>
</tr>
<tr>
<td>- After consent</td>
<td>Netherlands, Belgium, Switzerland, Spain</td>
</tr>
<tr>
<td>Core age group</td>
<td>55–69 years (range 50–74 years)</td>
</tr>
<tr>
<td>Biopsy indication</td>
<td></td>
</tr>
<tr>
<td>- Netherlands</td>
<td>PSA ≥4 or +ve DRE or TRUS at PSA ≥3; since 1997 PSA ≥3</td>
</tr>
<tr>
<td>- Finland</td>
<td>PSA ≥4 or +ve DRE or TRUS at PSA ≥3</td>
</tr>
<tr>
<td>- Sweden</td>
<td>PSA ≥3</td>
</tr>
<tr>
<td>- Italy</td>
<td>PSA ≥4 or +ve DRE or TRUS at PSA ≥2.5</td>
</tr>
<tr>
<td>- Spain</td>
<td>PSA ≥4</td>
</tr>
<tr>
<td>- Belgium</td>
<td>PSA ≥4 or +ve DRE or TRUS</td>
</tr>
<tr>
<td>- Switzerland</td>
<td>PSA ≥3</td>
</tr>
<tr>
<td>Biopsy procedure</td>
<td>(lateralized) sextant biopsies</td>
</tr>
<tr>
<td>Screening interval</td>
<td>4-years (87%)</td>
</tr>
<tr>
<td></td>
<td>2-years (13%)</td>
</tr>
</tbody>
</table>

PSA = prostate specific antigen (ng/ml), DRE = digital rectal examination, TRUS = transrectal ultrasound.

Table II. Results of the ERSPC.

<table>
<thead>
<tr>
<th></th>
<th>Screening group</th>
<th>Control group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of participants</td>
<td>72 890</td>
<td>89 353</td>
</tr>
<tr>
<td>Mean age (years)</td>
<td>60.9</td>
<td>60.7</td>
</tr>
<tr>
<td>Mean follow-up (years)</td>
<td>8.8</td>
<td>9.0</td>
</tr>
<tr>
<td>PC detected (%)</td>
<td>5 990 (8.2%)</td>
<td>4 307 (4.8%)</td>
</tr>
<tr>
<td>PC deaths</td>
<td>214</td>
<td>326</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>ITS analysis</th>
<th>Secondary analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mortality reduction</td>
<td>20%</td>
<td>30%</td>
</tr>
<tr>
<td>Metastatic disease reduction</td>
<td>25%</td>
<td>32%</td>
</tr>
</tbody>
</table>

PSA = prostate specific antigen, PPV = positive predictive value, PC = prostate cancer, ITS = intention to screen.

need to be screened (NNS) and another 48 men who need to be treated (NNT) in order to prevent one PC death. When adjusted for non-compliance, a 27% reduction in PC mortality was seen in men who were actually screened. After adjustment for the differences in stage distribution between the two arms, no difference was seen in treatment, which makes a mortality reduction solely caused by a treatment effect very improbable [6].

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A secondary analysis [8] was carried out according to the method described by Cuzick et al. [9] to assess the impact of adjustment for men assigned to the screening arm, who did no undergo screening (non-compliance) and for men assigned to the control arm, who sought opportunistic PSA-based screening (contamination). This analysis showed a further
enhancement of the PC-specific mortality reduction after adjustment for both non-compliance and contamination by up to 31%.

The effect of this secondary analysis on the rate of metastatic PC was analyzed by Kerkhof et al. [10]. The ITS analysis resulted in a significant reduction of 25% (RR 0.75, 95% CI 0.59–0.95, p = 0.02) for developing metastatic PC in the screening arm, with an even more distinct reduction of 32% (RR 0.68, 95% CI 0.49–0.94, p = 0.02) in the adjusted analysis reckoning non-compliance and contamination. This hence leads to an improvement in the relative risk of 7% in the secondary analysis. While the ITS analysis is diluted by non-compliance and contamination it shows the effect on the study population as a whole, where the secondary analysis has the ability to give a better adjusted estimate of the effect of screening at the individual level for those men who are actually screened.

In the meantime the results of the Göteborg screening trial have been published [3]. This trial was initiated as an independent study in 1994 but joined the ERSPC trial shortly thereafter by signing the ‘agreement of participation’, a research contract. The adjusted power calculation is based on the randomization of 20,000 men, aged 50–64 years, identified in the population registry in 1994. A power of 80% was predicted with a follow-up of 14 years and a participation rate of 76% to show a difference of 40% in prostate cancer mortality by screening. These conditions were met when the follow-up was complete up to the end of 2008. The results show a rate ratio for PC death of 0.56 (95% CI 0.39–0.82, p = 0.002) in the ITS analysis and of 0.44 (95% CI 0.28–0.68, p = 0.0002) after adjustment for non-compliance. This resulted in a NNS of 293 and a NNT of 12, and for attendees after adjustment for non-compliance a NNS of 234 and NNT of 15. The main differences with the ERSPC as a whole are the type of randomization, younger age, a shorter screen interval, and, most importantly, a longer follow-up due to the simultaneous randomization of all participants in 1994.

What is true and what is not true in the controversy around ERSPC?

So what are we to believe? This year, the ‘inventor’ of PSA, Richard Ablin, called PSA-based screening a “public health disaster” and pictured the idea of one man being saved by screening while 47 other men might experience loss of sexual function or urine leakage for no good reason [11]. The numbers in this example clearly relate back to the NNT outcome of the ERSPC. Are the future prospects really that bad and, if so, why should we try to decrease PC mortality at all?

Prostate cancer is a major public health problem being the most numerous cancer among men and the second most important cause of cancer related deaths with 192,280 and 27,360 incident cases respectively in the US in 2009 [12]. Worldwide numbers of PC, show an incidence of 679,023 and a mortality of 221,002 in 2002 [13]. With a seemingly achievable 35% mortality reduction, this could lead to the prevention of 77,350 men dying from PC, provided that the price we have to pay to do this is acceptable. In the US, a mortality reduction of 35%, would lead to PC going from a second to a fifth place in leading cancer related deaths [12].

Despite these large incidence numbers and a substantial disease-specific mortality reduction in the ERSPC, the effect on overall mortality will be minimal even if the PC mortality reduction would double, as seen in the Göteborg trial. Some critics [14] promote the view that overall mortality would be the appropriate end point. A trial addressing overall mortality as an end point would need several million participants to create enough statistical power to show an overall mortality reduction and this would thus be very unlikely to ever be accomplished. At present, the aim of health care systems worldwide is to gain improvements in mortality of different types of cancer that will in the end hopefully decrease the total burden of cancer mortality. The ERSPC study contributes to this process.

Some authors have suggested that ERSPC is not a coherent study and that pooled analysis is not justified, but should be replaced by a meta-analysis. These authors suggest that the ERSPC actually is a collection of seven studies with differing screening protocols and consider this to be a weakness [15,16]. Truth is, that agreement on a common data set with central data collection and agreements on mutually accepted small differences per country including the core age group (55–69 years), were already agreed upon in 1994 and 1995, at the time European cooperation was initiated. National regulations caused randomization protocols to differ among countries, which led to a population-based effectiveness trial in Finland, Sweden and Italy, where randomization took place before informed consent. In the Netherlands, Belgium, Switzerland, Spain and the 2005 late comer France, men were randomized after providing informed consent, also known as an efficacy trial. Irrespective of the way of randomization, population registries were used to identify trial subjects [1]. Validation of randomization to test for heterogeneity of outcomes between (groups of) centers was pre-planned as was defined in the published ERSPC monitoring plan [17] and was carried out with successful results [1]. Since in all centres a trend toward mortality reduction by screening can be observed,
the agreed differences in national protocols apparently do not exert major effects on the outcome. The described heterogeneity must be considered as a strength rather than a weakness of ERSPC.

Critical remarks have been made in various communications [15,18] about the effect on PC mortality being due to treatment instead of being due to screening. In a screening study cases in both arms should be treated similarly to rule out treatment as a confounder in mortality reduction. If treatment is reported to be more aggressive in the screening arm then inequalities in treatment choices between the two groups could be causing the mortality reduction instead of screening. Within ERSPC however, after correcting for stage and grade, no difference favoring more aggressive treatment in the screening arm could be found. The observed mortality reduction is therefore very unlikely to be solely caused by treatment effect [6]. This study also shows that the only difference in treatment that was found, was the combination of radiotherapy and endocrine treatment which is superior in high-risk patients and was applied more often in the control arm, so any effect was expected it might be in favor of the control arm. Noteworthy is, that treatment decisions in PC cases in both arms were left to regional care providers, as was recorded in the study protocol (www.erspc.org; publications). In practice, general practitioners were encouraged to refer patients to regional urologists and both were contacted in this respect beforehand. Also the Göteborg trial did not report treatment difference which could impact on screening as the determinant of outcomes.

Then, there is the issue of α-spending that could cause future analyses to lack power and become statistically invalid, because of the interim analyses that were performed previously. When sequential testing is performed in interim monitoring of clinical trials, the α-value should be adjusted accordingly at each look to preserve the overall type-I error (i.e. stating an event is significant when it is not). This was already anticipated in the monitoring plan [17] and described in the 2009 publication [1]; an α-spending curve (O’Brian-Fleming rule) of =1% each time was used, with a division of uneven weights and higher weight at the end. All three interim analyses of the ERSPC had their power adjusted for α-spending.

Others stated that the re-assurance of men with negative test results is not appropriate [16,19]. This is indeed a statement that embodies a continuous worry. Studies show that there is no PSA cut-off below which a man can be reassured that he has no PC. As was shown in the only empirical analysis of the performance characteristics of PSA, a cut-off value of 3.0 ng/ml misses 67.8% of biopsy detectable PC and 42.4% of potentially aggressive ones [20]. Low PSA levels can therefore not even rule out the presence of high-grade PC, although both risks of finding a PC on biopsy are directly related to PSA levels, also in the lower range.

For the ERSPC it was calculated that, of 48 867 men in six of the participating countries with an initial PSA <3.0 ng/ml, 5% was diagnosed with PC after a mean follow-up of nine years, of which 4.6% was confirmed to be high-grade [21]. Substantial numbers of cancers must be therefore assumed to be missed. Only long-term follow-up, as it is planned within the ERSPC, can shed light on their natural history and on the rate of their detection at subsequent screens. In addition to this, results of ERSPC Rotterdam [22] showed 9.4% of men who had initial negative biopsies to develop PC over an 11-year follow-up period, with the number of potentially missed cancers with a poor outcome in terms of progression-free survival (9%) and deaths from PC (2.4%) being very low. More aggressive screening may result in the detection of additional aggressive PC, but this would be at the cost of detecting a lot of potentially indolent PC, which will increase overdiagnosis, overtreatment and the NNT.

Does the benefit shown in the ERSPC match the damage? We expressed this as an important downside in our 2009 paper [1], when we addressed overdiagnosis and overtreatment as the most important adverse effects induced by screening. Overdiagnosis in the ERSPC was estimated to be no less than 50% in the screening group [7] and although this is a major concern, it is also one of the main achievements of the ERSPC to quantify and report on these events. Furthermore, the NNS and the NNT are time dependent and can be expected to become more favorable with longer follow-up, as the mortality reduction increases.

Unfortunately, PSA lacks the specificity to be a solid tool in determining a biopsy indication, resulting in large numbers of unnecessary biopsies and, at the same time, missing PC diagnoses in men with PSA values <3.0 ng/ml. The ideal screening tool for PC, which could identify potentially aggressive cancers in an early, curative stage, but could also avoid detection of cancers that would never cause any symptoms, let alone deaths, unfortunately does not yet exist. However, the unnecessary treatments can with some as yet unidentified risk for the patient, be delayed by offering “active surveillance”. About 25% of men in ERSPC have made this choice.

Since PSA testing can not distinguish lethal PC from indolent disease and a lowering of the PSA threshold to proceed to prostate biopsy would imply detecting more and more PC that would be over-diagnosed and potentially overtreated. Addressing this issue, Roobol et al. [23] have applied the PC
Riskcalculator (www.prostatecancer-riskcalculator.com) to reduce the number of unnecessary biopsies, while still detecting most clinically important PC cases. Including ultrasound volume, digital rectal exam and transrectal ultrasound together with the PSA value in a model, while applying an additional probability cut-off value of, for example, 12.5% to trigger a biopsy, resulted in a substantial increase of PPV in initial (from 29 to 38%), as well as repeat screening (from 19 to 25%). The predictive value for PSA alone showed an AUC of 0.64, which increased to 0.77 when the Riskcalculator was used. With this method, cancer diagnoses would be missed, but this would predominantly concern indolent PC (70–81%) and only a very small proportion of potentially important PC. Just increasing the PSA cut-off to >4.0 ng/ml for example, would also result in a considerable decrease in biopsies, but considerably higher numbers of missed PC diagnoses (1.8–2.6 times higher). This individualized screening algorithm might contribute to counteract two of the most negative side-effects of screening, namely unnecessary invasive testing and overdiagnosis with the related overtreatment. The fact that some cancers will always be missed by increasing test specificity must however be considered. It is unknown at present what proportion of cancers will escape all efforts and which cancers can be safely detected during a subsequent round of screening. Besides this, hopefully in the future, new markers and improved nomograms will become available to lead to a more selective detection of aggressive PC.

What are the “real issues” and how can they be dealt with?

As incidence rates are high and PC is one of the most frequent causes of cancer related deaths, it is a relevant public health problem to decrease the PC mortality provided that this can be done at an acceptable price. This ‘price’ should comprise of a number of considerations.

First, the quality-adjusted life years (QALY’s) and other costs and benefits of screening should be matched against the achievable decrease of PC mortality. A paper on this subject addressing the cost effectiveness and quality of life in the ERSPC is in preparation and will be updated with increasing follow-up.

Then, testing for PC should become more selective, resulting in detecting less non-aggressive cancers and decreasing the amount of overdiagnosis and resulting overtreatment. As discussed earlier, PSA alone is not an optimal marker for PC screening, but individualized screening by means of the ERSPC based Risk Calculator is a step in the right direction.

Figure 2. Riskcalculator predicting the risk of positive biopsy based on solely PSA. This figure shows the predicted risk of having a positive biopsy, solely based on the PSA-value, irrespective of any other values of ancillary tests. In this example a man with a PSA-value of 4.0 ng/ml has a predicted risk of 21%. The estimation is based on data from the first 6288 participants in the Rotterdam arm of the ERSPC.
These decision tools use risk modifying techniques to identify the cancers that will most likely be indolent and can be managed with active surveillance. An example of the application of the Riskcalculator (step 3) to three different men all presenting with a PSA value of 4.0 ng/ml is presented in Figures 2–4. The ERSPC based Riskcalculator among others, calculates the probability of having a positive biopsy (step 3) and of indolent cancer (step 6). The calculator was updated and validated [24] and used for the study by Roobol et al. [23] mentioned above. Nevertheless, better markers and more accurate, advanced nomograms should be developed in order to decrease overdiagnosis even more.

Next, continued follow-up of the ERSPC is needed, since until now about 22% of participants died, there are still many more events to be expected. In the Scandinavian SPG-4 study of clinically diagnosed locally confined PC [25], the difference in cumulative incidence of death due to PC between groups assigned to radical prostatectomy or watchful waiting, remained stable only after about ten years. In the Göteborg trial, even after 14 years of follow-up, the mortality curves still continue to diverge. This illustrates that the follow-up in the ERSPC study is still too short to draw definite conclusions and further follow-up has to be awaited. If longer follow-up shows a larger difference in PC mortality – which can be expected from the 2009 mortality curves – the NNS and NNT will decrease. With an assumed 35% relative risk reduction in the intention to screen analysis, the NNS would decrease to 806 and the NNT to 27. If a 40% reduction would be accomplished, this would lead to a NNS of 256 and NNT of 14. For those men with PSA values <3.0 ng/ml, longer follow-up will help quantify the predictive value of a negative result and to better be able to reassure men with certain characteristics.

These estimates are similar to the results of the Göteborg screening trial. If effects of similar size were shown in ERSPC as a whole, these findings are likely to be more relevant because of the size of the trial, the European multiple country setting, the fact that small protocol differences still produce identical trends, the possibility to study multiple aspect on how to screen best for PC and the ongoing analysis of QALY’s based on ERSPC findings.

Another important issue to study in this context, will be the screen detected PC that escape all efforts

Figure 3. Riskcalculator step 3. Prediction of the chance of a positive sextant biopsy in a man who was never screened before; PSA = 4.0 ng/ml, low risk. This figure shows the prediction of a positive biopsy, making use of the results of transrectal ultrasound (TRUS), digital rectal examination (DRE), prostate volume at ultrasonography and PSA-value. In this example a man with a PSA-value of 4.0 ng/ml, normal TRUS and DRE and prostate volume of 60cc were used, which results in a 7% chance.
in spite of early detection and treatment. We are cooperating internationally to identify the characteristics of such patients and the mechanism that causes these “escapes”. Again, the resulting knowledge can be used to improve testing and screening. There will always be a group of unavoidable “escapes” which, when defined and quantified, will contribute to resolving many controversies.

Finally, validated mechanisms for shared decision taking should be established. Results of the ERSPC have been adopted in several guidelines, including those of the European Association of Urologists [26], but so far no commonly accepted information material exists about the proper interpretation of the trial results and for men who wish to be informed about the possible risks and uncertainties. Effort should be taken at an international level to create validated decision aids.

So, what should we tell patients who wish to be screened, taking the contemporary evidence into account? This message has changed dramatically by the results of recent studies and should include the following statements. In the case of PC detected with screening, the chances of dying of the disease are decreased by at least 31%. The downside remains though, as long as we have to deal with a high chance of being diagnosed and treated for disease which otherwise may not harm you within a period of nine years or longer. However, when non aggressive disease is suspected, treatment can be avoided at least for some time.

**Conclusion**

In conclusion, we can say that the ERSPC shows a reduction of PC mortality for screened men of 20–31% at nine years of follow-up. In the Göteborg screening trial these figures amount to 44–56% at 14 years. The resulting overdiagnosis and overtreatment are major worries, but can be decreased by screening less aggressively and more selectively using available risk modifying calculators. Establishing population based screening for PC as a health policy, will depend on lowering the NNT, while focussing on methods for more selective screening and achieving an acceptable risk-benefit ratio.
We think screening will become an accepted health policy, but it will take time and work to be done. Key developments in the field of screening show, that PSA should be used as a marker within an algorithm instead of a single biopsy indicator, to achieve better predictions of positive biopsies. Nevertheless, we have to work hard to find new, better and more selective markers for screening purposes. It is important however to be aware of the fact that we will always miss cancers with screening and we have to learn to selectively miss the ones that would otherwise stay indolent and those that will always escape all efforts in spite of screening.

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**References**


The Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial: The prostate cancer screening results in context

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Abstract

Background. The Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial (PLCO) was conducted in sites around USA during a period of marked secular changes in the use of prostate specific antigen (PSA) screening for prostate cancer. Material and methods. Trends in prostate cancer incidence, stage at presentation and mortality are useful when interpreting the results from a screening trial that commenced in 1993 and enrolled participants through 2001. The last participants completed active screening in 2006. Incidence and mortality data published to date on PLCO need to be placed into the context of the secular trends. Additional data analyses have been conducted on subsets of the participants and these results can also enhance the interpretation of the trial. Additionally, the accompanying biospecimen repository has served as a rich research resource yielding informative findings. Results. The PLCO is best viewed as a trial comparing a regimented active annual screening program of PSA screening for six rounds, four of which had accompanying digital rectal examination (DRE) to patterns of screening that were occurring in the population in many academic and community settings across the USA. The epidemiology and molecular genetics of prostate cancer is becoming better understood and analyses of the PLCO resource have contributed. One approach to risk assessment utilizing genetic markers from selected members of the PLCO prostate cancer cohort has been developed. A modeling effort with CISNET-ERSPC-PLCO is underway to compare and contrast findings such as effects of different PSA thresholds and screening intervals. Conclusions. The information emerging from PLCO is useful to inform the debate around prostate cancer screening. An understanding of the biologic differences underpinning indolent and aggressive prostate cancer will better guide the future development of screening and treatment strategies.

Background

Prostate cancer has been the leading cause of cancer in males in USA for decades [1]. In the late 1970s near the inception of the Surveillance Epidemiology and End Results (SEER) Registry (http://seer.cancer.gov/), prostate cancer incidence was similar to lung cancer. The decline in smoking in males has led to a gradual decline in incidence in lung cancer. Prostate cancer screening appears to have contributed to the marked rise in incidence witnessed in the late 1980s and early 1990s. Although, prostate cancer incidence has fallen from its peak, it remains elevated compared to the pre-prostate specific antigen (PSA) era. Mortality from prostate cancer fell relatively rapidly after the introduction of PSA testing. Debate has raged as to the extent to which treatment advances and/or widespread PSA testing have contributed to this decline.

The Food and Drug Administration approved the PSA test in 1986 as a way to monitor men after treatment. It was then studied as a means of detection of prostate cancer [2]. The medical community recognized at the time that interest and uptake would be generated particularly as it was a non-invasive, relatively inexpensive, blood test. Many prominent members of the urologic community and the National Cancer Institute (NCI) met to discuss how best to assess the effect of the emergence of PSA testing. A prospective, randomized trial with a prostate cancer specific mortality endpoint was judged as the most definitive approach. Other outstanding screening questions for lung, colorectal and ovarian cancer screening were identified. A multi-modal screening trial was thought to have cost-efficiencies. Also, mature adults when receiving their medical care are...
evaluated for their risk of cancer as it continues to be the second leading cause of death in the United States. Their clinicians would benefit from information to guide screening choices and a multi-modal approach to assessment would better reflect the clinical reality [3].

**Material and methods**

The design of the PLCO focused on developing a randomized trial in males and females that would be adequately powered to assess the impact on cancer specific mortality of screening for lung and colorectal cancer in males and females, ovarian cancer screening in females and prostate cancer screening in males [4]. The trial initially set out to enroll individuals 60 to 75, reasoning that these were ages of high incidence of cancer. More elderly individuals had and still have higher rates of competing mortality and therefore it is more difficult to assess the effect of screening in this group and they were excluded from the study. As the trial progressed it became clear that the enrollment that was occurring, although more rapid in the younger age groups, was slow and to expand the numbers the age for entry was lowered to 55.

To reflect the geographic, racial and ethnic diversity of the US population a Request for Proposals was broadly solicited. The proposals were reviewed for capabilities of the sites to perform the recruitment, screening, retention and follow-up needed. The resulting sites were distributed throughout the continental US and Hawaii (http://prevention.cancer.gov/plco/centers). One poorly performing site was replaced early in the trial by the University of Alabama which has a strong emphasis on recruiting African-Americans from surrounding communities into trials. Efforts at another site, the University of Colorado Health Systems focused on Hispanic recruitment. A location at the Pacific Health Research Institute (now Pacific Health Research and Education Institute) was aimed at recruiting Asian and Pacific-Islanders. All sites including the NCI had the study approved by their local Institutional Review Boards. The PLCO participants signed an informed consent detailing the nature and risks from the screening interventions. The control participants were encouraged to continue to receive care from their usual health care providers. Screening was neither encouraged nor discouraged for them.

The resulting demographics of the enrolled male population can be seen in Table I. An analysis of the characteristics of the participants confirmed that the PLCO enrollees like many in clinical trials are “healthy volunteers” [5]. They are of higher socio-economic and educational attainment than the population at large and tend to have a better health profile. This needs to be recognized as one analyzes the results of the trial. Enrollment occurred from 1993 to 2001. Prostate cancer screening was with annual PSA testing for six years (T0–T5) and digital rectal examination (DRE) annually for four years (T0–T3). The threshold for an abnormal serum PSA was set at > 4 nanograms per milliliter (ng/ml). A reference laboratory was established at UCLA where all specimens were shipped on dry ice after centrifugation and serum separation. PSA tests were analyzed with the Tandem-R PSA assay until January 1, 2004 and with the Access Hybritech PSA after that (both assays were manufactured by Beckman Coulter). Additional blood specimens were drawn from screening arm participants and buccal cells were collected from control arm participants to establish a biospecimen repository [6]. Tissue samples were also collected from participants in both arms. Tissue microarrays were constructed and cores obtained from formalin-fixed paraffin embedded specimens.

| Table II. Tumor stage and Gleason score for all prostate cancers at 10 years. |
|-----------------|-----------------|-----------------|
| **Stage**       | **Screening Group** | **Control Group** |
| I               | 2 (0.1%)         | 15 (0.5%)       |
| II              | 1458 (97.2%)     | 2790 (93.8%)    |
| III             | 22 (1.5%)        | 56 (1.9%)       |
| IV              | 15 (1.0%)        | 79 (2.7%)       |
| Unknown         | 3 (0.2%)         | 34 (1.1%)       |

<table>
<thead>
<tr>
<th><strong>Gleason score on biopsy</strong></th>
<th><strong>Screening Group</strong></th>
<th><strong>Control Group</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>2-4</td>
<td>94 (6.3%)</td>
<td>137 (4.6%)</td>
</tr>
<tr>
<td>5-6</td>
<td>963 (64.2%)</td>
<td>1656 (55.7%)</td>
</tr>
<tr>
<td>7</td>
<td>318 (21.2%)</td>
<td>779 (26.2%)</td>
</tr>
<tr>
<td>8-10</td>
<td>98 (6.5%)</td>
<td>341 (11.5%)</td>
</tr>
<tr>
<td>Unknown</td>
<td>27 (1.8%)</td>
<td>61 (2.1%)</td>
</tr>
</tbody>
</table>

Table I. Demographics of male enrollees in the PLCO.

<table>
<thead>
<tr>
<th></th>
<th><strong>Screening Group</strong></th>
<th><strong>Control Group</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>percent</strong></td>
<td>n = 38343</td>
<td>n = 38350</td>
</tr>
<tr>
<td><strong>Age</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>55–59 yr</td>
<td>32.3</td>
<td>32.3</td>
</tr>
<tr>
<td>60–64 yr</td>
<td>31.3</td>
<td>31.3</td>
</tr>
<tr>
<td>65–69 yr</td>
<td>23.2</td>
<td>23.2</td>
</tr>
<tr>
<td>70–74 yr</td>
<td>13.2</td>
<td>13.2</td>
</tr>
<tr>
<td><strong>Race or ethnic group; self-reported</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-Hispanic white</td>
<td>86.2</td>
<td>83.8</td>
</tr>
<tr>
<td>Non-Hispanic black</td>
<td>4.5</td>
<td>4.3</td>
</tr>
<tr>
<td>Hispanic</td>
<td>2.1</td>
<td>2.1</td>
</tr>
<tr>
<td>Asian</td>
<td>4.0</td>
<td>3.9</td>
</tr>
<tr>
<td>Other</td>
<td>0.8</td>
<td>0.9</td>
</tr>
<tr>
<td>Missing data</td>
<td>2.4</td>
<td>5.0</td>
</tr>
<tr>
<td><strong>Family history of prostate cancer</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Once</td>
<td>32.8</td>
<td>31.9</td>
</tr>
<tr>
<td>Two or more times</td>
<td>9.4</td>
<td>9.8</td>
</tr>
</tbody>
</table>
At enrollment, all participants completed a baseline questionnaire that inquired about screening practices in the three year period prior to enrollment, demographic characteristics and potential risk factors for malignancy. Separate dietary questionnaires were also administered. The participants were asked if they had had prior PSA or DRE testing and the number of tests. After 1995, those participants who had more than one PSA blood test in the previous three years were excluded. During the conduct of the screening portion of the trial, participants in the control arm were also assessed for screening test uptake. A randomly selected group was queried every one to two years. For prostate cancer screening, the participants were asked if they had ever had a PSA blood test for prostate cancer or a digital rectal examination of the prostate. Those who answered yes were then asked when the most recent test was. The categories for response were “within the past year”, “1–2 years ago”, “2–3 years ago” and “more than three years ago.” The reasons for the test were also queried to determine if it was routine or for evaluation of a specific health problem. Those participants who had repeated PSA testing prior to entry were assumed to continue screening annually. This comprised 9.8% of the control group. A weighted average, of the percent responding both “within the past year” and routine, and the 100% estimate in the group screened frequently prior to enrollment, was used to provide an estimate of overall contamination. Assessment of compliance in the screening arm was determined by attendance at the annually scheduled screening appointments and an estimate of compliance was calculated by dividing that by the number expected.

Results

The trial was monitored from inception by an independent Data and Safety Monitoring Board. Reviews of the accumulating data were done every six months with regular planned interim analyses. Publication of prostate cancer specific mortality results for up to ten years from recruitment occurred in March 2009 [7]. Median follow-up was 11.5 years and vital status was known for 98% of participants at seven years and 67% at ten years. The decision to report was made as no evidence of difference between arms was emerging at that point but evidence of harms from diagnostic evaluations following screening and after treatment was noticed. Earlier publications had presented results from the baseline [8] and all screening rounds [9].

Six annual rounds of screening were conducted and at seven years, 2 820 prostate cancers and 50 prostate cancer specific deaths were noted in the screened group. In the control group, 2 322 cancers and 44 deaths were noted. The data at ten years (67% complete) showed 3 452 screened versus 2 974 control group cancers and 92 compared to 82 deaths. Of note, the excess number of prostate cancer cases persisted after completion of screening. Also, although 25% more prostate cancers were diagnosed in the active screening arm at seven years, mortality rates through seven to ten years were the same in each arm. Whether or not the small differences in stage and Gleason’s score between arms will result in differential survival in the future remains to be seen.

The percentage of patients having late stage disease [AJCC Stages III and IV, [10]] Table II, at diagnosis was low in both arms. At ten years, 3.5% of all screening arm subjects were clinical stage III and IV compared to 4.6% in the control arm. This compares with a 25% incidence of presentation of late stage disease prior to the advent of PSA testing and a rate of 4% in the population as a whole in 2002. A comparison of Gleason’s score on biopsy, revealed aggressive Gleason’s 8 to 10 in 8.4% of screened arm participants and 11.5% in the control arm participants. Additional follow-up on the entire cohort is continuing. Whether these differences in stage at diagnosis and in Gleason’s grade will translate into differences in prostate cancer specific mortality will be seen.

A separate analysis of the effect of the contamination on rates of prostate cancer has been conducted [11]. The rates of reported test usage increase if one includes any testing within the year compared with routine testing. Rates increased from 33 to 40% at study year 0 and from 46% at study year 5 to 54–55%. At year 0, 38% of men reported no history of PSA testing while at year 5, 15% did. Also, at year five, 18% reported testing one to two years earlier. Compliance with the screening protocol overall was 85% for PSA testing and 86% for DRE, lower than the study design estimate of 90%. Clearly, the men in the control arm were being screened but at a lower frequency and intensity than men in the screened arm.

To estimate what would have occurred in the absence of screening, SEER rates from 1985–1987 prior to the onset of the PSA era were utilized. Five year age groups and race (white, black, other) were constructed and the SEER rates were applied to the person-years at risk for control and screened arm men during the screening period of the trial (the first six years). A separate calculation was done utilizing SEER rates contemporaneous to the screening period of the trial. During the six screening years of the trial, 2 538 prostate cancers were identified in the screened arm and 1 958 in the control arm. In the screened arm there were an excess of 1 589 and in the control arm, 1 024 compared to the pre-PSA screening era. When one compares with the contemporaneous
SEER rates, 927 and 354 (screening: control) excess cancers were diagnosed. For the control arm, this reflects a 22% excess over the expected number if screening had been conducted in the control arm as in the populations covered by SEER.

Ancillary data analyses

The information available from the PLCO prostate cancer screening trial can be analyzed to improve the understanding of the role of other factors that influence the conduct of screening and evaluation for prostate cancer. A large fraction of screened men, initially have low PSA levels (2 ng/ml). In the PLCO, in men with baseline PSAs less than 1 ng/ml, 1.5% were found to have a PSA of more than 4 ng/ml by year 5, while in those with PSAs between 1.0 and 1.99 ng/ml the rate of progression was somewhat higher with 7.4% progressing by year five [12]. A total of 33.5% and 79% of men with initial PSA of 2.0 to 2.99 and 3.0 to 4.0 converted by year 5. This information could help to inform thresholds for positivity and screening frequency. Information on PSA velocity (PSAV) may also help to inform decisions as to when to biopsy. In 1 441 men enrolled in the PLCO who received ≥ 2 PSA screens, and were diagnosed with prostate cancer within one year of the last screen, PSAV was calculated using all available PSA levels [13]. Both PSA and PSAV were related to biopsy Gleason score. The median PSAV was 0.60 ng/ml per year for men with Gleason scores from 2 to 6 versus 0.84 ng/ml for men with Gleason scores from 7 to 10 (p < 0.001). Information such as this can inform watchful waiting and active surveillance approaches as well. Another issue is what happens after an initial negative prostate biopsy. The probability of having a repeat biopsy within three years of initial biopsy was 43% for 1 736 men with suspicious PSA levels after an additional round of screening [14]. An analysis of men who had an initial false positive result was done to assess the impact of this result on subsequent screening behavior. Given the subsequent high risk of repeat biopsy it was worrisome to note that in a multi-variable model being African American (p = 0.016 and having a high school education or less p = 0.007) were predictive of not returning for prostate cancer screening within the following year [15]. Additional effort may be warranted to facilitate compliance with screening in this group.

The impact of associated diseases and conditions can also be assessed in the PLCO cohort. Analysis within the PLCO has confirmed an inverse relationship between PSA concentration and body mass index [16]. Dietary factors and supplement use have been analyzed within the cohort. There was no overall association between dietary or supplemental intake of vitamin E, beta-carotene or vitamin C and prostate cancer risk [17, 18]. Also, no evidence was found substantiating the hypothesis that lycopene and tomato product intake affected risk of prostate cancer [19].

The PLCO biospecimen repository with high quality DNA specimens has contributed to our understanding of the molecular genetic factors that are associated with increased risk of prostate cancer. A locus within the 8q24 chromosome, rs6983267, was identified separate from the initially reported locus at rs1447295 [20]. Additional SNP analyses done in second stage replication scans confirmed three previously reported loci, two in 8q24 and one in HNF1B [21] and loci on chromosomes 7, 10 (2 loci) and 11 were highly significant. The loci on chromosome 10 include MSMB which encodes β-microseminoprotein, a primary constituent of semen and CTBP2, a gene with antiapoptotic activity. Additional fine mapping and functional analysis confirmed the strong association with prostate cancer risk of the rs10993994 locus in MSMB and gene expression was higher in cell lines with a CC or a CT genotype than with a TT genotype. [22].

Utilizing knowledge of SNPs to assess risk of malignancy is a developing endeavor. A project utilizing a population-based case-control study in Sweden and a nested case-control study from the PLCO developed a risk-prediction model utilizing SNPs and family history [23]. Men with 11 risk alleles (mode) and negative family history were considered at baseline risk and those who had ≥ 14 risk alleles and a positive family history had an odds ratio of 4.92 (95% CI: 3.64–6.64) for prostate cancer in the Swedish study and this was confirmed in the PLCO. This could be utilized to calculate a man’s absolute risk of prostate cancer. For example, a 65-year-old man in the US with a family history and ≥ 14 risk alleles, has a 41% risk of being diagnosed with prostate cancer compared with a population average of 13%. The utility of these types of assessments for screening and consideration of chemoprevention still need to be determined.

Discussion

The PLCO trial was conceived at the beginning of utilization of the PSA test for screening for prostate cancer. Rapid dissemination and widespread initial use of PSA testing for prostate cancer screening occurred in the years leading up to the launch of the trial. Subsequent to launch, regular PSA screening remained common in the community. The peak in prostate cancer incidence in males in the US coincided with the launch of the trial in the early 1990s [1]. Also, with the increasing incidence in the disease, a decrease in advanced stage disease was noted. Subsequent modeling was consistent with much of this decline in late stage
disease being a consequence of screening [24]. Several years after the trial launch, prostate cancer mortality in the US fell, going below pre-PSA rates by 2003. The results of the trial must be placed into this context.

When one looks at the incidence rates in the PLCO the control group actually has higher rates than compared with contemporaneous results from the SEER registry. This can be explained by the characteristics of the enrollees. As mentioned earlier, they represent a “healthy volunteer” who is of higher socio-economic status and better educated than average. This demographic undergoes screening more frequently. The prostate cancer specific mortality in both arms of the study is low.

A limitation of the PLCO could be the cut-off of > 4 ng/ml chosen as the threshold for referral for further evaluation. This was the commonly accepted threshold at the time of trial initiation. Subsequently, a trial of finasteride in prostate cancer prevention had as part of the design an exit biopsy in all men in the placebo arm regardless of PSA level. This demonstrated that overall, men with PSAs of less than 4 ng/ml had a 15% incidence of prostate cancer of which 14.9% was Gleason’s 7 + [25]. The prevalence of prostate cancer in men with PSAs of ≤ 0.5 ng/ml was 6% of which 12.5% were high grade. If PSA screening were to be implemented as an organized program, the ideal threshold value is unclear. Also, the PLCO investigators did not prescribe evaluations or therapy. Participants and their physicians decided on a course of evaluation of an elevated PSA and if prostate cancer were diagnosed the treatments were also determined in the same manner. It should be noted that these same diagnostic and treatment approaches were the ones employed during the period of rapid increase in prostate cancer incidence in SEER and also in the decrease in mortality seen in the US.

An analysis under the auspices of the Cancer Intervention and Surveillance Modeling Network (CISNET) http://cisnet.cancer.gov/ will compare results across ERSPC and PLCO. Differences in the screening approaches such as PSA threshold and screening interval, and populations will have to be noted when these comparisons are presented.

Conclusion and next steps

Continued collection of endpoint data in the PLCO is critical. An impact of the differential in Gleason’s grade between arms and the very small difference in stage may emerge. The analyses of CISNET-ERSPC-PLCO will be revealing. The molecular genetics of prostate cancer risk are better understood through contributions of the PLCO. Much more remains to be accomplished and the prostate tumor cores and TMAs from the PLCO and the matched pre-diagnostic biospecimens are available to the research community. Interesting understandings of the behaviors of indolent and aggressive disease may emerge.

The goal of all is to minimize the adverse impact of prostate cancer in our ageing society. Clearly, the mortality reductions achieved to date are to be applauded. However, they do come with a high rate of overdiagnosis. Treatment side effects are also substantial. Knowledge gained from watchful waiting and active surveillance approaches will be important. As discussed elsewhere in this monograph advances in the chemoprevention of prostate cancer have also been made.

The PLCO trial has contributed to this emerging database. However, it is important when analyzing this trial to place the results in the context of the times. When designing screening trials for the future these issues need to be considered.

Acknowledgements

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Declaration of interest: The author reports no conflicts of interest. The author alone is responsible for the content and writing of the paper.

References


REVIEW ARTICLE

Early detection of prostate cancer with emphasis on genetic markers

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Abstract

Background. The recent advances in genomic research have made it possible to identify several new genomic-based biomarkers for prostate cancer. In this review we evaluate these new markers and speculate about future scenarios. Results. Today 35 single nucleotide polymorphisms (SNPs) have been identified and independently validated to associate with prostate cancer. These SNPs are common in the population (>5%) but the effect of these SNPs in these regions on prostate cancer risk is modest with odds ratios typically ranging between 1.1 and 1.3. It is estimated that these markers explain 25% of the familial risk of prostate cancer. However, it is anticipated that additional 50–75 prostate cancer SNPs will be identified in the near future. The SNPs associated with prostate cancer so far are not associated with disease stage or outcome. There are several efforts to identify germline genetic markers that can be used as prognostic markers. There are also tumor-based methods that are promising in identifying new genetic markers that can be easily measured in plasma or urine. Conclusion. There are several new “genetic” markers that in the near future might be used in clinical routine. These markers are easy to measure and stable over time. However the challenge is not only to identify new biomarkers but the real test is to validate new biomarkers in several large well-characterized patient populations. This validation must be done together will all other known biomarkers at the same time as it not likely that one single marker is enough, but a panel of different markers. Today 2010 there are over 19 000 publications in the area of biomarkers and prostate cancer, but only one biomarker, PSA, is used in the clinic today!

Since the late 1980s Prostate Specific Antigen (PSA) has been used as a clinical biomarker initially used to follow treatment outcome but later as a tool to identify patients at a high risk of being diagnosed with prostate cancer.

Two recent studies have presented different outcomes as to whether PSA can serve as a tool for screening or not. The European study, ERPC, showed a 20% reduction in prostate cancer mortality compared to the non-intervention arm. The PLCO study did not show any reduction in mortality, probably due to contamination of the control group and relatively few events in the intervention group. Although it seems as if screening would affect the prostate cancer related deaths it has not led to general screening in most western countries mostly due to the fact that screening inevitably leads to overdiagnosis and overtreatment which also was shown in the ERCP study where 1 400 men had to be screened and 48 men had to be treated to avoid one death related to prostate cancer [1,2]. In an updated analysis of the Göteborg part of the ERPC, a 44% reduction in prostate cancer mortality was reported. This results reduces the numbers needed to screen to 293 and number needed to treat to only 12 [3].

Still this calls for novel biomarkers with a better specificity in order to better predict which men would benefit from further diagnostic interventions and eventually treatment. Biological markers associated with aggressiveness would be very valuable in order to better be able to individualize treatment.

In this review we are focusing on genetic markers that can be measured in blood, either from DNA or plasma/serum. Genetic markers measured in urine or tumors are covered in other articles. We have the following definitions of a genetic marker:
Germline (inherited) genetic markers

Point mutations/copy number variation in the human genome. A single nucleotide polymorphism (SNP) is an inherited mutation that is present in more than 1% of the population. These markers can be analyzed from any normal tissue in the body but in most cases DNA from white blood cells are used.

Germline genetic markers and prostate cancer risk

A family history of prostate cancer is one of the strongest risk factors and twin studies suggest that as much as 42% of the disease risk is explained by heritable factors [4]. Attempts to decipher the inheritable component of prostate cancer based on candidate gene association studies and genome-wide linkage studies in multiple case families have suggested numerous prostate cancer susceptibility genes and loci. However, an inability to replicate reported linkage and association findings suggest that prostate cancer is genetically complex with multiple common low-penetrance genes involved in prostate cancer predisposition [5].

Recently, genome-wide association studies (GWAS) have emerged as a powerful method to identify genomic low-risk susceptibility regions for complex diseases including cancer [6]. Through genotyping platforms that explore hundreds of thousands of single nucleotide polymorphisms (SNPs) simultaneously it is possible to screen the complete genome for common genetic variation associated with the disease of interest. In 2006 the first prostate cancer susceptibility region was identified at chromosome 8q24. Subsequent GWAS and region-focused studies have revealed five distinct linkage disequilibrium blocks harbouring prostate cancer susceptibility alleles at 8q24 [7–13]. The 8q24 region has also been shown to harbour susceptibility alleles for breast cancer [14], colorectal cancer [15], bladder cancer [16], and ovarian cancer [10]. The 1.2-Mb sequence at 8q24 containing all observed risk alleles does not code for any known genes and the biologic mechanisms underlying these associations are unknown. The oncogene c-MYC is the closest distal gene to this region and it has been suggested that the observed associations reflect long-range control of myc expression. To date, 38 distinct genetic loci harbouring prostate cancer risk alleles have been identified and consistently replicated (Table I). In general, the effect of variants in these regions on prostate cancer risk is modest with odds ratios typically ranging between 1.1 and 1.3. It has been estimated 17 that hitherto identified variants together explain approximately 22% of the familial risk of prostate cancer and it is anticipated that many more prostate cancer susceptibility variants will be identified in the future. All these studies have been conducted on European/North American populations making the translation to other ethnic groups uncertain. However, a recent Japanese study showed that x/x of known prostate cancer susceptibility loci was confirmed in Japanese men [18]. In addition five new loci was reported and it is unknown if these loci are specific to the Japanese population or not.

Germline genetic markers and disease aggressiveness

To date there is no reliable way of predicting whether prostate cancer will be an aggressive, fast-growing disease or a non-aggressive, slow-growing type of cancer. In general, a combination of tumor staging (using the tumor-node-metastasis staging system [19]), tumor grading (using the Gleason scoring system [20]) and diagnostic PSA serum levels are used to classify patients into different prognostic risk groups to guide clinicians in treatment decisions. In genetic association studies, prostate cancer patients are commonly classified as having a more aggressive form of the disease if they fulfill any of the following criteria: 1) disease spread outside of the prostate gland, or presence of cancer in the lymph nodes or other metastatic sites; 2) presence of poorly differentiated cancer as indicated by a high Gleason score (i.e. 4+3=7 or higher); or 3) a serum PSA level associated with a high likelihood of extensive disease (i.e. >20 ng/ml).

Several studies have explored the capacity of established prostate cancer risk variants to distinguish between less aggressive and more aggressive disease [7–9,21–43]. Overall, results are inconclusive, with some studies reporting stronger associations for some of these variants among more aggressive prostate cancer patients, while others did not. In a large replication study from the PRACTICAL (Prostate cancer association group to Investigate cancer associated alterations in the genome) consortium, which evaluated genetic variants at chromosome 3p12, 6q25, 7q21, 10q11, 11q13, 19q13 and Xp11 among 7 370 prostate cancer cases and 5 742 controls, no association with tumor grade was observed for any of the explored variants [42]. Fitzgerald and coworkers assessed the same seven variants and an additional six variants at chromosome 7p15, 8q24, 10q26, and 17q12 in a population-based study comprising 1 308 cases and 1 267 controls for association with family history and clinical features of more aggressive disease [43]. No association was observed between any of the evaluated risk variants and a composite measure of disease aggressiveness; however, two variants, rs10993994 at 10q11...
and rs5945619 at Xp11 (p=0.03) were nominally significantly associated with Gleason score. Most of the published studies exploring established risk variants with respect to prostate cancer aggressiveness had several limitations including small sample size, heterogeneous definition of aggressive disease across multiple study populations, and reliance on clinical grading and staging of tumors. To address these limitations Xu and coworkers evaluated 20 established risk variants in 17 distinct genomic regions among 5,895 prostate cancer patients of European descent who underwent radical prostatectomy for treatment of prostate cancer. Based on the entire prostate gland each tumor was uniformly graded and staged using the same protocol. For 18 of the 20 variants explored no significant difference was observed in risk allele frequencies between patients with more aggressive and less aggressive disease. Two variants were significantly associated with disease aggressiveness; SNP rs2735839 downstream of the kallikrein 3 gene (\(KLK3\), \(p=8.4 \times 10^{-9}\)), the gene coding for PSA, and SNP rs10993994 in the microseminoprotein beta gene (\(MSMB\), \(p=0.046\)). Since these risk alleles have been shown to strongly associate with higher PSA levels among population controls [25,44,45], it is possible that the observed association with aggressive disease may partly reflect a PSA detection bias.

<table>
<thead>
<tr>
<th>dbSNP No.</th>
<th>Chromosome</th>
<th>Gene*</th>
<th>Risk Allele†</th>
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<td>C</td>
<td>1.19</td>
<td>Eeles et al. 2008 [25]</td>
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*These genes are within the linkage-disequilibrium block defined by the associated variant. THADA denotes thyroid adenoma associated isoform 1, EHB1P1 the EH domain binding protein 1, ITGA6 the integrin alpha chain 6, EEFSEC the elongation factor for selenoprotein translation, PDLIM5 the PDZ and LIM domain 5 isoform d, FLJ20032 the hypothetical protein LOC54790, SLC22A3 the solute carrier family 22 member 3, JAZF1 the juxtaposed with another zinc finger gene 1, LMTK2 the lemur tyrosine kinase 2, SLC25A37 the mitochondrial solute carrier protein, NKKX3-1 the NK3 transcription factor related locus 1, MSMB the beta-microseminoprotein isoform a precursor, CTBP2 the C-terminal binding protein 2 isoform 2, HNF1B hepatocyte nuclear factor 1 homeobox B, PPP1R14A the protein phosphatase 1, regulatory inhibitor, KLK3 the kallikrein 3 gene, TNRC6B the trinucleotide repeat containing 6B isoform 2, BIK the BCL2-interacting killer, NUDT11 the nudix-type motif 11.

† Risk alleles as defined from published data cited in the column.
**Somatic (tumor) genetic marker**

Acquired genetic mutations/rearrangement in a tumor during the development of the tumor. These changes can be detected from DNA or RNA from the specific tumor. New techniques make it possible to detect these somatic mutations (DNA) or copies of gene expression (RNA) directly in blood plasma.

Since the 1970s it has been known that elevated levels of free circulating DNA can be detected in blood in patients with malignant disease [46]. It was also shown in the same paper that the levels correlated with metastatic status and that the levels decreased after therapy. Chun et al. described 2006 that plasma DNA level is predictive and highly accurate to assess the risk of positive biopsy outcome in prostate cancer. Increased levels of cDNA have also been shown in patients with metastatic disease, although they also found elevated levels in patients with benign prostatic hyperplasia [47]. Papadopoulou et al. showed that cell-free DNA could be used to distinguish between patients with prostate cancer and those not diagnosed with the disease [48]. Cherepanova et al. has also shown that patients with prostate cancers have elevated levels in blood in comparison to patients with benign diseases of the prostate [49]. The utility of detecting tumor-specific rearrangements in plasma is currently limited by the heterogeneity of solid tumors. In an attempt to map structural variation in breast cancer, none of 24 tumors harbored any identical rearrangements [50]. Therefore, for most cancer types, the primary tumor needs to be investigated first, which limits the possibility to use circulating DNA for early detection. Although it would be possible to detect individual-specific tumor rearrangements directly in plasma or serum it is not practically feasible due to costs since tumor DNA constitutes <30% of the total amount of circulating nucleic acids [51]. Unlike other adenocarcinomas, prostate tumors harbors a rearrangement (TMPRSS2-ERG gene fusion) that occur in 50% of cases [52]. These rearrangements may be detectable in blood and could be of diagnostic and perhaps also of prognostic value. Further studies are needed to assess this as a clinically useful marker.

**miRNA**

MikroRNAs are small, up to 22 nucleotides long, non-coding functional RNAs. It is estimated that 1 000 such small RNAs exist and they are all expressed in a tissue specific manner suggesting that they could be used as markers for various conditions. miRNAs are known to regulate gene expression. Porrka et al. identified 51 different miRNA being either up- or down from an expression profile of 319 genes encoding miRNA in prostatic cancer tissue as compared to normal prostatic tissue. Twenty two of these were downregulated in all prostate cancer samples and 15 were only downregulated in castration resistant specimens. It has also been demonstrated that miRNA profiles were consistent with prostate cancer disease process [53]. miRNA plays a biological role that may imply their correlation with diagnosis and therapeutic outcome, and miRNA are possible to detect in plasma samples.

**Identification of somatic mutations in prostate tumors**

With the introduction of massive parallel sequencing it is now possible to screen for somatic mutation in the entire coding parts of the genome (all genes) or even the entire genome in 10–100 of tumors. This has been demonstrated to be successful in several other tumors as breast cancer, colon cancer and lung cancer in which several new key genes and pathways have been identified. If protein products from these altered genes are secreted in either the urine or the bloodstream new biomarkers might be identified.

**Conclusion**

There are several promising new “genetic” markers that in the near future might be used in clinical routine. These markers are easy to measure and stabile over time. We foresee the following areas as most promising:

1. **Identifying high-risk populations using a combination of prostate cancer susceptibility alleles (SNPs).**

   Individually, each risk variant has a modest effect on disease risk and they will clearly not be useful for individualized risk prediction. However, risk profiles based on a combination of risk variants lead to an appreciable increased risk of disease [32] and there is potential for the predictive power to increase considerably as more risk variants are detected [54]. Combining the first 28 prostate cancer SNPs in the Swedish CAPS study, the top 8% of the population had three times of more increased lifetime risk of prostate cancer. These high-risk men can be selected for targeted screening or chemoprevention.

2. **Using prostate cancer susceptibility alleles (SNPs) in current risk calculators for prostate cancer.**

   These risk variants might be used in combination with current risk calculation aiding clinicians when to do prostate biopsy or not.

3. **The identification of new prognostic markers both germline genetic variants and other biomarkers based somatic mutations in the tumors are highly warranted.**

   However the challenge is not only to identify new biomarkers but the real test is to validate
new biomarkers in several large well-characterized patient populations. This validation must be done together will all other known biomarkers at the same time as it not likely that one single marker is enough, but a panel of different markers. Today 2010 there are over 19 000 publications in the area of biomarkers and prostate cancer, but only one biomarker, PSA, is used in the clinic today!

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References


Introduction

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We are facing tremendous dilemmas in the field of prostate cancer detection and treatment. Of all prostate cancers diagnosed, many do not need treatment while others are lethal, even when found and treated in a localized clinical stage. The main challenge today is not so much how to treat, but whom to treat.

Can we reliably identify patients who do not need aggressive treatment? Do we have the right tools at hand to identify lethal prostate cancer? As clinicians, we rely on clinical factors such as prostate-specific antigen (PSA), clinical stage and biopsy Gleason score. Based upon those, patients can be classified using risk stratification models of whom the D’Amico risk groups are amongst the most frequently used. Such models are convenient for physicians, as treatment guidelines are built around them. Nevertheless, these risk stratification models fail to provide accurate risk predictions. Improved, individual risk predictions can be achieved using nomograms. However, no matter how refined these tools are, they only provide statistical estimations with a certain degree of uncertainty. What we really need is individualized patient care using patient-specific tools.

Ways to overcome these hurdles have emerged in the last couple of years.

Imaging modalities have improved tremendously. Multiparametric magnetic resonance imaging (MRI) not only delivers extremely detailed anatomical images, but also provides tissue-specific information by using magnetic resonance (MR) spectroscopy, dynamic contrast enhanced (DCE) MRI and diffusion weighted (DW) MRI. These modalities help us to discern benign from malignant tissues. Furthermore, our understanding of prostate cancer biology has improved tremendously. Based upon this knowledge, novel positron emission tomography (PET) tracers like $^{18}$F-FDHT, probing the androgen (AR) signaling axis, have been developed. The role of MRI and PET will be discussed in detail in the papers by Heijmink and Fox.

Besides imaging, even more individualized information can be gained from the tumor itself. Serum PSA still is the most important biomarker for the detection and follow-up of prostate cancer. PSA based screening can reduce disease specific mortality but coinciding unnecessary testing and over diagnosis warrant further research for more specific biomarkers. Attractive, because non-invasive, are urine-based tests. Numerous, very interesting candidate tumor markers have been identified and are presently being tested. The prostate cancer antigen 3 (PCA3) test, the TMPRSS2–ERG fusion gene and their combination have been subject of many studies showing encouraging results. Finally, most of the information can be derived from the cancer tissue itself. On one side, correct tissue handling and reporting after biopsy taking or surgical removal is vital, as outcome prediction and thus tumor marker validation is fully dependent on this. On the other side, careful tissue collection and internal quality control of the tissues is of extreme importance in the development of tumor marker development. Those issues will be further elaborated in the contributions by Roobol, Berney and Montironi.

Better identification of those patients who do not need treatment and those who harbor a potentially lethal form of prostate cancer have a far-reaching impact on global healthcare. Undoubtedly, further development of modern imaging modalities and tumor markers are key in this process and will change the face of prostate cancer management forever. Truly individualized prostate cancer care is on the horizon.
State-of-the-art uroradiologic imaging in the diagnosis of prostate cancer

STIJN W. T. P. J. HEIJMINK, JURGEN J. FÜTTERER, STEPHEN S. STRUM, WIM J. G. OYEN, FERDINAND FRAUSCHER, J. ALFRED WITJES & JELLE O. BARENTSZ

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Abstract

In the diagnostic process of prostate cancer, several radiologic imaging modalities significantly contribute to the detection and localization of the disease. These range from transrectal ultrasound (TRUS) and magnetic resonance imaging (MRI) to positron emission tomography (PET). Within this review, after evaluation of the literature, we will discuss the advantages and disadvantages of these imaging modalities in clarifying the patient’s clinical status as to whether he has prostate cancer or not and if so, where it is located, so that therapy appropriate to the patient’s disease may be administered. TRUS, specifically with the usage of intravenous contrast agents, provides an excellent way of directing biopsy towards suspicious areas within the prostate in the general (screening) population. MRI using functional imaging techniques allows for highly accurate detection and localization, particularly in patients with prior negative ultrasound guided biopsies. A promising new development is the performance of biopsy within the magnetic resonance scanner. Subsequently, a proposal for optimal use of radiologic imaging is presented and compared with the European and American urological guidelines on prostate cancer.

With a total of 217,730 new cases estimated for 2010, prostate cancer (PC) now accounts for 28% of all new male cancers diagnosed in the USA [1]. In their lifetime, one in six men will be clinically diagnosed with having PC, although many more men are found to have histological evidence of PC at autopsy [2–4]. Presently, approximately 1 in 10 men will die of PC [5,6]. The ever-aging population and wider spread use of the prostate-specific antigen (PSA) test [7,8], as well as the tendency to apply lower cut-off levels for this test [9], will further increase the diagnosis of this disease [10].

An elevated PSA level, abnormal changes in PSA level (i.e. PSA dynamics) such as PSA velocity or doubling time, or an abnormal digital rectal examination are biologic indicators signaling an increased risk of PC. With the improvement and wider range of curative therapies, detection and subsequent exact localization of PC have become increasingly important because of their influence on treatment strategy [11,12]. Two such affected treatments are laparoscopic (robotic) radical prostatectomy and intensity-modulated radiation therapy (IMRT [13]). The urologist’s inability to palpate the operating field during laparoscopic surgery makes it even more crucial to know where the cancer is located. Similarly, the urologist must know whether the cancer is near a neurovascular bundle since this affects the decision of whether or not to perform nerve-sparing prostatectomy [14]. IMRT also necessitates accurate PC localization. While giving a standard dose to the prostate, a higher (i.e. boost) dose can be given to any dominant intraprostatic lesion(s) as these lesions regularly appear to be the sites of recurrent disease [15]. Furthermore, precision radiation dosimetry will decrease radiation complications, particularly...
rectal wall toxicity [16], thereby likely diminishing the development of post-radiation rectal cancer [17].

In order to determine the optimal treatment for the individual patient, it is necessary to evaluate all patient and cancer characteristics. Most often used for this purpose are laboratory values (PSA level and dynamics), the results of digital rectal examination (clinical staging), and histopathological prostatic biopsy findings (Gleason score). However, imaging may play an important role in detecting and localizing areas most reflective of the actual aggressiveness of the cancer. This directly influences the assessment of the patient and may lead to important changes in treatment strategy which can mean the difference between treatment success and failure.

Currently, a spectrum of imaging modalities is available to clinicians for tackling detection- and localization-related problems. To provide optimal and cost efficient patient care, these techniques should be used in the appropriate clinical context to aid clinicians in detecting and localizing PC.

This review 1) presents an overview of the currently available imaging methods to aid in PC detection and localization. 2) Additionally, a scheme is proposed for optimal evidence-based use of imaging in detecting and localizing prostate cancer and a critical comparison is made between this scheme and the most recent guidelines as put forward by the American Urological Association (AUA) and European Association of Urology (EAU).

**Literature search**

Relevant articles were retrieved using combinations of both Medical Subject Headings (MeSH) and free search terms in the MedLine® (WebSPIRS Version 5.12, Build 20060224, Ovid Technologies) and Pubmed (U.S. National Library of Medicine) online search engines.


Reference lists of selected articles were further analyzed for relevant articles.

**Prostate cancer detection and localization in reference to prostate anatomy and essential prostate cancer characteristics**

In order to effectively apply the various imaging modalities, it is important to first understand both the normal prostate anatomy and the distribution and intrinsic characteristics of PC.

**Normal anatomy as related to PC localization**

On the basis of its embryological origins, the prostate is anatomically divided into three zones that are eccentrically located around the urethra: the innermost transition zone (TZ), the central zone, and the outermost peripheral zone (PZ) [18,19]. In older patients, the former two cannot be distinguished radiologically due to compression of the central zone by benign prostatic hyperplasia (BPH) in the TZ and together they are referred to as the central gland; this as opposed to the outer gland, which comprises the PZ. Furthermore, the prostate is craniocaudally divided into apex (the caudal one-third), mid-gland, and base (the cranial one-third).

**Anatomical distribution of PC**

Up to 70–80% of PC is located in the PZ [20] and overall analysis of these cancers has shown homogeneous distribution across the entire PZ [21], with over half of the prostates containing two or more distinct cancer foci [22]. Nevertheless, while up to 20–52% of all PC originate in the TZ, only a small (3.6–25%) percentage of these cancers [21,23] occur solely in the TZ as many will have concurrent PZ cancer foci [20,24,25].

**Pathological grading of PC aggressiveness**

Presently, the most widely used histological scaling system for PC aggressiveness is the Gleason score [26,27], which consists of two numbers: a primary and secondary Gleason grade reflecting the two grades most frequent in the specimen. Each Gleason grade is assigned a value between 1 and 5, the higher numbers indicating a more aggressive cancer. The prognostic value of the Gleason grading system is well-documented [28,29].
Patients clinically at risk for prostate cancer: Radiological imaging to detect and localize primary PC

Transrectal ultrasound (TRUS): Reliable, although not perfect, daily-use modality

Grayscale TRUS. Today, in regular clinical practice, prostate biopsies are performed under TRUS guidance. Even though the traditional ultrasound appearance of PC is a PZ hypoechoic lesion (Figure 1A, B), other conditions such as prostatitis and prostatic intraepithelial neoplasia may also present as hypoechoic lesions [30,31]. It is important to note that over 40% of PC lesions are isoechoic (Figure 1C, D) while only 5% are hyperechoic [32]. Therefore, targeting only hypoechoic areas is not an optimal approach for successful PC detection [33] and various biopsy protocols that sample tissue at standard locations (i.e. systematic biopsy) within the prostate have become the most common biopsy technique [34]. The number of cores taken per session varies across institutions. Recently, however, emphasis has been put on adequate tissue sampling from more laterally located PZ regions [35,36] and on the relative unimportance of biopsying the central gland [37]. Despite the use of extended systematic biopsy protocols, there is still an approximately 20% chance that the Gleason score at prostatectomy will differ from that at biopsy to a clinically relevant degree [38]. Recently, it was observed that biopsies performed with an endfire probe obtained a significantly higher biopsy rate compared with side-fire probes [39]. PC detection rates have varied from 19–40% [40,41] and repeat biopsy sessions are often necessary [42]. Localization sensitivity varied widely between 39–75% (Table I).

Doppler TRUS. Because increased blood flow due to neovascularity is one of the characteristics of PC, this is a means of targeting lesions. In a study of 96 patients with lower urinary tract symptoms and PSA levels over 4 ng/ml [43], the degree of Doppler signal correlated with the microvessel density and Gleason score of a lesion. One study achieved Doppler imaging-based detection rates of 40% [44]. Power Doppler TRUS could improve the localization specificity [45]. However, a drawback of Doppler imaging is the high inter-observer variability [46,47], reflected in the widely spread sensitivity and specificity figures in the literature (27–98% and 46–84%, respectively) (Table I).

Figure 1. (A) Axial gray-scale transrectal ultrasound image (Aplio™, Toshiba) of the prostate of a 65-year-old man (PSA level 19.02 ng/ml, biopsy Gleason score 6, normal digital rectal examination). A hypoechoic lesion was observed in the right peripheral zone (arrows). (B) Histopathology of the prostatectomy specimen confirmed the presence of a Gleason 3 + 4 adenocarcinoma (T). (C) In another patient (69 years, PSA level 3.59 ng/ml, biopsy Gleason score 7, normal digital rectal examination), the axial gray-scale image did no show any echogenic abnormality while at histopathology (D) a number of cancer foci (T) were reported.
Contrast-enhanced TRUS. An innovation is the application of gas-filled microbubble contrast agents, such as Levovist® (Schering, Berlin, Germany) and SonoVue® (Bracco, Milan, Italy) [48]. These microbubbles remain intravascular, thereby enhancing the visibility of the vascular tree in and around the prostate. This improves the ability to detect PC and to thus target areas more representative of the aggressiveness of PC. In experienced hands, it is reported that compared with systematic biopsy, targeting only lesions with pathological enhancement after contrast administration requires less than half the number of biopsy cores to obtain the same diagnostic yield [49–51]. A recent randomized clinical trial comparing systematic biopsy and contrast-enhancement targeted biopsy confirmed these findings [52]. In addition, contrast-enhanced TRUS biopsies on average detected significantly more aggressive cancers compared with systematic biopsy. Therefore, we can speculate that by using this technique the difference in Gleason score between biopsy and prostatectomy specimens would most likely diminish. If the latter is confirmed by future studies, pre-therapeutic risk assessment of patients will increase in accuracy. Disadvantages of using contrast agents are the longer duration and higher degree of invasiveness of the examination; however, the risk of hypersensitivity to the substance is rare and most adverse events are minor and self-resolving [53]. Sensitivities and specificities of PC detection using contrast-enhanced TRUS varied between 48–94% and 46–88%, respectively (Table I). A preliminary study suggests that a 14-day pre-biopsy course of dutasteride, a dual 5α-reductase inhibitor, causes a relative high reduction in blood flow in healthy prostate tissue compared with cancer tissue and could increase the diagnostic yield of contrast-enhanced TRUS targeted biopsy [54].

Sonoelastography. Transrectal sonoelastography is a new non-invasive technique that analyzes the compression characteristics of prostate tissue. A study by König et al. of 404 men undergoing biopsies based on real-time sonoelastography revealed a detection rate of 37.4% [55]. In a comparative study, Pallwein et al. found a significantly higher per core detection rate for sonoelastography-targeted biopsy compared with systematic biopsy. Sonoelastography-targeted biopsy was 2.9 times more likely to detect cancer [56]. A drawback of the latter study was the heterogeneity of the population since more than half of the patients had already undergone one or more negative biopsy sessions. A study comparing real-time sonoelastography with radical prostatectomy reported a localization sensitivity of 88% [57].

<table>
<thead>
<tr>
<th>Imaging modality</th>
<th>Periods of publication, range</th>
<th>Number of patients, range</th>
<th>Accuracy (%)</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>PPV (%)</th>
<th>NPV (%)</th>
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Tables I. Overview of studies that examined the diagnostic performance of localizing prostate cancer by imaging modality.

PPV, positive predictive value; NPV, negative predictive value; MRSI, magnetic resonance spectroscopic imaging; PET, positron emission tomography; FDG, 18-fluorine-labelled 2-fluoro-2-deoxy-D-glucose, 11 C-choline, carbon-11 labeled choline.
Sonoelastography-based targeted biopsy improves the diagnostic yield it is not yet clear whether it can replace systematic biopsy [58]. Future randomized studies are required to determine the true value of sonoelastography in prostate cancer detection and localization.

**Computed Tomography (CT) scanning: Inadequate soft tissue contrast and radiation burden**

The literature search resulted in identifying only one recent study on the ability of CT scanning to document histologic PC sites within the prostate gland. This study revealed that contrast-enhanced helical CT scanning was able to detect only 58% of the 102 histologic PC sites documented by TRUS-guided biopsies in 25 patients [59]. In general, CT scanning has inadequate soft tissue contrast resolution to discern the subtle tissue changes due to PC (Figure 2A) and, therefore, should not be used for PC detection and localization. An additional disadvantage of CT is that it involves ionizing radiation.

**Endorectal Magnetic Resonance Imaging (MRI) and Magnetic Resonance Spectroscopic Imaging (MRSI): High soft-tissue resolution, radiation-free, but costly and time consuming**

T2-weighted imaging. Contrary to CT scanning, MRI has a high soft-tissue contrast resolution. The use of an endorectal coil (ERC) combined with other external coils at 1.5 tesla (T) increases soft-tissue contrast significantly and is now the accepted standard for MRI of the prostate [60]. A drawback is the extra time required for insertion and checking of the position of the disposable ERC, as well as substantial expense.

On MRI, PC typically appears as an area of low signal intensity within the brighter, healthy PZ using a T2-dominated sequence [61–63] (Figure 3A–E). In the central gland, PC is not as clearly discernable because the central gland generally has lower signal intensity than the PZ and is more inhomogeneous due to BPH-induced architectural changes that may mimic PC. A recent study showed that a homogeneous low T2 signal intensity and lenticular shape were significantly associated with presence of central...
gland PC [64]. It was reported that higher Gleason score cancers had lower signal intensities (relative to muscle) compared with low Gleason score cancers [63]. T2-weighted imaging can be performed in multiple planes or as a three dimension (3D) volume acquisition [65]. Comparing T2-weighted MRI with prostatectomy specimens, MR attained high (52–83%) sensitivities in PC localization, while specificities were somewhat lower (46–88%) (Table I).

A study that directly compared endorectal MRI with digital rectal and TRUS-guided biopsy localization revealed significant incremental value from MRI [66]. In patients subjected to multiple prior negative TRUS-guided biopsies, anatomical MRI by means of T2-dominated acquisition plays an important role. In this patient population, an 83% sensitivity and 50% positive predictive value for MRI have been established [67].

Postbiopsy hemorrhage causes areas of low signal intensity on T2-weighted imaging, thereby making prostate cancer detection more difficult. However, it was shown recently that the amount of hemorrhage was significantly lower in areas of cancer compared with healthy tissue [68].

MRSI. Additionally, MRSI (Figure 3G) can be added to the protocol to provide metabolic information based on the citrate, choline, and creatine levels, and their ratios within the prostate. This is highly informative since the ratio between choline and citrate alters during the transformation from healthy to malignant prostatic cells [69,70] and an increasing choline/H11001 creatine/citrate ratio was correlated with higher Gleason scores [71]. Presently, 3D MRSI of the entire prostate can be performed [72], thereby aiding in the diagnosis of central gland PC. The addition of 3D MRSI to MRI has increased localization accuracy, particularly by raising specificity up to 91% [73]. However, a limitation of MRSI is its low spatial resolution. Compared to systematic biopsy, PC localization by means of MRI and MRSI was found to be more sensitive (67% and 76% versus 50%) but less specific (69% and 57% versus 82%) than systematic biopsy [74]. With whole-mount section histopathology as standard of reference, 3D MRSI had a significantly larger area under the receiver operating curve (AUC) of 0.80 in localizing cancer, compared with T2-weighted MRI [75]. Adding the combination of T2-weighted imaging and MRSI to clinical data was shown to have the highest accuracy (AUC 0.85) in predicting the probability of a patient having insignificant prostate cancer [76], significantly higher than that of clinical nomograms. A recent multi-institutional American College of Radiology Imaging Network study raised doubts on the additive value of MRSI over T2-weighted imaging alone [77]. However, potential factors for this result were the selected
prostatectomy population, the small average cancer focus size, and the inclusion of centers without any previous MRSI experience.

**Diffusion weighted imaging (DWI).** DWI is a novel non-invasive technique that measures the fractional anisotropy of water molecules within the prostate which is expressed in apparent diffusion coefficient (ADC) mapping. Thereby, cancer tissue is deemed to result in a more restricted movement of water molecules and thus producing lower ADC values (Figure 3C, D) [78,79]. A recent study in 38 patients, performed at 1.5T with an ERC observed that the mean ADC values of regions of interest placed within prostate cancer tissue was significantly lower than those placed within healthy prostate tissue [80]. In preliminary studies, combining this technique with MRSI [80] or T2-weighted imaging [81] significantly improved the localization accuracy. A recent study in 37 patients revealed a significant increase in sensitivity from 51% for T2-weighted imaging to 71% for combined T2-weighted and DWI reading [82]. In a recent multiparametric analysis, DWI was the best-performing parameter [83]. Preliminary studies at 3T show promising results [84–86]. The b value used appears to affect the PC localization accuracy, as in a preliminary study imaging with a b value of 2000 s/mm² was shown to have a significantly higher accuracy compared with 1000 s/mm² [87], possibly due to a fall in the signal-to-noise ratio. At biopsy, DWI may aid in differentiating between low-risk and high-risk patients [88].

**Dynamic contrast-enhanced MRI [83,89].** To further enhance localization accuracy of MRI, one may use contrast agents. Dynamic contrast-enhanced endorectal MRI, in which the contrast agent concentration is followed in time [90], is able to

Figure 4. A comparison of the image quality between axial endorectal coil (ERC) MRI at 1.5 T (A) and 3 T (B) in the same patient (age: 58 years, PSA level: 2.7 ng/ml, Gleason biopsy score: 6, normal digital rectal examination). The visibility of the internal architecture of the central gland (*) increased and the capsule (arrowheads) is better delineated at a field strength of 3 T and the tumor (T) as outlined by the histopathology (C) is also better appreciated (arrows).
discriminate between healthy prostatic tissue and PC [91]. Early contrast enhancement and high (relative) peak enhancement are the most accurate predictors of PC of the PZ, while fast washout of contrast agent and high permeability of the blood vessels (Figure 3B, F) are most sensitive for central gland PC [92,93]. A recent study showed that the AUC for localizing PC increased significantly from 0.68 with regular anatomical MRI to 0.91 by applying contrast agent [75]. However, limitations of using contrast agents are the higher costs and possible adverse reactions, of which the most serious, anaphylaxis, is rare [94,95].

**Multiparametric imaging.** Combining any number of these techniques (‘multiparametric imaging’) has shown to increase the ability of MRI to detect and localize prostate cancer (Figure 3A–G) [96–98].

**High-field imaging.** An important future direction is the use of higher magnetic field strengths (e.g. 3T) [99–101] (Figure 4). Compared with body array coil MRI, the higher resolution obtained with ERC MRI at 3T significantly improved PC localization accuracy [102].

**Biopsy.** Another development is to directly biopsy the prostate by means of MRI [103]. Preliminary results of direct MR-guided transrectal biopsy of suspicious lesions on pre-biopsy MRI in patients with prior negative or inconclusive TRUS-guided biopsy results demonstrated the feasibility of MR prostate biopsy without complications. Nevertheless, disadvantages of the biopsy device are its limited reach, particularly towards the base of the prostate, and procedure duration [104]. In a study of 68 patients with at least two prior negative TRUS biopsy sessions, MR guided biopsy established cancer in 59% [105].

**Positron Emission Tomography (PET) Scanning:**

**Metabolic information yet not sufficiently discriminatory from benign disease**

**FDG.** The utility of PET scanning with fluorine-18-labelled deoxyglucose (FDG) in detecting PC is compromised by the relatively low uptake of FDG by prostate cancer cells [106] and significant overlap with marker uptake by BPH. Moreover, reports of FDG uptake correlating with PC aggressiveness have been conflicting, although FDG uptake was substantially higher in metastasized compared to organ-confined primary cancers [107]. A further drawback is that the normal urinary FDG excretion results in high bladder activity which obscures pathological FDG uptake in the prostate. Generally, FDG PET is not recommended for evaluation of the prostate as sensitivities are as low as of 4–64% with a specificity in the order of 50% [108–110].

**11C-choline.** Another tracer, carbon-11-labelled choline (11C-choline), accumulates in prostatic cells and has the advantage that, unlike FDG, it is not excreted via the urinary tract, and thereby does not influence the visualization of the prostate [111]. Furthermore, the prostate is the only organ in the pelvis to accumulate 11C-choline. The 11C-choline uptake was higher in PC compared with BPH, but the difference was not significant [112]. In a direct comparison between 11C-choline PET and MRSI, a significant linear correlation was observed between the maximum standardized uptake value (SUV) of 11C-choline and the MRSI metabolite ratios. Also, 11C-choline PET was more accurate than MRSI in accurately predicting the laterality (i.e. left- or right-sidedness) of the cancer: 81% (13/16) versus 50% (8/16), respectively [113]. Recently, it was shown that 11C-choline preferentially detected more aggressive prostate cancer foci [114]. Drawbacks are the high costs of 11C-choline and the short half-life of 11C-choline (20 minutes). This latter precludes application of 11C-choline in centers without cyclotrons. Nevertheless, the results of the first two studies combining 11C-choline PET/CT scanning were encouraging, with a sensitivity of 66% and specificity between 81–84% on a sextant basis [115,116]. However, the high rate of false negative findings was a concern. A direct preoperative comparison between 11C-choline PET, FDG PET, and MRI in 43 patients showed that 11C-choline outperformed FDG PET in localizing prostate cancer but that MRI was superior to both [117].

**Other radiopharmaceuticals.** A preliminary PET/CT study using fluoro-18-choline (18F-choline) demonstrated its feasibility, but reported its inability to distinguish cancer from BPH [118]. In a small population of both primary and recurrent disease, dual-phase 18F-choline showed that areas of malignancy had stably high or increasing uptake while benign areas had decreasing uptake [119]. Thereby, this technique may aid in differentiating malignant from benign prostatic tissue. In a double-tracer study, 11C-acetate PET was more sensitive than FDG, showing consistently increased uptake in PC lesions [120]. A further advantage was that 11C-acetate did not
accumulate in the urine. Again, a considerable uptake overlap was described between normal prostatic tissue, BPH and PC [121].

ProstaScint® scanning: No place in regular clinical practice

ProstaScint® (Cytogen, Princeton, NJ) is an Indium-111 labeled monoclonal mouse antibody specific for prostate-specific membrane antigen. A significant association between the PSA level and detection of ProstaScint® activity in the prostate was reported [122]. A recent study revealed sensitivities between 37–87% and specificities between 0–50%, concluding that the scan could not be used to reliably localize prostate cancer foci within the prostate [123]. In a single study of only seven patients in which the results of ProstaScint® fusion with CT scanning were correlated with systematic biopsy a sensitivity and specificity of 79% and 80%, respectively, were found [124]. In 47 of 51 (92%) preoperative patients at high risk of metastatic disease an increased ProstaScint® activity in the prostate was observed [125].

A drawback is that the antibodies clear slowly from the vasculature and muscle. Blood and bone marrow activity may cause false-positive findings. In regular clinical practice, this modality has no place in primary prostate cancer detection and localization.

Conclusions and discussion

In summary, new developments in ultrasound imaging (Doppler imaging and particularly the application of contrast agents) have proved capable of increasing the PC detection rate with fewer biopsy cores necessary as well as detecting relatively more aggressive cancer foci. This is a substantial improvement for the patient, who will have to undergo fewer biopsies. In addition, treatment guidance is improved since more representative areas are discovered at biopsy and thus the subsequent diagnostic process can be more accurately performed. TRUS remains the primary imaging tool because of its ease-of-use and its role in guiding prostate biopsy. However, TRUS accuracies varied widely among studies, in part due to the inherent high inter-observer variation, particularly in Doppler imaging.

MRI achieves high accuracy rates, particularly when functional information from dynamic

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Figure 5. Proposed scheme for optimal use of imaging in patients at risk of prostate cancer. Abbreviations: PSA, prostate-specific antigen; DRE, digital rectal examination; TRUS, transrectal ultrasound; PPA, pelvic phased-array coil.
contrast-enhanced MR and MRSI are added. A multiparametric approach was shown to optimize the diagnostic accuracy. This compensates for the longer examination time and the discomfort of the use of an ERC. Nevertheless, on a cost-effectiveness basis, MRI cannot be performed in all patients at risk of PC [126]. In patients with one or more prior negative TRUS-guided biopsy sessions and continuing suspicion of PC, MRI can provide valuable additional information for PC detection and localization and thereby reduce the future number of biopsies the patient must undergo. Direct MRI guided biopsy is a novel method of performing targeted prostate biopsy.

CT scanning does not play a role in PC detection or localization because of its low soft-tissue resolution and radiation burden. This also applies to PET scanning due to its high costs and invasive nature, as well as the availability of alternative imaging modalities. PET scanning may possibly be used in instances in which TRUS-guided biopsies are negative and absence of evidence of PC on MRI. In addition, combining or fusing PET scanning with, for instance, MRI may be of additional value.

Proposals for optimal usage of imaging

Scheme

Based on the abovementioned, the authors propose the following scheme for patient care in patients at risk for prostate cancer (Figure 5).

Comparison with AUA and EAU guidelines [127–129]

Both associations recognize that the stage migration during the PSA era necessitates more accurate techniques in detecting and localizing prostate cancer. Use of TRUS to guide biopsy is regarded as the standard of reference. The EAU’s guideline, however, does not mention any contrast-enhanced Doppler imaging based biopsy strategies. This is in contrast to the data presented in our review. Neither CT scanning nor MRI is recommended or mentioned in relation to prostate cancer diagnosis. The latter is in contrast with our proposal in which MRI is used in patients in whom no cancer was found on first TRUS biopsy but with persistently high or rising PSA levels.

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State-of-the-art radiologic imaging in prostate cancer


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State-of-the-art radiologic imaging in prostate cancer


ORIGINAL ARTICLE

Developing imaging strategies for castration resistant prostate cancer

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Abstract
Recent advances in the understanding of castrate-resistant prostate cancer (CRPC) have lead to a growing number of experimental therapies, many of which are directed against the androgen-receptor (AR) signaling axis. These advances generate the need for reliable molecular imaging biomarkers to non-invasively determine efficacy, and to better guide treatment selection of these promising AR-targeted drugs. Methods. We draw on our own experience, supplemented by review of the current literature, to discuss the systematic development of imaging biomarkers for use in the context of CRPC, with a focus on bone scintigraphy, F-18 fluorodeoxyglucose (FDG)-positron emission tomography (PET) and PET imaging of the AR signaling axis. Results. The roadmap to biomarker development mandates rigorous standardization and analytic validation of an assay before it can be qualified successfully for use in an appropriate clinical context. The Prostate Cancer Working Group 2 (PCWG2) criteria for “radiographic” progression by bone scintigraphy serve as a paradigm of this process. Implemented by the Prostate Cancer Clinical Trials Consortium (PCCTC), these consensus criteria may ultimately enable the co-development of more potent and versatile molecular imaging biomarkers. Purported to be superior to single-photon bone scanning, the added value of Na18F-PET for imaging of bone metastases is still uncertain. FDG-PET already plays an integral role in the management of many diseases, but requires further evaluation before being qualified in the context of CRPC. PET tracers that probe the AR signaling axis, such as 18F-FDHT and 89Zr-591, are now under development as pharmacodynamic markers, and as markers of efficacy, in tandem with FDG-PET. Semi-automated analysis programs for facilitating PET interpretation may serve as a valuable tool to help navigate the biomarker roadmap. Conclusions. Molecular imaging strategies, particularly those that probe the AR signaling axis, have the potential to accelerate drug development in CRPC. The development and use of analytically valid imaging biomarkers will increase the likelihood of clinical qualification, and ultimately lead to improved patient outcomes.

About 32,050 men will die of prostate cancer in the United States in 2010 [1], the majority after the transition to a castration resistant state, the invariably lethal form of the disease [2]. The hallmark of castration resistant prostate cancer (CRPC) is evidence of tumor growth despite castrate levels of serum androgens [3]. Several important insights into the molecular pathogenesis of CRPC, including mechanisms of androgen receptor (AR) signaling, have now been elucidated, leading to the development of new and more potent therapies targeting the AR pathway [4]. These novel agents include inhibitors of CYP17, an enzyme required for androgen synthesis; direct AR-antagonists that prevent nuclear translocation; inhibitors of HSP90 which protects AR from degradation; inhibitors of histone deacetylases which is required for optimal AR mediated transcription, and tyrosine kinase inhibitors. A number of these drugs appear to durably repress disease growth, even after numerous prior hormonal treatments and chemotherapy [5–7].
Despite these advances there remains a pressing need for continued drug development in this arena. The difficulty in introducing new prostate cancer drugs to the market is well documented. Among the challenges confronted is the disease’s heterogeneous clinical course, which complicates eligibility and response criteria for clinical trials. This problem has been addressed in part through the use of the “clinical-states” framework for organizing the natural history of the disease [8], but substantial impediments persist. Notably, the chief manifestation of metastatic disease (i.e., bone metastases) is notoriously difficult to monitor. Furthermore, multiple mechanisms have been implicated in AR signaling reactivation, which in part accounts for the non-uniform response to AR directed therapy [9]. These obstacles highlight the need for biomarker development in CRPC. Molecular imaging with PET has the potential to address this need through its versatility, non-invasiveness and quantitative capabilities.

The biomarker roadmap

A biomarker is “a factor that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention” [10]. Biomarkers can be subclassified into various categories, including: (1) predictive/risk markers, (2) pharmacodynamic markers, and (3) biologic response/progression markers. The roadmap for biomarker qualification requires rigorous standards for trial design, data capture, reporting, and analysis [11]. Imaging biomarkers, like any assay, should be measured in an analytic test system with well-established performance characteristics. The validated imaging test must then undergo qualification, which is the evidentiary process of linking a biomarker with a biological process or clinical endpoint, in other words, establishing “fitness for purpose” [12]. A validated imaging test qualified for use in one disease entity, or cancer-type, may not qualify for use in other settings. Similarly, an assay that is validated for predicting risk may not be useful as a marker of progression.

Imaging biomarkers of response/progression are often used as endpoints in oncologic trials. Overall survival (OS) is regarded as the gold standard of clinical endpoints, defined as the time from the start of study treatment to the date of death of any cause. Because survival endpoints can take years to reach, regulatory authorities, such as the Federal Drug Administration (FDA) in the United States, allow for accelerated approval in certain life-threatening diseases. Accelerated approval is usually based on an endpoint “reasonably likely to predict” clinical benefit. It is distinct from a surrogate endpoint which, as defined by Prentice, must meet the following criteria: 1) treatment has a statistically significant impact on the true endpoint; 2) treatment has a statistically significant impact on the surrogate endpoint; 3) the surrogate endpoint has a statistically significant impact on the true endpoint; and 4) the full effect of the treatment on the true endpoint should be captured by surrogate endpoint [13]. Surrogate endpoints commonly incorporated in oncologic clinical trials include objective response rate (ORR), time to progression (TTP) and progression free survival (PFS) [14]. ORR is defined as the proportion of subjects with a predefined amount of reduction in tumor burden, often assessed on the basis of radiologic criteria, as in Response Evaluation Criteria in Solid Tumors (RECIST) [15]. Unfortunately, RECIST has a limited role in CRPC drug development, and is only applicable to the assessment of soft-tissue disease [16]. TTP is often a composite endpoint defined as the time from the start of study treatment to the date of the first documentation of objective tumor progression/relapse, initiation of other cancer therapy, or death as a result of the tumor, whichever comes first. PFS is similar to TTP, but also includes death from any cause. These composite endpoints face difficulties in terms of data collection and analysis, and are susceptible to subjective bias. The precise definition of TTP and PFS, specifically, the measurement of progression or relapse, continues to evolve as new biomarkers for progression are established.

The broader oncology community in the United States is attempting to address the need for qualified biomarkers through collaborative efforts between the FDA, the National Cancer Institute (NCI) and other regulatory bodies, with the formulation of consortia such as the Oncology Biomarkers Qualification Initiative (OBQI) and the AACR-FDA-NCI Cancer Biomarkers Collaborative [17]. Meanwhile, tangible measures to improve patient outcomes have been undertaken by the prostate cancer clinical trials community. Specifically, the Prostate Cancer Clinical Trials Consortium (PCCTC) was created in 2006, supported by the Prostate Cancer Foundation and the US Department of Defense, for the purpose of carefully designing and conducting phase 1 and 2 multicenter clinical trials. Memorial Sloan-Kettering Cancer Center (MSKCC) is the coordinating center for the consortium, which is currently comprised of 13 prostate cancer research centers.


**Bone scintigraphy: a “Gold Standard”?**

The PCWG2 recognized that trial eligibility and end points based solely on the presence and regression/progression of measurable lesions (target lesions as defined by RECIST) would shift the emphasis from bone metastases (considered unmeasurable) to lymph nodes, which occur in only 20-25% of prostate cancer patients. Bone metastases, on the other hand, are the primary cause of morbidity and mortality in the CRPC population, developing in 80-90% of patients [20]. Typical sequelae include pain, hematologic disorders, fracture, and neurologic compromise [21]. Given the predominance of bone involvement, the uncertainty surrounding the clinical significance of PSA as a marker of “response” or progression [22], and the increased availability of cytostatic agents, reliable methods to ascertain progression in bone are of increasing importance.

The X-ray has been used for the detection of metastatic prostate cancer in bone since the early days of “skiagraphy” [23]. The plain radiograph still plays an important role in the assessment of skeletal metastases, but has largely been supplanted by bone scintigraphy [24]. 99mTc-methylene diphosphonate (MDP) and similar radiolabeled phosphate analogues, introduced for bone scanning in the early 1970’s, are incorporated into the hydroxyapatite crystalline lattice and collagen matrix [25]. Uptake is a function of blood supply, rate of bone turnover or osteoblastic activity, quantity of mineralized bone, capillary permeability, fluid pressure and local acid/base balance. Bone scanning is highly sensitive for blastic metastases and allows for quick appraisal of the entire skeleton. It is widely available, relatively inexpensive and reimbursable, making it the preferred modality for assessing bone metastases. The main shortcoming of the bone scan is that it depicts secondary changes rather than directly imaging the tumor. As such, early metastases may be missed and sensitivity for detection of osteolytic disease is lacking. Regression of disease is nearly impossible to verify on account of lingering uptake in healing bone, despite eradication of tumor. Moreover, response assessment is confounded by the flare phenomenon, a major obstacle that can occur up to 12 weeks post effective treatment [26]. Thus, the declaration of scintigraphic progression during this period should be restrained in comparison to later time points. Changes in intensity or minor changes in extent of existing lesions are non-specific features and should not be considered determinants of progression at any time point.

Recognizing the variability in bone scan interpretation between observers, the PCWG2 emphasized the need for standardization of reporting, as a requirement.

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**Figure 1.** Example of scintigraphic progression per PCWG2: Two or more new lesions appear on first post-12 week assessment (arrows), followed by at least 2 additional new lesions on confirmation scan more than 6 weeks later.
to begin studies designed to generate the evidence toward qualification. The simplified approach recommended by the PCWG2 requires the emergence of two or more unequivocal metastatic lesions, beyond the flare period, in order to declare progression. New lesions seen at the first post-flare window reassessment require a confirmatory scan performed 6 or more weeks later demonstrating at least two additional new lesions (Figure 1). PCWG2 discourages the performance of follow-up bone scanning prior to 12 weeks after start of therapy, unless clinically indicated. These criteria have been operationalized through standardized data collection forms that are now incorporated into PCCTC clinical trials (Figure 2). These forms enabled the analytical validation of the PCWG2 progression endpoint so that the clinical qualification process in the context of phase III clinical trials for the novel antiandrogens, abiraterone and MDV3100 could begin.

We hypothesized that the utility of the bone scan could be strengthened by employing a quantitative measure of disease burden to integrate into statistical analyses. An example of such a metric is the Bone Scan Index (BSI) [27], which measures the total skeletal tumor burden in ordinal terms. Developed by Larson and colleagues in the 1990’s, the BSI was shown to be prognostic for survival, and the role in the assessment of “response” and “progression” is under investigation [28,29]. The manual BSI measurement while time consuming and tedious, was shown to be highly reproducible. Automated methods for interpreting bone

Figure 2. The analytically validated PCWG2 bone scan assessment form currently undergoing clinical qualification in phase 3 registration trials.
scans that can produce results within seconds and with 100% reproducibility are under development and may prove useful for calculating the BSI [30–32]. Though promising, the automated platform itself must first be validated against the manual technique before its prognostic value can be determined.

Additional efforts to standardize the assessment of bone disease include the proposed criteria from MD Anderson (MDA), the PET Response Criteria in Solid Tumors (PERCIST) by Wahl and colleagues, and the updated RECIST 1.1 publication [33–35]. The latter only addresses osteolytic lesions with soft tissue components that are infrequent in prostate cancer. The MDA report proposes a multimodality approach that incorporates bone scan, X-ray, CT and MRI. The recently proposed PERCIST is presumed to apply equally to bone and soft tissue lesions alike, given that the functional information derived from PET is largely independent of tissue type. As was the case with the proposed PCWG2 bone scan progression criteria, both the MDA and PERCIST proposed criteria will require analytical validation before they can begin qualification testing. Until PET and other molecular imaging biomarkers are qualified in the context of CRPC, rigorously standardized bone scanning may for now serve as the groundwork by which these other modalities are tested.

**Positron Emission Tomography (PET)**

Several PET tracers have shown promise as potential biomarkers in CRPC [36]. 18F-Sodium Fluoride (NaF) is a high affinity bone seeking agent that if employed in lieu of the single photon 99mTc agents could enhance traditional bone scanning. 18F-FDG-PET is a marker of tumor glycolytic rate (Warburg effect [37]) with established benefits in several contexts [38–40], while the role in prostate cancer management is still considered “investigational”. Additional metabolic agents such as 18F-FACBC, 18F-Choline, and 11C-methionine have been studied extensively in prostate cancer, but are beyond the scope of this review. Finally, 18F-FDHT and novel tracers such as 89Zr-J591 are under development for probing the AR signaling axis.

18F-sodium fluoride (NaF) PET

Ironically, NaF was developed for bone scanning as a single photon agent prior to the advent of the 99mTc phosphonates [41], but is being reinvestigated as a PET tracer. Possible advantages of NaF over 99mTc-agents are attributable to the higher affinity for osteoblastic activity, and the superior imaging characteristics of PET. Several studies have suggested that NaF performs better than 99mTc-agents for the detection of metastases, particularly when combined with the anatomic information derived from CT. Even-Sapir et al. compared planar bone scintigraphy, bone SPECT, NaF PET, and NaF PET/CT in patients with localized high-risk or metastatic prostate cancer. The reported sensitivity and specificity for detection of bone lesions was higher for NaF PET/CT (100% and 100%, respectively) than for planar bone scanning (70% and 57%), bone SPECT (92% and 82%) or NaF PET (100% and 62%) [42]. These results seem to favor NaF PET/CT over traditional bone scanning; however, the study included a mixed population and did not include a standard comparator. Further investigation of NaF in the context of rigorously controlled prospective trials is needed before it can be recommended to replace the single photon bone scan, which is less expensive and more widely available.

**18F-FDG: Imaging tumor glycolysis**

Despite the apparent advantage of NaF-PET over single photon bone scanning, it remains an indirect

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**Figure 3.** (A). Kaplan-Meier survival curves for 22 patients with low (≤6.10) and 21 patients with high (≥6.10) SUVmax, p = 0.002. Reprinted with permission from Meirelles et al. [29]. (B). 69-year-old male with CRPC. MIP and axial images shows markedly FDG-avid bone lesions in the thoracic spine, SUVmax 16.8. The patient died within 18 months after the scan.
method of imaging bone metastases. FDG, on the other hand, has the benefit of directly assessing tumor metabolism, and is useful for both bone lesions and soft tissue lesions. These characteristics raise the possibility of developing a “response” biomarker that occurs earlier than TTP or OS, without sacrificing the emphasis on bone metastases. This in turn could lead to shorter approval times for novel CRPC therapies. Initial studies of FDG-PET in prostate cancer examined heterogeneous patient populations [43, 44]. The less-than-favorable results highlighted the need to study patient populations controlled for clinical state, disease progression, therapy, scanning algorithm and clinical endpoints. Subsequent studies adhering to this approach have suggested a role for FDG-PET as an outcome measure in CRPC [45, 46]. With respect to the context of prognosis, one study [29] found an inverse relationship between FDG-PET SUVmax and survival of CRPC patients (median survival 14.4 vs. 32.8 months if SUVmax >6.10 vs. ≤6.10, p = 0.002) (Figure 3). A combination of SUVmax and a nomogram for progressive prostate cancer dichotomized patients into a high versus low risk group (median survival 14.4 vs. 34.6 months, p = .015) that was more prognostic than either alone. In this same study, of 105 FDG-positive bone lesions that were negative on bone scan, 84 (80%) lesions eventually turned positive on follow-up bone scan, indicating that FDG-PET bone findings are clinically relevant. A similar inverse relationship with survival was also shown for BSI (14.7 vs. 28.2 months if BSI >1.27 vs. <1.27; p = 0.004); however, only SUVmax was an independent factor in multivariate analysis.

Paralleling the approach to develop the BSI, our group evaluated a single quantitative measure of tumor burden on PET, termed the SUVmax-avg, which is an average of the 5 lesions (bone, lymph node or soft tissue) with the highest SUVmax. We showed the value of SUVmax-avg as a prognostic factor for survival and treatment response [46, 47]. Nevertheless, while a RECIST-like target lesion analysis has merit, it is feasible and perhaps desirable to perform a total lesion analysis that better estimates the true tumor burden and has potential for capturing inter-lesional heterogeneity. This daunting task could be mitigated by semi-automated applications of acquisitions and for interpretation that are now in clinical testing. PET VCAR (Volume Computer Assisted Reading) is one such program that was co-developed by Larson and colleagues in collaboration with GE Healthcare Systems. PET-VCAR is based on the fiduciary marker of the skeleton [48], a count-based edge recognition program [49], and introduction of novel parameters to associate with clinical outcomes (total lesion glycolysis or gross metabolic volume) [50]. The application bookmarks regions of interest and propagates them from one time point to another, improving analysis and workflow. These features, in addition to accelerating the interpretation process, may also

Figure 4. (A). CRPC patient with multiple osteoblastic metastases. Sagittal fused PET/CT and PET images (FDG top row, FDHT bottom row) demonstrate prominent FDG and FDHT uptake, consistent with a “Glycolysis/AR Concordant” phenotype. (B). Second CRPC patient with multiple osteoblastic metastases. Sagittal fused PET/CT and PET images (FDG top row, FDHT bottom row) demonstrate intense FDHT uptake and relatively low level FDG uptake, consistent with an “AR Predominant” phenotype.
Imaging Castration Resistant Prostate Cancer

Imaging the Androgen Receptor Signaling Axis

FDHT is an analog of the primary ligand of AR, dihydrotestosterone (DHT), and is thus a rational candidate for imaging of the AR [51]. The feasibility of FDHT-PET imaging in CRPC patients and displacement of DHT by antiandrogens are already established [52,53]. Quantitative kinetic models of FDHT uptake as a measure of AR expression in human tumors, in vivo, were recently elucidated [54]. These studies were the foundation for FDHT-PET as a vital component of broader AR imaging strategies in CRPC; however, continued investigation is needed to further determine its optimal context of use. As a step towards this goal, we have studied more than 100 CRPC patients with baseline FDHT-PET and FDG-PET assessments prior to entry into clinical trials, and the majority of these patients have undergone early and late post-treatment scans. Facilitated by the PET-VCAR software, this imaging strategy has suggested diverse metabolic phenotypes of CRPC on both a patient and lesional basis, which we prefer to classify as either “AR Predominant”, “Glycolysis Predominant” or “AR/Glycolysis Concordant” [55] (Figures 4 and 5). This classification scheme may have prognostic and treatment predictive implications, as associations with specific molecular determinants in the tumor itself are being explored.

One specific context for FDHT-PET was its use as a pharmacodynamic response indicator in the phase 1 trial of the next generation anti-androgen MDV3100 [5]. The study demonstrated antitumor effects in patients with CRPC, with PSA declines by ≥50% in 56%, soft tissue regression in 22%, bone disease stabilization in 56%, and conversion from unfavorable to favorable circulating tumor cell counts in 49% of patients. The pharmacodynamics of MDV3100 was evaluated by measuring the change in FDHT uptake after start of treatment.

Figure 5. (A) Axial fused PET/CT and CT images show an example of a discordantly positive nodal mass on 18F-FDG (top, crosshairs), negative on 18F-FDHT (bottom). (B) In the same patient, axial images show an example of a discordantly positive bone lesion on 18F-FDHT (bottom, crosshairs), negative on 18F-FDG (top). (C) VCAR derived bar graph of total lesional SUVmax for the same patient demonstrating substantial heterogeneity in lesion avidity.
(displacement) in a subset (n = 22) of patients receiving dosages ranging from 60 mg to 480 mg per day. A clear reduction in FDHT uptake was seen in all patients (~20-100%), with some indication that those receiving lower dosages had a smaller reduction (mean decrease <50%) than those receiving dosages in the higher range (mean decrease ≥50%). No appreciable difference in FDHT uptake was seen in the higher dose range, despite substantial differences in serum levels of MDV3100. This suggests that the maximal effect of the drug may be reached before reaching the maximum tolerated dose (Figure 6). Based on the findings of this pilot study, FDHT-PET appears useful as a pharmacodynamic marker in discrete contexts. Further optimization of the imaging with a form of background correction is ongoing. Interestingly, these same 22 patients also underwent FDG-PET scans to assess tumor glycolysis. Modulation of tumor glycolytic rate was evidenced by reductions in FDG metabolism with declines of SUVmax-avg of 25% or more in 45% of cases. The fact that the across-the-board reduction of FDHT uptake did not parallel the FDG-PET response seems consistent with FDHT-PET as a pharmacodynamic marker, as opposed to a response indicator. Nevertheless, it is possible that FDHT-PET may prove to be a response-indicator in other settings. For instance, with drugs that decrease androgen synthesis (e.g. CYP17 inhibitors), but do not directly target the AR, a reduction of FDHT uptake may signal a true cytostatic or cytotoxic response.

While changes in FDHT uptake do reflect AR ligand-receptor interaction, there remains a disconnect between the detection of AR occupancy and the effects of the drug on downstream AR signaling. An investigative agent, 89Zr-J591 [56], is hypothesized to distinguish between these two entities. J591 is an antibody that binds to an external epitope on prostate specific membrane antigen (PSMA) that has been studied extensively for both imaging and radioimmunotherapy purposes [57]. Androgen deprivation has been shown to upregulate PSMA expression. As such, changes in PSMA expression detected by 89Zr-J591 are proposed to reflect the downstream effects of AR inhibition [58,59]. 89Zr-J591 and similar downstream imaging agents will hopefully improve our understanding of the biology of CRPC and its escape mechanisms. If successful, these

Figure 6. Pharmacodynamics of MDV3100. (A) Sagittal fused PET/CT and PET images 1 h after administration of FDHT at baseline and 4 weeks after start of MDV3100 therapy show a reduction in FDHT accumulation in tumor within the vertebrae, compared with the cardiac and aortic blood pool, in which FDHT metabolites circulate bound to serum proteins. (B) Percentage change in FDHT average maximum standard uptake value (SUVmax) from baseline to 4 weeks by dose. At baseline, all 22 patients had at least one FDHT-avid lesion that could serve as index lesions: 17 patients had five index lesions, three had three index lesions, and two had one index lesion. At baseline, the median FDHT SUVmax average was 7.81 (IQR 4.9–9.6). Reprinted with permission and modification from Scher et al. [5].
non-invasive AR probes, in tandem, will give us the capacity to discern four key aspects of targeted therapy: the presence of the target, the ability of the drug to localize to the target, the ability of the drug to inhibit downstream target effects, and the ability of the drug to modulate tumor viability.

CONCLUSION

Recent advances in the understanding of prostate cancer biology have lead to the development of much needed experimental therapies for CRPC with proven efficacy, several of which target the AR signaling axis. Qualified imaging biomarkers are sorely needed to facilitate the continued development and approval of these drugs. The bone-tropic nature of metastatic CRPC justifies the emphasis on rigorously standardized bone scanning as the gatekeeper through which more potent and versatile imaging biomarkers are co-developed. In concert with the metabolic information derived from FDG, molecular imaging of the AR signaling axis promises to reveal important insights into CRPC that will, hopefully, result in improved patient outcomes.

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References


Pathology in prostate research: Optimizing the pathological data

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Abstract
Pathology remains the gold standard for the diagnosis and local staging and grading of prostate cancer. However, as in any discipline, there are variations in national standards and protocols leading to possible significant intra-observer variations. This can significantly impact on the data supplied to clinical trials. Diagnostic and grading criteria. Error rates in the diagnosis of prostate cancer have improved but the possibility that diagnostic error may be discovered has to be addressed in any research series. Major changes in Gleason grading have occurred in the past 40 years and this may lead to suboptimal application of grades in research cohorts, falsely raising the prognostic power of new biomarkers. Tumor measurements and staging criteria. Further information that may provide additional prognostic information include various measures of tumor extent and peri-neural invasion in biopsy specimens. Standardization of measures of tumor extent is necessary to give more useful assessments of prognosis. In radical prostatectomy specimens there are a number of other staging measurements which might be applied, including tumor volume, margin status, extra-capsular extension and nodal positivity though many of these variables are interdependent. Conclusion. Appropriate utilization of such pathological material will produce improved cohorts in which it will be possible to test new biomarkers with increased rigor.

Pathological data in clinical research

Validation of the diagnosis
The pathological diagnosis of prostate cancer on needle biopsy is still recognized to be a challenging area. Retrospective analyses of cases has revealed error rates of between 7–20% [1,2] though modern immunohistochemical methods may have reduced this [3]. Nevertheless, recent series still reveal significant errors in both the under-diagnosis and over-diagnosis of cancer [4]. Specialist uropathologists have been shown to have lower error rates than non-specialists. Error rates will also probably increase in older series. However it should be realized that review of old cases may lead to ethical questions of whether the patient should be informed of such changes if cases are reviewed rather than case notes abstracted. This may be handled in a number of ways. In one retrospective series, the patients were informed, after 20 years if their diagnosis of prostate cancer was incorrect [1]. No legal action resulted from this (personal communication from the author). A second similar series had built-in anonymization to their results and was given ethical approval that the patients would not be informed of such changes [2]. These issues have to be addressed before the commencement of any review.

Gleason scoring
Gleason scoring, devised over 40 years ago, remains the most powerful predictor of prostate cancer behavior in any biopsy or resection specimen [5,6]. However, significant changes have occurred in Gleason scoring since it was devised in the late 1960s and early 1970s [7]. This has resulted in significant shifts in Gleason scoring, invariably to higher scores. The reasons for this are many, but include the recognition that the lowest Gleason scores probably do not represent cancer, meaning they are no longer diagnosed. The ISUP 2005 consensus conference made a number of significant changes to Gleason scoring. Unfortunately these were not fully validated, and it remains uncertain whether traditional or ‘revised’ Gleason scores
scoring is a better predictor of behavior [8], especially for the crucial cut-off between Gleason pattern 3 and Gleason pattern 4. It should also be recognized that the modern criteria are a ‘consensus’ and therefore there may be variations between different laboratories and different pathologists in how Gleason grading is calculated [9]. The results of this Gleason drift is the so called ‘Will Rogers’ phenomenon, where survival curves appear to show improvement over time due to upgrading of the disease [10].

Examples of this include the calculation of Gleason score from a biopsy series. Some pathologists will calculate a composite score, taking into account the appearances in every core with cancer. Other groups report the ‘worst’ score seen in any of the cores sampled. A second example is the calculation of tertiary grade [11]. There was no consensus at the meeting in radical prostatectomy specimens as to whether the worst pattern seen, if it was a tertiary element should be incorporated into the main Gleason score.

Unfortunately it is not yet possible to state on the optimal method. However there is evidence that abstraction of notes from a retrospective series will provide much poorer information than a single pathologist reviewing to modern criteria [12]. This is important in any biomarker assessment since merely using abstracted information from multiple centers will considerably under-estimate the power of Gleason scoring and therefore lead to a falsely more powerful assessment of a given tumor biomarker.

Therefore for both retrospective and prospective studies there is considerable variation how Gleason score has been and is calculated, and any utilization of Gleason score should include a description of the methodologies used.

**Tumor extent and staging in diagnostic samples**

Estimates of tumor extent are being increasingly used in estimation of recurrence and progression risk and should therefore be used in multivariate analyses. Unfortunately multiple methods of assessment are available leading to non-comparability of large series. Tumor extent in TURP specimens may be measured counting the number of positive chips or by visual estimates of involved tissue though one paper suggests these are comparable [13].

Measurements of tumor extent are prognostically more important in prostate biopsies, and most series have shown that tumor content of prostate biopsies yields prognostic information on multivariate analysis. A systematic review [14] has shown that multiple methodologies for core measurement and assessment preclude proper analyses. However, tumor extent does appear in nomograms and tables and some assessment of tumor extent should be regarded as standard practice in the evaluation of new biomarkers on needle biopsies [15].

**Perineural invasion**

The importance of reporting perineural invasion prostate cancer is still controversial. A recent review concluded that variations in study design precluded meta-analysis [16] but that the weight of evidence suggested that in localized disease, the presence of perineural invasion might favor the use of more radical therapy.

**Pathologic data in radical prostatectomy specimens**

As in biopsy specimens, details of the methods by which Gleason grade is calculated should be regarded as an essential criterion in any multivariate assessment of disease progression after RP in clinical trials. However there are other important assessments of disease extent which are not routinely of importance in biopsy specimens. The International Society of Urological Pathologists has recently published guidelines to standardize the acceptable approaches to radical prostatectomy processing [17–21].

a. **TNM Stage.** The major decision in radical prostatectomy (RP) specimens is to distinguish between tumors limited to the prostate (organ confined, pT2) or involving extra-prostatic tissues (pT3) [22]. In radical prostatectomy specimens tumor volume measurements and subdividing the category of organ confined tumors (pT2) does not appear to provide useful independent prognostic information as it is less informative than other prognostic parameters such as stage, capsular involvement, margin positivity and lymph node status. Assessments of tumor volume in this scenario have yielded contradictory information [23,24].

b. **Margin status.** Many studies have reported on the prognostic significance of involved margins and the extent of positivity is also of prognostic significance [25,26].

c. **Seminal vesicle involvement (SVI, pT3b) is a poor prognostic factor after radical prostatectomy [27].**

d. **Vascular invasion.** The presence of vascular invasion is an independent predictor of biochemical recurrence following radical prostatectomy [28].

e. **Nodal positivity is a poor prognostic factor and the diameter of the largest metastasis appears to be more predictive of cancer-specific survival than the number of positive nodes alone. However the presence of extranodal extension was not prognostic [29,30].**
Conclusions

There is an urgent need for new biomarkers to predict outcome in prostatic carcinoma. However the search for such markers must be backed up with full and thorough assessment of all pathological criteria which might compete with a novel assessment. There is a concern that novel markers may appear more promising if measured in series with suboptimal or incomplete pathological assessments. Utilization of the maximum amount of pathological data will obviate these concerns and hopefully lead to biomarkers progressing from the research laboratory into clinical practice.

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References


REVIEW ARTICLE

Pathology in prostate research: Optimizing tissue quality

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Abstract
The collection of tissue from the prostate gland for research creates unique challenges in the identification of cancer and in preserving pathological material. Value and uses of formalin fixed tissue. Formalin fixed paraffin embedded (FFPE) tissue is often available in abundance after pathological processing and reporting of specimens but is limited in value for detailed molecular tests. Tissue micro-array if carefully performed is a helpful technique for examining many FFPE specimens with immunohistochemical or fluorescence in situ hybridization tests. Value and uses of frozen tissue. The collection of fresh tissue prior to formalin fixation and later validation samples of fresh prostate cancer is difficult as prostate cancer is very difficult to identify macroscopically on cut prostate specimens. Also, the act of manipulation and dissection of the gland while fresh and without compromising surgical margins is challenging. Methods which have been used to dissect the fresh prostate gland and also collect fresh tissue from other prostatic specimens are discussed. The ethical challenges of collecting research tissue without compromising patient care are discussed. Conclusions. Prostate cancer tissue banks, particularly of frozen tissue are still relatively few in number. Enhanced collection methods which do not prohibit full pathological examination are available but require expertise to maximize their potential.

Formalin fixed paraffin embedded tissue
Formalin fixed and paraffin embedded tissue (FFPE) allows a variety of molecular techniques to be assayed after histological diagnosis. Storage can be undertaken at room temperature and the vast majority of epitopes will remain stable even after 10–20 years or more in paraffin blocks. A number of factors may affect this. Firstly if another fixative such as Bouin’s is used, this may cause loss or different expression of markers [1]. Secondly, after FFPE blocks have been cut onto slides there is a loss of strength of signal with some antigens. For instance, HER-2 is known to degrade within a few weeks of sections being cut [2]. It has been suggested that if unstained slides are stored then they are preserved from degradation by storing at a low temperature or an oxidation free environment [3].

Tissue microarrays (TMA) allow multiple specimens to be examined on a single slide while preserving their identity and also allowing a single immunohistochemical experiment to be performed on the same tissue [4,5]. It has proven a valuable technique for high throughput of multiple tumors but nevertheless requires careful technical preparation. Areas for microarray need to be identified on adjacent Haematoxalin and Eosin sections, preferably by a pathologist. This is especially true for prostate carcinoma where diagnosis is often challenging, especially in low grade disease. Secondly the heterogeneity of tumor needs to be reflected in sampling. This will include sampling of the index tumor. As Gleason scoring has shown, prostate cancer shows unique heterogeneity. At least three areas of tumor should be sampled and all grades of tumor, if different grades are present [6]. TMA is a technique frequently used in TURP and radical prostatectomy specimens. However recently it has been shown to be possible to create TMAs from biopsy material as well as TURP and RP specimens [7].

Fresh tissue
Molecular profiling techniques such as real-time PCR and microarrays require the harvesting of high quality tissue, yet the process of formalin fixation
results in non-specific degradation of delicate intracellular molecules, particularly RNA [8,9]. Therefore although essential for cell culture studies, the collection of fresh tissue is also greatly preferable for DNA and especially RNA studies. However, the collection of fresh prostatic tissue, especially tumor tissue is far more challenging than tissue collection of most other tumors. A major challenge is to identify tumor at gross examination which is often very difficult or impossible in prostate cancer. Thus, morphological verification is absolutely mandatory. It must in no way compromise the diagnostic or prognostic information than can be extracted from the tissue. Therefore the ability to extract fresh tissue depends upon the surgical procedure undertaken.

TRUS biopsy

18 gauge needle biopsies are too small to identify cancer macroscopically, and inevitably the whole of the core is needed for histopathological processing. Due to the insensitivity of PSA screening and lack of precision of needle biopsies, only about 30–40% of TRUS biopsy procedures lead to the identification of cancer [10]. Methods of collecting fresh tissue from TRUS include the collection of extra needle cores for research. However this may pose ethical problems, especially if cancer is identified in the cores, while the specimens taken for routine FFPE processing are negative. It is likely to be useful only in extensive or high grade tumors. Some groups have attempted to use tissue imprints from biopsy cores [11] by transferring the superficial cells onto a surface. However, this also will tend to identify only high grade tumors.

TURP collection

As few pathology departments process all the tissue removed at TURP, there is usually scope to collect fresh tissue from these operations. However this is usually hyperplastic tissue from the transition zone or non-neoplastic central zone tissue. Although it might be considered an adequate ‘normal’ control tissue, it must be remembered that most of the tissue is not from the peripheral zone, where the majority of prostatic cancers arise. Techniques for sampling fresh tissue from TURP specimens include sampling the middle of a TURP chip while processing the outer portions routinely to correlate the morphology with the fresh tissue [12]. Occasionally prostatic cancers are operated upon using this method, especially in fast growing tumors which are not amenable to radical therapy, in cases of bladder outflow obstruction. In these cases, abundant tumor tissue is available. However, it should be remembered that these tumors are unlikely to be representative of the full spectrum of prostate cancer. They are usually hormone pre-treated and occupy an unusual part of the prostate cancer spectrum, being tumors that have spread rapidly locally but have not yet resulted in death by metastatic spread.

Radical prostatectomy specimens

The harvesting of validated prostate tissue from radical prostatectomy specimens, probably represents the optimal method of the accrual of tissue from low risk prostate cancer. However the macroscopic dissection of the prostate gland is difficult and critical attention needs to be paid to margin status and capsular invasion. Therefore any dissection should always be conducted by a pathologist according to an agreed protocol.

A number of methods are currently in use to collect fresh tissue. Some centres use 18 gauge biopsies on the fresh gland before fixation in formalin [13]. This has the advantage of less distortion of the gland and minimal disruption of the surgical margin. However disadvantages are that little tissue is sampled and immediate correlation with macroscopic internal appearances of the gland are not possible. Furthermore, it is very difficult to localize the tumor as the majority of prostate cancers at radical prostatectomy are currently T1c tumors, i.e. non-palpable disease.

Another technique includes immediate sectioning of the gland with the dissection of suspicious areas of cancer from the center of the specimen where there is less danger of the disruption of surgical margins [14,15]. Disadvantages include the subsequent potential distortion of the specimen in formalin rendering assessment challenging, and the fact that increasingly prostate cancers are too small to identify macroscopically. A method has been described for harvesting tissue from cut surfaces without compromising surgical margins [16].

Radical cystectomy specimens removed for urothelial carcinomas are also a potential source of prostate tissue. Although small prostate cancers may be found in many cases, they represent a potential source of ‘normal’ controls and do not require such rigorous assessment of margin status of the prostate.

Storage of fresh prostate tissue for research

Due to rapid autolysis, tissue should be stabilized as soon as possible after removal from the patient. Common methods include snap freezing of tissue and storage at −70°C. Cooling the specimen immediately in a theater by placing it on ice may be helpful. However for transport this is often cumbersome and the
development of an RNA stabilization solution RNAalater may used if RNA is to be analysed [15]. A disadvantage of RNAalater is that it impairs the interpretation of morphology. Correlation of all tissue with either frozen sections or adjacent FFPE tissue is important if cancer is to be properly validated [17]. When harvesting for cell culture, morphological verification with frozen section or RNAalater fixed material is not possible as the harvested tissue needs to remain fresh. This may also be the case with tissue intended for proteomic analysis. A morphological validation can then be carried out using cytological sampling technique [18].

Ethical issues

The ethical issues involved in the collection and use of fresh tissue will vary greatly between countries [19–21]. However they are underpinned by an ethos that no patient should suffer harm from the research, and that their confidentiality must be ensured. Ethical approval in the country of origin should always be obtained, and depending on the procedure and country, consent by the patient is required. Harm is conceivable if the patient undergoes unnecessary procedures: for instance research orientated TRUS biopsies with no diagnostic use or intent. The patient may undergo indirect harm if research manipulation of tissue renders a specimen difficult to interpret for margin status.

Conclusions

Harvesting the prostate, especially cancer tissue, for research creates problems not encountered in other organs and cancers, mostly secondary to the unique methods by which prostate cancer is diagnosed and sampled. However by using the variety of techniques suggested above and with cooperation of local pathologists it is possible to collect both frozen and FFPE tissue banks which can be used for future research programs.

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Role of histopathology and molecular markers in the active surveillance of prostate cancer

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Abstract

Surgery or radiation therapy remain the standard curative treatments for newly diagnosed prostate cancer patients. Nonetheless, these aggressive treatments are associated with decreased quality of life with altered sexual and urinary functions. The objective was a systematic review of active surveillance protocols to investigate the role of histopathology and molecular markers in the active surveillance of prostate cancer. Medline was searched using the following terms: prostate cancer, active surveillance and expectant management.

Selection criteria, follow-up strategies and outcomes

Using modern risk stratification, several centres have gained significant experience in identifying patients with a low risk of prostate cancer progression and have adopted an active surveillance program with delayed curative therapy. Interestingly, only limited numbers of patients under active surveillance require additional treatment. Recent data suggest that delayed treatment does not appear to alter the clinical outcome among those highly selected patients. The future and conclusions. A better understanding of the molecular determinants of prostate cancer behaviour would not only enable healthcare professionals to identify which cases need aggressive treatment but, perhaps more importantly, would also indicate potential targets for the development of novel therapeutic strategies.

The aim of active surveillance (AS) of early prostate cancer is to individualise therapy by selecting for curative therapy only patients with significant cancers. Patients with favourable tumour characteristics in terms of clinical T stage, Gleason score and serum prostate specific antigen (PSA) testing are closely monitored using serum PSA kinetics and repeat prostate biopsies. The choice between continued observation and radical treatment is based on evidence of disease progression, with progression defined in terms of ‘upgrading’ at repeat biopsy and PSA doubling time (PSADT). The aim is to identify cases for treatment long before any symptoms or overt clinical signs of tumour progression are evident [1].

AS should be distinguished from ‘watchful waiting’. The latter involves relatively lax observation with late, palliative treatment for those who develop symptoms of progressive disease, whereas AS involves close monitoring with early, radical treatment in those with signs of progression [1–3].

Selection criteria

A critical factor for successful AS is the best possible selection of patients with prostate cancer with low risk of progression (Table I). Patients with an identifiable low risk of progression are most likely to be safely observed and treated only when necessary. Epstein et al. introduced prostate biopsy criteria to predict insignificant prostate cancer (PCA) in the radical prostatectomy (RP) specimens [4]. In addition to the original Epstein criteria, multiple selection or entry criteria, based on preference or on experience and not always obtained on hard data, have been published [1,4–9] (Table II). The most common clinical data used to define low-risk prostate cancer include a Gleason score ≤6 (no pattern 4 or 5 disease), PSA...
Table I. Prostate cancer aggressiveness risk strata and related care options.

<table>
<thead>
<tr>
<th>Early Stage Prostate Cancer Aggressiveness Category</th>
<th>Measures of prostate cancer severity</th>
<th>Prostate Cancer Care Options</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low risk</td>
<td>Biopsy Gleason Score ≤ 6 T1 or T2a</td>
<td>Active surveillance, prostatectomy, brachytherapy, or external radiotherapy</td>
</tr>
<tr>
<td>Intermediate risk</td>
<td>Clinical Stage ≤ 6 T1 or T2a or b 10 – 20</td>
<td>Prostatectomy, external radiotherapy with adjuvant androgen suppressive therapy, or brachytherapy</td>
</tr>
<tr>
<td>High risk</td>
<td>Serum PSA &lt; 10 7 T1 or T2a or b 10 – 20</td>
<td>External radiotherapy with adjuvant androgen suppressive therapy or prostatectomy</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8 – 10 T1 or T2a, b, or c Any PSA</td>
</tr>
</tbody>
</table>

Table II. Entry criteria for active surveillance (authors in alphabetic order).

<table>
<thead>
<tr>
<th>Source</th>
<th>Entry criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dall’Era et al. [1] (Most common clinical criteria)</td>
<td>Gleason score 6 No Gleason pattern 4 or 5 PSA level &lt; 10 ng/ml and stable PSA kinetics ≤ 35% single core involvement ≤ 35% positive cores</td>
</tr>
<tr>
<td>D’Amico et al. [5]</td>
<td>PSA level ≤ 10 ng/ml No Gleason pattern 4 or 5 Clinical stage T2a or lower</td>
</tr>
<tr>
<td>Epstein et al. [4]</td>
<td>Clinical stage T1c PSA density &lt; 0.15 ng/ml/cm³ No Gleason pattern 4 or 5 &lt; 3 positive cores ≤ 50% cancer per core</td>
</tr>
<tr>
<td>Patel et al. [6]</td>
<td>Clinical stage T3 or lower Gleason sum ≤ 7</td>
</tr>
<tr>
<td>PRIAS* (Van den Bergh et al.) [9]</td>
<td>Clinical stage T1c–T2b No Gleason pattern 4 or 5 PSA density &lt; 0.20 ng/ml/cm³ PSA level &lt; 10 ng/ml Fewer than three positive cores</td>
</tr>
<tr>
<td>Soloway et al. [7]</td>
<td>Clinical stage T2 or lower PSA level &lt; 15 ng/ml No Gleason pattern 4 or 5 ≤ 50% cancer per two positive cores</td>
</tr>
<tr>
<td>Van As et al. [8]</td>
<td>Clinical stage T1–T2a Gleason sum ≥ 7 (3 + 4) PSA level &lt; 15 ng/ml ≤ 50% of biopsy cores positive</td>
</tr>
</tbody>
</table>

*PRIAS, Prostate Cancer Research International Active Surveillance.

level ≤ 10 ng/ml, and clinical stage T1 to T2a disease. Other characteristics which have been used include PSA kinetics (stable) before diagnosis, PSA density (PSAD) < 0.15 ng/ml/cm³, percent positive cores at biopsy < 33%, and the extent of cancer in any core < 50% [1]. The percentage of core involvement and percentage positivity of the biopsies are both dependent on the length and number of cores respectively. Measurements of cancer length may be more helpful. Prospective studies comparing entry criteria for AS protocols with subsequent disease progression and treatment patterns are needed to clarify the best candidates for AS.

Issues on the role of prostate biopsies in patient selection

Some authors have shown that a non-negligible proportion of patients who meet the entry criteria for AS actually harbour aggressive or locally advanced disease if they are submitted to RP [10]. Ploussard et al. provided a detailed analysis evaluating, for the first time, the misclassification rate in patients who could be suitable for an AS program according to different biopsy schemes [10]. All of their patients were submitted to a 21-core first biopsy mapped by location. The authors found that patients who could have been selected for AS programs based on a 12-core biopsy scheme showed higher rates of unfavourable prostate cancer characteristics at RP compared to patients who would have been included only in a 21-core biopsy scheme (overall unfavourable prostate cancer: 28.6 – 35.9% vs. 14.0 – 17.6%, respectively). Interestingly, among patients without cancer evidence in the 12-core scheme but with cancer diagnosed only at the 21-core biopsy, roughly 16% showed unfavourable disease at RP, defined as Gleason score 8 or category pT3 or higher. These data showed that a certain proportion of patients initially submitted to AS actually harbour aggressive disease at the time of diagnosis. The data by Ploussard et al. [10] could help reduce the misclassification risk by introducing high density biopsy strategies in the initial management of patients submitted to AS.

Follow-up strategies to detect prostate cancer progression

Even though different AS follow-up strategies have been adopted (Table III) [6 – 8, 11 – 15], the criteria are somewhat similar. Besides a regular repeat biopsy, regular PSA level testing, digital rectal examination (DRE) and optional transrectal ultrasound studies are warranted [15].
The detection of prostate cancer progression in a patient selected for AS remains a continuing challenge. What will serve as the best parameter to correctly identify patients that progress to more aggressive cancer in order not to miss the window of curability is still a matter of debate. At present, the choice between radical treatment and continued observation is based on evidence of disease progression, with progression defined in terms of ‘upgrading’ at repeat biopsy and PSADT.

Prostate cancer ‘upgrading’ at repeat biopsy is a major criterion for active treatment [6–8, 11–15, 17]. The study by van As et al. used PSA kinetics profiles, progression of Gleason grade, and increased percentage of cancer per core as indicators to stop AS in patients with low-risk prostate cancer [8]. Interestingly, in the cohort of Klotz et al., only 4% of patients were treated because of progression of Gleason grade alone [16]. The greatest trigger for intervention in the Toronto cohort remained the PSADT, with 21% of the cohort having a PSADT < 3 years [17].

Outcomes

Multiple studies have reported their experience with AS, but the value of most studies is limited by a relatively short follow-up time (Table IV) [6–8, 11–15, 17–19]. However, recent data suggest that delayed treatment does not appear to alter the clinical outcome among those highly selected patients. In a recent study by van As et al. it was found that 20% of patients received delayed radical treatment after a median follow-up of 22 months. Within this time frame no patient developed metastatic disease or died of prostate cancer [8]. Hardie et al. reported similar findings at a median follow-up of 42 months [14]. Approximately 91% of the patients had a Gleason score ≤6 and 73% a PSA level < 10 ng/ml. All patients revealed organ-confined disease in the RP specimen.

The future

AS provides an ideal opportunity for healthcare professionals to improve their understanding of the basis for the extraordinary variation in prostate cancer behaviour. A better understanding of the molecular markers of prostate cancer behaviour would not only enable healthcare professional to identify which cases need treatment but, perhaps more importantly, would also indicate potential targets for the development of novel therapeutic strategies.

Molecular markers

Multiple susceptibility genes and many additional mechanisms involved in carcinogenesis and cancer progression have been discovered [20–23]. However, no single biomarker capable of improving the common clinical parameters included in the currently used predicting models has yet been identified in any prospective active surveillance series.

The Prostate CAncer gene 3 (PCA3) assay is a novel tool that might aid in the diagnosis of prostate cancer and that might indicate the significance of the disease [24, 25]. The PCA3 urinary assay might be used to guide biopsy decisions in: (i) men with an elevated serum total prostate specific antigen (tPSA) level and one or more previous negative biopsies; (ii) men with a normal tPSA level and a family history of prostate cancer; (iii) men with an elevated tPSA level (2.5–10 ng/mL) and no previous biopsy; (iv) men with an elevated tPSA level and a concomitant urinary condition. In addition, in men diagnosed

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### Table III. Predicting progression during active surveillance (authors in alphabetic order).

<table>
<thead>
<tr>
<th>Source</th>
<th>PSA</th>
<th>DRE</th>
<th>TRUS</th>
<th>Rebiopsy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carter et al. [11, 12]</td>
<td>Every 6 months</td>
<td>Every 6 months</td>
<td>No mention</td>
<td>Yearly</td>
</tr>
<tr>
<td>Dall’Era et al. [13]</td>
<td>Every 3 months</td>
<td>Every 3 months</td>
<td>6–12-month interval</td>
<td>Every 12–24 months</td>
</tr>
<tr>
<td>Hardie et al. [14]</td>
<td>Every 3–6 months for 2 years, then every 6 months if PSA is stable</td>
<td>Every 3–6 months for 2 years, then every 6 months if PSA level is stable</td>
<td>Optional</td>
<td>At 12–18 months</td>
</tr>
<tr>
<td>Klotz et al. [15]</td>
<td>Every 3 months for 2 years, then every 6 months if PSA level is stable</td>
<td>Every 3 months for 2 years, then every 6 months if PSA level is stable</td>
<td>Optional</td>
<td>At 6 months</td>
</tr>
<tr>
<td>Patel et al. [6]</td>
<td>Every 3 months for 1 year, then every 6 months</td>
<td>Every 3 months for 1 year, then every 6 months</td>
<td>At 6 months</td>
<td>At 6 months</td>
</tr>
<tr>
<td>Soloway et al. [7]</td>
<td>Every 3 months for 2 years</td>
<td>Every 3 months</td>
<td>No mention</td>
<td>At 6–12 months, afterwards when indicated</td>
</tr>
<tr>
<td>Van As et al. [8]</td>
<td>Year 1: monthly Year 2: every 3 months Afterwards: every 6 months</td>
<td>Every 3 months for 2 years, then every 6 months</td>
<td>No mention</td>
<td>At 18–24 months, then biannually</td>
</tr>
</tbody>
</table>

DRE, digital rectal examination; TRUS, transrectal ultrasound; PSA, prostate-specific antigen.
Table IV. Treatment criteria (authors in alphabetic order).

<table>
<thead>
<tr>
<th>Source</th>
<th>Treatment criteria</th>
<th>Median follow-up, months</th>
<th>Percentage of patients with treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carter et al. [11,12]</td>
<td>Gleason score ≥7 on rebiopsy, any pattern 4/5, &gt;2 cores involved, &gt;50% any single core involved</td>
<td>23</td>
<td>31</td>
</tr>
<tr>
<td>Dall’Era et al. [13]</td>
<td>Gleason score ≥7 on rebiopsy, rising PSA, increase in volume by biopsy parameters</td>
<td>24</td>
<td>21</td>
</tr>
<tr>
<td>Ercole et al. [18]</td>
<td>Increase in tumor volume, Gleason score progression, urinary symptoms, change of DRE, patient preference</td>
<td>48</td>
<td>7.8</td>
</tr>
<tr>
<td>Hardie et al. [14]</td>
<td>Rising PSA, clinical judgment</td>
<td>42</td>
<td>14</td>
</tr>
<tr>
<td>Klotz et al. [15]</td>
<td>PSADT &lt;2 years Gleason score ≥8 Update 2001: PSADT &lt;3 years Gleason score ≥7 (4 + 3)</td>
<td>64</td>
<td>34</td>
</tr>
<tr>
<td>Patel et al. [6]</td>
<td>Gleason score increase, PSA &gt;0.75 ng/ml per year, increase DRE/TRUS detected lesion, increase biopsy volume</td>
<td>44</td>
<td>35</td>
</tr>
<tr>
<td>Roemeling et al. [19]</td>
<td>PSADT</td>
<td>40</td>
<td>29</td>
</tr>
<tr>
<td>Soloway et al. [7]</td>
<td>Gleason score increase, PSA and PSADT increase, stage progression, increase biopsy volume, patient preference</td>
<td>45.3 (mean)</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Van As et al. [8]</td>
<td>PSAV &gt;1 ng/ml per year Gleason score ≥4 + 3 or &gt;50% cancer per core</td>
<td>22</td>
<td>20</td>
</tr>
</tbody>
</table>

PSA, prostate-specific antigen; PSADT, PSA doubling time; PSAV, PSA velocity; DRE, digital rectal examination; TRUS, transrectal ultrasound.

With prostate cancer, the PCA3 assay could aid in the decision of whether active therapy is needed or active surveillance is appropriate. In a study by Tosoian et al. in patients with low risk prostate cancer who were carefully selected for active surveillance PCA3 score was not significantly associated with progressive disease in the short term [26]. While there was a trend toward higher PCA3 scores in patients with high grade disease on surveillance biopsy, significant overlap between the groups prevented the identification of a threshold PCA3 score for clinical use. Therefore, the true value of the test in the setting of AS remains unclear at this point.

Conclusions

AS is a new strategy that aims to individualise therapy by selecting only those patients with significant cancers for curative therapy. Patients with favourable tumour characteristics are closely monitored using serum PSA concentrations and repeat prostate biopsies [27]. The choice between radical treatment and continued observation is based on evidence of disease progression, defined in terms of the PSADT and ‘upgrading’ at repeat biopsy. AS provides an excellent opportunity for studies to identify molecular markers of prostate cancer behaviour and of assessment therapeutic agents.

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References


REVIEW ARTICLE

Tumor markers in prostate cancer I: Blood-based markers

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Abstract

The introduction of total prostate specific antigen (total PSA) testing in blood has revolutionized the detection and management of men with prostate cancer (PCa). The objective of this review was to discuss the challenges of PCa biomarker research, definition of the type of PCa biomarkers, the statistical considerations for biomarker discovery and validation, and to review the literature regarding total PSA velocity and novel blood-based biomarkers. Methods. An English-language literature review of the Medline database (1990 to August 2010) of published data on blood-based biomarkers and PCa was undertaken. Results. The inherent biological variability of total PSA levels affects the interpretation of any single result. Men who will eventually develop PCa have increased total PSA levels years or decades before the cancer is diagnosed. Total PSA velocity improves predictiveness of total PSA only marginally, limiting its value for PCa screening and prognostication. The combination of PSA molecular forms and other biomarkers improve PCa detection substantially. Several novel blood-based biomarkers such as human glandular kallikrein 2 (hK2), urokinase plasminogen activator (uPA) and its receptor (uPAR), transforming growth factor-beta 1 (TGF-β1); interleukin-6 (IL-6) and its receptor (IL-6R) may help PCa diagnosis, staging, prognostication, and monitoring. Panels of biomarkers that capture the biologic potential of PCa are in the process of being validated for PCa prognostication. Conclusions. PSA is a strong prognostic marker for long-term risk of clinically relevant cancer. However, there is a need for novel biomarkers that aid clinical decision making about biopsy and initial treatment. There is no doubt that progress will continue based on the integrated collaboration of researchers, clinicians and biomedical firms.

In Western societies, prostate cancer (PCa) is the most common solid malignancy and the second leading cause of cancer death in men [1]. The wide availability of total prostate-specific antigen (PSA, formal name human kallikrein 3, hK3) revolutionized PCa screening and ushered in the PSA era resulting in a decrease of PCa metastasis and death. However, the ubiquitous application of PSA screening has also led to over-detection and overtreatment. The lifetime risk of PCa diagnosis is estimated at ~18%, whereas that for death from PCa is ~3%. In addition, PSA is neither cancer specific nor a surrogate for the biologic behavior of PCa. An elevated PSA level can reflect the presence of cancer but can also be caused by benign prostatic hyperplasia (BPH), infection, and/or chronic inflammation. Virtually all prostate epithelial cells, whether normal, hyperplastic or cancerous, synthesize PSA. Therefore, there has been a concerted effort to discover and validate novel PCa biomarkers. In this review article, we first discuss the challenge of PCa biomarker research, types of PCa biomarkers, and the statistical considerations for biomarker discovery and validation. Then, we discuss the limitations of measuring total PSA and its derivatives such as total PSA velocity (total PSAV) and different molecular forms (i.e. free PSA, BPSA, pro-PSA, and intact PSA). Moreover, we briefly discuss several promising novel blood-based biomarkers for PCa diagnosis, staging, prognostication, and monitoring, i.e. human glandular kallikrein 2 (hK2),

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urokinase plasminogen activator (uPA) and its receptor (uPAR), transforming growth factor-beta 1 (TGF-β1); interleukin-6 (IL-6) and its receptor (IL-6R).

**Biomarker challenge in prostate cancer**

A PubMed Search on “prostate cancer” AND (“biomarker” OR “marker”) in English language yielded 3016 hits (accessed 8/8/2010). The number of articles published on PCa biomarkers has increased steadily over the years. Despite this plethora of biomarkers reported to be “promising”, only one biomarker, (i.e. total PSA in blood) is routinely used by urologists. Why are PCa biomarkers not living up to their promise? For one, there are remarkable analytical and regulatory barriers to the application of biomarkers in PCa care [2,3]. These include but are not limited to the status of intellectual property protection, availability of standard reference materials for the assay, complexity of assay format, implementation of quality control to assure reproducibility and accuracy, sufficient market testing size to assess methods of commercialization, lack of clear guidelines for good manufacturing/laboratory practice and quality control requirements for all phases of biomarker development, cost and effort required to accumulate clinical data under appropriately designed and Institutional Review Board-approved prospective trials, and the interval required for resolution of patent issues, assay standardization, validation, testing, and regulatory approval.

Besides analytical and regulatory barriers, the lack of PCa biomarker use in daily clinical practice is also a result of poor application of statistics and study design. There has been a lack of effective and efficient strategies to determine which biomarker candidates justify the great investment of time and money required for assay development, optimization and demonstration of analytical robustness. There are now guidelines intended to ensure that biomarker studies conform to some basic standards of design and reporting [2,3]. For a new biomarker to be clinically useful, it has to answer a clinically relevant question and provide information that is not available in a more simple and cost-effective way. Any new biomarker needs to provide a benefit over these standard criteria or at least improve their accuracy. Before a biomarker assay can be implemented in the community setting, it needs to address four concepts: “better, easier, faster and cheaper” [2].

Conceptually, the development of new biomarkers should be a process that is similar to therapeutic drug evaluation. In 2002, the National Cancer Institute’s Early Detection Research Network developed a five phase approach to systematic discovery and validation of biomarkers (Figure 1) [2–6]. This schema is not only an intellectual process but also provides a clear scale by which researchers, patients, and investors can evaluate the status of the biomarker in the development process. The expected failure rate of biomarkers in development can be expected to be similar to the one of drugs. A large concerted effort is required to advance the field of PCa biomarker through systematic discovery, verification, and validation – each step coupled with adequate statistical analysis.

**Refining the definition of prostate cancer biomarker**

According to the National Institute of Health (NIH) in the USA, a biomarker is a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes or pharmacutical responses to a therapeutic intervention [7]. Cancer biomarkers are either produced by the tumor or by the body in response to the tumor. Six different types of biomarker can be differentiated in PCa:

1) **Detection/screening**: this biomarker is used for evaluating patients with either risk factors for or symptoms of PCa.

2) **Diagnostic**: this biomarker can help classical histopathological characteristics in assessing presence or absence of cancer.

3) **Prognostic**: this biomarker is used to predict the outcome of patients based on different risk of recurrence or progression thereby allowing individualized management.

4) **Predictive**: this biomarker is used to predict whether the treatment (drug or other therapy) will be effective, and/or to monitor the effectiveness of the treatment. It can help identify the best treatment modality.

5) **Therapeutic target**: this biomarker can help identify the patients who will benefit from a particular treatment regimen. It identifies the molecular targets of novel therapies and is affected by therapy. As of now there is no such blood-based biomarker in clinical use for prostate PCa.

6) **Surrogate endpoint**: this biomarker is used to substitute for a clinical endpoint and/or to measure clinical benefit, harm or lack of benefit or harm. Surrogates could replace traditional endpoints, such as mortality due to disease or the recurrence or relapse of disease. Biomarkers can reduce time factors and costs for Phase I and II clinical trials by replacing clinical endpoints.

For the purposes of the current review, we will focus on blood-based biomarkers that are the end result of a bioassay, for processing biological material from humans, expressed quantitatively or categorically. Tissue-based and urine-based PCa biomarkers are discussed in separate reports.
Statistical considerations for biomarker discovery and validation

An issue that has received less attention is the degree to which research on biomarkers has made sufficient use of clinically relevant statistics, such as the assessment of predictive accuracy, decision analysis, and/or experimental methodology. Most biomarkers do not provide sufficient information to be used independent of other information. The optimal use of biomarkers lies in incorporating it in a model that also includes standard clinical data [3,8–12]. To determine the value of a new biomarker, it is not sufficient to show that it is significantly related to the outcome, statistically significant in a multivariable model including the standard clinical and pathologic factors, or more significant than the standard clinical and pathologic factors. A variable that is statistically significant in a multivariable model might not improve the model’s predictive accuracy. P-value and odds/hazard ratio do not meaningfully describe a biomarkers’ ability to classify patients. For a biomarker to be potentially clinically useful, it is necessary to show that adding the biomarker to an existing model based on the most important clinical and pathologic factors improves the predictive accuracy (discrimination and calibration) of the model [3,11,13–16].

One major issue with model development is the need for appropriate validation. There are two general types of validation: internal validation on original dataset and external validation on independent dataset [2,3]. External validation on a different data set allows for evaluation of the generalizability of the risk prediction tool to wider populations than originally reported. Finally, methods that incorporate clinical consequences such as decision curve analysis are crucial to the evaluation of biomarkers. Several methods are available including decision curve analysis which combines simplicity with efficient computations [17–20].

Total PSA and its limitations

Neoplastic cells produce somewhat lower and varying tissue levels of PSA compared to benign epithelial cells although both conditions cause total PSA elevation in the blood [3]. Therefore, it has been suggested that total PSA should be considered as a marker of BPH-related prostate volume, growth, and outcome rather than a reliable marker of PCa [3]. Moreover, some aggressive PCa’s do not produce PSA.

PSA levels are inherently variable thereby affecting the interpretation of any single result [21]. Variation in total PSA includes both analytical (i.e. pre-analytical sample handling, laboratory processing, assay performance, and standardization) and biological variation (i.e. metabolism, renal elimination, medication, physical and sexual activity, size and integrity of the prostate). Oscillations up to 20–30% in the total PSA range 0.1–20 ng/ml may be due to biologic variation [22,23]. Furthermore, the use of different detection assays may be another important cause of variation. Differences in assay standardization can give an artefactually high or low estimate of total PSA and total PSAV [24–26]. Assays are not interchangeable and caution should be exercised when comparing results from different commercial total PSA assays. Patients and physicians should be aware of which assay was used each time. 

<table>
<thead>
<tr>
<th>Phase</th>
<th>Goals/aims</th>
<th>Experimentation</th>
<th>Sample details</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preclinical Testing</td>
<td>Exploratory; nominate and rank candidate biomarker profiles</td>
<td>Preclinical study for hypothesis generation</td>
<td>Possible bias: small size and convenience sampling</td>
</tr>
<tr>
<td>0</td>
<td>Develop an assay with clinically reproducible results</td>
<td>Reproducibility and robustness of assay; No assessment of benefit</td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>Test on small sample to determine benefit</td>
<td>Marker optimization, establish prediction rules, determine cut-offs</td>
<td>Sample population assay developed from candidate biomarker profile</td>
</tr>
<tr>
<td>II</td>
<td>Determine operating characteristics &amp; internal validation</td>
<td>Retrospective design be the target population</td>
<td>Sample population should be the target population</td>
</tr>
<tr>
<td>III</td>
<td>External validation</td>
<td>Retrospective or prospective, Generalizability, Impact on clinical decision-making</td>
<td>Multi-institutional, large study</td>
</tr>
<tr>
<td>IV</td>
<td>Assess whether biomarker reduces the burden of disease</td>
<td>Post-approval reporting and testing for other disease processes or disease stages</td>
<td></td>
</tr>
</tbody>
</table>

Figure 1. Modification of the structured phase-approach to the systematic discovery, evaluation, and validation of biomarkers [2,3].
a total PSA measurement is performed, and an effort should be made to use the same assay at the next visit. In addition, studies of total PSA kinetics over time using different assays should be interpreted with caution. Of note, there is an expected 20% lower value when the World Health Organization standard is adopted and laboratories should be obliged to mention the name of the PSA assay used on the lab report as well as stating the assay specific reference range and the type of master-calibration (i.e. WHO standardized or traditional calibration) [27–29].

The effect of previous BPH treatment on total PSA remains mostly unpredictable. For example, the effect of commonly used 5-α-reductase inhibitors on the predictive value of total PSA kinetics for tumor progression is uncertain. Because 5-α-reductase inhibitors are known to decrease the PSA level with ~50% and mostly suppress the benign components of PSA secretion, they may enhance the utility of total PSA [30]. In addition, by shrinking the prostate gland, finasteride has been suggested to increase the likelihood of detecting a small cancer on needle biopsy [30].

This large normal variability of total PSA requires larger changes between two consecutive measurements to distinguish pathological changes from changes resulting from analytical and biological variations. Nixon et al. calculated the coefficient of variation over two weeks and demonstrated that a change between two total PSA measurements of approximately 25% indicated a significant change [31,32]. Bunting et al. reported a critical difference, defined as the minimum percent change between two consecutive measurements that suggests a significant change beyond the normal variation, close to 60% over a time period of one year [33]. Bruun et al. recently assessed the long-term variability of the different forms of PSA at several different total PSA levels in a randomly selected population of asymptomatic and apparently healthy men whose total PSA levels were <2.0 ng/mL at the end of the 8-year observation period [34]. They found that the total intra-individual variation of total PSA was much less than that reported by Bunting et al. [33] and somewhat higher than the intra-individual variation for either free PSA or percent free PSA. This suggests that free PSA concentration in blood may vary less than complexed PSA concentration, which is the major contributor to total PSA. One explanation is that free PSA and complexed PSA may have different elimination pathways, and hence different elimination rates [35–38].

**Optimal total PSA cut-off values**

No single total PSA cut-off separates men at high risk for PCAs from men at low risk, nor men affected with high-grade disease from those with low-grade disease. At a total PSA cut-off of ≥4 ng/mL, a significant number of PCAs remain undetected [39–42]. In addition, intervention at lower total PSA levels has been proposed to improve patient outcomes [43,44]. Catalona et al. found that 22% of men with a normal digital rectal examination (DRE) and a serum total PSA level between 2.6 and 4.0 ng/ml have PCAs, and 81% of them have organ-confined disease [45]. Data from the Prostate Cancer Prevention Trial (PCPT) revealed that as many as 15% of men with normal DRE and a serum total PSA less than 4.0 ng/ml have PCAs [39]. Among men with total PSA levels =0.5, 0.6–1.0, 1.1–2.0, 2.1–3.0, and 3.1–4.0 ng/ml, PCAs was detected in 6.6%, 10.1%, 17.0%, 23.9%, and 26.9%, respectively. Moreover, approximately 25% of these men had a tumor with Gleason score of 7 or higher. These and other investigators demonstrated that increasing levels of total PSA are associated with increasing probability of PCa risk within the 0–4.0 ng/ml interval [39,41,46]. There is no total PSA threshold at age 62–91 below which PCAs can be ruled out with high specificity [39]. No single total PSA cut-off separates men with “significant” (high grade, high volume) cancer from those with low-grade, possibly insignificant cancer. Similar to PCA presence, high-grade cancer can be found in men with low total PSA levels.

On the other hand, as of now, there is no evidence that lowering the total PSA threshold below 4 ng/ml improves the long-term survival in men with PCAs. Lowering the total PSA threshold combined with decreasing the age of total PSA screening may be beneficial for men who are at an increased risk for PCAs (i.e. strong family history of PCAs and/or African-American race). However, consideration must be given to the possibility that lowering the total PSA threshold could result in unnecessary biopsies and an increased detection of insignificant cancers. Finally, determination of the optimal, institution-specific, and management-guiding threshold involves not only clinical and epidemiologic features but should also consider the social and psychological implications of prostate biopsy and possible PCA detection.

The difficulty in selecting a cut-off to define what constitutes an abnormal total PSA suggests that total PSA is most useful as a continuous variable, providing a spectrum of prostate cancer risk. Therefore, we prefer to include serum total PSA levels in an overall estimate of the risk of cancer, inform the patient of his particular risk, then make a shared decision about a biopsy [39–41,47–51]. Nam et al., for example, developed a model that predicts an individual’s risk for PCAs in a cohort of 3108 men who underwent a prostate biopsy for the first time [51]. This model comprises factors that can be easily determined at
the time of screening such as age, ethnicity, family history of PCa, the presence of urinary symptoms, total PSA, percent free PSA, and DRE. Addition of all these risk factors improved the predictive accuracy of a base model from 0.62 to 0.74. The main advantage of this and other predictive tools [47] is that clinicians can assess PCa risk on an individual basis and make management decisions. However, despite the reasonable accuracy, similar to all predictive tools, the exact probability cut-off for undergoing or foregoing a biopsy is left with the patient and his treating physician and should be individualized.

Long-term prediction of the future risk of prostate cancer using total PSA

Several studies have suggested that total PSA levels are associated with the risk of PCa years, or even decades, before its diagnosis. The first long-term prediction study, which reported that total PSA levels >2.5 ng/ml predicted diagnosis of PCa over the subsequent decade was limited by the small number of cancer cases (n = 44) and by the degradation of total PSA in archived serum samples [52]. In a prospective study involving a large number of cases, the lead time between total PSA levels ≥4 ng/ml and the subsequent clinical diagnosis of PCa was estimated at 5.5 years [53]. Similarly, Fang et al. studied the risk of PCa diagnosis in a cohort of 549 men following a baseline total PSA measurement at age 40–60 while providing a median follow-up of 13 years [54]. They concluded a total PSA value above the age-adjusted median carried a relative risk of subsequent cancer diagnosis of 3.6.

Two larger studies extended prediction models to lower total PSA ranges and longer follow-up intervals. Loeb et al. examined 1178 men in their 40s who had risk factors for PCA [55]. The risk of subsequent PCa diagnosis was 14.6-fold higher for men with a baseline total PSA level between 0.7 and 2.5 ng/ml compared to men with total PSA <0.7 ng/ml. Lilja et al. assessed PCa risk among 21 277 men younger than 50 years when they attended the Malmö Preventive Medicine study (MPM), a cardiovascular risk assessment study [56]. The investigators measured total PSA levels in archived plasma obtained from 462 participants diagnosed with PCa within a median of 18 years from start of the study and from 1 222 matched controls. Total PSA level at age 44–50 was very strongly associated with the likelihood of developing PCa up to 25 years later. The odds ratio for a PCa diagnosis at a total PSA value of 0.51–1.0 ng/ml was 2.51 compared to total PSA ≤0.50 ng/ml, which roughly corresponded to the population average. The odds ratio increased to 7.02 for a total PSA of 1.0–1.5 ng/ml, and further up to 19.01 for a total PSA of 2.01–3.0 ng/ml compared to a total PSA ≤0.50 ng/ml. In a follow-up study, the authors have further shown that total PSA level at age 44–50 predicts the likelihood of developing advanced PCa, defined as either locally advanced (clinical T3 or higher) or metastatic disease at the time of diagnosis [57]. In another analysis of the MPM-study cohort, the prognostic accuracy of PSA (both total PSA and complexed PSA, described below) decreased with age [58]. The authors hypothesized that these findings result from a greater prevalence of BPH (and therefore of non-cancer-related total PSA increase) among older men.

An analysis of the same cohort demonstrated that PSA at 60 is an extremely strong predictor of the risk of prostate cancer metastasis (AUC 0.86) and death (AUC 0.90) by age 85. Almost all deaths (90%) occurred in men in the top quartile of PSA levels (>2 ng/ml); men with PSA below the median (<1 ng/ml), had an extremely low risk of clinically relevant prostate cancer by age of 85 (0.5% risk of metastasis, 0.2% risk of death from prostate cancer). This suggests that at least half of men can be exempted from prostate cancer screening at age 60, with early detection efforts focusing on a sub-group of men at elevated risk [59].

In summary, these studies indicate that men who will eventually develop PCa have increased total PSA levels years or decades before the cancer is diagnosed. These total PSA levels may reflect the long duration of prostate carcinogenesis or could reflect a causal role of total PSA in PCa development and/or progression. A total PSA measurement before age 50 could help risk-stratify men for frequency and/or type of later PCa screening; a PSA at 60 could determine which men need to continue with screening.

Prostate-specific antigen derivatives

Enhancing the diagnostic accuracy of total PSA, particularly specificity, is critical, since higher specificity would reduce the number of biopsies performed in men not affected by PCa. Several different strategies have been investigated, including the use of age-specific total PSA cut-offs, total PSA density, total PSA density of the transition zone, total PSA velocity (total PSAV), and the measurement of various molecular forms of PSA [47,60–62]. In this section, we will focus on total PSAV and the measurement of various molecular forms of PSA.

Total PSA velocity

Total PSAV refers to the serial evaluation of serum total PSA concentration over time [63,64]. Different methods of calculating total PSAV are available.
(e.g. based on the first and the last measured values or on a regression line through all available measurements, based on normal or logarithmic values), but only small differences in predictive value have been found among these derivatives. Connelly et al. found that using all available total PSA measurements in a linear regression analysis should be the method of choice for calculating total PSAV [65]. When using the first and last measurements only, these should at least be separated by a sufficiently long time period.

Carter et al. showed that patients with BPH demonstrated a linear increase in total PSA levels over time, whereas patients with PCa had an initial linear increase with a subsequent exponential rise that occurred approximately five years before cancer detection [63]. In men with an initial total PSA level between 4 and 10 ng/ml, a total PSAV cut-off value of 0.75 ng/ml per year provided a sensitivity and specificity for PCa of 79% and >90%, respectively. If the initial total PSA concentration was less than 4 ng/ml, the specificity of total PSAV remained >90%, but the sensitivity dropped to 11%. These results were questioned using relatively short total PSA intervals of one and two years [66]. Subsequently, Carter et al. showed that total PSAV values are only useful if a minimum of three consecutive measurements are taken over a two year period [67]. While the specificity of total PSAV is high, its sensitivity is too low to advise against prostate biopsy in a patient with an elevated total PSA level who is otherwise healthy and a good candidate for curative therapy. Other limitations of total PSAV include imprecision due to biological and analytical intra-individual variability (see section entitled “Total PSA and its limitations”) and total PSA stability.

Prospective screening studies have reported that total PSAV does not appear to add diagnostic value for PCa detection beyond that of a single total PSA level. In an analysis of PCPT data, Thompson et al. found that when total PSAV was used alone, it was an independent predictor of PCa presence and aggressiveness [40]. However, when total PSAV was adjusted for the effect of total PSA and other standard variables, it lost independent predictive value. Similarly, the first two screening rounds of the Rotterdam section of the ERSPC found that total PSAV did not improve accuracy when combined with total PSA in the prospective setting [68,69]. A recent analysis from the Prostate, Lung, Colon, and Ovarian (PLCO) cancer screening trial showed that although total PSAV was an independent predictor of high-grade disease, addition of total PSAV to total PSA only slightly increased its performance for prediction of high-grade tumors [70]. Using a large population-based cohort of men in early middle age who were likely to have a low incidence of BPH, Ulmert et al. found no benefit to calculate total PSAV or the velocity of any other PSA form over total PSA for long-term PCa prediction [71]. Of note, the predictive value of total PSAV alone was 71.2%, while the predictive value of a single total PSA was higher (77.1%) and the combined model including both total PSAV and total PSA did not alter the predictive accuracy. The observed lack of additional predictive value for total PSAV indicates that total PSA levels do not increase sharply before PCa diagnosis but rise gradually and slowly over many years, also in those men who later present with advanced cancer.

Several studies have shown that that a high pre-treatment total PSAV is strongly associated with a poor disease-specific survival following diagnosis and could help identify men with low total PSA values who are at increased risk of harboring a potentially lethal tumor [72–75]. Carter et al. found a strong association between survival and higher total PSAV as early as 10–15 years before diagnosis in the Baltimore Longitudinal Study of Aging project [75]. Based on these findings, they proposed that a total PSAV threshold of 0.35 ng/ml per year be used in screening men with low total PSA levels to increase the detection of potentially lethal tumors still in the window of curability. These data have prompted debate as to whether this would suffice as evidence to warrant the National Comprehensive Cancer Network to recommend a prostate biopsy if the total PSAV is greater than 0.5 ng/ml per year [76]. However, in analysis of the PCPT data, Vickers and colleagues showed that biopsy men with low PSA but elevated PSAV, led to a large increase in unnecessary biopsies without detecting an important number of clinically significant cancers [77].

D’Amico et al. reported that men with a pre-operative total PSAV greater than 2.0 ng/ml per year had a 9.8-fold increased relative risk of death from PCa than men with a lower total PSAV [72]. This analysis is compromised by a low number of events; accordingly, it is impossible to tell whether PSAV adds predictive value to standard predictors such as stage, grade and absolute level of PSA. In a more recent study, these investigators reported that total PSAV was also significantly associated with the risk of cancer-specific mortality following external beam radiation therapy [73]. Conversely, using data from 267 Scandinavian men with localized PCa and baseline total PSA levels <50 ng/ml, Fall and colleagues found that, although prognostically relevant, baseline total PSA levels and relative total PSAV in the first two years following diagnosis were not able to predict accurately which patients would have a lethal PCa outcome [78]. Several other studies have found that PSAV does not aid prediction
in men treated by radical prostatectomy [79,80] or conservatively [81].

It may be that the observation period necessary for obtaining a valid calculation of total PSAV that is not disturbed by considerable short-term fluctuations is be too long, or that number of total PSA measurements is too high for use in clinical practice. In addition, total PSAV may not correlate with early tumor progression, but could be a mere indicator of aggressive disease for which the window of curability has already closed. Furthermore, a quickly rising total PSA is more common in men with a high starting total PSA level [82]. This proportion of men is expected to be much smaller in a screened cohort than in a clinically diagnosed cohort. PSAV is a practical parameter after treatment, when PSA is a sensitive measure of cancer; its value in men with a prostate, in whom rises in PSA may be cause by benign disease, remains to be established.

**PSA molecular forms**

Improvements in measuring PSA isoforms have allowed the measurement of free PSA and its ratio to total PSA [83–85]. Other forms include complexed PSA, which is a measure of how much PSA in serum is bound to α2-macroglobulin, α1-protease inhibitor, or α1-antichymotrypsin. Currently there is no assay commercially available which specifically measures the complex of α2-macroglobulin with PSA.

The FDA has approved the use of percent free PSA testing [i.e. (PSA/total PSA) x 100] as an adjunct to total PSA in men with a serum total PSA concentration between 4 and 10 ng/ml. A higher percent free PSA value indicates a lower probability of finding PCa on biopsy and raises the likelihood that the elevation in total PSA is due to the presence of BPH [86,87]. In a multicenter, prospective trial, Catalona et al. reported that when a percent free PSA of <25% is used for triggering a sextant prostate biopsy, it yielded a 95% sensitivity for PCa detection and increased the specificity by 20% over PSA alone [86]. The area under the curve for percent free PSA was significantly higher than that for total PSA (AUC = 0.72 versus AUC = 0.53). However, in response to the realization that sextant biopsies misclassify up to one third of patients who have PCa as without cancer, a more recent evaluation of the utility of percent free PSA in patients undergoing extended 10- or 12-core biopsy has suggested a lower diagnostic efficiency of percent free PSA [88]. While most investigators agree that percent free PSA can improve the diagnostic performance of total PSA between 4 and 10 ng/ml, the most appropriate percent free PSA cut-off value remains debatable. Catalona et al. determined that with a percent free PSA cut-off of less or equal to 27%, they were able to obtain a sensitivity of 90% and avoid 18% of unnecessary biopsies in men over the age of 50 with a total PSA of 2.6 to 4.0 ng/ml [45]. In addition, 83% of these cancers were clinically significant. Finally, data on the utility of percent free PSA for the prediction of pathologic grade and stage of PCa is inconclusive.

Data on the usefulness of free PSA to predict clinical outcomes is inconclusive. Graefen and coworkers failed to detect an independent association of preoperative free PSA with biochemical failure in 581 unscreened patients who underwent radical prostatectomy for clinically localized CaP [89]. In contrast, Shariat and colleagues found that lower preoperative serum free PSA is an independent predictor of advanced pathologic features, biochemical progression, and patterns of aggressive disease progression in 402 consecutive men treated with radical prostatectomy for clinically localized CaP who had total PSA levels less than 10 ng/ml [84].

There are three distinct cleavage isoforms of free PSA in the serum: pro-PSA, BPH-associated PSA (BPSA), and intact free PSA [90]. The precursor of PSA is a 261 amino acid pre-pro-protein. Subsequent processing by human glandular kallikrein 2 (hK2) and other proteases produces the active 237 amino acid mature PSA [90]. Studies have shown that higher levels of pro-PSA are associated with PCa. In men with PSA levels between 6.0–24.0 ng/ml, the [-2]proPSA fraction was found to be significantly higher in men with PCa [90,91]. Moreover, authors demonstrated the utility of the pro-PSA to free PSA ratio for screening patients with PSA levels between 2.5–4.0 ng/ml and between 4.0–10.0 ng/ml [92]. Elevated pro-PSA to free PSA ratios have also been associated with aggressive pathological features and decreased biochemical disease free survival after radical prostatectomy [93,94]. A new automated tool using the [-2]proPSA assay with a percent free PSA based artificial neural network was capable of detecting PCa and more aggressive disease with higher accuracy than total PSA or percent free PSA alone [95]. In a recent prospective cohort of men enrolled into active surveillance for PCa, serum and tissue levels of pro-PSA at diagnosis were associated with need for subsequent treatment [96]. The authors hypothesized that the increase in the ratio of serum pro-PSA to percent free PSA might be driven by increased pro-PSA production from “premalignant” cells.

Molecular forms of PSA may differ in their in-vitro stability properties and information about the pre-analytical conditions is therefore essential for proper clinical interpretation. For proper measurement of [-2]proPSA, blood samples should be centrifuged within three hours of blood draw. Serum may be stored at room temperature or refrigerated...
BPH-associated PSA (BPSA) is formed by the internal cleavage of free PSA between Lys182 and Ser183. BPSA is expressed in nodular hyperplasia limited to the transitional zone of men with BPH. BPSA can be detected in semen, blood and prostate, and its levels correlate with transitional zone volume and obstructive voiding symptoms [90,98]. BPSA seems to be a promising marker of BPH since it has been shown a direct association between its secretion and the volume of the transition zone [98]. As such, BPSA is a better predictor of prostate enlargement than total and free PSA [99]. In addition, BPSA is not affected by age and is significantly higher in the presence of BPH symptoms. Adjusting the level of free PSA for BPSA resulted in 13–17% improvement in specificity compared to free PSA alone while maintaining a sensitivity of 90–95% [100].

Combination of PSA molecular forms for improved cancer detection

It is most unlikely that a single biomarker will have the single decision as to a diagnosis and/or a prognosis of a particular pathology. The future of cancer profiling relies on the combination of a panel of complimentary biomarkers that can give accurate molecular staging and indicate the likelihood of aggressive behavior [10,11,101,102].

The group of Vickers and Lilja has developed a statistical model that predicts prostate biopsy outcomes based on age, DRE and a panel of four kallikrein markers – total PSA, free PSA, intact PSA and hK2. Using data from the randomized prostate cancer screening trial in Göteborg, Sweden [one centre of the European Randomized Study of Prostate Cancer screening (ERSPC)], they estimated that for every 1000 previously unscreened men with elevated total PSA, use of the model to determine biopsy would reduce biopsy rates by 573, while missing only a small number of cancers (31 of 152 low-grade cancers and 3 of 40 high-grade cancers) [15]. These findings were subsequently replicated in an independent cohort (reduction in biopsy by 513 per 1000 men with elevated total PSA, missing 54 of 177 low-grade cancer and 12 of 100 high-grade cancers) [103]. These findings have also been verified in men who recently have undergone previous screening, with resultant improvements in predictive accuracy [104,105]. Recently, Gupta et al. demonstrated that the panel of four kallikrein markers can predict the outcome of prostate biopsy in men who had previously undergone prostate biopsy during previous screening [106]. This model in addition to age and DRE substantially improved the predictive accuracy of a base model (comprising of total PSA, age and DRE), for both low- and high-grade cancers.

Emerging blood-based biomarkers for both prostate cancer detection and prognostication

Human Kallikrein 2 (hK2)

Human kallikrein related peptidase 2 is a secreted serine protease from the same gene family as PSA [107]. They share an 80% sequence homology and are both primarily expressed in the prostate gland [107]. Despite these structural similarities, hK2 and total PSA differ in their enzymatic activity. The levels of hK2 in prostate tissue, plasma, semen, and serum are less than 2% that of total PSA, although hK2 mRNA transcript expression represents half that of total PSA. Similar to total PSA, serum hK2 is present in two forms in the blood: one bound to various protease inhibitors, and the other (preponderant) free in the circulation. Several studies have shown that, when used in conjunction with free and total PSA, serum hK2 could improve the discrimination of men with PCa from men without cancer [108–110]. It has also been suggested that hK2 could predict poor differentiation, extra-capsular extension and biochemical recurrence in patients treated with radical prostatectomy [111–113]. However, this finding has not been validated by other authors [114]. The usefulness of hK2 for the preoperative staging of localized PCa therefore remains controversial. As mentioned above (see “Combination of PSA molecular forms for improved cancer detection” section), the addition of hK2 to three other kallikreins (total, free, and intact PSA) improved the prediction of prostate biopsy results in men with elevated total PSA (increase of predictive accuracy from 68–72% to ~83%) [15]. Considering the risk of PCa at 20%, the number of biopsies would have reduced by half, missing only 3 of 40 high-grade tumors [15].

Emerging blood-based biomarkers for prostate cancer prognostication

Urokinase Plasminogen Activation (uPA)

The urokinase plasminogen activation (uPA) axis represents a potential target for PCa markers by being involved in various phases of tumor development and progression though degradation of the extra-cellular matrix. The serum protease uPA may play a role in cancer progression by binding to the uPA receptor (uPAR) and consequently converting plasminogen to plasmin, which activates proteases related to the degradation of extracellular matrix proteins [115].
Immunohistochemical staining of radical prostatectomy specimens revealed that overexpression of both uPA and its inhibitor (PAI-1) were associated with aggressive PCa recurrence [116]. In patients with a total PSA level above 2 ng/ml, soluble uPAR and free PSA measured in serum before prostate biopsy improved the regression model accuracy for prediction of PCa [117]. Steuber et al. recently showed that uPAR fragments were significant predictors of PCa on biopsy specimens of patients with an elevated PSA [117].

Both uPA and uPAR might also have a prognostic value. Elevated circulating levels of uPA and uPAR have been linked to PCa stage and bone metastases [116,118–120]. In a study of 429 patients treated with radical prostatectomy, preoperative plasma uPA was a strong predictor of biochemical recurrence after surgery. Both preoperative uPA and uPAR were associated with features of aggressive biochemical recurrence such as development of distant metastasis suggesting an association with occult metastatic disease at time of local therapy. Moreover, elevation of plasma uPA and uPAR levels in PCa patients seemed to be partly caused by local release from the prostate. Larger multi-institutional studies are under way to validate the potential role of uPA and uPAR as markers of metastatic PCa.

**Transforming Growth Factor-Beta 1 (TGF-β1) and Interleukin-6 (IL-6)**

TGF-β1 is a growth factor involved in the regulation of several cellular mechanisms including proliferation, immune response, differentiation and angiogenesis [121]. TGF-β1 has been shown to promote cell progression in PCa models and its local expression has been associated with higher tumor grade, tumor invasion and metastasis in PCa patients [122,123]. Several studies have shown that increased levels of circulating TGF-β1 were associated with cancer progression, occult and documented metastasis and biochemical progression in PCa patients [123–125].

IL-6 is a cytokine with variable effects on immune and hematopoietic mechanisms. In vitro and in vivo studies have shown that both IL-6 and its receptors (IL-6R) were expressed in PCa [126,127]. Several authors reported that elevated serum levels of IL-6 and IL-6R were associated with metastatic and hormone refractory disease, and suggested that IL-6 could predict progression and survival of PCa patients [128,129].

Based on these findings, Kattan and associates developed and internally validated a prognostic model that incorporates plasma TGF-β1 and IL-6R into a standard nomogram for prediction of biochemical recurrence following radical prostatectomy [14]. This combination of serum markers and classical clinical parameters improved the predictive accuracy by a statistically and prognostically substantial margin (increase in predictive accuracy from 75 to 84%). However, before a biomarker can become useful in daily clinical management, it needs to be externally validated in an independent cohort of patients (Figure 1) [2,3]. Therefore, in a multi-institutional dataset of 423 patients treated with radical prostatectomy, Shariat et al. confirmed that plasma levels of TGF-beta1 and IL6-SR considerably enhance the accuracy of the standard preoperative nomogram for the prediction of biochemical recurrence (accuracy of clinical features plus biomarkers 87.9% versus 71.1% for clinical features alone; p < 0.001). Such prognostic models refine our ability to identify patients at a high risk of biochemical recurrence after radical prostatectomy who may benefit from inclusion into perioperative clinical trials and/or intensified follow-up protocols.

**Endoglin**

Endoglin, or CD 105, is a transmembrane glycoprotein that is typically expressed by human vascular endothelial cells. Functionally, it is a cell-surface co-receptor for transforming growth factor β1 (TGF-β1) and β3 (TGF-β3) [130] that modulates cellular responses to TGF-β in the early steps of endothelial cell proliferation. Its critical role in angiogenesis has prompted investigators to evaluate the role of Endoglin in cancer progression and metastasis. In PCa, Endoglin is preferentially found on new, immature blood vessels and immunohistochemical analysis supports an association between Endoglin expression and disease progression [131]. Urine levels of Endoglin may distinguish patients with PCa and may help in the staging of the disease [132]. In addition, pre-operative plasma Endoglin were found to be associated with metastasis to regional lymph nodes [133], established features of biologically aggressive PCa such as higher pathologic Gleason sum, and biochemical recurrence following radical prostatectomy [134]. Use of pre-operative plasma Endoglin could help decide whether and how extensively to perform a lymphadenectomy as well as preoperative identification of patients at risk for disease progression. This would help select patients for neo-adjuvant and/or adjuvant therapy or enrollment into clinical trials. Moreover, Endoglin may be valuable as a surrogate biomarker for occult metastatic disease in patients with presumed organ-confined disease. Further investigation is needed to validate Endoglin as a useful biomarker in men with PCa and to elucidate the mechanistic role of this biomarker in the progression of PCa.
Combination of blood-based biomarkers for prostate cancer prognostication

A biomarker may reflect disruption of a biochemical pathway by a particular mechanism. Given the complexity of the molecular abnormalities associated with PCa, it is improbable that a single marker can accurately segregate tumors of similar clinicopathologic phenotypes into distinct prognostic categories. Therefore, combinations of independent, yet complementary markers, may provide a more accurate prediction of outcome compared to a single marker [62].

Shariat et al. found that the addition of a panel comprising pre-operative plasma levels of TGF-β1, soluble IL-6R, IL-6, Endoglin, vascular endothelial growth factor (VEGF), and vascular cell adhesion molecule-1 (VCAM-1 or CD 106) [120,121,123, 133–137] improved the predictive accuracy the Kattan pre-operative nomogram [9] by 15.0% (i.e. 71.6 to 86.6%) [11,102]. This increase substantially exceeds accuracy gains obtained from the consideration of detailed pathologic descriptors of prostate cancer at radical prostatectomy. Svatek et al. confirmed the strong predictive value of pre-operative levels of the candidate biomarkers after adjusting for the effect of post-operative features [101]. Addition of pre-operative levels of the candidate biomarkers improved the accuracy of the base model (i.e. total PSA, surgical margin status, extracapsular extension, seminal vesicles invasion, lymph node involvement, and pathologic Gleason sum) for prediction of biochemical recurrence by a statistically and prognostically significant margin (79 to 86%, p < 0.001). Predictive tools integrating biomarker levels could constitute the new standard for counseling patients regarding their risk of recurrence following curative therapy and for designing clinical trials to test neo-adjuvant and/or adjuvant treatment strategies in high-risk patients. However, while prediction of biochemical recurrence is important, prediction of response to therapy as well as metastasis and survival is more important for the management of PCa patients [138].

Blood-based biomarkers for monitoring of prostate cancer treatment

Total PSA

Treatment monitoring is the most accepted clinical application for total PSA. Total PSA is used for monitoring response to local treatments such as radical prostatectomy, various methods of radiation therapy, and other local therapies including cryosurgery, as well as systemic treatments such as androgen deprivation therapy and chemotherapy. Post-treatment total PSA levels can provide invaluable information about the effectiveness of the therapy given and the existence of residual cancer in men treated with local therapy with curative intent. In such patients, rising total PSA levels can signal cancer activity well before any clinical signs of recurrence appear. This lead-time can be further increased by months and even years.

In patients treated with systemic therapy, post-therapy total PSA changes are a seemingly perfect outcome measure because they are easily assessable, quantitative, reproducible, and inexpensive; this is true regardless of whether this outcome measure is applied to evaluate drugs in the clinical trial setting or in clinical practice. Critical to the successful application of total PSA measurement as an endpoint are the therapeutic objectives of the trial and the mechanisms of action of the treatment administered. Criteria were proposed to screen for treatment effects in prostate cancer clinical trials on the basis of the hypothesis that total PSA declines reflect significant cell kill in response to agents that cause reduction in overall tumor burden. With the recognition that short-term declines in total PSA levels might simply reflect the effect of the drug on the marker, and not on cell growth or survival, it is recommended that the declines be documented on more than one occasion and, equally importantly, over time. Indeed, different total PSA-based outcomes would be required for different classes of drug. For example, drugs that are not anticipated to kill cells would not be expected to produce declines in total PSA. Similarly, ‘differentiating effect’ might produce an initial rise in total PSA that might be an indication that the drug is actually working. A single set of outcomes would not only be inapplicable to agents that act via diverse mechanisms, but could also be misleading.

Unfortunately, the significance of post-therapy changes in total PSA have been misinterpreted as indicators of tumor response, and/or as a measure of clinical benefit. An additional misconception is that the demonstration of a particular degree of decline in total PSA, in a proportion of patients, is an indication that the treatment prolongs life, and as such, should be used as an endpoint for accelerated drug approval.

The use of a post-therapy decline in total PSA as an outcome measure has been justified in part by statistical analyses exploring the relationship between the defined outcome measure and survival [139]. Most of these reports include multivariate techniques, and in some, the resultant models were validated on independent data sets. These early reports did not assess randomized comparative trials showing a survival benefit, a necessary but not sufficient condition in which to explore measures of surrogacy [139,140].

There is no clear demonstration that a post-therapy total PSA change can account for all of the
Blood-based biomarkers for prostate cancer

also important to note that there remains significant and angiogenesis and growth factor inhibitors. It is through immunization strategies where the effect on rise in total PSA before a decline is observed, or act through immunization strategies where the effect on total PSA levels might be delayed or not occur at all, and angiogenesis and growth factor inhibitors. It is also important to note that there remains significant variation in the methodologies and assays used by different laboratories, which makes it difficult to compare results between groups. All of these factors must be considered carefully to ensure that a drug is not discontinued prematurely on the basis of a total PSA rise that is not relevant to the drug under study.

Conclusions

The introduction of total PSA in clinical practice has resulted in early detection and reduced mortality from PCa [141]. However, PCa screening remains controversial, because of the risk of overdiagnosis reduced mortality and overtreatment and the inability to detect a significant proportion of dangerous tumors. A large concerted effort has been made to improve and/or monitoring the activity of PCa and to guide molecular targeted therapy and/or assess therapeutic response. An integrated approach with blood-based measurement of different molecular forms of PSA in combination with genetic and urine biomarkers hold the promise of improving screening for and diagnosis of PCa. Panels of blood-based biomarkers will allow a fingerprinting of the tumors biologic behavior resulting in individualized therapy and monitoring. In addition, the emergence of new therapeutic approaches for PCa cannot flourish without a set of markers to serve as prognosticators, predictors, therapeutic targets, and/or surrogate end points.

Declaration of interest: Dr. Shariat holds patents for soluble FAS and Biomarker Nomograms. Dr. Lilja holds patents for free PSA, intact PSA, and hK2 assays.

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Blood-based biomarkers for prostate cancer


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Tumour markers in prostate cancer II: Diagnostic and prognostic cellular biomarkers

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Abstract

The main goal of prostate cancer tissue biomarkers is to improve diagnostic and prognostic accuracy. A particularly important question is whether the cancer needs immediate treatment or if treatment can be deferred. It is highly unlikely that a single biomarker that provides comprehensive prognostic information about a newly diagnosed prostate cancer will be forthcoming. Despite extensive research efforts, very few biomarkers of prostate cancer have been successfully implemented into clinical practice today. This can be partly explained by a lack of standardised methods for performance and interpretation of immunohistochemistry, but also by poor study design with insufficient biomaterial or inappropriate statistical analysis. Also appropriate cohorts to test prostate cancer biomarkers do not exist. It must be kept in mind that unsuccessful integration of new biomarkers in nomograms can also be explained by the good performance of the clinical and pathological base model with serum PSA as the only independent biomarker. A new biomarker must be powerful enough to improve this prediction model and not merely replace. Material and methods. In this report, we focus on diagnostic and prognostic cellular biomarkers in prostate cancer, recent advances and future aspects by reviewing currently available literature. Results. Similar to other malignancies, the proliferation marker Ki-67 seems to be a prognostic tissue biomarker and a strong candidate for integration in prediction models. Circulating tumour cells are promising markers of response to treatments in patients with metastatic disease. Conclusion. Important technical advances together with histological techniques of antibody or probes conjugated with different fluorophores will certainly improve standardisation and make immunohistochemical biomarker research more reliable and precise in the future. Cellular biomarker studies are also expected to change in the future towards a complexed individualised profiling of human tumours with integrative analysis using different technologies, genome-wide scanning and expression profiling.

A biomarker can be defined as a molecular test to provide additional information over current clinical data. There is a need for biomarkers in prostate cancer for several reasons:

1. to improve cancer detection and staging;
2. to identify subclasses of prostate cancer;
3. to predict outcome after treatment;
4. to select patients for different treatment options.

The introduction of the well established biomarker prostate-specific antigen (PSA) testing has impacted the detection rate of prostate cancer and is responsible for down-staging at diagnosis, with the vast majority (approximately 80%) of newly diagnosed tumours being localised to the prostate. Gleason score and clinical stage at the time of diagnosis are important factors to predict prognosis and outcome after therapy but additional accurate and reliable biomarkers are warranted. An increasing proportion of tumours fall into the category of clinical stage T1c, low Gleason score (6 or less) and PSA of less than 10 ng/ml and may be considered as indolent cancers. Despite extensive research efforts, very few biomarkers of prostate cancer have been successfully implemented and used in clinical practice today. In fact, the only prostate cancer biomarker routinely used in prediction models is PSA in blood.

The search for diagnostic or prognostic tissue biomarkers in prostate cancer was predominantly based on immunohistochemistry and a large number
of tumour markers with prognostic information were proposed. However, a vast majority of these are not used in clinical practice, probably due to lack of standardised methods to perform and interpret immunohistochemistry, but also due to inadequate study design with insufficient biomaterial and inappropriate end points (e.g., biochemical recurrence instead of death) or misleading statistical analysis. The influence of tumour heterogeneity is yet another unsolved problem, especially when only a small part of the tumour volume is available for examination; i.e., in the case of prostate biopsies. Biomarker studies after radical treatment often lack sufficient end point data and only those from radical prostatectomy have plentiful tissue. Diagnostic tissue specimens are all that is available for those treated by radiotherapy, hormone manipulation or active surveillance. Subsequently, many reports on promising prognostic tissue biomarkers were never successfully reproduced and validation studies are still missing for most prostate cancer tissue biomarkers.

In 2005, the Statistics Subcommittee of the NCI-EORTC Working Group on Cancer Diagnostics encouraged transparent and complete reporting of prognostic tumour marker research by publishing the REMARK document “Reporting recommendations for tumor MARKer prognostic studies”, which led to a general reduction in the number of studies describing new immunohistochemical markers [1]. More recently, a systematic and comprehensive review recently focused on the use of classical and novel biomarkers as prognostic risk factors for localised prostate cancer and highlighted the poor quality and heterogeneity of studies, which render much of the results inconclusive [2].

In the era of "omics" (genomics, proteomics, metabolomics, pharmacogenomics, etc.), tissue-based biomarker research has evolved dramatically. Recent advances in high-throughput technology, microarrays and bioinformatics have opened the door to gain much more information from an individual tumour and to develop multiplex tests to obtain a complete characterisation and to predict the behaviour of prostate cancer.

In this report, we focus on a selection of established and promising tissue biomarkers in prostate cancer, recent advances and future aspects. Areas of research based on novel and complexed technology like proteomics and genomics will not be covered in this report.

**Diagnostic prostate cancer markers**

The diagnosis of prostate cancer in histopathological specimens is mainly based on a combination of architectural and cellular atypia. In the vast majority of cases a confident diagnosis can be made based on morphology alone. However, in some cases the morphological findings are insufficient for a conclusive diagnosis, either because the atypia is too mild or the atypical focus too small. In a review of the literature, atypia suspicious for cancer was found to have been reported in an average of 5% of needle biopsy series [3]. The diagnostic accuracy can be improved in some of these cases by immunohistochemistry using one or several biomarkers, either on consecutive sections or using a staining cocktail.

**High molecular weight cytokeratin**

Invasive prostate cancer almost invariably lacks basal cell layer. The cytokeratin profile of basal cells of prostatic glandular epithelium differs from that of luminal cells. The basal cell layer can be labelled with antibodies against high molecular weight cytokeratin or cytokeratin 5. In rare cases (0.3%) there is an aberrant labelling of cancer cells by high molecular weight cytokeratin but the band-like basal cell distribution is not seen [4]. More problematic is that benign lesions such as partial atrophy may be negative for high molecular weight cytokeratin [5]. Thus, it is important that results with this biomarker is interpreted with great caution and carefully correlated with conventional morphology.

**p63**

An alternative to high molecular weight cytokeratin as basal cell marker is p63, a homologue to p53. This biomarker can be used either alone or in a cocktail with high molecular weight cytokeratin [6]. Similar to high molecular weight cytokeratin false negative stains of benign glands occur and correlation with morphology is of paramount importance. Aberrant diffuse expression of p63 in prostate carcinoma has been described [7]. The sensitivity of p63 and high molecular weight cytokeratin is comparable [8].

**AMACR**

Alfa-methylacyl-CoA-racemase (AMACR) is expressed in cancer and usually negative in benign glands [9]. Staining for AMACR in atypical cases with negative basal cell markers can convert the diagnosis from atypical to cancer in 50% of needle biopsy cases reviewed by an expert uropathologist. The sensitivity varies between 80–100% in small atypical foci on needle biopsy [10]. However, variants of prostate cancer that are more difficult to diagnose are unfortunately less often positive: 62–68% of foamy gland prostate cancers and 70–77% of pseudohyperplastic prostate cancers [11]. AMACR is also positive in PIN and occasionally in benign lesions such as atrophy and adenosis, hence, similar to basal cell markers, the results have to be interpreted with caution and carefully compared with hematoxylin and eosin stained sections.
Prognostic tissue biomarkers

Tissue biomarkers may be of value to predict the effect of certain treatments but also as a general prognostic tool. The natural history of prostate cancer has shown that the molecular determinants may change with time by clonal selection of tumour cell populations towards a more aggressive phenotype. However, we also know that some prostate cancers show an aggressive behaviour already at an early stage and there is an urgent need to identify tumours that need immediate treatment.

Kallikreins

The human tissue kallikrein KLK gene locus consists of 15 genes on chromosome 19q13-4 [12]. The best known of these genes is kallikrein 3 (KLK3), also known as prostate-specific antigen (PSA), that is a widely used clinical tumour marker in serum for detection and monitoring of prostate cancer progression. PSA is produced in luminal cells of the benign epithelium and in most tumour cells. Thus it has a prostate-specific but not a tumour specific expression, which explains the low specificity of serum PSA measurement to detect small tumours in patients with benign prostatic hyperplasia (BPH). Human kallikrein type 2 (hK2) is similar to PSA in structure and is overexpressed in tumour cells [13]. In an immunohistochemical study hK2 was expressed in every cancer, and the expression incrementally increased from benign epithelium to primary cancer and lymph node metastases [14]. Lintula et al. demonstrated that the ratio of hK2/PSA mRNA increased with grade of tumour [15]. Using a large tissue microarray (TMA) containing samples of 3,261 prostatectomy specimens Erbersdobler and co-workers showed that the loss of PSA expression in tissue samples of prostate cancer is associated with adverse pathologic features and clinical outcome but is not an independent prognostic factor for PSA recurrence after prostatectomy [16]. Immunohistochemical studies on other kallikrein family members have also been suggested to be of prognostic value in prostate cancer patients but validation studies are still lacking.

Prostate-specific membrane antigen (PSMA)

The prostate specific membrane antigen (PSMA) is a cell surface membrane protein. It is an attractive antigen for antibody-based diagnostic and therapeutic intervention in prostate cancer, since it is highly restricted to the prostate and overexpressed in all tumour stages [17]. In a TMA study, high PSMA expression was independently associated with PSA recurrence in a high-risk cohort and thus might provide insight into the additional use of adjuvant therapy in this group of patients [18]. Again, validation on other cohorts is required and further evaluation of this marker is needed to determine whether or not it has clinical utility for prostate cancer detection or treatment monitoring.

Ki-67

The Ki-67 protein is well known and widely used to assess the tumour proliferation rate and numerous studies have shown Ki-67 to be a prognostic marker in prostate cancer patients treated by radical prostatectomy, radiotherapy or androgen deprivation therapy (ADT). In a cohort of 808 patients diagnosed by TURP treated conservatively, Ki-67 expression was assessed immunohistochemically in two laboratories, by two different scoring methods and the results compared with cancer-specific and overall survival [19]. Using both methods, Ki-67 provided additional prognostic information beyond that available from Gleason score, PSA and tumour extent. Although it was confirmed to be the most promising biomarker, confirmatory studies need to occur in TRUS biopsy material in order for prospective studies to implement use into routine practice and prediction models.

Androgen receptor

Expression of kallikreins and several other gene products in the benign and malignant prostate gland are dependent on the androgen receptor (AR) function. Studies in animals and humans have demonstrated that AR plays a key role in prostate cancer progression with an overexpression in castration-resistant prostate cancer (CRPC) [20]. However, expression of AR may be of prognostic value also in hormone-naïve prostate cancer. Using a total of 640 cases in a TMA setting, Li et al. correlated levels of AR expression with some well-established clinical and pathologic parameters in patients treated with radical prostatectomy [21]. High levels of AR status correlated with proliferation (high Ki-67 index) and a high level of AR expression was an independent predictor of decreased biochemical recurrence-free survival. The prognostic role of AR in prostate cancer was recently confirmed in a study from an independent group who described that increased nuclear AR expression in either the diagnostic biopsy and/or radical prostatectomy specimen, from patients with advanced disease, was associated with a reduced time to prostate cancer-specific mortality [22]. Simple immunohistochemical analysis of AR may not be sufficient to evaluate AR function, because tumour cells may harbour differences in the AR genotype with variation in the length of CAG repeat, mutations and increased copy number.
Gene fusions

The bioinformatic tool Cancer Outlier Profile Analysis (COPA) was developed to analyse DNA microarray data for outlier genes and used when they presented the first evidence of current rearrangements in common epithelial tumours as demonstrated by fluorescence in situ hybridisation (FISH) [23]. Gene fusions involving members of the erythroblast transformation-specific (ETS) family of transcription factors were reported to occur in prostate cancer and with the gene fusion of the 5’-untranslated region of the androgen-regulated gene TMPRSS2 and the ETS family gene ERG as the predominant finding (approximately 90%). Several studies have confirmed the presence of TMPRSS2–ERG fusions in 36–78% of prostate cancers from PSA screened surgical cohorts. Conflicting results have been reported regarding the prognostic significance of prostate cancers harbouring TMPRSS2-ETS gene fusions. Duplication of the fusion TMPRSS2 to ERG sequences was shown to identify fatal human prostate cancer in a cohort on 445 men conservatively treated for prostate cancer [24] but in a recent TMA study of 521 cases of clinically localised prostate cancer, Fine and co-workers found that TMPRSS2–ERG fusion was associated with low Gleason scores and not with high-grade morphological features [25]. Taken together, it seems like the TMPRSS2–ERG fusion gene is an early event related to development of prostate cancer rather than a marker for progressive disease. Duplication of the gene may be merely an indicator of overall genetic instability, aneuploidy and hence, poor prognosis. Several strategies for therapeutically targeting ETS fusions have been identified and are currently being pursued.

PTEN

The phosphatidylinositol 3-kinase (PI3K) is a frequently activated signal transduction pathway in prostate cancer cells [26] and the most common mechanism of PI3K activation is deletion of the gene encoding the phosphatase and tensin homologue (PTEN) protein. PTEN deletion has been reported to be more frequent in metastatic than in organ-confined prostate cancer [27]. Decreased expression of PTEN and consequent activation of the PI3K pathway members in prostate cancer tissue samples has been correlated to higher Gleason grade, advanced stage, and development of androgen resistance [28,29]. These discoveries have already been shown to have clinical impact and several phase 1 and 2 clinical trials with specific agents targeting key activated proteins in the PI3K pathway are ongoing in prostate cancer patients [30]. A recent finding that may have impact on future studies is that PTEN deletion, cooperates with aberrant ERG expression to promote prostate cancer progression [31]. Use of molecular biomarkers related to the PI3K pathway, may facilitate future selection of patients for these novel therapeutic regimens.

p53

The tumour suppressor gene p53 is mutated in half of human malignancies and has been intensively studied in numerous cancer models. Many of these studies have suggested that p53 may be of prognostic value in prostate cancer after different treatments. In a more recent study on patients treated conservatively for prostate cancer, p53 remained prognostically significant in a multivariate model though the risk ratios were less strong compared to Ki-67 [32].

SPINK1/TATI

Tumour-associated trypsin inhibitor (TATI), which is alternatively called pancreatic secretory trypsin inhibitor (PSTI) or serine protease inhibitor Kazaltype 1 (SPINK1), is expressed in various normal and malignant tissues, and is known as a prognostic tumour marker as reviewed by Paju and Stenman [33]. TATI was first shown to be overexpressed in high-grade prostate cancer [34] and later, outlier expression of SPINK1, the gene coding for the TATI protein was identified exclusively in a subset of ETS rearrangement-negative cancers (approximately 10% of total cases) [35]. A series of in vitro and in vivo experiments revealed SPINK1/TATI to be associated with prostate cancer aggressiveness and with invasive growth in a prostate cancer cell line (22RV1) with outlier expression. It was thus shown that SPINK1 outlier expression defines an aggressive molecular subtype of prostate cancer (approximately 10% of cases) not attributable to known gene fusion events. Further support of the potential role of SPINK1 expression as a prognostic biomarker was recently demonstrated by Leinonen et al. who studied a cohort of men with endocrine-treated prostate cancer [36]. Additional work is needed to understand the tumour biology behind the role of SPINK1/TATI as a tissue biomarker in prostate cancer.

MSMB and EZH2

MSMB (also known as prostate specific protein of 94 amino acids (PSP94)) is expressed in benign and malignant prostatic epithelium and is along with prostate specific antigen (PSA) and prostate acidic phosphatase (PAP) one of the three most abundant proteins in human seminal plasma [37]. Additionally, MSMB, located at chromosome 10q11.2, has recently been reported as an important candidate gene for prostate
cancer susceptibility [38,39]. Several groups have employed a variety of approaches in both tissue and serum samples to demonstrate decreasing levels of MSMB in prostate cancer compared to normal controls, prompting the suggestion that MSMB may be a promising biomarker for prostate cancer. In a large TMA study of almost 1,000 patients with localized prostate cancer, MSMB was found to be an independent predictor of recurrence after radical prostatectomy; however, addition of MSMB did not importantly improve the performance of existing predictive models [40]. Molecular mechanisms behind the decreased expression of MSMB in prostate cancer is not yet fully clarified. In advanced, castration-resistant prostate cancer decrease expression of MSMB was found to correlate with an increased expression of the Polycomb gene (PcG) protein EZH2 (enhancer of zeste homolog 2), which represses transcription via trimethylation of histone H3 on Lys27 (H3K27) [41]. EZH2 has also been attributed the role of a useful tissue biomarker in prostate cancer [42]. Furthermore, EZH2 has also been suggested to silence expression of E-cadherin by trimethylation of H3 lysine 27, providing a functional link between dysregulation of EZH2 and repression of E-cadherin during cancer progression.

Epigenetic silencing of genes by EZH2 and activation of members of the PcG group may be common oncogenic events in pathogenesis of metastatic solid tumours and provide justification for development of small molecule inhibitors of the PcG chromatin silencing pathway as a novel therapeutic modality for treatment of metastatic prostate cancer [43].

**Heat shock proteins**

Heat shock proteins (HSPs) are molecular chaperones, protecting cells against stress-related injury [44]. Although HSPs are important for the function of normal cells, cancer cells frequently overexpress HSPs in response to stress and may thereby gain a survival benefit. It has been demonstrated by proteomics that prostate cancer overexpress HSP60 and HSP70 [45]. In a validation study using immunohistochemistry, HSP60 was an independent predictor of biochemical recurrence after radical prostatectomy in multivariate analysis including extraprostatic extension, margin status, seminal vesicle invasion and Gleason score, while HSP27 correlated with outcome in univariate analysis [46]. Interestingly, HSPs are potential therapeutic targets in prostate cancer patients. Inhibition of the function of HSPs may decrease tumour cell survival by blocking the antiapoptotic activity of HSPs [47]. HSP90 is suggested to play a key role in prostate cancer through interaction with AR and recently designed mitochondrial Hsp90 chaperones are potentially attractive therapeutic targets in advanced prostate cancer [48].

**DNA methylation**

Epigenetic events that can affect gene expression without altering the actual sequence of DNA include phenomena such as DNA methylation, chromatin remodeling, histone modification and RNA interference [49]. Many gene promoters are associated with GC rich regions of the DNA known as CpG islands. Abnormal methylation of CpG islands located within gene promoters is associated with decreased transcriptional activity and it occurs in many types of cancers. Abnormal methylation of genes such as those involved with control of cellular growth or detoxification is believed to have a critical role in early stages of PCA progression. Already in 1994, glutathione S-transferase P (GSTP1) hypermethylation was introduced as a central part of prostate carcinogenesis [50]. GSTP1 is a member of a large family of glutathione transferases that function to protect cells from oxidative insult thus, the biological rationale for selecting this marker is its role in preventing damage to cells by neutralising free radicals. GSTP1 has been extensively studied in prostate cancer, and its reduced expression, due predominantly to promoter hypermethylation, is the most common epigenetic alteration associated with this disease. Several studies have shown a high sensitivity for this marker to detect the presence of both PIN and prostate cancer, an ability to distinguish these from BPH, and a prevalence of methylation in the range of 60–80% in prostate cancer. Strengths of GSTP1 as a clinical marker are the ability to quantitate the methylation status of the GSTP1 gene in biopsy/prostatectomy tissues and in cells derived from serum, urine, and seminal plasma. Recent studies using quantitative real-time methylation sensitive PCR demonstrate that epigenetically modified genes are candidate markers for early detection and post-treatment monitoring of prostate cancer, however it is more likely that urine and serum will be used instead of tissue samples. This role of hypermethylated genes as promising biomarkers was recently reviewed by Ahmed who emphasised that in addition to clinical validation, assays for methylated genes must be robust, simple, sensitive, specific, and made available at affordable costs [51].

**HER2**

Overexpression, or gene amplification of the human epidermal growth factor receptor 2 (HER2) is evident in 20–25% of breast cancers and correlated with disease outcome. Validated methods for determination of HER2-status by immunohistochemistry and/or FISH are used to identify patients with breast cancer who are eligible for treatment with trastuzumab, an HER2-targeted monoclonal antibody that inhibits the proliferation of tumour cells and induces tumour
cell death through multiple mechanisms. HER2 has also been linked to the clinical progression of CRPC. In a recent meta-analysis, Serpa Neto and collaborators [52] investigated the prognostic impact of HER2 over expression in patients with prostate cancer and its correlation with other pathological and clinical variables. Several databases were searched for studies of HER2 protein expression in primary prostate cancer tissue. The overall relative risk of death in those with HER2 over expression in the primary tumour was 1.63 (95% CI 1.47–1.82, p<0.0001) and the authors found a consistent association of HER2 over-expression with death and recurrence. The clinical usefulness of HER2-status in prostate cancer patients still has to be proven.

NXX3.1 and MYC

Losses of 8p and gains of 8q are two of the most common chromosome aberrations in prostate cancer [53]. Gene products related to these loci are candidates as new important biomarkers in prostate cancer. NXX3.1 (8p21) is an androgen-regulated transcription factor that regulates the proliferation rate of prostatic luminal epithelial cells and functions as a tumour suppressor gene [54]. Experimental work from different groups have demonstrated the importance of NXX3.1 in development of prostate cancer, but studies on its role as a tissue biomarker have generated conflicting results.

The well-known oncogene MYC resides on the short arm of chromosome 8. MYC is a well-known regulator of proliferation and biologic activity in prostate cancer cells, and its amplification is associated with the presence of PIN and poor clinical outcome of prostate cancer [55]. Similar to NXX3.1, additional studies are needed to understand how MYC can be clinically used as a tissue biomarker in prostate cancer.

Validation and implementation of tissue biomarkers

Examples of statistical models to predict outcome in prostate cancer patients are the clinical nomograms (www.nomograms.org) [56]. These models incorporate known prognostic variables, such as PSA, Gleason grade, extracapsular extension, lymph node involvement, and surgical margins to estimate the probability of disease recurrence following prostatectomy and other treatments. Unsuccessful integration of new biomarkers in nomograms can be explained by the already high performance of the clinical and pathological base models with strong independent biomarkers. Several questions must be satisfactorily met before a new biomarker can be implemented:

1. Can the marker be measured accurately and reproducibly? The value of each biomarker must be tested in large annotated series of patients with reliable independent validation sets. The assay must be robust and reproducible.

2. Does the marker provide additional information to that already available to the clinician? An important step in evaluating the value of a new marker is to compare the predictive accuracy of a model including only standard clinical variables with that of a model including standard clinical variables plus the new marker [57]. The statistical analyses must be robust and demonstrate a significantly improvement in receiver operation characteristics (ROC) analyses or Concordance Index (CI) when the new biomarkers is added to the base model.

3. Is the marker associated with outcome in the sort of patients to whom the marker would be applied to in clinical practice? Before we use a marker in clinical practice, we need to know whether it will improve clinical outcome. In other words, we need evidence that measuring the marker would change the decision a doctor would have made in the absence of the marker, and that this changed decision will benefit the patient. If an assay failed to give reproducible results, it would clearly be of little clinical value and would not be worthy of subsequent research.

Conclusions and future aspects

It is unlikely that a single biomarker will provide all information we need to tell how aggressive a newly diagnosed prostate cancer is. No immunochemical, or genetic marker is currently used to differentiate between aggressive and non-aggressive prostate cancers.

Recent discoveries in genetics, proteomics and bioinformatics using new high-throughput arrays indicate that new multiplex tests will soon be launched and some even commercially available, however, the above mentioned criteria for evaluation of new biomarkers must always be strictly followed. Tissue biomarker research will change in the future as illustrated in a recent report by Taylor and co-workers who used different new technologies for integrative genomic profiling of human prostate cancer and identified new candidate biomarkers in prostate cancer [58]. Technologies of genome-wide scanning such as gene expression profiling, comparative genomic hybridisation (CGH), and single nucleotide polymorphism (SNP) arrays are now also applicable to nucleic acids extracted from archival tissues on large series of prostate cancer patients.
Development of TMA for large-scale analysis and automated image analysis systems for more precise quantitation and a direct transformation of scoring data to biostatistical analysis are now progressively replacing the subjective, semiquantitative manual scoring previously performed by pathologist. These important technical advances together with histological techniques of antibody or probes conjugated with nanoparticles (quantum dots) and other fluorescent conjugates will certainly improve the standardisation and make immunohistochemical biomarker research more reliable and precise in the future.

Another challenge is to identify a specific tissue biomarker for prostate cancer stem cells. Today it seems like a combination of different markers is needed to identify a cancer stem cell population. Uglokov et al. [59] performed immunohistochemical analysis of proposed stem cell markers (CD44, CD133, Oct4, SOX2 and EZH2) in benign and malignant prostatic tissues and suggested that suggest that combined expression of embryonic stem cell markers EZH2 and SOX2 might identify potential cancer stem cells as a minor (<10%) subgroup in CD44+ prostatic adenocarcinoma. A new and different approach is aldehyde-dehydrogenase (ALDH) – based sorting of human prostate cells by flow cytometry (ALDEFLUOR assay) which is used to simultaneously select putative prostate cancer stem cells and metastasis-initiating cells [60]. Analysis of ALDH activity of clinical prostate cancer samples may thus become useful for the stratification of prostate cancer patients at risk of developing metastatic disease.

An important question that remains to be answered is the cellular origin of human prostate cancer. Using a combination of immunohistochemical and biochemical studies in vitro and in vivo, an American group recently provided evidence that prostate tumours originate from the basal cell layer and not from the luminal cell compartment as earlier believed [61]. Their discoveries may impact future strategies in search for new prostate cancer biomarkers.

Another complex but highly interesting area of research with great expectations in the near future relates to microRNA and other forms of non-coding RNAs which hypothetically can act as important regulators of other biomarkers, but can also function as biomarkers themselves and as therapeutical targets [62].

Circulating tumour cells (CTC)

The shedding of tumour cells into the circulation is a necessary condition for metastatic spread. Recently, the enumeration of circulating tumour cells (CTC) using the CellSearch™ system which has been cleared by the US Food and Drug Administration as a prognostic clinical biomarker that can be used to monitor the effectiveness of therapy in patients with metastatic prostate cancer [63]. Although changes in CTC counts over time are predictive of survival, it is the molecular profiling of these cells that offers insight into the biological status of the tumour. Effective molecular profiling of CTC is complicated by the relatively low cell numbers involved and by dilution from non-tumour material because the current enrichment procedures cannot eliminate all contaminating leukocytes. Further, as patients with advanced disease may shed tumour cells from multiple sites, the CTC detected may have diverse characteristics, further diluting potential molecular biomarkers and complicating their predictive utility. In a study of 77 patients with progressive castration-resistant disease, Scher and his group have shown that CTC numbers can be monitored in a routine clinical laboratory setting and that cells confirmed by immunohistochemistry to have features of prostate cancer can be sampled for genetic profiling by FISH [64]. FISH could be done on CTC (success rate >87%) supporting its use in the routine management of progressive castration-resistant prostate cancer [65]. The CTC technology is currently evaluated and validated in clinical trials as a predictor and surrogate end point of treatment response.

In conclusion, the search for new cellular biomarkers in prostate cancer has resulted in a proliferation of tumour marker studies. In order for markers to be successfully incorporated into clinical practice, we need evidence that measuring the marker would change the decision a doctor would have made in the absence of the marker, and that this changed decision will benefit the patient.

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References
Prostate cancer tissue biomarkers


**REVIEW ARTICLE**

**Tumour markers in prostate cancer III: Biomarkers in urine**

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**Abstract**

The serum PSA test still is the most important biomarker for the detection and follow-up of prostate cancer. PSA-based screening can reduce disease specific mortality but coinciding unnecessary testing and overdiagnosis warrant further research for more specific biomarkers. Numerous studies of both serum and urine-based prostate cancer biomarker candidates have been presented the last ten years. However, biomarkers for identifying the most aggressive subsets of this malignancy are still missing. Being non-invasive, urine-based tests might be suitable for both clinical and (mass) screening purposes, but also for prediction and to gain prognostic information. Protein-based, DNA-based and RNA-based urine biomarkers have been developed and tested. **Protein markers in urine.** Data on protein-based urine biomarkers (i.e. Annexin A3, matrix metalloproteinases and the urinary:serum PSA ratio) show up to now contradictory results and further studies are warranted to be able to assess their clinical value in which the cost aspect should not be overlooked. **DNA markers in urine.** Studies on DNA-based urine biomarkers focus on hypermethylation of gene panels with GSTP1 hypermethylation being the most promising individual marker. Larger prospective clinical studies of single markers and gene panels are however needed to validate their clinical utility. **RNA markers in urine.** RNA-based urine biomarkers are by far the most developed. The PCA3 test, the TMPRSS2–ERG fusion gene, transcript expression levels of GOLPH2, SPINK1 and their combination have been subject of many studies showing encouraging results. **Conclusion.** Up to now urine-based biomarkers represent a promising alternative or addition to serum-based biomarkers. Prospective studies in a multivariate setting, including larger sample sizes and avoiding attribution bias caused by preselection on the basis of serum PSA are however required.

The serum PSA test still is the most important biomarker for the detection and follow-up of prostate cancer. The test is well tolerated, quick, cheap and standardised. Physicians are familiar with test results and can easily translate those into a certain risk level for having the disease or into the risk of tumour progression.

Recently it has been shown that screening with the use of the serum PSA test can reduce disease specific mortality [1–3]. This very important gain comes however with considerable costs such as 70–80% of potentially unnecessary prostate biopsies, depending on the PSA cut-off level used [1]. Next to this PSA-based screening leads to overdiagnosis; i.e. detection of “non-life threatening” disease, often resulting in overtreatment [4].

New biomarkers for the detection and staging of prostate cancer are therefore an absolute must, and numerous studies of both serum and urine-based prostate cancer biomarker candidates have been presented the last decade. However, biomarkers for identifying the most aggressive subsets of this malignancy are still missing. Being non-invasive, urine-based tests might be suitable for both clinical and (mass) screening purposes, but also for prediction and to gain prognostic information. Urine-based tests can roughly be divided into three groups; protein-based, DNA-based and RNA-based markers. This report will cover last years development in this area and highlight the most promising urinary markers in prostate cancer.

**Protein markers in urine**

Initially most research focused on the serum-urinary PSA ratio with conflicting results as compared to total serum PSA alone. In a prospective multicentre trial Irani et al. investigated the clinical relevance of the
urinary:serum PSA ratio in enhancing the specificity of serum PSA in the detection of prostate cancer [5]. In patients with a PSA level of 4–10 ng/ml, receiver operating characteristic curves (ROC) showed that the urinary:serum PSA ratio had a larger AUC (0.63) than did total PSA (0.55) or free-to-total PSA ratio (0.60). In a later prospective study, urinary:serum PSA ratio was significantly different, between patients with prostate cancer and those with benign prostatic hyperplasia, with ROC analysis showing a diagnostic cut-off for urinary PSA of > 150 ng/ml, with a sensitivity of 92.5% [6]. Promising results from these two studies were contradicted by Pannek et al. who failed to show any improvement in prostate cancer detection or staging in a cohort of 110 men [7].

AnxIII A3 (ANXA3) is a calcium-binding protein with decreased production in prostate cancer cells. Quantification by ANXA3 using Western blots of urine samples showed significantly lower values in prostate cancer patients as compared with BPH patients, resulting in an improved sensitivity at high specificities compared with total PSA [8]. The combination of PSA and urinary ANXA3 showed an AUC of 0.81 in the overall cohort.

Several studies have demonstrated a role of matrix metalloproteinases (MMPs) in growth, invasion and metastatic spread in prostate cancer and other malignancies [9]. In a recent study of 103 prostate cancer cases compared with 45 healthy controls, Roy et al. showed that MMP9 in urine was an independent predictor of prostate cancer on multivariate analysis and that prostate cancer could be detected with a specificity of 82% and a sensitivity of 74% with regard to the presence of any MMP in urine [10].

As concluded by Ploussard and de la Taille [11], more recently described urinary protein markers like delta-catenin [12], the heptatocyte growth factor (c-met) [13] and thymosin β15 [14] have been evaluated in different pilot studies but none of these proposed new prostate cancer markers have yet been validated in independent studies.

The availability of proteomic platforms allowing the analysis of hundreds of peptides simultaneously protein-based urinary marker research has evolved enormously during the last decade. However the complexity of peptide analysis in urine samples was illustrated by Adachi et al. [15] who demonstrated the occurrence of more than 1 500 proteins in the normal urine proteome. A comprehensive review on proteomics and prostate cancer was recently published by Goo and Goodlet [16] and this area of research will not be further described in this report.

Metabolomics is a new approach to identify and separate metabolites using technology similar to proteomics. These small molecules are often the final products of biochemical activity in molecular pathways and metabolomics could potentially provide opportunity to develop diagnostic and prognostic evaluation to stratify patients to choose the best kind of treatment. Using a combination of high-throughput liquid and gas chromatography-based mass spectrometry, Sreekumar and colleagues identified 1 126 metabolites across 262 clinical samples (plasma, tissue and urine) and proposed sarcosine, an N-methyl derivative of the amino acid glycine, to be an important biomarker for prostate cancer progression and metastasis [17]. Other groups, using somewhat different methodology, have failed to reproduce these findings [18] and the value of sarcosine as an important biomarker in prostate cancer is still under debate.

Taken together, contradictory results are reported on protein-based urinary markers and further studies are warranted to be able to assess their clinical value in which the cost aspect should not be overlooked.

DNA markers in urine

Hypermethylation at various gene loci has been associated with most malignancies, and several DNA methylation markers have been investigated in prostate cancer. The loss of glutathione-S-transferase P (GSTP1) expression as a result of promoter hypermethylation is the most common molecular alteration reported in prostate cancer [19]. Initial studies reported promising results with high sensitive and moderate specificity in assays detecting GSTP1 hypermethylation in urine samples, whereas subsequent studies have shown conflicting results in terms of predictive accuracy as recently reviewed by Ploussard and de la Taille [11]. Later reports have mainly focused on hypermethylation of gene panels again showing contradictory results [20–22]. A multiplexed, quantitative methylation-specific PCR assay consisting of the three different methylation markers, GSTP1, RARB and APC was recently tested in a prospective multicentre study of post digital rectal examination (DRE) urine samples from 178 patients with prostate cancer and 159 controls [23]. The predictive accuracy (area under the curve, AUC) of the assay for detecting prostate cancer was 0.72, but this was only a marginal gain in predictive capability with respect to biopsy outcome as compared to total PSA and DRE alone. Results this far have suggested that gene methylation might serve as a useful marker in prostate cancer, and that GSTP1 hypermethylation is the most promising individual marker. Larger prospective clinical studies of single markers and gene panels are needed to validate their clinical utility.

RNA markers in urine

RNA-based urinary tests are by far the most developed. The well known differential display clone 3 or
PCA3 test is already commercially available under the trade name Progensa® PCA3 (Gen-Probe, San Diego, Ca) [24].

The PCA3 test has been subject of many studies virtually all showing superiority of the PCA3 score to the serum PSA level in predicting biopsy outcome when comparing ROC curves. Especially the high specificities in the range of 80 to 90% are impressive and can be helpful in avoiding unnecessary biopsies. Looking into more detail at test performance characteristics and the study cohort used is however warranted. Sensitivity and specificity of the PCA3 test in men with PSA levels in the so-called grey zone (4–10 ng/ml), representing those men that actually would benefit from an additional test are not that convincing. In a comprehensive review of Vlaeminck-Guillem et al. the test performance in the PSA grey zone is summarised [25]. The specificity when applying different, study dependent, PCA3 cut-off values is indeed impressive ranging from 71 to 93%. However sensitivities are in the range of 53 to 84% [24,26–28] and question its clinical value. Data for men with previous negative biopsies on the basis of an elevated PSA level and/or abnormal DRE result show a similar picture. Again specificities are high and repeating of unnecessary biopsies will without doubt be avoided with the use of the PCA3 test. However with corresponding sensitivities ranging from 47 to 75% one can question whether this is desirable, especially in these men [26–30].

Even more important to realise when interpreting these data is the fact that studies of new diagnostic markers are subject to attribution or assignment bias. Usually a more or less arbitrarily chosen cut-off value is used as a “gold standard” to determine the indication for the decisive test, a prostatic biopsy, and the assumption is made that no cancers are present below that cut-off value. This assumption has been proved wrong by findings in the control arm of the Prostate Cancer Prevention Trial (PCPT), where more than 5,000 men were biopsied independent of their PSA status. As an example: a PSA cut-off value of 4.0 ng/ml, a commonly used biopsy threshold, missed about 75% of all biopsy-detectable prostate cancer [31]. Most reported studies on PCA3 are in men previously screened with PSA and selected on the basis of an elevated PSA level. When calculating sensitivity and specificity all biopsy detectable prostate cancer cases present below the PSA cut-off are thus ignored. Resulting sensitivity and specificity percentages are actually relative sensitivity and specificity values.

Recent a side study within the ERSPC Rotterdam evaluated the value of PCA3 as a first line screening test. The study design was chosen as such that more than 80% of men had a biopsy indication. Prostate biopsy was indicated if the PSA level was ≥ 3.0 ng/ml and/or the PCA3 score was ≥ 10. With doing so the attribution bias was avoided as much as reasonably possible [32]. Based on ROC analyses of 721 men all biopsied, PCA3 performed marginally better than total PSA in predicting biopsy outcome. AUCs of PSA and PCA3 were 0.58 and 0.64 (p=0.143) respectively. A sensitivity of 85% coincided with a PSA cut-off level of 1.0 ng/ml and a PCA3 score cut-off of 20. Specificities were similar and relatively low; 27% and 28% respectively. Again high specificities coincided with low sensitivities in both PSA and PCA3. For example a specificity of 75–80%, reached with a PSA cut-off of 4.0 ng/ml or a PCA3 score cut-off of 60 rendered sensitivities of 24% and 39% respectively. These data indicate that the PCA3 test, although to a somewhat lesser extent suffers from similar weaknesses as the serum PSA test. There is no cut-off value at which sensitivity and specificity achieve a reasonable balance.

The relationship between the PCA3 score and parameters of cancer aggressiveness has also been studied and differ by outcome. Some studies [33] report a positive relationship between PCA3 scores and parameters of more serious disease, while other studies could not find such a relationship [34]. Another RNA-based urinary biomarker comes from gene fusions which occur in approximately 50% of the prostate cancer cases. The most common fusion in prostate cancer is between the strong androgen-regulated TMPRSS2 gene transcriptional promoter and the oncogene ERG. This fusion results in an androgen-regulated TMPRSS2–ERG fusion gene. In 108 men with prostate cancer biopsied on the basis of an elevated PSA level (≥ 3.0 ng/ml) Hessels et al. analysed the fusion transcripts in urinary sediments. Again sensitivity was low (37%) with a very high specificity of 93%. No significant relationship was found between the presence of the fusion transcripts and Gleason score in prostate biopsies [35]. This was confirmed by Rice et al. who detected ERG mRNA in urine samples from 237 men [36].

Combining the PCA3 test and the TMPRSS2-ERG fusion test seems a way to improve diagnostic accuracy. A study done in 105 men showed that the PCA3 alone had an AUC of 0.65, while the combination of PCA3 and TMPRSS2-ERG increased the AUC to 0.77. Adding the PSA level to this multivariate approach resulted in an AUC of 0.80 [37].

If a multiplex analysis might improve the sensitivity of urine-based tests, without sacrificing specificity was evaluated by Laxman et al. [38] who performed quantitative PCR after whole transcriptome amplification in urine samples from 257 patients, including 152 men with prostate cancer and 105 with negative biopsy results. Different mRNA biomarkers were evaluated based on bioinformatic analysis and in univariate
and multivariate analyses, transcript expression levels of GOLPH2, SPINK1, PCA3, and TMPRSS2–ETS fusion significant predictors of prostate cancer, with an AUC significantly greater than that of the PCA3 score alone (0.758 versus 0.662). SPINK1 expression in urine samples was higher in TMPRSS2–ERG-negative than in TMPRSS2–ERG-positive samples, suggesting the mutual exclusivity of SPINK1 expression and ETS fusions similar to tissue biomarker studies [39]. Validation studies are to be presented.

Conclusion
Urinary biomarkers for prostate cancer are subject of ongoing research and represent a promising alternative or addition to serum-based biomarkers. Prospective studies including larger sample sizes and avoiding attribution bias caused by preselection on the basis of serum PSA are however required. Looking at test performances combinations of different, both serum and urinary-based markers in a multiplex setting will most likely help in resolving the current problems in the (early) detection and staging of prostate cancer.

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References


Introduction: Therapy with curative intent

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Prostate cancer is a disease that is curable when it is still localized to the prostate gland. The rapid developments in early detection as well as surgical- and radio therapeutic modalities have led to an increasing number of patients undergoing curative treatment. Still, there are no scientifically unobjectionable randomized studies that have compared surgery with radiotherapy. Comparisons between these two treatment options are therefore solely based on outcome data from treatment series in which patients have been categorized with respect to commonly recognized risk factors such as T stage, Gleason score and prostate specific antigen (PSA). Guidelines in most countries state that the two treatment modalities have comparable outcomes with regard to cure and therefore the choice of treatment should be in the hands of patient. In the UK, patient recruitment to the ProtecT (Prostate testing for cancer and Treatment) trial was recently completed [1]. This study is expected to provide important information about the three treatment modalities compared: active monitoring, radiotherapy and radical prostatectomy, respectively, in low-risk prostate cancer. Before the results from this trial are obtained, there is an obvious responsibility for treating clinicians to initiate, and participate in, additional prospective randomized trials evaluating the efficacy of different therapeutic interventions in patients with localized prostate cancer.

Adjuvant radiotherapy after radical prostatectomy has shown excellent results in terms of significantly reducing the risk of biochemical progression [2], improving biochemical progression-free survival and local control [3] as well as overall survival [4]. One still unanswered question is whether this type of adjuvant therapy can be replaced by “early salvage” radiation therapy, i.e. at the first sign of PSA relapse. Randomized trials are ongoing on this theme and will hopefully provide clarity on this in the next few years: RAVES (Radiotherapy adjuvant versus early salvage) (NCT00860652), RADICALS (Radiotherapy and androgen deprivation in combination after local surgery) [5] and GETUG-17 (Adjuvant versus salvage radiotherapy) [6].

Another important therapeutic comparison that has to be investigated in curative treatment of patients with localized prostate cancer is radical prostatectomy plus adjuvant radiotherapy versus radical radiotherapy. Prospective randomized trials in this area are expected to start in the near future.

The value of neoadjuvant, concomitant and adjuvant endocrine therapy in conjunction with radiotherapy has been investigated in several randomized trials. The results from these have been updated in a systematic review and meta-analysis [7]. However, one important question still unanswered is whether dose-escalated radiotherapy can be fully offset or exceed the effect of combined hormonal- and radiation therapy. We look forward to the initiative also to such studies in the near future.

Trials evaluating outcome of different surgical procedures such as open radical prostatectomy versus robotic assisted laparoscopic prostatectomy are important. One trial currently ongoing on this theme is the Swedish Prospective LAPPRO trial [8]. This trial aims to compare the two surgical techniques in aspects of short- and long-term functional and oncological outcome, cost effectiveness and quality of life, supplying new knowledge to support future decisions in treatment strategies for prostate cancer.

The rapid development in radiation therapy techniques [9,10] and trials regarding different dose-escalation- and dose-fractionation regimens are ongoing and are expected to shed more light to their place in future curative recommendations in the different prostate cancer risk groups [11].

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REVIEW ARTICLE

Radical retropubic prostatectomy: A review of outcomes and side-effects

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Abstract

Background. Radical prostatectomy (RP) is worldwide probably the most common procedure to treat localized prostate cancer (PC). Due to a more widespread use of Prostate-Specific Antigen (PSA) testing, patients operated today are often younger and have organ confined disease justifying a more preservative surgery. At the same time, surgical technique has improved resulting in lower risk of permanent side-effects. This paper aims to give an overview of results from modern surgery regarding cancer control and side-effects. A brief overview of the history is given. Material and Methods. A literature research identified recently published papers focusing on outcome and side-effects after RP. Results. One large randomized study (SPCG-4) compared RP and watchful waiting (WW). The study showed that RP was superior to WW in preventing local progression (RR = 0.36), distant metastasis (RR = 0.65) and death from PC (RR = 0.65). Observational studies also show a better outcome for men treated with RP compared to WW. Peri-operative mortality after RP is low in most material around 0.1%. The risk of stricture of the vesico-urethral anastomosis has decreased with improved technique from historically 10–20% to a low incidence of around 2–9% today. Also the risk of incontinence has declined with improved technique. However, while the rates of severe incontinence is usually very low, as many as 30% still report light incontinence after long-term follow-up. Erectile dysfunction (ED) is still a frequent side-effect after RP. This risk is dependent on age, pre-operative sexual function, surgical technique and other risk factors for ED such as smoking, diabetes, etc. In selected subgroups the risk of ED is low. Inguinal hernia is a more recently described complication after open retropubic RP with a post-operative incidence of 15–20% within three years of surgery. Conclusion. RP is an effective method to achieve cancer control in selected patients. With modern technique it is a safe procedure with a low risk of permanent side-effects except for ED.

Prostate cancer (PC) is a major public health problem. It is one of the most commonly diagnosed cancers and one of the most common causes of cancer-related death in developed countries [1,2]. The estimated incidence of PC in the USA was nearly 192 280 new cases in 2009 [1] and 345 900 new cases in Europe in 2006 [3]. From being a rare disease in the 19th century, the incidence has increased rapidly during the past century, especially during the last two decades. A man’s lifetime risk of a PC diagnosis has more than doubled from 8% in the early 1980s to almost 18% today [4]. The discovery of the Prostate-Specific Antigen (PSA) test in the 1970s [5–7] is likely to explain most of this increase, reflected in that the greatest increase in incidence constitutes of non-palpable tumors. Screening with digital rectal exam and/or the PSA test offers detection of early stage, clinically localized and potentially curable disease. These tumors can be managed in different ways, however, radical prostatectomy (RP) is today considered gold standard for treating localized PC [8,9]. The frequency of curative treatment for early-stage disease with surgery as primary treatment has increased and evolved enormously since the late 1980s [10–14]. Though providing an effective means of treating localized disease [14], feared side-effects of RP are urinary incontinence and impotency. The surgeon’s technique put priorities on cancer control, continence and potency [15]. The main indication of RP is patients with low- and intermediate-risk localized PC (cT1a-T2b and Gleason score 2–7 and PSA ≤20) and a life expectancy >10 years [9]. However, treating high grade as well as locally advanced tumors have also been reported with favorable outcome [16].

The aim of the present paper was to give an overview of results from modern surgery regarding cancer control and permanent side-effects. A brief
overview of the history is given. The study did not intend to compare different ways of performing RP or RP with other treatment modalities.

Material and methods

A literature research through April 5, 2011 identified recently published papers focusing on outcome and side-effects after RP. Medical electronic databases were used (MEDLINE as well as the Cochrane Library to identify systematic reviews). Information was also derived from guidelines. Keywords for the literature search included: radical prostatectomy, surgical technique, mortality, side-effects, impotence, incontinence and inguinal hernia. Data extraction and quality assessment were made by the authors.

Results

History

Billroth performed the first perineal prostatectomy for palliation of obstructive PC in the 1860s [17] followed by Young in the beginning of the 20th century [18]. In the early 1930s the procedure was replaced by transurethral prostatic resection for this indication [19]. The first retropubic prostatectomy was performed by Millin in 1945 [20]. However, many of the tumors were detected late. The discovery of the PSA changed this and prostatectomy could be regarded as a means of treating early disease. In the early 1980s, Walsh described in detail the anatomy of the prostate, the dorsal venous complex and the neuro-vascular bundles [21] and he also performed the first nerve-sparing radical retropubic prostatectomy (RRP) [22]. These efforts led to improvements in postoperative continence and potency as well as reducing peroperative mortality [22]. Peroperative blood loss is aimed at being minimized and the known postoperative complications may be reduced to low levels in expert hands [23]. Modifications and improvements of surgery, anesthesia and pre- and postoperative care have been made throughout the years, with a decrease in complication rates [24]. RP can be performed either as open retropubic surgery, laparoscopic surgery, robotic-assisted laparoscopic surgery as well as perineal surgery. Evidence is lacking which technique is superior as regards oncological and functional results as well as cost-effectiveness [9]. Observational studies have compared different modalities [25], but no randomized controlled trial exists to date. A large Swedish study (LAPPRO) is currently ongoing comparing open retropubic prostatectomy with robotic-assisted laparoscopic prostatectomy [26].

Outcome

The three primary outcomes of RP, cancer control, continence and potency, are achieved to a high extent today. RP is associated with excellent cancer control in men with organ confined disease up to 10 years of follow-up [27]. Prostate cancer-specific survival in localized disease has been reported to be 86–99.6% at 10 years [9,28–30], 82–90% at 15 years [31,32] and 76% at 30 years [33].

The benefits of RP as compared to expectant management have been reported in one large prospective randomized trial (SPCG-4) judged to be of good quality [34]. This Scandinavian landmark study, reported by Holmberg et al., randomized (in 1989–1999) 695 men with clinical stage T1-2 tumors to either watchful waiting or RP. During a median of 10.8 years of follow-up, RP significantly reduced the risk of local progression (RR = 0.36), the risks of metastasized disease (RR = 0.65) and diseasespecific mortality (RR = 0.65, 95% CI 0.45–0.94; p = 0.03) [14]. There was a statistically significant difference in overall mortality rates for men <65 years randomly assigned to RP as compared to watchful waiting (RR 0.59, 95% CI 0.41–0.85, p = 0.004) [14]. However, the number needed to treat (NNT) to “cure” a single case of PC with RP was calculated to 10–19. This rather high NNT could be explained by a high risk of over-treatment (treating too many indolent cancers) or treating too advanced cancers where local treatment no longer is effective. The latter explanation is supported by the fact that in the SPCG-4 trial, the majority of prostate cancers were palpable tumors. However, there is no doubt that also in the SPCG-4-trial there is a high rate of overtreatment emphasizing the demand for a very low rate of permanent side-effects to justify RP in these men. There are three major clinical trials (PIVOT, ProtecT and START) ongoing that investigate the outcomes of RP vs. expectant management for PSA-detected tumors.

Observational studies

In a cohort study of men aged 55–74 years with non-metastasized disease diagnosed during 1971–1984, RP was compared with external beam therapy and observation. Kaplan–Meier estimates for 10-year disease-specific and overall survival were in favor of RP (86% for disease-specific survival, 95% CI 84–88% and 69% for overall survival, 95% CI 67–71%) [29].

In another observational cohort study (including 44 630 men aged 65 to 80 years) of men with low and intermediate risk localized PC, the adjusted hazard ratio for overall mortality comparing RP with observation was 0.50 (95% CI 0.47–0.53) [35].
The natural history of today’s prostate cancers that are often PSA-detected is not completely understood. Stattin et al. reported 10-year outcomes of men in the National Prostate Cancer Register of Sweden Follow-up Study that included men aged ≤70 years at diagnosis, local tumor stage T1-2, Nx or N0, Mx or M0, and PSA <20 ng/ml. For men with low-risk PC, the cumulative 10-year PC-specific mortality was 2.4% (95% CI 1.2–4.1%) among men on conservative management and 0.4% (95% CI 0.13–0.97%) among men who underwent RP [30]. However, non-randomized studies have limitations and should be interpreted with caution because of the risk of selection bias and comparison between treatment modalities is beyond the scope of this review.

Side-effects

Short-term side-effects. Thirty-day mortality. We performed a nationwide population-based record-linkage study in which the 30-day mortality after RP was low, 0.11–0.13% [36], a finding consistent with previous studies based on modern series [31,37–43]. Co-morbidity [44] and increasing age are factors associated with higher risk of perioperative death [41,45–46]. Hospital volume and surgeon volume may also affect the outcome [37,44]. As indicated by our study group [36] and others, ischemic heart disease and pulmonary embolism still seems to constitute the majority of causes of deaths following RP [47–50].

Long-term side-effects. All treatments for PC at different stages have side-effects. The most well-known and bothersome postoperative complications after RRP are urinary incontinence, impotence and stricture of the vesico-urethral anastomosis [51,52]. Large improvements in surgical technique have been made during the past three decades and the risk of side-effects has fallen and together with the earlier detection of PC this results in RP today being a procedure with much lower morbidity [28]. However, while at the same time earlier detection provides a higher chance of cure and treatment with less side-effects, it also implies a higher risk for over-treatment and, in the case of permanent side-effects that still can follow, a long-time suffering for the individual. Due to inconsistency in reporting the outcomes in the literature, the incidence of post prostatectomy urinary incontinence ranges from 0% to 87% [53–57] and postoperative potency rates vary from 11% to 87% [53]. It has been shown that sexual function can continue to improve even beyond two years postoperatively [58,59]. At 52 months after RP, 88% have been reported to suffer from erectile dysfunction and 31% from urinary leakage [60].

However, it should be noted that already before surgery, the prevalence of erectile dysfunction is high in population-based studies. Long et al. have reported that 64% suffered from erectile dysfunction overall pre-operatively; 43% of patients younger than 65 and 84% of patients over 65 [61]. Salonia et al. showed that only 43% of 234 men with localized PC candidates for bilateral nerve-sparing RP had a normal erectile function pre-operatively [62]. Risk factors for postoperative impotence include non-nerve sparing surgery, the surgeon’s ability to master the technique, age of the patient, baseline sexual function, and risk factors for erectile dysfunction including diabetes, hypertension and smoking [63].

The average incidence of severe incontinence is, in most studies, not greater than 5% [55,64], however, severe urinary leakage has a huge negative impact on a patient’s quality of life.

The risk of stricture of the vesico-urethral anastomosis has decreased with improved technique from historically 10–20% [65] to a low incidence at around 2–9% today [9].

Quality of Life. Studies have shown that men reported similar (or indicated even better) quality of life after RP compared to men assigned to watchful waiting [52,66]. Better mental health scores and health-related-quality of life scores for men having undergone RP has also been reported compared with men having undergone radiotherapy [66] as well as compared with a control group of men without cancer [67].

Inguinal hernia. Inguinal hernia (IH) as a postoperative complication to RP is less well known and was first reported by Regan et al. in 1996 [68]. Later reports indicate an incidence of IH of 12% to 21% within two to three years after RRP [69–74]. The background annual incidence of inguinal hernia is approximately 0.5% [75]. These results have recently been confirmed from the SPCG-4 study, a prospective randomized study between RRP and watchful waiting with a follow-up of over 12 years [14], where 9.3% developed IH after RRP within 48 months as compared to 2.4% in a group of non-operated men with PC and 0.9% in a population control group [76]. Today IH can definitely be considered a complication to RRP.

The lower mid-line incision per se has been suggested to be causative [69–74]. In a study from 2008, Koie et al. reported a postoperative IH incidence of only 2.9% in a group of 272 patients where RRP was performed through a so called “mini-laparotomy” incision of only 6 cm [77]. Matsubara et al. also reported an IH incidence of 1.8% after
radical-perineal prostatectomy where the whole procedure is performed through a perineal incision and consequently there is no abdominal incision at all [78]. In a recent study of 946 patients after robot-assisted laparoscopic radical prostatectomy (RALP) 5.8% developed an IH within 48 months as compared to 12.2% after RRP. There was no statistical difference in IH incidence after RALP as compared to a population control. Thus, the length, and possibly the placing, of the abdominal incision seems to affect the development of postoperative IH [76].

Concluding remarks

It is 150 years since the first RP was performed. Enormous improvements in surgical technique have been achieved especially during the last 20 years. It is nowadays a safe procedure with excellent cancer control in men with localized disease and few side-effects apart from ED. The main challenge in the future is to further decrease the risk of sexual dysfunction and to avoid over-treatment.

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References


Radical Retropubic Prostatectomy


Curative radiation therapy in prostate cancer

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Abstract
Radiotherapy has experienced an extremely rapid development in recent years. Important improvements such as the introduction of multileaf collimators and computed tomography (CT)-based treatment planning software have enabled three dimensional conformal external beam radiation therapy (3DCRT). The development of treatment planning systems and technology for brachytherapy has been very rapid as well. Development of accelerators with integrated on-board imaging equipment and technology, for example image-guided radiation therapy (IGRT) has further improved the precision with reduced margins to adjacent normal tissues. This has, in turn, led to the possibility to administer even higher doses to the prostate than previously. Although radiotherapy and radical prostatectomy have been used for the last decades as curative treatment modalities, still there are no randomized trials published comparing these two options. Outcome data show that the two treatment modalities are highly comparable when used for low- and intermediate-risk prostate cancer.

Keywords: Curative radiation therapy – the rationale for dose escalation – choice of radiation boost techniques

Several randomized trials have shown that a dose-response relationship exists for curative radiotherapy of prostate cancer. A comprehensive summary of these are found in a recent meta-analysis of Viani et al. [1]. Most outcome data are reported with respect to the D’Amico risk classification [2]. In patients with intermediate-and high-risk prostate cancer doses in the range of 70–72 Gy are generally not sufficient to achieve ablation of the tumor [3–7]. These tumors require radiation doses in the range of 78 Gy and above [1]. There also appears to be a dose-response relationship even in low-risk prostate cancer, i.e. these tumors also need dose escalation [1].

Dose escalation is currently being used with different approaches at different centers. The majority of these use external radiation therapy alone based on modern technologies such as intensity modulated radiation therapy (IMRT) and IGRT, enabling radiation doses in the range of 78–81 Gy, or even higher, with conventional fractionation combined with selected radiation boost regimens [1]. Other centers use a combination of 3DCRT, IMRT or IGRT in combination with high dose-rate (HDR) brachytherapy, the latter technique used in the context of radiation boost [8–12]. Such combined therapy achieves doses > 116 Gy [8]. Two randomized studies have compared the effect of external beam radiotherapy alone with external beam radiotherapy plus HDR brachytherapy boost [13,14]. Early data from one of these studies have been published [14]. These show the benefit of combination external beam radiotherapy plus HDR brachytherapy. However, the external radiation therapy alone utilized a lower biological dose compared with the combination therapy concept. This hampers the comparison. A systematic review with meta-regression analysis has recently been carried out in which observational studies on external beam radiation therapy (EBRT), seed implantation brachytherapy, and HDR brachytherapy were included [15]. The selection for EBRT included only studies with cohorts treated with at least 75 Gy. The conclusions drawn from this
analysis are that the combination of external beam radiotherapy and HDR brachytherapy results in a superior biochemical control as well as overall survival [15]. However, there is still a need for randomized controlled trials comparing the outcome of radiation therapies with different dose fractionation schedules, radiation boost techniques and total doses to the prostate and organs at risk.

**Neoadjuvant, concomittant and adjuvant endocrine therapy**

The value of combination treatment – radiotherapy and endocrine therapy – has been studied in multiple randomized trials [16,17]. These have in various degrees shown the benefit of this concept with respect to local control, distant metastasis-free survival, disease-free survival, cancer specific mortality and overall survival. However, it is important to take some factors in consideration. The majority of trials have exclusively included patients with high-risk disease, i.e. patients with locally advanced and/or poorly differentiated cancer and/or high risk of lymph node metastatic disease. Radiotherapy was generally given at doses that, with today’s knowledge, are not sufficient to achieve cure. Radiation was in most studies given with whole pelvic fields to 50 Gy and a subsequent radiation boost to the prostate [16]. One of the major arguments against the combination concept (radiotherapy combined with endocrine treatment) is that the radiotherapy used de facto needed androgen deprivation therapy to compensate, in part, for the inadequate radiation doses that could be achieved with the radiation therapy techniques used at that time. Some observational studies support the assumption that hormonal neoadjuvant and adjuvant treatment can be excluded from the curative treatment provided that adequate radiation therapy is given [18]. The concept of neoadjuvant, concomittant and adjuvant endocrine treatment will most certainly be challenged by the concept of curative treatment with high-dose radiotherapy as monotherapy (dose-escalated 3DCRT or IMRT/IGRT) or with combination therapy (3DCRT/IMRT/IGRT plus HDR brachytherapy boost) to the primary tumor.

**Adjuvant and early salvage radiotherapy**

The value of adjuvant radiotherapy after radical prostatectomy has been investigated in three large randomized trials [19–21]. Two of these have shown overall survival benefit in the order of 10%, while the third [21] is not yet mature for overall survival evaluation. All studies showed, in comparison to no postoperative radiotherapy, statistically significant differences in disease-free survival, metastasis-free survival, local progression and cancer-specific mortality at advantage for radiotherapy. The question whether the postoperative treatment is best served in a strictly adjuvant setting or if this treatment could also well be given as early salvage radiotherapy has to be answered. Randomized studies on this concept are under way. The majority of centers have not yet adopted adjuvant radiotherapy into their clinical routine practice. The main reason for this is that the results from the adjuvant trials have not been obtained until quite recently. At least half of the patients experiencing recurrence after radical prostatectomy (about 30%) should be considered for early salvage radiotherapy. Still there are no conclusive data on optimal fractionation schedule and the total dose needed in this setting. Questions also remain regarding the efficacy of salvage radiotherapy in patients who never reach undetectable prostate-specific antigen (PSA) after surgery and/or have already reached a PSA level over 1 ng/ml before salvage radiation therapy and/or with tumor invasion of seminal vesicles at surgery. Pending the results from prospective trials the nomogram by Stephenson and coworkers is useful when discussing the benefit and possible side-effects of salvage radiotherapy for patients experiencing recurrent disease [22].

**Treatment of locally advanced disease – the SPCG7/SFUO3 trial**

The treatment of choice in patients with locally advanced prostate cancer has over the years been endocrine therapy, either as castration therapy or treatment with antiandrogen as monotherapy. This paradigm was challenged in the Scandinavian SPCG7/SFUO3 randomized phase III trial comparing endocrine therapy with and without local radiotherapy, followed by castration on progression [23]. The trial recruited 875 patients with locally advanced prostate cancer (T3; 78%; PSA < 70; N0; M0) between February 1996 and December 2002. The patients were randomly assigned to endocrine treatment alone (three months of total androgen blockade followed by continuous endocrine treatment using flutamide), or to the same endocrine treatment combined with curative radiotherapy. The primary endpoint was prostate-cancer-specific survival, and analysis was by intention to treat. After a median follow-up of 7.6 years, 79 men in the endocrine alone group and 37 men in the endocrine plus radiotherapy group had died of prostate cancer. The cumulative incidence at 10 years for prostate-cancer-specific mortality was 23.9% in the endocrine alone group and 11.9% in the endocrine plus radiotherapy group (difference 12.0%, 95% CI 4.9–19.1%), for a relative risk of 0.44 (0.30–0.66). At 10 years, the cumulative incidence for overall mortality was 39.4% in the endocrine alone group and
29.6% in the endocrine plus radiotherapy group (difference 9.8%, 0.8–18.8%), for a relative risk of 0.68 (0.52–0.89). Cumulative incidence at 10 years for PSA recurrence was substantially higher in men in the endocrine-alone group (74.7% vs. 25.9%, \( p < 0.0001; \) HR 0.16; 0.12–0.20). After five years, urinary, rectal, and sexual problems were slightly more frequent in the endocrine plus radiotherapy group. The addition of local radiotherapy to endocrine treatment, thus, halved the 10-year prostate-cancer-specific mortality, and substantially decreased overall mortality in patients with locally advanced or high-risk local prostate cancer.

This is the only trial that has, so far, shown an overall survival benefit in patients with localized prostate cancer and, in light of these data, endocrine treatment plus radiotherapy should be implemented as the new evidence-based (level 1) standard treatment in patients with locally advanced disease [23].

**Unanswered questions and developmental areas within radiation therapy of prostate cancer**

As mentioned above, the early radiotherapy trials included treatment with whole-pelvic fields with a subsequent radiation boost to the prostate [16]. However, with the introduction of conformal radiation therapy techniques during the 1990s and that only patients with low risk for metastatic disease or patients with N0 disease were accepted for curative treatment, whole-pelvic radiotherapy was abandoned in most centers. With the knowledge of the shortcomings of surgical lymph node staging [24] there is now a re-awakening of adjuvant radiation treatment of regional lymph nodes, especially with the advent of conformal techniques such as IMRT and intensity modulated arc therapy (IMAT) [25] and in patients with high risk (> 15%) of having lymph node metastatic disease [26–29]. With these techniques it is now possible to administer radiotherapy to the regional lymph nodes in a more conformal manner, minimizing unwanted radiation dose to organs at risk such as bladder and bowel.

Another area that has attracted much of attention recently is radiobiology of prostate cancer and radiation dose fractionation. Ample data exist to show that this tumor differs from several others by a low level \( \alpha/\beta \) value, speaking in favor of hypofractionation [8,30]. Several studies have now been designed on this concept. Arcangeli et al. have recently published data from the first randomized trial [31]. A Swedish-Danish randomized hypofractionation trial has recruited over 400 patients with intermediate-risk prostate cancer patients comparing EBRT 2 Gy per fraction to 78 Gy with 6.1 Gy in 7 fractions to 42.1 Gy (Widmark, personal communication). It is important to note that the hypofractionation concept is based on the assumption that prostate cancer is often a slowly proliferating malignancy. However, some tumors also harbor poorly differentiated cancer cells which may not be suitable for hypofractionation [32,33]. The outcome of ongoing and future trials will, hopefully, clarify which patients benefit from hypofractionation.

**Radiotherapy versus radical prostatectomy**

Prostate cancer is different from many other malignancies in that it is one of the few cancers in which radiotherapy is a major curative treatment option primarily. Radical prostatectomy is the other curative treatment modality and patients are therefore asked to participate in the decision-making between these two options. Paradoxically, there are still no comparative, randomized studies on the concept radiotherapy versus surgery as primary treatment in prostate cancer – a situation which not seldom contributes to a frustrating situation for the patient as well for his partner when choosing therapy. Active monitoring is a valid option for patients with low-risk (T1c, Gleason score \( \leq 3 + 3 = 6 \) and PSA \( \leq 10 \)) prostate cancer. The first study on this theme is the ProtecT (Prostate testing for cancer and Treatment) trial undertaken in the UK, in which men with clinically localized prostate cancer were randomized to radiotherapy, radical prostatectomy or active monitoring [34]. This study has recruited extremely well and has now been closed for inclusion according to protocol. Over 1500 participants agreed to randomization (63% of those eligible) with annual follow-up at over 90%. The first results are expected in 2015 and overall survival data will be obtained in due course.

In patients with intermediate- and high-risk prostate cancer the main options, radiotherapy and surgery, remain. One Swedish randomized trial (neoadjuvant endocrine therapy plus radical prostatectomy versus neoadjuvant endocrine therapy plus external beam radiotherapy with HDR brachytherapy boost) included 89 patients with HRQoL as main end-point. The data will be published in 2011. To our knowledge, no other prospective randomized trials comparing these modalities are being undertaken.

**Discussion**

There are very good reasons to expect that the role of radiation therapy will increase in coming years,
warranting further randomized studies in this area. Healthcare authorities have an important task in supporting and commissioning such trials to provide sufficient evidence to guide practice and improve patient outcomes. Strong growth is expected in the areas of dose escalation, both with external beam radiation therapy as monotherapy and combination treatment with external therapy and brachytherapy. In all dose escalation protocols, the need for minimizing the margins of surrounding normal tissue is imperative. The need for improved positioning is becoming increasingly important. The trend toward higher fractions per dose, hypofractionation, will remain, highlighting the need for adequate visualization of the prostate and its positioning with techniques such as IGRT. The need for IMRT will increase for treatment of regional lymph nodes in patients with high-risk disease.

The trend towards higher radiation doses to the prostate and the balance between efficacy and toxicity will place increasing demands on technical developments, their adequate use and understanding of their limitations.

Data from the early 1990s have clearly shown that radiotherapy doses of 70 Gy and below are in the majority of cases insufficient for eradication of the cancer [3–7,35], and four randomized trials comparing 64–70 Gy with 74–80 Gy have shown improved outcome with dose escalation [36–39]. Several studies have thereafter shown a correlation between residual cancer and the risk of local progression, metastatic disease and increased cancer-specific mortality [3,40–42]. This has also recently been shown in a biopsy study from the above mentioned SPCG-7 trial in which residual cancer was verified in 22% of patients after more than three years and that this presence was associated with the above negative factors including increased cancer-specific mortality [43]. Notable is that the residual cancer was poorly differentiated (Gleason sum 8) in all patients with recurrent disease [43]. Data from our own experience have shown that residual cancer is associated with poor overall survival (Ljung et al., unpublished).

The difficulties of implementing prospective randomized studies in radiation therapy as well as surgery are obvious, especially for the fact that at least 8–10 years of follow-up is needed to detect significant differences in overall survival. The difficulties in comparing outcome data from non-randomized studies, especially from different centers, are even more obvious, not only because of patient selection but also because different definitions have been used over the years to define biochemical free survival. The difficulties are compounded by the fact that PSA levels not only reflect residual disease within the prostate but also metastatic progression, particularly in patients with intermediate- and high-risk prostate cancer. An alternative endpoint would be to utilize transrectal ultrasound (TRUS)-guided biopsy mapping of the prostate two to three years after completion of radiotherapy, especially when comparing the outcome of different doses and dose fractionation schedules. Analysis of residual cancer is, by definition, a surrogate endpoint, but may have the potential of being more accurate than serum-PSA in assessing local radicality of a given radiation treatment.

The importance of randomized controlled trials comparing modern radiation therapy and radical prostatectomy cannot be stressed enough. The ProtecT trial in the UK will provide important information on the outcome and potential side-effects of these two modalities in comparison with active monitoring. However, it will take many years before mature data on overall survival become available. In the meantime it is important to continue the prospective evaluation of new radiation therapies and surgical treatment concepts in large and well conducted randomized controlled trials.

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References


Curative radiation therapy in prostate cancer


ORIGINAL ARTICLE

Four and five dimensional radiotherapy with reference to prostate cancer – definitions, state of the art and further directions – an overview

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Abstract

Radiotherapy (RT) always requires a compromise between tumor control and normal tissue side-effects. Technical innovation in radiation therapy (RT), such as three dimensional RT, is now established. Concerning prostate cancer (PC), it is reasonable to assume that RT of PC will increase in the future. The combination of small margins, a movable target (prostate), few fractions and high doses will probably demand dynamically positioning systems and in real time. This is called four dimensional radiotherapy (4DRT). Moreover, biological factors must be included in new treatments such as hypofractionation schedules. This new era is called five dimensional radiotherapy, 5DRT. In this paper we discuss new concepts in RT in respect to PC.

Radiotherapy (RT) always demands a compromise between tumor control and side-effects. Undesirable side-effects are the result of dose delivered to non-tumor tissue or healthy organs, and can be reduced if the irradiated volume is minimized. However, due to different errors in the radiotherapy chain, and tumor movements, dose to healthy tissue/ organs cannot be totally avoided. Radiotherapy will therefore always be associated with some degree of side-effects [1].

Radiotherapy is and will be an important modality in the treatment of PC. There are several randomized studies showing a clear survival benefit of RT in the treatment of PC and RT shall be considered alone or adjuvant to surgery and hormone therapy [2–4].

The goal of developments in radiation therapy is twofold: (1) improve tumor control and/or (2) decrease side-effects. Suit 2002 [5] has listed up 20 major contributions or steps in the development of radiotherapy from the development of the gantry to computer tomography based dose planning and three dimensional radiotherapy (3DRT).

3DRT became available in the beginning of the 1990s. The Nordic collaboration in the CART program (computer aid in radiotherapy) was an important contribution to this development, see Figure 1 [6]. In these systems the anatomy of the target could be more complex and the margin surrounding the tumor viewed in three dimensions. Compensation for dose absorption in surrounding organs/tissue (such as the pelvis) became better. Possibly, 3DRT is one of the greatest single improvements in radiation therapy.

Even if 3DRT can account for a more complex anatomy, it is still difficult in clinical practice to modulate the beam in the axial direction. Wedges can compensate for some inhomogeneous contours, but “banana-shaped” or concave targets remain difficult to treat with appropriate dose distributions.

The introduction of Intensity modulated radiotherapy (IMRT), or later developments VMAT (volumetric modulated arc therapy) or Rapid Arc, made it possible to treat banana-shaped targets (lymph node volumes, prostate with seminal vesicles). Proton beams have used since the 1940s, but the accessibility has been low. In recent years, however, the number of facilities increased, and thus possibilities to treat more patients. Because of limitations of space, and the complexity of these techniques, the interested reader is referred to books such as “The Technical

It has always been important to be aware of and control movements of the target and patient. In the use of IMRT and protons this is even more important. Displacement errors during IMRT treatments can significantly change the outcome of treatment. Considering that the treatment time for IMRT is two to three times longer than for conventional 3DRT and the steep dose gradient in protons, the need for improved and continuous patient/target positioning is obvious.

With better positioning of the target and smaller margins this problem can possibly be reduced. Positioning problems associated with 3DRT and IMRT has led to the development of Image Guided Radiation Therapy (IGRT). This development has accelerated during recent years and IGRT has initiated a new era in radiation therapy, namely “four-dimensional radiation therapy” (4DRT).

One major disadvantage of 3DRT is that all treatments are based on the anatomy as defined by the treatment planning CT prior to the actual treatment planning. In an optimal 4DRT system positioning information during irradiation is feedback to the treatment planning system for online corrections. In the clinic this is very complicated and maybe unnecessary to compensate for small movements.

In the case of prostate cancer, and most other tumors, the development of the treatment protocol involves five critical steps [1,7]. The first step involves the diagnostic procedures including determination of the spread of the tumor (e.g., prostate only or lymph node involvement). Next, what is the best treatment for each patient. Third, if radiation therapy is selected, the target(s) must be accurately defined. Fourth, the treatment plan must be optimized (e.g., 3DRT, protons or IMRT) and, finally, the treatment must compensate for inter/intra-treatment variations in position of the patient and the prostate (QA, quality assurance).

In this article we will discuss the last three steps from a 4DRT point of view and discuss necessary developments and improvements of radiation therapy in general and radiation therapy of prostate cancer in particular for the next dimension in radiation therapy – namely 4DRT.

We will also discuss the concepts of biological modeling of the physical dose distribution in 4DRT, and this will be referred to as “five dimensional radiotherapy”, 5DRT. This is the first time the concept of radiobiology will be integrated with the physical dose, and this is possible because of the exceptional control of the dose distribution using 4DRT.
Material and methods

The 4DRT and certainly the 5DRT era is in its formative stage. In April 2005 there were approximately 20 references found on 4D radiotherapy (RT) or four-dimensional RT (depending on search items) in “Pubmed”. On the Internet there were approximately 5,000 references and this reflects the fact that there were multiple 4D projects globally. In 2010 there are approximately 18,500 Internet hits and approximately 400 hits on Pubmed, although some are not fully appreciable.

There are also numerous publications and projects concerning radiotherapy and movement in the 3DRT milieu and aspects from selected articles will be discussed. Several of these articles could be classified as a 4DRT-publication, but the awareness of the technique has been minimal (that is, the keywords on certain articles do not necessarily include 4D, even though the article itself contains 4D information). This makes it possible that we might miss important contributions in our discussion.

Results & discussion

There is no clear definition of 4DRT. 4DRT can be considered as the logical progression forward from 3DRT. 3DRT treatment is often referred to as any treatment using a 3D based dose-planning system and computer tomography (CT). Since 3DRT is a static situation, 4DRT should be considered to be any treatment which dynamically compensates for target or patient movements during radiotherapy. On the other hand 5DRT is a stand alone concept merging physical and technical aspects of RT with radiobiology. It is of course possible to perform 3DRT and include biological information, but it is impossible to have a definition including all combinations. We suggest that the most advanced technology is used as part of the definition (5DRT > 4DRT > 3DRT). It is also important to distinguish the six geometric dimensions (X, Y, Z and rotation) from the different levels (dimensions) of radiotherapy. Although they are linked they describe different aspects of RT.

The definition of 4DRT does not include treatments were motion has been taken in account by merely adding a margin around the tumor. With this definition in mind, many of the current standard treatments cannot be considered as being true 4DRT although they have the technical possibilities to do online corrections.

The first articles in which the term 4DRT is found is in the late 1990s [8]. There are also authors with a slightly different approach, namely that of including time in a biological model, but today most authors define the motion of the target and its effect on the physical dose distribution as the fourth dimension. This is in concordance with the 4D imaging literature [9]. We suggest that biological considerations of the physical dose distribution should be defined as the fifth dimension if this is applied to the treatment directly (that is not just during follow-up), and we will briefly discuss the concept.

Most radiotherapy publications refer to 4DRT in relation to moving targets due to respiratory movement (lung, liver and breast) and prostate cancer is only briefly discussed [9]. The development follows the evolution of the computer tomography [10,11]. However, the concerns about positioning and moving targets is as old as that of radiotherapy. It is also notable that most of the problems discussed in the literature today, were outlined by the Nordic CART project in the 1970s (Figure 1). This relates to the fact that the development of modern radiotherapy is closely linked to the development of computers and imaging devices – 4DRT could hardly be done using laser beams and skin marks.

Two publications worth mention are the entire issue of Seminars in Radiation Oncology [11] dealing with moving targets and 4DRT and a recent review by Bucci et al. [12]. These articles present state of the art procedures compensating for moving targets during radiotherapy.

For the clinically oriented reader, literature about moving targets and techniques for dealing with the problem is sometimes confusing for two basic reasons. First, techniques like 3DRT, IMRT, IGRT and 4DRT are used as if they were independent techniques. In clinical practice 3DRT and 4DRT are the level of treatment precision required, and IMRT, IGRT and are the techniques used to achieve that precision. Second, frequently, positioning only refers to the final step in the treatment chain, but in reality, the 4DRT concept includes all steps from dose planning to the last moment of the treatment.

4D computer tomography (4DCT) and treatment planning

Motion of the target during the dose planning CT can change the shape of the target significantly (Seminars), and this is summarized in Figure 2. Two targets (A and B) are scanned with five CT-slices (1-5). Below are the two resulting 3D images based on the scans. Due to different motion pattern two totally different shapes of the target will be stored in the treatment planning system (A1-5 and B1-5). If we use a high precision RT technique with small margins, this error alone can drastically effect the treatment and it is important to monitor target and
patient position throughout the whole radiotherapy chain [13].

If we assume that the movement is +/- 2 cm (a most common margin) and we divide it in steps of 5 mm, we have nine separate locations of the structures. If the treatment plan has three portals the number of combinations in this very simple example is $9 \times 9 \times 9 = 729$. It is not possible to produce different dose plans for all these combinations. This emphasizes the fact that in clinical practice it is not and it will not be possible to perform ultimate 4DRT, because the number of combinations is too great and probably are not clinically relevant.

Four dimensional imaging devices are today well established and as conventional CTs were a major step forward for RT, 4DCTs will be important for RT in the future. This also stresses the importance of a nomenclature and standards for 4DRT in order to stimulate technical development [10-12].

An interesting new field of avoiding dose to OAR is the introductions of spacers separating the OAR from the target area, thus reducing the dose to the OAR [13]. These must of course be used on the CT and can possible also reduce the motion of the prostate.

Prostate motion

The prostate can move relative to the beam as a part of a patient movement or internally. With laser positioning, displacement errors of several centimeters can be noted [14]. Figure 3 refers to a 20 minute recording of prostate movements, using an electromagnetic positioning implant (Raypilot, Micropos Medical). The maximum error in this example is approximately 14 mm. Usually, prostate movements are minimal (millimeters), but large errors can occur and this is important with fewer fractions and/or long treatment times.

Electronic portal imaging (EPI), cone computer tomography (cone CT) and IGRT

EPI is probably the most commonly used motion detecting device in clinical practice and is the basis of IGRT. Contrast in an EPI image can be enhanced by using markers [15]. Markers in the target-volume (tumor) will also increase the resolution, since EPI does not visualize soft tissue. EPIs are easy to use and give an x-ray verification of the treatment. It is possible to monitor the motion continuously, but this requires manual inspection (although automatic systems have been developed). The quality of EPI in high voltage therapy is sometimes very poor for all portals. EPIs cannot detect movements axial to the beam, but this is seldom a problem for a treatment field. An additional disadvantage of the EPI is that personnel must leave the room during positioning of the patient.

Cone CT was first discussed in isotope applications in the 1980-1990s. Today it is used for RT and the principle is to use the whole detecting area of the EPI (not just a small pencil beam such as that in a standard CT). One can use the treatment beam, but it is also possible to improve the image quality by using standard x-ray tubes mounted on the treatment gantry (e.g., Electra Synergy). Cone CTs are closely linked to IMRT and the development of VMAT and Rapid Arc but do not solve the problem shown in Figure 3. It is possible to visualize the body, bone and target, but it is not possible in clinical practice to perform 4DRT. The largest disadvantage with cone CT is that it cannot detect motion during treatment.

Respiration and gating

Most of the work on 4DRT is on motion due to respiration (lung, liver and breast). In this field the problem of Figure 2 is obvious. If one assume that a certain filling of the lung is proportional to the position of the target, it is possible to monitor the respiration and use the flow of air as a surrogate for target.
position. Once the position is known you can treat in two principle different ways, see Figure 3. Either you follow the tumor and treat continuously or you can treat only in well defined periods of the respiration cycle. The latter will be more time consuming, but is probably easier to plan and perform. However, it is important to notice that the accelerator must have the possibility to start and stop irradiating with small time intervals.

IMRT, VMAT, Rapid Arc and 4DRT

As mentioned above, IMRT made it possible to treat banana-shaped targets such as lymph node volumes and prostate with seminal vesicles. One problem with IMRT is the larger volume of healthy tissue receiving low dose irradiation. Hall and Wuu (2003) have estimated that this increased volume of normal tissue irradiation, will almost double the incidence of second malignancies [17]. VMAT and Rapid Arc can be considered as IMRT with an infinite number of portals. The low-dose volume using VMAT and Rapid Arc seems to lower and treatment time is shorter. Possible advantages in different tumors are currently being investigated [16].

The major step in dose escalation and side-effect reduction is to reduce the Planning Target Volume (PTV), and IMRT, Rapid Arc and VMAT makes it possible to have a uniformly small margin around any shaped target. On the other hand – small margins stress the importance of continuous positioning monitoring and management of the deviation of the position.

Real time radiotherapy

In order to perform real time corrections in clinical practice, the positioning device must determine the position of the target without human interference. This can be done by using continuous diagnostic x-rays with auto-detection of the target or marker, optical systems (only surface), implanted isotopes, or by using electromagnetic positioning devices (EMP). These techniques have their advantages and disadvantages, but EMP is the only technique which can determine a target position in the body, such as the prostate, without adding more ionizing radiation to the patient.

There are two EMP systems available, Calypso Medical and Micropos Medical [18]. Figure 4 describes one EMP system. A transmitter is implanted near the target and an external antenna (in the treatment table) tracks the signal and thus the position of the target can be determined at sub-millimeter levels. EMP is non-ionizing and the system can therefore be used with personnel in the room.

In the treatment of prostate cancer, the combination of small margins, a moving target, few fractions and high doses requires very high precision, and probably demands more than one positioning system. At a minimum, one of these systems should be real time and continuously monitoring.

Figure 3. In-vivo prostate movements during 20 minutes. The maximum deviation in 3D is 14 millimeters.
Radiotherapy in 4D and 5D for PC – an overview

The aim of 5DRT is to, in the clinic, to integrate biological factors and the physical dose distribution over time (fractionation). Five dimensional radiotherapy, is also essential for understanding side-effects of radiotherapy. For PC better understanding of the nature of PC in individual patients will also improve the selection of patients for curative treatments or watchful waiting [21].

For PC, 5DRT is important due to the biological nature of PC and all the possible radiation treatments with different fractionation and doses available. However, if 4DRT has recently been established the era of 5DRT has, possibly, just begun. For example, PC is considered to be a slowly proliferating cancer during treatment [22]. However, recently Thames et al. showed a 6% decrease in biochemical control if the treatment time was extended by one week [23].

During the last decade IMRT in a 3DRT milieu has become very common. One of the goals for QUANTEC (Quantitative Analyses of Normal Tissue Effects in the Clinic) is to summarize 3D dose volume data into constraint parameters to improve IMRT dose planning [24]. However, as pointed out by QUANTEC, data collected from 3DRT is not optimal for the judgment of individual dose plans, since NTCP (Normal Tissue Complication Probability) data extracted from 3DRT are population-based estimates based on a single planning CT with no account for anatomic variation (compared to 4DRT) [25].

The QUANTEC group outlines several other problems which can relate to 5DRT, such as drug interactions, comorbidity and individual radiosensitivity. Methodological problems with side-effect sensoring and determination of the delivered dose to the OAR or target in fractionated RT is also highlighted [24].

The group identified research priorities reflecting the shortcomings of 3DRT data and summarizes that clinical judgment should not be replaced by NTCP or other constraint parameters for the individual patient [27]. It is also notable that the parameters are extracted from 3DRT 1.8–2Gy/fraction and other fractions schedules such as hypofractionation, should be adjusted to conventional fractionation [26]. However, it is not self evident that the Linear Quadratic (LQ) formula which contains no volume parameter is optimal for this conversion for OAR with a non-homogeneous dose distribution.

**Biological models and 5DRT**

The era of Radiobiology started at Radiumhemmet, Karolinska Hospital, by Magnus Strandquist which combined time and fractionation in the “Stradquist diagram” [19]. Today the most common calculation is the Linear Quadratic (LQ) and BED (Biological Effective Dose) formula introduced by John Fowler [20] using the alfa/beta value. One disadvantage of this particular formula is that it is not suited for heterogeneous dose distributions. Lennernäs et al. have further developed the LQ/BED formula to incorporate heterogeneous dose distributions, making the it suitable for 4DRT. This formula is called the Dose Volume Inhomogeneity–BED (DVIC-BED).

Five dimensional radiotherapy (5DRT) is defined as any biological parameter (i.e., sensitivity to RT, drug interactions or resistance to RT) that can alter the outcome of radiotherapy. The 5DRT concept is important to consider in high dose treatments of PC such as high dose rate brachytherapy or hypofractionation. The aim of 5DRT is to, in the clinic, to integrate biological factors and the physical dose distribution over time (fractionation). Five dimensional radiotherapy, is also essential for understanding side-effects of radiotherapy. For PC better understanding of the nature of PC in individual patients will also improve the selection of patients for curative treatments or watchful waiting [21]. For PC, 5DRT is important due to the biological nature of PC and all the possible radiation treatments with different fractionation and doses available. However, if 4DRT has recently been established the era of 5DRT has, possibly, just begun. For example, PC is considered to be a slowly proliferating cancer during treatment [22]. However, recently Thames et al. showed a 6% decrease in biochemical control if the treatment time was extended by one week [23].

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**Conclusion**

Radiotherapy is an important modality in the treatment of PC. It can be used either alone with or without dose escalation, or in combination with surgery
or hormone therapy. It is reasonable to assume that RT of PC will increase in the future. The development of techniques for 4DRT are important techniques for optimizing RT for PC and to improve constraints for IMRT. Furthermore, the combination of small margins, a moving target, few fractions and high doses will probably require more than one positioning system and at least one of these should be real time. The development and research in 5DRT should be accelerated in order to improve risk assessment for an individual patient. It is important to evaluate new technologies in respect to exciting guidelines, but it is equally important it is that new technologies are investigated and also easier used in the clinic if found to be of advantageous for individual patient [28].

Declaration of interest: Bl Lennernäs, Sten Nilsson and Seymour Levitt are founders of Micropos Medical.

References
Hypofractionation for radiotherapy of prostate cancer using a low alfa/beta ratio – possible reasons for concerns? An example of five dimensional radiotherapy

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Abstract
It is very attractive, due to the assumed low alfa/beta ratio of prostate cancer (PC), to construct new treatment schedules for prostate cancer using only a few large fractions of radiation (hypofractionation). This will widen the therapeutic window since the ratio for PC might be lower than that of the organs at risk (OAR). PC is an extremely variable disease and often contains both highly and poorly differentiated cells. It is reasonable to assume that different cells have different patterns of radiosensitivity, i.e. alfa/beta ratios and proliferation. In this study we will simulate the effect on the outcome of the treatment with different fractionations and different ratios.

Material and methods.
In this simulation we use an extension of the Linear Quadratic (LQ)/Biological Effective Dose (BED) formula called the dose volume inhomogenity corrected BED (DVIC-BED). In the formula the tumour volume is divided in 50 subvolumes (step of 2%) and it is possible to calculate the relative effect of the treatment with different ratios (1.5, 4 and 6.5) in different subvolumes.

Results.
The simulations demonstrate that only a small portion (5 – 10%) of cells with a higher ratio will dramatically change the effect of the treatment. Increasing the total dose can compensate this, but this will on the other hand increase the dose to the OAR and also the risk for severe side effects.

Conclusion.
These simulations highlight possible reasons for concerns about the use of hypofractionation for pathologically heterogeneous tumours, such as prostate cancer, and also demonstrate the need for testing new treatment schedules using both high and low ratios.

Prostate cancer (PC) is one of the most common types of cancer in western countries [1]. Modern research and development in surgery and radiation treatment techniques have contributed to significantly improved outcomes for patients who are diagnosed with PC. In recent years hypofractionated curative radiotherapy of PC has been discussed in order to reduce costs, side effects and possibly improve local control by dose-escalation [2].

In the beginning of the 1990s it was shown that the alfa/beta for PC cell-lines might be lower than that of other tumour cell-lines [3–5]. During the past decade evidence for low alfa/beta ratio derived from clinical data has been published, and several retrospective studies have shown that the ratio could be as low as 1.5 Gy [6,7].

It thus became very attractive to construct new treatment schedules using a few large fractions of radiation since one gains more cell kill effect per dose if the alfa/beta is low. If the ratio for the tumour is lower than that of the surrounding late reacting tissue, one will widen the therapeutic window.

However, PC is common an extremely variable disease [8]. PC in each patient is also heterogeneous and very often PC shows both highly and poorly differentiated cells in the same biopsy. Recently the hypothesis that PC is a slowly proliferating tumour has been questioned and this could alter the estimation of the alfa/beta ratio.

The assumption of a low alfa/beta ratio implies that certain patients will benefit from hypofractionation. However, it is also reasonable to assume that the variable degree of differentiation implies different ratios in some areas of the tumour. Thus the ratio in some parts of the tumour is similar to that of other common cancers.
In this study we present a simulation of external beam hypofractionation with different degrees of tumour differentiation in different volumes (more radioresistant clones varying from 1% to 100%) of the PC. We will demonstrate that using a low ratio when converting a conventional treatment may be problematic since there is a risk of underdosage if only a small part of the tumour volume contains more poorly differentiated cells.

**Material and methods**

**Calculation model**

DVIC-BED is an extension of the LQ model, and was initially developed for non-uniform dose distributions with non-standard fractions such as in high dose brachytherapy [9,10]. However, it can easily be modified for both different doses and different radiobiological factors such as the alfa/beta ratio.

The principle of DVIC-BED is to calculate the biological effect in several sub-volumes (with different a/b ratios or dose) of the target and then to add these sub-effects and express the sum as a dose as if the target had received this uniform dose. The DVIC-BED model is a combination of the linear-quadratic (LQ)-formula and the principle of the critical volume (tissue-rescuing unit) model [10] and the Biologically Effective Dose concept (BED). The formula has been described previously [10].

If the number of clonogenic cells is c (c_t at a specific time), the number of fractions is n, fraction dose d, total dose D and survival fraction SF and E for the effect after several fractions, the following expression for tumour control probability (TCP) can be derived:

\[
\text{TCP} = e - c_t 
\]

\[
c_t = c_0 \times SF 
\]

\[
SF = e^{(-\alpha D - \beta dD)} 
\]

\[
E = -\ln(e^{(-\alpha D - \beta dD)}) 
\]

If one assumes that the clonogenic cells are homogeneously distributed in the tumour from the beginning, one can divide the tumour into several sub-volumes (v). The probability for cure will then depend on the number of clonogenic cells in each volume according to:

\[
\text{TCP} = e^{-(c_0/v \times e^{E1})} + \ldots + (c_0/v \times e^{Ev}) = e^{-(c_0/v \times (e-E1 + \ldots + e-Ev))} 
\]

To compare different simulated treatments the Biologically Effective Dose is calculated:

\[
\text{BED} = E/\alpha = D(1 + d/(\alpha/\beta)) 
\]

In order to calculate the BED in a target with an inhomogeneous dose distribution it is possible to combine Equation 4 and 5 to the expression:

\[
\text{BED} = -(\ln(e^{E1} + e^{-E2} + \ldots e^{-Evn/vn})/\alpha 
\]

The advantage of using this formula is that it is based on radiobiologically related formulas such as the LQ/ BED and that different fractions and treatments can be summed independent of the homogeneity of the dose distribution or radiobiological parameters.

**Alfa/beta ratio**

The α/β ratio and α-values have been published by Eklöv (4.14, α = 0.12) for 60Co and DeWeese (3.7–10.9, (=0.064–0.115) among others for both high and low dose rate irradiation [3–5]. Although these values were determined in vitro PC can be assumed to have both a low α value and a low α/β ratio [3–7]. Recently, the ratio has been a subject for discussion by Brenner, Hall and Fowler and the ratio proposed was as low as 1.5. In the simulations different combinations of the alfa, beta ratio are used.

**Simulation and presentation**

In this study we will alter fractionation and radiobiology in eight simulations, see Table I. The first (1) simulation is 3–>54Gy with the same dose and ratio in all subvolumes; second (2) a 3–>54Gy with 2 Gy underdose in 1–100% of the tumour volume treatment; third (3) a 3–>54Gy treatment with change of ratio from 1.5 to 4 in 1 to 100% (step; 2%) of the volume; and fourth (4) a change of ratio from 4 to 6.5. In simulation 1–4 the ratios have the same alfa (0.12) and for comparison a calculation using a different alfa (0.036) is performed. In a separate simulation for comparison, the factors are the same as simulation (2) but with the fractionation 6.4–>32Gy, simulation (6), and the use of a different alfa for different ratios (0.12 for ratio 1.5 and 0.036 for ratio 4).

The simulation was performed on an Apple Computer, iMac, using Chipmunk BASIC (http://www.nicholson.com/rhn/basic/). Graphic presentation was performed using EXCEL 2004 for Mac, Microsoft, USA. In simulation 6 and 7 the alfa value differed in the two ratios used. This means that the conversion to DVIC-BED (Equation 6) in absolute terms is only valid on the left side of the Figure 2. However, it is the relative effect which is important in this presentation.
Results

All simulations are summarised in Table I and the effects are presented in Figures 1 and 2. It is important to remember that different ratios will give different BED's (or DVIC-BED). In Figures 1 and 2 it is the relative effect for each curve (slope of the curve) which is important – not the absolute value.

Simulation one and two were performed for control of the method. Simulation one is the standard treatment with no underdosage and shift in ratio. Simulation two shows the effect of an underdose in 1–100% of the volume. With 100% underdosage, the treatment is 36 Gy/2 rather than 54 Gy/3, and this will of course have a significant effect on the treatment.

In simulation 3–7 the drastic effect of using a low ratio for treatment conversion can be seen. To the left in the figure all subvolumes have a low ratio (1.5 or 4 with a different alfa). To the right it has shifted to a higher value (with the same alfa). It can be seen that if a treatment is constructed based on a low ratio, it will rapidly lose in total effect if only a small portion (less then 5–10%) of the volume has a higher ratios.

The effect is as drastic as treating a subvolume of the tumour with an inadequately low total dose (simulation 2). On the other hand, if one uses a ratio of 4, the treatment will be much less sensitive to small portions of tumour cells with higher ratios. The last simulations (8) in Figure 2 show that the effect is present even with larger fractions (6.4 Gy x 5) being used.

Discussion and conclusion

PC is an extremely variable disease. Prostate cancer in one patient is often heterogeneous and usually demonstrates both well and poorly differentiated cancer areas in the same biopsy. It is reasonable to assume that different cells have a different radiobiology including the alfa/beta ratio as compared to most common cancers.
that this study will highlight the relative effect related to the presence of even small number of poorly differentiated cells with a possibly lower alfa/beta ratio. By using a proper (higher) total dose the relative suboptimal effect of large fractions of irradiation on poorly differentiated cells will be diminished. This will, on the other hand, increase the dose given in high dose per fraction to the OAR.

PC has a lower alfa/beta ratio compared to other tumours and therefore fractionation may not be the optimal factor to alter compared to other tumours. As mentioned above, by using a proper total dose the relative suboptimal effect on poorly differentiated cells will be diminished.

In order to have some built in safety margin in the hypofractionation schedule, one must prescribe a proper dose for poorly differentiated cells and thus one must utilise physical and technical factors as well to protect OAR, namely; the physical factors of the beam (photons, electrons, protons) or technical factors (number of fields, intensity modulation, position).

It has been hypothesised since 1990 that PC is non-proliferating during RT [11]. However, recently Thames et al. showed a 6% decrease in biochemical control if the treatment time was extended one week [12]. These examples emphasises the importance of radiobiology as a dimension (e.g. five dimension radiotherapy, 5DRT) that must be considered along with all other technical and physical factors in the RT of PC [13] and in hypofractionation in particular.

Recently Arcangeli et al. showed a 12% improved 3-year freedom from biochemical failure (FFBF) using hypofractionation in high risk cancers [14]. At a glance this could contradict the presented simulations – but it actually does not. Assuming 100 cells and 90% with a ratio of 1.5, these cells will be destroyed more effectively and reduce the PSA by 90% using hypofractionation. The problem relates to the remaining 10%. These cells might be poorly differentiated; more dangerous, and have a higher ratio. Therefore they might not be eradicated. Moreover, it is well known that poorly differentiated PC cells produce less PSA [15]. The use of biochemical control can thus be an illusion of a more effective treatment with respect to survival (or local control) of the patient in 10–20 years. In the paper of Arcangeli it is also shown that despite the more effective reduction in PSA, the local recurrence rate was four times more common in the hypofractionated arm.

It is important to note that we are not claiming that hypofractionation is not appropriate for PC. However, by using an alfa/beta lower than the numeric value of the fraction dose it might be inappropriate for the poorly differentiated cells, and this will influence local control and survival. Although the PSA may decrease, this decrease may not reflect the actual effect on all cells in the tumour and a number of the poorly differentiated cells may not be eradicated.

Our recommendation is that, until the radiobiology of PC is fully understood in each patient, not to use a lower alfa/beta ratio for PC below the ratio of organs at risk (OAR e.g. rectum and bladder). One should perform “sensitivity analysis” such as this simulation (with different ratios and total doses) prior to using a new fractionation schedule in the clinic. One should also consider compensating for the lesser effect on poorly differentiated cells by delivering a higher total dose. However, this will also increase the dose to the OAR and this is the dilemma in tumours with low ratios – the lower ratio will diminish the fractionation as a method for decreasing side effects leaving the physical factors of the beam (photons, electrons, protons) or technical factors (number of fields, intensity modulation, position) as the only methods to improve the treatment.
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Supplementary material available online

Appendix can be found at http://www.informahealthcare.com/doi/abs/10.3109/0284186X.2011.562536
Natural history of prostate cancer, chemoprevention and active surveillance

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Prostate cancer is a disease with an extreme span of clinical presentations and consequences. Locally advanced and metastatic disease is a major health concern and cause of death in industrialised countries. However, only a minority of the initial, microscopic foci of cancer cells in prostatic glands progress to symptomatic and potentially lethal disease. Most elderly men harbour such indolent cancer foci and die from other causes unaware of their existence. The risk of progression from microscopic prostate cancer to clinical illness is influenced by environmental and life-style factors, such as diet, interacting with hereditary genetic factors, but the exact biological events and interactions are difficult to unravel [1].

Recent and current research on the natural history of prostate cancer

Research on the natural history of prostate cancer has mainly focused on asymptomatic, localised tumours. Information on prognostic factors is of profound importance when deciding the treatment strategy for a man with recently diagnosed localised prostate cancer. Since the introduction of PSA testing, the detection of small, well differentiated prostate cancers has increased dramatically [3,4]. Most patients with such low-risk tumours do not need any therapy at all. However, no definition of “clinically insignificant” prostate cancer in living men (as opposed to autopsy studies) can predict which cancers will be clinically important and which will not. Since the last WHO consultation on prostate cancer in 2004, active surveillance with selective delayed treatment has evolved to be the method of choice for
reducing overtreatment of low-risk prostate cancer. The critical issues which patients should receive treatment and when were outlined by Peter Albertsen at the WHO International Consultation on Prostate Cancer in Stockholm, 8–10 September 2010, and are summarised in this issue of Acta Oncologica [5].

**Active surveillance for low-risk prostate cancer**

There is no doubt that the adverse effects would vastly overshadow the benefit if all men diagnosed with low-risk tumours were treated with curative intent. Unfortunately, as long as we continue to biopsy palpably benign prostates, we will continue to detect such clinically insignificant prostate cancers in huge numbers. It seems unlikely that research will in the near future lead to methods for accurate prediction of which localised prostate cancers will develop to life-threatening disease and which will not. Thus, the concept of active surveillance with repeated assessments of the tumour and the patient is here to stay. Active surveillance is proven effective for reducing overtreatment, but knowledge is scarce about the risk of missing the window of curability and of issues related to quality of life. We are thus obliged to do further research on which patients should be recommended active surveillance, how it should be performed, and when radical therapy should be initiated – if ever. Some specific areas of uncertainty are listed below:

1. **Which criteria define the tumours best managed with active surveillance?** The optimal tumour criteria vary with the age and co-morbidity of the patient. The current criteria are based on Gleason score, tumour extent and PSA values, but they are largely arbitrary. Most urologists and oncologists agree that patients with a life expectancy of more than ten years should be recommended immediate treatment if there is a substantial amount of cancer with Gleason pattern 4 or 5 in the prostate biopsies. However, Gleason grading is observer-dependent and the definitions of the Gleason grades vary over time. Also, the prognostic significance of the extent of cancer in the biopsies is not well studied. Sampling of the prostate with transrectal biopsies commonly underestimates the Gleason score and the tumour volume compared to prostatectomy specimens [6,7]. More aggressive tumours in the anterior aspect of the prostate may be missed with routine biopsies [8]. The optimal number of biopsies and the location of these for accurate sampling are still to be defined. The value of the new variants of magnetic resonance imaging for assessing the local tumour in potential candidates for active surveillance is unclear, but recent results are promising. Likewise, the total PSA value, the PSA density and the ratio of free to total PSA are all of importance, but optimal cut-off values for recommending active surveillance are unknown. Hopefully, new biomarkers that add prognostic information will appear on the scene within the next few years.

2. **What parameters should be followed during active surveillance?** The monitoring of the cancer must herald progression before the disease becomes incurable. Most likely, the chance for cure is decreased substantially when tumour progression is obvious with digital rectal examination or transrectal ultrasound. We know little of the risks for tumour dedifferentiation over time or for that poorly differentiated areas of cancer are not sampled at the initial biopsies. Repeat biopsies are incorporated in most follow-up schedules for active surveillance, but how they should be performed and how the results should be interpreted is not studied systematically. Increasing PSA is the most common reason for initiating deferred treatment [9]. One problem with PSA as a marker of tumour progression is that poorly differentiated tumours produce less PSA than slowly growing, well-differentiated tumours. Another problem is that many patients with low-risk localised prostate cancer also have benign prostatic hyperplasia, which may contribute to most of the PSA measured in blood serum. A small, but comparatively rapidly progressing cancer in a large gland may therefore not give rise to a short PSA doubling time before metastases occur. Furthermore, PSA may fluctuate for various reasons which may lead to unnecessary intervention or anxiety. The PCPT and REDUCE studies indicate that 5-alpha-reductase inhibitors may be used to stabilise the PSA derived from the benign hyperplasia and enhance the utility of PSA to detect progressive cancer [10,11], but their role during active surveillance remains to be defined.

3. **What is the optimal assessment interval during active surveillance?** For the majority of patients tumour progression is very slow and biannual or even annual assessment is probably adequate. The crucial issue is, however, how short the intervals must be to detect more rapid progression during the “window of curability” for the small minority of patient that harbour lethal tumours. Our knowledge on the biology of disease progression from localised prostate cancer to metastatic diseases is still poor.
4. Can the risk of disease progression be reduced? Several pharmacological and non-pharmacological interventions, such as physical activity and dietary modification, are theoretically interesting, but randomised studies are necessary for their evaluation. The potential role of 5-alpha-reductase inhibitors is discussed later in this issue by Roger Rittmaster [12].

5. How does active surveillance affect quality of life? What is the psychological impact of having an untreated cancer, of slowly rising or fluctuating PSA values, and of the uncertainty of what the next scheduled visit will lead to? Is delayed treatment associated with more side-effects than immediate curative treatment? Delayed treatment may decrease the chance for nerve-sparing surgery and include adjuvant therapy because of a more advanced tumour stage. How are patients affected when deferred treatment with curative intent turns out to be initiated too late, at a time when the disease has already spread?

Chemoprevention

Large, randomised studies of chemoprevention with vitamins and trace elements have reported negative results and one study with an anti-inflammatory drug was terminated prematurely because of toxicity [12]. It may be questioned if it is the right way forward to initiate further studies on chemoprevention for prostate cancer targeting men in the general population. Selecting high-risk populations by the family history of prostate cancer, genetic testing or other biomarkers may be more efficient.

Primary chemoprevention of prostate cancer must start decades before the age at which symptomatic disease becomes common in the male population. Even secondary chemoprevention, e.g. for patients on active surveillance for low-risk tumours, must most likely include prolonged treatment before positive effects are clinically apparent. It is therefore essential that studies on chemoprevention do not focus on “prostate health” only. For the average middle-aged man there are many more threats to future health than prostate cancer, of which cardiovascular diseases are the most important. Smoking men with a low-risk prostate cancer will probably increase their chance of longevity more by stopping smoking than by subjecting themselves to a radical prostatectomy. Any intervention that reduce prostate cancer risks but have negative effects on cardiovascular morbidity or mortality may cause more harm than good. It is therefore of major interest that cholesterol-synthesis inhibiting statins, widely used to prevent cardiovascular disease, also may reduce the risk of prostate cancer [13]. Randomised studies of statins with prostate cancer as the primary endpoint are still lacking, though.

As Roger Rittmaster points out in his review later in this issue of Acta Oncologica, the term chemoprevention implies that a disease is being prevented [12]. For a disease like prostate cancer with its abundant preclinical form, preventing disease progression to clinical disease may be as important as preventing cancer initiation. The 5-alpha-reductase inhibitors finasteride and dutasteride reduced the detection of prostate cancer in two large, randomised trials [14,15]. Their effect on the poorly differentiated cancers with the highest potential of becoming life-threatening is, however, at best small. On the other hand, reducing the detection and progression of low-risk tumours may still be beneficial to the patients, since a cancer diagnosis per se and the side-effects of (unnecessary) radical treatment may reduce their quality of life. Which patient groups that could be recommended 5-alpha-reductase inhibitors for such risk reduction was discussed at the WHO meeting, but no consensus was reached.

Conclusions

The natural history of small prostate cancers is usually very protracted and most of them will never become clinically important. There is no need to diagnose cancers that will never lead to symptoms. Unfortunately, prostate cancer is commonly incurable when it has progressed to a stage that is obviously clinically significant. At present and most likely for decades to come, early detection of asymptomatic tumours followed by curative treatment is and will be the most effective measure for reducing morbidity and mortality of prostate cancer. Research on prognostic factors and strategies that reduce overtreatment of clinically insignificant cancers is therefore essential. Pharmacological risk-reduction and active surveillance with selective delayed intervention for low-risk tumours are two very important research fields. An expert session was devoted to these topics at the WHO International Consultation on Prostate Cancer in Stockholm, 8–10 September 2010. Summaries from this session are published in this issue of Acta Oncologica [5,12].

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References


When is active surveillance the appropriate treatment for prostate cancer?

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Abstract

Background. The incidence of prostate cancer has increased dramatically worldwide during the past few decades in part because of increased testing for prostate specific antigen (PSA). The aggressive use of this screening tool has resulted in the identification of many localized prostate cancers a majority of which are relatively low volume, low grade tumors. Older autopsy studies have documented that incidental prostate cancer is quite common especially in older men. The finasteride chemoprevention trial confirmed these findings. Many prostate cancers are not destined to progress to clinically significant tumors. Several case series have documented the natural history of clinically detected prostate cancer. The progression of disease identified by PSA testing is less certain. These studies uniformly show that many men with low grade tumors can survive for over two decades in the absence of treatment. Furthermore, randomized clinical trials have shown only a modest ten year survival advantage for those men undergoing either surgery or radiation. Results. As a consequence, men with low risk of disease progression may wish to consider active surveillance as a treatment option. To date, several case series have documented that men following an active surveillance protocol that includes regular PSA testing and periodic re-biopsy have an excellent outcome. The majority of these men have not demonstrated evidence of progression during the first decade of follow-up and among those that have the majority have undergone either surgery or radiation without compromise of their long-term outcome. Unfortunately, until better biomarkers become available, the outcome of any individual patient defies accurate prediction. Conclusion. Men with newly diagnosed prostate cancer must weigh the risk of disease progression against the potential efficacy and safety of treatment when making a decision whether to consider active surveillance as an appropriate treatment.

When is active surveillance the appropriate treatment for prostate cancer?

During the past three decades, the diagnosis of prostate cancer has increased substantially worldwide. The rise in incidence rates has been most dramatic in countries that have aggressively embraced testing for prostate specific antigen (PSA). In USA, for example, the annual age-adjusted prostate cancer incidence rates have almost doubled from 1980 to 2010 [1]. In 2009, over 192 000 men were told that they had prostate cancer. In the UK where PSA testing is much less widespread the incidence trends parallel those of USA, but at significantly lower numbers. Prostate cancer is diagnosed at a rate that is 2.5 times higher in USA when compared to the UK [2]. The higher incidence rates have occurred primarily among younger men where the rate ratio between USA and the UK is even higher. Among men age 45–54 years the ratio is 8.2; for men age 55–64 years the ratio is 6.7.

These numbers contrast dramatically with the number of prostate cancer deaths recorded worldwide. In 1991 over 33 000 men died from prostate cancer in USA [1]. Similar prostate cancer mortality rates were recorded in the UK. Since then there has been a steady decline in prostate cancer mortality in both countries, however the decline in USA has been significantly greater [2]. This is especially true for those men aged 75 years or over. Between 1994 and 2004 the age-adjusted prostate cancer mortality rate declined in USA by 4.17%, a rate four times higher when compared to the UK. In 2009, over 27 000 men died from prostate cancer in USA.
Which prostate cancers are clinically significant?

The dramatic differences between prostate cancer incidence rates and mortality rates have led many researchers and clinicians to question whether all newly diagnosed prostate cancers pose a clinical threat. For prostate cancer, the ratio of incidence to mortality is now nearly 8:1. This compares with a ratio of only 1.3:1 for lung cancer and 2.1:1 for colorectal cancer. There is no universally accepted definition of clinically significant or insignificant disease, but cancer volume, clinical stage and tumor grade at diagnosis have consistently predicted long-term clinical outcome. As a consequence of widespread PSA testing, there has been a major stage shift towards localized disease. Many men are now diagnosed with low volume, low grade disease often presenting with only one or two cores positive containing Gleason 6 disease. Epstein et al. suggested four criteria to define clinically insignificant disease: tumor volume <0.5 ml, PSA density <0.15, no Gleason pattern 4 or 5 disease and the presence of less than 3 mm of tissue in a single needle core [3]. Although not prospectively validated, most clinicians believe that only tumors greater than 0.5 cm$^3$ are clinically significant.

Although well documented by older autopsy series, several recent publications have demonstrated that prostate cancer is a fairly common finding especially among older men. Researchers designing the finasteride chemoprevention trial estimated the prevalence of prostate cancer among men age 55–70 years to be 6% and powered the trial to detect a 25% reduction in prostate cancer [4]. After seven years of follow-up, prostate cancer was detected in 24% of men in the control group and 18% of men in the treatment group. Most of the cancers identified by this study were Gleason 6 tumors and were present in men with serum PSA levels below 4.0 ng/ml.

All prostate cancers will progress, but many prostate cancers progress at remarkably slow rates. As a consequence, competing medical hazards often play a more dominant role in patients’ estimates of their long-term prognosis. Some men have aggressive disease that may benefit from early detection and intervention, but many others harbor cancers that grow slowly and never progress to clinical significance.

Several key studies have helped shape our understanding of the natural history of prostate cancer progression. Between 1989 and 2004, Johansson and colleagues published a series of four articles that documented the outcomes of untreated prostate cancer in a population based cohort of patients diagnosed with prostate cancer in Sweden [5]. No screening for prostate cancer took place during the period when this study population of 648 consecutive cases was assembled. Initially the authors found relatively low five and ten year mortality rates among men with clinically localized disease and challenged the use of aggressive initial treatment for all patients with low grade early stage prostate cancer. Long-term follow-up of the study cohort, however, suggested a rising mortality rate from prostate cancer for those men surviving 15–20 years following diagnosis.

In 1994 Chodak et al. published a report describing the results of conservative management of clinically localized prostate cancer [6]. Unlike the Johansson report, this study consisted of a pooled analysis of 828 case records from six non-randomized studies published during the decade preceding the report. Patients with poorly differentiated cancers had a significantly lower cancer-specific survival rate (34%) when compared with men who had well or moderately differentiated cancers (87%). In addition, men with poorly differentiated tumors were much more likely to develop metastases when compared to men who were diagnosed with well differentiated disease.

In 1998 and 2005, Albertsen et al. reported long-term outcomes of a competing risk analysis of 767 men diagnosed between 1971 and 1984 who were managed expectantly for clinically localized prostate cancer [7]. The results of this study are presented in Figure 1. Few men (4–7%) with Gleason 2 to 4 tumors identified by prostate biopsy had progression leading to death from prostate cancer within 20 years of diagnosis. Men with Gleason 5 and 6 tumors identified by prostate biopsy experienced a somewhat higher risk of death from prostate cancer when managed expectantly (6–11% and 18–30%, respectively). Men with Gleason scores 7 and 8–10 tumors identified by prostate biopsy experienced a very high rate of death from prostate cancer regardless of their age at diagnosis (42–70% and 60–87%, respectively). Very few of these men of any age survived more than 15 years.

Unfortunately, these studies do not reflect the impact of widespread testing. Since the introduction of PSA screening, more than one million additional men have been diagnosed with prostate cancer in USA. Prior to PSA testing it was rare to diagnose prostate cancer before age 55 years. Since then there has been a dramatic increase in prostate cancer incidence among men in their late 50s and 60s and it is not uncommon to diagnose prostate cancer in men in their late 40s and early 50s. Compared to 1986, the relative incidence of prostate cancer is 1.91 times greater among men aged 60–69 years, 3.64 times greater among men aged 50–59 years and 7.23 times greater among men younger
than 50 years [8]. Using computer models of incidence and mortality, Draisma et al. estimate that PSA testing has advanced the date of diagnosis by approximately 12.3 years for men age 55 years and by 6.0 years for men age 75 years [9].

Recently Grace Lu-Yao et al. explored the ten year outcomes of men over age 65 years with newly diagnosed localized prostate cancer [10]. They assembled a cohort of over 14 500 men and found that after a median follow-up of seven years most men were either alive or had died of causes other than prostate cancer (Figure 2). Ten-year prostate cancer mortality was 5.9% (95% CI, 3.6–8.2) and 6.2% (95% CI, 4.3–8.1) for men aged 66–69 years and 70–74 years, respectively, diagnosed with moderately differentiated disease. These results were much more favorable than the 15–24% mortality rates reported in the studies cited earlier. Similar improvements were observed in poorly differentiated disease. Ten-year cancer-specific mortality for T1c (screen detected) and T2 (palpable) disease was 9.8% and 12.3%, respectively, for moderately differentiated cancer (adjusted HR = 0.63,
Figure 2. Competing risk of death by age at diagnosis, cancer stage, and grade. Panel A: Moderately-differentiated (Gleason 5–7) cancer. Panel B: Poorly-differentiated (Gleason 8–10) cancer.
poorly differentiated cancer (adjusted HR \( p < 0.001 \)), and 22.2% and 25.5\%, respectively, for poorly differentiated cancer (adjusted HR = 0.74, \( p < 0.001 \)). The use of chemotherapy (1.6\%) and surgical or radiological intervention for spinal cord compression (0.9\%) was uncommon. They concluded that contemporary men over age 65 years with screen-detected prostate cancer had survival outcomes significantly better than those in the pre-PSA era, and had a low-risk of developing cancer related complications that require palliative surgery, radiation, or chemotherapy.

Historically, tumor grade has been the most powerful predictor of clinically significant disease. When Gleason originally developed his scoring system based on lower power evaluation of glandular architecture, men were usually diagnosed following a transurethral resection, an open prostatectomy to treat obstructive urinary symptoms or a needle biopsy of a prostate nodule. Up until 2000 most pathologists used all five patterns described by Gleason, but since then many have become increasingly hesitant to grade any malignant glands lower than pattern 3. During the past decade there has been a steady inflation of Gleason scores such that all low grade tumors previously recorded as Gleason score 2–5 are now classified as Gleason score 6 and many Gleason score 6 tumors are now classified as Gleason score 7. Changes in the application of the Gleason scoring system have become so widespread that clinical outcomes are significantly improved if historical classifications are replace by contemporary classifications [11]. It is unusual for men with contemporary Gleason 6 tumors to have clinically significant progression of their disease.

**Active surveillance as a treatment alternative**

The rationale for selecting active surveillance over immediate surgical intervention or radiation therapy reflects the changing understanding of the risk posed by screen detected disease. Prior to the advent of PSA testing, most men presented with clinically advanced disease that often required palliative intervention. Men presenting with localized, low grade cancers frequently had slow progression of their disease and often succumbed to competing hazards [5,7]. The recent publication of results from two large randomized trials on prostate cancer screening have shown that in contemporary practice prostate cancer deaths are infrequent during the first ten years following screening and that as many as 48 men must be identified and managed to prevent one prostate cancer death [12,13].

Interest in active surveillance also reflects a greater understanding of the relative impact of intervention. Aggressive treatments make sense only if they are effective. Unfortunately, data supporting the efficacy of surgery and radiation are limited to two randomized trials both accruing men identified with prostate cancer on the basis of clinical disease rather than as a consequence of PSA testing. The Scandinavian trials of both surgery and radiation have shown that some men with localized prostate cancer live longer as a consequence of treatment, but the impact on prostate cancer mortality is modest [14,15]. At ten years, 19 men in the surgery trial and ten men in the radiation trial required treatment to prevent one prostate cancer death. Most patients participating in these trials were diagnosed on the basis of clinical findings and therefore had greater tumor volume when compared to contemporary screen detected patients. As a consequence the relative impact of treatment on contemporary patients is likely to be much less because the threat posed by clinical progression is much less.

These considerations have led clinicians at several academic medical centers to propose criteria that identify men who have a very low risk of disease progression and who may therefore want to consider a treatment strategy that defers immediate intervention in favor of a strategy that monitors disease progression and initiates treatment only when a tumor shows signs of becoming clinically significant. They have relied on concepts originally developed by Epstein to propose the following criteria: a) men who present with prostate biopsies that demonstrate prostate cancer in two cores or less, b) neither core has more than 50\% involvement with disease and c) tumor histology contains no Gleason pattern 4 or 5. Active surveillance is most appropriate for men over 70 years old who have a life expectancy of 15 to 20 years or less, but can also be followed by any man who is willing to defer immediate treatment because he believes the risks associated with more aggressive treatments are not justified by the potential benefit. Active surveillance does not imply that no treatment will ever be necessary. Should additional clinical information suggest that the risk of disease progression has increased, more aggressive treatments are still available.

Several researchers have published outcomes from large observational cohorts of men who have selected active surveillance as a treatment alternative. Protocols differ slightly among institutions but usually restrict entry to men meeting the criteria outlined above. Most researchers following men on an active surveillance protocol recommend that repeat biopsies and serum PSA values be checked regularly. Protocols differ in the timing of these repeat studies. Some researchers state that a repeat biopsy should be performed within one or two months of the original positive biopsy. Others
suggest that a repeat biopsy should be performed at one year, while still others suggest that repeat biopsies can be deferred for as long as two years following the initial biopsy. Disagreement also surrounds the frequency of PSA testing. Some clinicians sample PSA every three months while others check them less frequently.

Klotz et al. recently published an update of the clinical outcomes of 450 men enrolled in an active surveillance program in Toronto [16]. After a median follow-up of 6.8 years (range 1 to 13 years) overall survival was 78.6%. The ten year prostate cancer actuarial survival was 97.2%. Most of the patients enrolled in this protocol had a low risk of disease progression. Seventeen percent of patients had Gleason 3+4=7 disease. All of the men in the latter group were older than age 70. Patients were initially followed every three months with a serum PSA level and a repeat biopsy was performed six to 12 months after the initial diagnostic biopsy and every three to four years thereafter until age 80. If the PSA doubling time was less than three years, the Gleason score increased on a repeat biopsy or there was evidence of clinical progression, patients were reclassified as having higher risk disease and were offered more aggressive therapy. After ten years of follow-up the probability of death from a competing medical condition was 18.6 times more common than prostate cancer among the patients enrolled in the study.

Six other research groups have also published their active surveillance series [17–22]. A total of 2500 patients have been enrolled and more than 200 have been followed for over ten years. All of the series rely on PSA kinetics and information obtained at repeat biopsy to identify those patients who are at a higher potential risk of disease progression. To date, the disease specific survival associated with active surveillance protocols is 99.7%.

In addition to data from case series, data from a large historical prospective cohort study also support the concept of active surveillance. Shappley et al. recently examined the consequences of deferred treatment as initial management by reviewing the outcomes of 3331 men diagnosed from 1986 to 2007 who were enrolled in the Health Professionals Follow-up Study [23]. Of these men, 342 (10.3%) initially selected no active treatment. Of these, 174 (51%) remain untreated after a mean follow-up of 7.7 years. The remainder were treated an average of 3.9 years after diagnosis. Older men and men with low risk cancer at diagnosis were more likely to defer treatment. Prostate cancer mortality did not differ among men selecting active treatment when compared to those who elected to defer treatment at the time of diagnosis.

Active surveillance, however, carries several risks. In the Klotz cohort 30% of patients have been offered more aggressive treatment because they were reclassified as having higher risk disease. Of these men, half have evidence of biochemical failure on the basis of a rising PSA. Many other patients find active surveillance disquieting. As many as one quarter of men enrolled in active surveillance programs have abandoned these programs after two years in favor of more aggressive treatment on the basis of psychological stress alone. These men have no clinical evidence of disease progression, but feel that the psychological stress of worrying about disease progression outweighs the risk of developing a complication associated with more aggressive treatment. In the SPCG-4 trial, Johansson et al. reported that patients randomized to watchful waiting showed more anxiety, depression and a lower overall sense of well being when compared to those men who underwent surgery [24].

Summary
The introduction of PSA testing has dramatically increased the incidence of localized prostate cancer. Many of these newly diagnosed cancers are low volume and low grade and pose minimal threat of progression over ten years. Active surveillance is an experimental approach to managing these cases, especially for those men over age 65 years who have a life expectancy of 10–15 years. While one randomized trial has been initiated, the data supporting this approach are limited to observational cohort studies with follow-up periods of ten years or less. While not confirmed in Albertsen et al.’s observational cohort, Johansson et al. documented an increase in cancer specific mortality among men identified with palpable disease and followed beyond 15 years [5,7].

The criteria used to select and monitor men on active surveillance have not been validated. Neither PSA values nor prostate biopsies provide definitive information concerning disease progression. PSA values are known to vary widely. In a retrospective analysis of an unscreened population of 972 men with a median age of 62 years, Eastham et al. demonstrated substantial year to year variation in PSA levels [25]. Furthermore, it is unclear whether changes in Gleason score on follow-up biopsies reflect disease progression or simply reflect sampling variation. Until better biomarkers for indolent disease are identified, men considering active surveillance should carefully weigh the potential of disease progression that persists for 20 years or longer no matter how small or well differentiated the disease appears initially against the potential of complications associated with treatment that often occur shortly after the treatment has been completed.
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References

Chemoprevention of prostate cancer

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Abstract
Over the past two decades, many more men are diagnosed with prostate cancer then die of the disease. This increase in diagnosis has led to aggressive treatment of indolent disease in many individuals and has been the impetus for finding a means of reducing the risk of prostate cancer. In the past decade, there have been eight large trials of prostate cancer risk reduction using dietary supplements, 5α-reductase inhibitors, or anti-estrogens. The only two trials which have demonstrated efficacy are those involving 5α-reductase inhibitors: the PCPT (finasteride) and REDUCE (dutasteride). This review examines prostate cancer risk reduction, with emphasis on conclusions that can be drawn from these two landmark studies.

Epidemiology
Prostate cancer is a heterogeneous disease. Whereas most prostate cancers behave indolently and are undiagnosed during life, it is still the second most common cause of cancer death in men. Autopsy studies have indicated that as many as 30% of men in their 30s will have microfoci of prostate cancer with the prevalence increasing by about 10% with each decade of life [1]. With the advent of widespread PSA testing, especially in North America, the incidence of prostate cancer diagnosis has greatly increased [2]. At present approximately one in six men will be diagnosed with prostate cancer in regions where PSA testing is widespread, and 3.5% of men will die of the disease. Because up to 90% of men diagnosed with prostate cancer are treated with aggressive therapy (radical prostatectomy, radiation, or androgen ablation) [3], cancers that are considered pathologically insignificant are still clinically significant, once they are diagnosed. Even if PSA screening is conclusively shown to decrease the death rate from prostate cancer, it is unlikely that treating low volume, low-grade prostate cancer will contribute to this reduction. Therefore, reducing the overdiagnosis and overtreatment of low-grade prostate cancer is a key benefit of prostate cancer chemoprevention.

Definition of chemoprevention
The term “chemoprevention” implies that a disease is being prevented. With respect to prostate cancer, “risk reduction” is a more appropriate terminology. Nevertheless, because of its widespread use, “chemoprevention” will continue to be used throughout this manuscript. Primary chemoprevention refers to reducing the risk of cancer development. Secondary chemoprevention involves reducing the risk of progression of cancer that is already present. Because many older men have foci of undiagnosed prostate cancer, chemoprevention involves both primary and secondary cancer risk reduction. This distinction is important, because any successful prostate cancer chemopreventative agent will also need to be a treatment of localized prostate cancer.

Targets for prostate cancer chemoprevention
The two principal targets for prostate cancer chemoprevention have been inflammation and hormonal stimulation of the prostate [4,5]. Inflammation has been associated with the development of lung cancer in smokers, hepatic cancer in chronic hepatitis and bowel cancer in inflammatory bowel disease. In the prostate, inflammation is associated with prostate cancer precursor lesions such as...
proliferative inflammatory atrophy (PIA) and is believed to increase genetic instability leading to prostate carcinogenesis. Studies involving rofecoxib (a cyclooxygenase-2 inhibitor) and the antioxidants, selenium and Vitamin E, have all been designed to test whether decreasing inflammation in the prostate would reduce the risk of prostate cancer.

Other trials have involved agents designed to reduce hormonal stimulation to the prostate. Androgens are known to stimulate both benign and malignant prostate growth. Neither benign prostatic hyperplasia nor prostate cancer has been described in eunuchs. However, abolishing androgen production and blocking the androgen receptor are unrealistic methods of chemoprevention, because of the side-effects of hypogonadism. On the other hand, testosterone, the major circulating androgen, must be converted to dihydrotestosterone by the 5α-reductase enzymes in order to be active in the prostate. Both finasteride and dutasteride, 5α-reductase inhibitors, target this pathway and have been the subject of successful chemopreventative trials described below.

The role of estrogens in human prostate cancer is not well defined. In some rodent models, exogenous estrogens can synergize with androgens to promote prostate cancer [6,7]. In humans the role of estrogens in prostate carcinogenesis is unclear. Clinical trials of estrogens and antiestrogens are difficult to interpret, because of the indirect effect of these agents on testicular and adrenal androgen production.

Prostate cancer risk reduction studies

Nearly all of the major prostate cancer risk reduction studies started in the last ten years are now complete (Table I). The only successful ones have involved 5α-reductase inhibitors: PCPT (finasteride) and REDUCE (dutasteride). The unsuccessful trials will be briefly reviewed in the order of their completion, followed by a more detailed discussion of the PCPT and REDUCE trials.

ViP trial (rofecoxib) (http://www.clinicalstudyresults.org/documents/company-study_783_0.pdf)

Based on an association of COX-2 overexpression with increased angiogenesis and decreased apoptosis in the prostate, the VIP trial planned to enroll 15 000 men at increased risk of prostate cancer based on an elevated PSA (2.5–10 ng/ml), in order to test whether the COX-2 inhibitor rofecoxib could reduce the risk of prostate cancer. The trial was terminated after 4 741 men were enrolled, because rofecoxib was withdrawn from the market due to an excess of ischemic cardiac events in another study. Therefore, the efficacy of this approach to prostate cancer risk reduction was never tested.

SELECT trial (Selenium and Vitamin E Cancer Prevention Trial)

Selenium and Vitamin E had been associated with a reduction in prostate cancer in post-hoc analyses of two large studies [8,9]. Prostate cancer was not a pre-defined endpoint in either study. In SELECT, 35 000 men over age 50 were randomized to receive 200 μg/d selenium, 400 IU/day Vitamin E, both or neither. Among the four groups, 1 758 cancers were diagnosed. After a median follow-up of 5.5 years, the trial was terminated for lack of efficacy [10].

Table I. Prostate cancer prevention studies (in order of completion).

<table>
<thead>
<tr>
<th>Agent (Trial Name)</th>
<th>Population Studied</th>
<th>Number of Subjects</th>
<th>Date Concluded</th>
<th>Results</th>
<th>Sponsor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Finasteride (PCPT)</td>
<td>Age ≥ 55 PSA ≤ 3.0</td>
<td>18,882</td>
<td>02/2003</td>
<td>24.8% decrease in cancers</td>
<td>Southwest Oncology Group</td>
</tr>
<tr>
<td>Rofecoxib (ViP Trial)</td>
<td>Age 50–75 PSA 2.5–10</td>
<td>15,000 planned</td>
<td>09/2004</td>
<td>Trial cancelled (drug toxicity)</td>
<td>Merck</td>
</tr>
<tr>
<td>Vitamin E and C (Physicians Health Study II)</td>
<td>Age ≥ 50 PSA 2.5–10</td>
<td>14 641</td>
<td>08/2007</td>
<td>No benefit from either agent</td>
<td>NIH, Wyeth</td>
</tr>
<tr>
<td>SeleniumVitamin E (SELECT)</td>
<td>Age &gt; 50 PSA ≤ 4.0</td>
<td>35 533</td>
<td>10/2008</td>
<td>No benefit from either agent</td>
<td>Southwest Oncology Group</td>
</tr>
<tr>
<td>Dutasteride (REDUCE)</td>
<td>Age ≥ 50 PSA 2.5–10</td>
<td>8 231</td>
<td>01/2009</td>
<td>23% decrease in cancers</td>
<td>GlaxoSmithKline</td>
</tr>
<tr>
<td>Soy, Selenium, Vitamin E</td>
<td>HGPIN</td>
<td>325</td>
<td>2010</td>
<td>No Benefit</td>
<td>NCI Canada</td>
</tr>
<tr>
<td>Selenium</td>
<td>Age ≥ 40 PSA ≤ 10 HGPIN</td>
<td>435</td>
<td>05/2010</td>
<td>No significant benefit</td>
<td>U.S. National Cancer Institute</td>
</tr>
<tr>
<td>Toremifene</td>
<td>Age ≥ 50 PSA ≤ 10 HGPIN</td>
<td>1 590</td>
<td>05/2010</td>
<td>No significant benefit</td>
<td>GTx</td>
</tr>
</tbody>
</table>
Chemoprevention of prostate cancer

Physicians’ Health Study II (Vitamin E and C)

In this study, 14,641 male physicians over age 49 were randomized to receive 400 IU Vitamin E, 500 mg Vitamin C or placebo for up to ten (median 7.6) years. There were 1,008 prostate cancers diagnosed on-for-cause biopsies, with no difference among the groups [11].

Prostate Cancer Prevention Study for Men with High-grade Prostatic Intraepithelial Neoplasia (toremifene vs placebo; ClinTrials.Gov identifier: NCT00106691)

This trial randomized 1,590 men with high-grade PIN and no cancer on biopsy to 20 mg toremifene, a selective estrogen receptor modulator, or placebo daily for three years [12]. Repeat biopsies were done after one, two and three years. In a press release on May 24, 2010, the sponsor GTx announced that toremifene reduced the incidence of prostate cancer by a non-significant 10.2% (p = 0.385).

L-Selenium Based Chemoprevention of Prostate Cancer Among Men with High-grade Prostatic Intraepithelial Neoplasia (ClinTrials.Gov identifier: NCT00030901)

This trial recruited approximately 465 men aged 40–80 with high-grade PIN on biopsy, who were treated with 200 μg selenium or placebo for three years. End-of-study biopsies were performed on any man not diagnosed with prostate cancer on for-causet biopsy during the study. This study showed similar incidences of prostate cancer in the two study arms, as reported during a plenary session at the 2010 Annual Meeting of the American Urological Association.

5α-reductase inhibitors for prostate cancer chemoprevention

Rationale. Testosterone is the principal circulating androgen, but it must be converted to dihydrotestosterone (DHT) in the prostate to stimulate prostate growth. DHT is intrinsically about twice as potent as testosterone, and it binds more tightly to the androgen receptor [13,14]. There are two enzymes responsible for conversion of testosterone to DHT, Type 1 and 2 5α-reductase [15]. Type 2 5α-reductase predominates in benign prostate tissue, and men born with Type 2 5α-reductase deficiency have small prostate glands and have never been reported to develop prostate cancer [16]. Type 1 5α-reductase is upregulated in prostate cancer, especially in high-grade or advanced disease [17,18]. Both finasteride, a selective type 2 5α-reductase inhibitor (5ARI), and dutasteride, a dual 5ARI, have proven effective in the treatment of benign prostatic hyperplasia by reducing androgen stimulation to the prostate [19]. Based on the knowledge that prostate cancers have not been reported in eunuchs or men with 5α-reductase deficiency, finasteride and dutasteride have both been considered logical candidates for prostate cancer chemoprevention.

Finasteride and the Prostate Cancer Prevention Trial (PCPT)

The PCPT was funded by the U.S. National Cancer Institute and coordinated by the Southwest Oncology Group. It was initiated in 1993 at over 200 sites in USA and concluded in 2003. The key inclusion criteria were men ≥ 55 years old, PSA ≤ 3.0 ng/ml, a normal digital rectal examination (DRE), and no suspicion of prostate cancer [20]. There were no baseline biopsies.

Men were randomized to 5 mg finasteride/day or placebo for seven years. Annual PSA measurements and digital rectal examinations (DREs) were done and a prostate biopsy (≥ 6 cores) was recommended for a PSA > 4.0 ng/ml or a suspicious DRE. PSA values were doubled in the finasteride arm in Years 1–3, and multiplied by 2.3 after Year 3 to compensate for the mean decrease in serum PSA with finasteride.

Ultimately, 18,882 men were randomized, and 9,060 men (48%) were included in the final analysis (Table II) [21]. The analysis included all men who had an interim biopsy for prostate cancer or who had an end-of-study biopsy (modified crude rate). Forcause biopsies were done in 39% of the participants, and 52% of the cancers were diagnosed on for-causet biopsies. There were 15% fewer for-causet biopsies in the finasteride group. Finasteride demonstrated a 24.8% reduction in the primary endpoint, the prevalence of prostate cancer during the seven-year period (18.4% vs. 24.4% of participants, p < 0.001). Looking only at for-causet biopsies, there were 10% fewer cancers in the finasteride group (26.5% vs. 29.5%; p = NS). The reduction in overall cancer incidence was entirely due to a reduction in low-grade cancers (Gleason score ≤ 6), and there was an increase in moderate to high-grade cancers: 280 (6.4%) in the finasteride group and 237 (5.1%) in the placebo group (p = 0.005). The increase in Gleason 7-10 cancers was almost entirely due to their increased detection in for-causet biopsies. In the end of study
biopsies, there were 92 and 89 Gleason 7-10 cancers in the finasteride and placebo groups, respectively.

These results indicate some limitations of the PCPT trial. Less than 50% of randomized men were included in the final analysis, and over half of the prostate cancers were detected on for-cause biopsies. There were fewer for-cause and end-of-study biopsies in the finasteride arm, suggesting that finasteride affected the decision to be biopsied. Subsequent analyses have demonstrated that finasteride improved the sensitivity of both PSA and DRE to detect prostate cancer, including high-grade cancers [22,23]. Furthermore, if high-grade cancers were present at radical prostatectomy, they were more likely to be detected in the finasteride arm than the placebo arm [24]. Also, finasteride decreases prostate volume (24% lower in the finasteride arm at the time of biopsy), improving the detection of both low and high-grade cancers [25,26].

Based on the increased utility of PSA for prostate cancer detection and prostate volume reduction, one would predict that finasteride would increase cancer detection rates, especially on for-cause biopsies. Four separate post hoc analyses have now been conducted to attempt to account for these factors in determining the true effect of finasteride on overall and high-grade cancer [27–30]. The results have ranged from a 0.88 odds ratio for high-grade cancer with finasteride when prostate volume changes were included to a 27% relative risk reduction when the results from subsequent radical prostatectomies were used instead of prostate biopsies. Because these analyses were retrospective in nature and involved many assumptions, they must be considered hypothesis generating rather than providing definitive answers. Although these analyses suggest that finasteride did not cause a net increase in high-grade cancers, they also suggest that it was less effective in reducing the risk of high-grade cancer, compared to low-grade cancer.

In summary, in the PCPT finasteride causes a highly significant reduction in overall and low-grade prostate cancers. Although induction of high-grade cancer cannot be excluded, the literature suggests instead that finasteride likely led to an earlier detection of high-grade cancer, which was less extensive than in the placebo group [24]. Some authors have challenged the efficacy of finasteride in the PCPT by focusing on the non-significant 10% reduction in prostate cancer in for-cause biopsies only [31]. To do so ignores the factors discussed above. The PCPT was never designed or powered to assess the effect of finasteride in a situation where cancers are only detected on for-cause biopsies.

**Dutasteride and the Reduction by DUtasteride of prostate Cancer Events (REDUCE) trial**

The initial interest in dutasteride for prostate cancer risk reduction came from two observations.

The first was the finding that the ratio of type 1 to type 2 5α-reductase was increased in prostate cancer, suggesting that inhibition of both isoenzymes might be important [18]. The recent observation that both isoenzymes are increased in localized high-grade prostate cancer compared to low-grade cancer or benign tissue emphasizes the potential relevance of dual 5α-reductase inhibition [32]. The second observation came from Phase 3 dutasteride trials for benign prostatic hyperplasia, in which the incidence of prostate cancer was 51% less in the dutasteride arm compared to placebo (27 vs 55 cancers) [33]. Although this was a post-hoc observation of adverse event data, such a decrease had not been seen in similarly designed trials with finasteride [34,35].

The REDUCE trial was designed in 2002, and the first patient was enrolled in early 2003 [36]. It was a multinational, randomized, placebo-controlled trial designed to test the ability of dutasteride to reduce the risk of biopsy-detectable prostate cancer in men at high risk of being diagnosed with the disease. The key entry criteria were men aged 50–75, PSA 2.5–10.0 ng/ml, prostate volume < 80 ml, and a single, negative prostate biopsy of 6–12 cores done independent of the study and taken within six months prior to study enrollment. After a one-month placebo run-in to assess symptoms of benign prostatic hyperplasia and prostatitis, 8,122 men were randomized to dutasteride or placebo for four years and took at least one dose of study drug [37]. Repeat, study-mandated prostate biopsies were taken after two and four years; for-cause biopsies could be done at any time. For-cause biopsies during Months 19–24 and 43–48 replaced the Year 2 or 4 study-mandated biopsies, and hence did not increase a subject's chance of being diagnosed with prostate cancer. Protocol-independent prostate biopsies were those for-cause biopsies done during Months 1–18 and Months 25–42. Key differences between the REDUCE and PCPT trials are shown
in Table III. Because the trials had different study designs and involved different patient populations, they should be viewed as providing complementary results.

Overall, prostate cancer was diagnosed in 858 men in the placebo group (25.1%) and 659 men in the dutasteride group (19.9%) with a relative risk reduction of 23% (p < 0.0001) (Table IV) [37]. Gleason 7-10 cancers were diagnosed in 220 men in the dutasteride group (6.7%) and 233 men in the placebo group (6.8%) (p = 0.81). In the subset of Gleason 8-10 cancers, there were 29 cancers in the dutasteride group and 19 cancers in the placebo group (p = 0.15). During Years 1–2 there were 17 and 18 Gleason 8-10 cancers in the dutasteride and placebo groups, respectively. However, during Years 3–4 there were 12 Gleason 8-10 cancers in the dutasteride group and only one in the placebo group (of 2,343 biopsies). Although induction of high-grade cancer cannot be excluded, one possible explanation for the paucity of Gleason 8-10 cancers in the placebo arm in Years 3–4 was the fact that 141 more Gleason 5-7 cancers were diagnosed in the placebo arm during Years 1–2. Because men with cancer were removed from treatment, there was no opportunity for those cancers diagnosed during Years 1–2 to be reclassified or upgraded during Years 3–4. Support for this hypothesis comes from an active surveillance study in which 105 men with Gleason 4-7 cancers entered into an active surveillance study [38]. Eight (8%) were upgraded to Gleason 8 cancers on re-biopsy a median of 22 months later. A similar rate of upgrading of lower grade cancers diagnosed during Years 1–2 in the REDUCE trial could explain the “missing” Gleason 8-10 cancers in the placebo group during Years 3–4. Furthermore, in the CombAT study [39], a 4,800-patient, 4-year BPH study comparing dutasteride and tamsulosin monotherapies with the combination of the two, in which all biopsies were done for cause, there was no evidence of an increase in high-grade cancers in the two dutasteride arms compared to the tamsulosin monotherapy arm (Roehrborn CR, Nickel JC, Andriole GL, Gagnier RP, Black L, Wilson TH, Rittmaster RS. Dutasteride improves the outcomes of benign prostatic hyperplasia when evaluated for prostate cancer risk reduction: a secondary analysis of the REduction by DUtasteride of prostate Cancer Events (REDUCE) trial., In Press, European Urology, 2010).

### Table III. Key differences between the PCPT and REDUCE study designs.

<table>
<thead>
<tr>
<th>Drug</th>
<th>PCPT</th>
<th>REDUCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duration (years)</td>
<td>Finasteride</td>
<td>Dutasteride</td>
</tr>
<tr>
<td>Age range</td>
<td>7</td>
<td>4</td>
</tr>
<tr>
<td>Entry serum PSA (ng/ml)</td>
<td>3.0</td>
<td>2.5–10.0</td>
</tr>
<tr>
<td>Baseline biopsies</td>
<td>No</td>
<td>Yes (6–12 cores)</td>
</tr>
<tr>
<td>Study-mandated biopsy timing</td>
<td>Year 7</td>
<td>Years 2 and 4</td>
</tr>
<tr>
<td>Study-mandated biopsy cores</td>
<td>6 (6 cores in ~80%)</td>
<td>10</td>
</tr>
</tbody>
</table>

Regardless of the etiology of the increase in high-grade cancers in the PCPT and REDUCE, it is important that such cancers be rapidly diagnosed when they occur. Both finasteride and dutasteride have been shown to enhance the utility of PSA for the diagnosis of high-grade prostate cancer [22,40]. Both medications increase the area under the receiver operator characteristics (ROC) curve for PSA detection of prostate cancer. In the REDUCE trial, any rise in PSA after six months of dutasteride treatment characterized a group of men with an increased likelihood of cancer overall, high-grade cancer and pathologically significant cancer (modified Epstein criteria), compared with men whose PSA was stable or continued to decrease [40]. This relationship was markedly attenuated in the placebo arm. The combination of a reduction in the diagnosis of low-grade cancer and enhanced detection of high-grade cancer should lead to a reduction in the overdiagnosis and overtreatment of indolent prostate cancers.

Because most men over age 50 harbor foci of prostate cancer, one could argue that the main effect of 5ARIs is to reduce the risk that biopsy-undetectable cancer will grow to biopsy-detectable cancer. 5ARIs also reduce the incidence of high-grade PIN, considered to be a marker of increased risk of prostate cancer on a subsequent biopsy. In the PCPT finasteride reduced the incidence of high-grade PIN without cancer by 15% [41]; in REDUCE dutasteride reduced the incidence of high-grade PIN without cancer by 30% [37]. This data suggest that 5ARIs reduce the stimulus to prostate cancer formation and will decrease the need for follow-up biopsies.

Whether or not 5ARIs have a beneficial effect on some high-grade cancers, clearly the greatest effect is the reduction in low-grade cancers. Although some authors have called such cancers “insignificant”, up to 90% of prostate cancers undergo some form of aggressive treatment [3]. For example, in the REDUCE trial, there were 32% fewer treatment interventions for prostate cancer in the dutasteride arm [42].

Men with an elevated PSA are at increased risk not only for prostate cancer, but also for BPH and its complications. In the finasteride arm of PCPT, urinary retention occurred 33% less often, there were...
47% fewer transurethral prostate resections, and 29% fewer urinary tract infections, compared to the placebo arm [21]. In the dutasteride arm of REDUCE acute urinary retention was reduced by 77%, BPH-related surgery by 73%, and urinary tract infections by 41% [37]. Because men in REDUCE had higher baseline PSA levels than those in PCPT, they were at a higher risk of BPH complications, and hence these results are not directly comparable between the two studies.

Side-effects of 5ARIs

Dutasteride and finasteride have similar side-effect profiles, the most common drug-related adverse events being related to sexual function. In a one-year comparative trial, new instances of impotence were noted in 9% of the finasteride group and 8% of the dutasteride group; new instances of decreased libido were noted in 6% of the finasteride group and 5% of the dutasteride group [43]. In the PCPT, erectile dysfunction occurred in 67% of the finasteride group and 61% of the placebo group. Decreased libido occurred in 65% of the finasteride group and 60% of the placebo group [21]. Age had a much greater effect on sexual dysfunction in the PCPT than did dutasteride use, with sexual function deteriorating in both groups over the seven years of the trial [44]. In the four-year REDUCE study, new instances of decreased libido occurred in 5.1% of the dutasteride group and 2.9% of the placebo group [37]. New instances of erectile dysfunction occurred in 9.0% of the dutasteride group and 5.7% of the placebo group. Four point three percent of the dutasteride group and 2.0% of the placebo group dropped out due to drug-related side-effects. Gynecomastia occurred in 4.5% of the finasteride arm of the PCPT over seven years and 1.9% of the dutasteride arm of REDUCE over four years, with placebo rates being about half those of the 5ARIs. If decreased libido or erectile dysfunction is going to occur due to 5ARIs, it usually happens within the first six to 12 months of treatment, with rates in the active and placebo groups being similar thereafter [45]. On the other hand, new instances of gynecomastia with 5ARIs occur at a low, but steady rate in excess of placebo.

There have been no life-threatening or serious side-effects proven to be related to either finasteride or dutasteride. Both can occasionally be associated with allergic-type skin reactions. In REDUCE, there was an excess of events in the composite term “heart failure” in the dutasteride vs. the placebo group (30 vs. 16; 0.7% vs. 0.4%); such an increase has not been seen with dutasteride in other studies.

Balancing the benefits and risks of 5ARIs for prostate cancer risk reduction – Who should be treated?

Neither dutasteride nor finasteride has yet to be approved in the EU or USA for reducing the risk of prostate cancer. The PCPT and REDUCE trials indicate that both drugs are effective in reducing biopsy-detectable prostate cancer, although the populations in which each was tested were different. Although the rates of prostate cancer in the placebo groups of the PCPT and REDUCE were similar, the likelihood of a prostate cancer diagnosis in the REDUCE population is much greater outside of a clinical trial. Men with serum PSAs less than 3.0 ng/ml are biopsied infrequently, and biopsying such men yields a low rate of detection of potentially lethal cancers [46]. On the other hand, men with a negative biopsy and elevated PSA are men that are followed closely, often have a rising PSA, and are likely to be re-biopsied. In REDUCE, 72% of men in the placebo group had a rising PSA after Month 6 [40]. Hence, this is a population at high risk of a prostate biopsy and resultant prostate cancer diagnosis and most likely to benefit from prostate cancer risk reduction.

How about men with a high PSA but below the biopsy threshold? Schroder et al. have shown in the ERSPC prostate cancer screening study that men with a baseline PSA \( \geq 1.5 \) ng/ml were 3.6 and 7.1 times more likely to be diagnosed with high-grade and low-grade cancer, respectively, than men with a baseline PSA < 1.5 ng/ml [47]. Treatment with a 5ARI in this population will likely reduce the number of men in whom a biopsy is indicated and will reduce the overdiagnosis and overtreatment of low-grade prostate cancers. Such men would already be candidates for 5ARI treatment if they have symptomatic BPH, and prostate cancer risk reduction would be

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Table IV. Summary of subjects, biopsies and cancers in REDUCE.

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>Dutasteride</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subjects randomized</td>
<td>8,122</td>
<td>4,049</td>
<td>4,073</td>
</tr>
<tr>
<td>Subjects Biopsied</td>
<td>6,729 (83%)</td>
<td>3,305 (82%)</td>
<td>3,424 (84%)</td>
</tr>
<tr>
<td>Number of biopsies</td>
<td>12,024</td>
<td>5,956</td>
<td>6,068</td>
</tr>
<tr>
<td>Protocol-independent biopsies (percent of biopsies)</td>
<td>810 (6.7%)</td>
<td>344 (5.8%)</td>
<td>466 (7.7%)</td>
</tr>
<tr>
<td>Total cancers (% of men biopsied)</td>
<td>1,517 (22.5%)</td>
<td>659 (19.9%)</td>
<td>858 (25.1%)</td>
</tr>
<tr>
<td>Cancers diagnosed on protocol-independent biopsies</td>
<td>98 (6.5%)</td>
<td>41 (6.2%)</td>
<td>57 (6.6%)</td>
</tr>
</tbody>
</table>
an added benefit. Fewer men would undergo aggressive treatment for prostate cancer, reducing the frequency of radical prostatectomy, radiation therapy and androgen ablation.

5ARIs not only decrease the incidence of low-grade cancer, but enhance the detection of high-grade cancer. It is likely that 5ARIs remove some of the background noise in PSA levels, by suppressing PSA production from benign prostate tissue and indolent prostate cancers. In both the PCPT and REDUCE trials, men whose PSA rose while taking a 5ARI were more likely to have a clinically significant prostate cancer [40,48]. One could hypothesize that dutasteride and finasteride may serve as bioassays for clinically significant prostate cancer (cancers whose growth is no longer being controlled by the 5ARI) with PSA being the readout. On the other hand, the percent decrease in PSA with dutasteride during the first six months of REDUCE did not predict overall or high-grade cancer detection during the study. To monitor PSA in men taking a 5ARI, reasonable guidance is to wait six months to establish a new PSA baseline. Confirmed PSA rises from a new baseline should prompt consideration for a prostate biopsy. Continued PSA monitoring is essential in any man taking a 5ARI, if the benefits of increased PSA utility are to be realized.

While it cannot be ruled out, it is unlikely that either dutasteride or finasteride induces the growth of high-grade cancer. In the PCPT, where PSA drove many biopsies, there was an increase in detection of Gleason 7-10 cancers, but only in the for-cause biopsies. In REDUCE, where PSA-driven biopsies were uncommon, there was no overall increase in Gleason 7-10 cancer detection. These results are consistent with the increased utility of PSA for detecting high-grade cancer. Both agents reduce prostate volume, making cancers easier to detect. When prostate volume at the time of biopsy was included in a logistic regression in the PCPT, the odds ratio for Gleason 7-10 cancers in the finasteride arm was 0.88 [27]. When this same adjustment was done in REDUCE, the odds ratio for Gleason 7-10 cancers in the dutasteride arm was 0.62 [49]. Nevertheless, it is clear that both 5ARIs are more effective at reducing the risk of low-grade cancer than high-grade cancer.

One concern is that use of 5-ARIs will cause more high-grade cancers to be missed by preventing PSA increases in such men [50]. There is little evidence to support this hypothesis. In REDUCE, if only men with a rising PSA are biopsied, there will be about twice as many men with Gleason 7-10 cancers who would not be biopsied in the dutasteride arm, compared to the placebo arm. However, normally a rise of 0.35–0.75 ng/ml/year is required for rebiopsy according to most guidelines in men not taking a 5ARI. In REDUCE, if one required a 1 ng/ml rise in PSA from Month 6 to the final PSA before biopsy in the placebo arm, the number of Gleason 7-10 cancers that would not be diagnosed would be similar in both arms [40]. In the dutasteride arm these are presumably cancers whose growth is being controlled, preventing a rise in PSA.

Neither the PCPT or REDUCE were of sufficient duration to assess whether 5ARIs reduce deaths from prostate cancer, because of the prolonged natural history of biopsy-detected prostate cancer. However, aggressive prostate cancers eventually escape even total androgen ablation, and there is no reason to suspect that such cancers would be controlled by 5ARIs. On the other hand, there is also no reason to suspect that cancers that progress during 5ARI therapy would not respond to more aggressive androgen ablation. Even advanced prostate cancers often respond to more complete androgen ablation after GnRH agonists alone have failed.

In addition to the beneficial effects of 5ARIs in reducing the risk of prostate cancer and high-grade PIN, both finasteride and dutasteride are effective treatments for men with symptomatic benign prostatic hyperplasia (BPH). They not only improve urinary symptoms related to an enlarged prostate, they reduce the risk of acute urinary retention and the need for BPH-related surgery.

Although only 10–15% of men taking 5ARIs are likely to experience new or worsening sexual adverse events, the high prevalence of sexual dysfunction in elderly men makes this issue more challenging. It is one thing to tell a man with an elevated PSA and a negative biopsy that his risk of needing another biopsy, and potentially being diagnosed with cancer, will be reduced by taking a 5ARI. It is a more difficult proposition to expect an asymptomatic man with a mildly elevated PSA to take a medication to reduce the risk of a disease he would prefer not thinking about.

In summary, both finasteride and dutasteride have been shown to reduce the risk of biopsy-detectable prostate cancer. The concern over induction of high-grade cancers and lack of regulatory approval have prevented their widespread use for this indication. Dutasteride is now being submitted for approval worldwide for prostate cancer risk reduction. In the near future we will learn if it meets the strict benefit:risk balance required for a cancer risk reduction indication.

Future directions

There have been many epidemiological or retrospective studies suggesting that different dietary factors, supplements or medications may reduce the risk of prostate cancer [51]. For example, there has been
increased emphasis on statins as a potential class of drugs for prostate cancer risk reduction. However, the data on primary prevention of prostate cancer with statins has been less convincing than their effects on reducing the risk of prostate cancer progression or advanced prostate cancer [52,53]. Complicating the interpretation of these studies is evidence that statins suppress PSA. It is impossible to be certain whether this represents a primary effect on prostate cancer or a primary effect on PSA leading to an ascertainment bias in identifying disease occurrence or progression. The stakes are high, because appropriately designed and powered studies on prostate cancer risk reduction are expensive and take many years to achieve definitive results.

If 5α-reductase inhibitors are going to be widely accepted for prostate cancer risk reduction, they will need the approval of regulatory agencies. Continued research is also needed on the issue of high-grade cancers. With respect to dutasteride, the 3-year REDEEM study, comparing dutasteride to placebo in 302 men with low volume, Gleason 6 cancers being followed by active surveillance [54], will provide data on the rate of upgrading to higher Gleason scores, as well as the utility of dutasteride in an active surveillance setting. This results of this study are scheduled to be published in early 2011. There still remains many unanswered questions regarding 5α-reductase inhibitors, such as whether they can be safely used in men who have never had a biopsy in order to better select men for biopsy, the ideal age at which such therapy should be initiated for prostate cancer risk reduction, and whether they should be continued indefinitely or stopped after a man reaches a certain age.

Prostate cancer can now be counted among the few cancers whose risk can be lowered through dietary or medicinal means. The prospects are excellent for improved management of this disease, especially with respect to the overdiagnosis and overtreatment of indolent prostate cancers that are unlikely to cause death or disability in the absence of treatment.

Declaration of interest statement. Dr. Rittmaster is an employee of GlaxoSmithKline, Inc. GlaxoSmithKline is the manufacturer of dutasteride. Dutasteride and finasteride are approved for the treatment of benign prostatic hyperplasia. Neither medication is approved for prostate cancer risk reduction.

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Chemoprevention of prostate cancer


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EDITORIAL

Management of advanced prostate cancer – new drugs

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Androgen deprivation therapy by chemical or surgical castration remains the cornerstone in the management of advanced disease. Although initially effective, the effect of currently available androgen deprivation therapies is transient and most patients develop progressive disease despite low levels of testosterone. It is generally believed that tumour progression is associated with continued signalling via the androgen receptor pathway through mechanisms such as androgen receptor mutation, androgen receptor amplification, ligand-independent androgen receptor activation or enhanced local production of androgens. The recognition of the antiandrogen withdrawal response, which demonstrates the continuous importance of the androgen receptor signalling pathway in castrate resistant prostate cancer, has stimulated the development of new exciting androgen targeting therapies.

MDV3100, which is a nonsteroidal compound and RD162, are examples of two new orally available interesting agents that target the androgen receptor with higher affinity than bicalutamide [1]. MDV3100 has shown promising phase I/II activity [2] and is currently evaluated in a placebo-controlled randomised phase III trial in patients progressive on docetaxel therapy. EPI-001 represents from a mechanistic point-of-view, a conceptually new and interesting compound. It binds to the N-terminal domain of the androgen receptor and has a new mode of action as it targets the transactivation of the androgen receptor, regardless of the presence of androgen [3,4]. Other efforts are made to further improve the blockage of testosterone production. Abiraterone acetate is an interesting, potent small molecule that irreversibly inhibits cytochrome p17 which catalyzes two key reactions in androgen biosynthesis [5]. This compound has been evaluated in a phase III trial in castrate-resistant patients previously treated with docetaxel (results are pending) and a second phase III trial is ongoing in patients who have not received prior ketoconazole or chemotherapy.

Since the data from the TAX 327 [6] and SWOG 9916 [7] trials were presented in 2004, every-three-week docetaxel has become a standard treatment for patients with castrate resistant prostate cancer. Indeed, these were the first trials demonstrating significant and principally important overall survival benefit using cytotoxic therapy for this patient category. Although significant, the survival benefits achieved by docetaxel-based therapy are rather small, improving the overall median survival compared to mitoxantrone with approximately three months, to less than 20 months [6,7]. Clearly, more effective regimens continue to be needed.

The last years, efforts have been made to improve the effects of docetaxel by either adding an agent to docetaxel/prednisone, develop more effective front line therapy or to find an effective second line therapy. Recently cabazitaxel, a novel taxane with a favourable low affinity to PGP (multidrug resistance P-glycoprotein) in combination with prednisone was approved by the FDA following a phase III trial demonstrating an overall survival benefit of 2.4 months as compared to mitoxantrone, in patients previously treated with docetaxel-containing chemotherapy [8]. If approved also by EMA, cabazitaxel is the reasonable second line option and furthermore the comparator for subsequent second line trials. The epothilone analogues are examples of other chemotherapeutics with promising phase I/II data under current evaluation [9,10].

Our understanding of the complex molecular pathogenesis of prostate cancer has continued to
expand and several novel drugs that target specific molecules in pathways involved in cell signalling, proliferation, apoptosis, angiogenesis, and immune modulation are also under intensive investigation. Examples of candidate progression pathways include epidermal growth factor (EGFR) signalling, vascular endothelial growth factor (VEGF) signalling pathways, phosphatidylinositol 3-kinase (PI3K) / Akt mammalian target of rapamycin (mTOR) pathway as well as the insulin-like growth factor pathway.

Multiple tyrosine kinase inhibitors with a typical antiangiogenic profile, such as sunitinib and sorafenib are evaluated in prostate cancer but data are yet limited. So far, their efficacy appears to be modest or the toxicity significant [11–15]. Of note are the reported discordant radiological and PSA evaluations, indicating that PSA might not be valuable as a marker for response for these compounds and furthermore that the antitumoural effects caused by these substances in prostate cancer are not completely understood [11–15]. The anti-VEGF antibody bevacizumab has been evaluated in several phase II studies with promising results when given in combination with docetaxel regimen. An awaited large phase III frontline trial, CALGB 90401, was recently reported evaluating the addition of bevacizumab to standard docetaxel regimen. Unfortunately, this trial failed to meet the primary endpoint of demonstrating an overall survival benefit following the addition of bevacizumab [16] and the place for this compound in the treatment arsenal remains unclear.

Src and src-family kinases represent interesting target molecules since they are involved in multiple signalling pathways central to prostate cancer development and in the pathogenesis of bone metastases. For example is the value of adding the src-inhibitor dasatinib, which has also activity against bcr/abl, to standard docetaxel regimen, under evaluation in an ongoing phase III trial [17]. Other examples of novel targeted agents under investigation in advanced prostate cancer include the mTOR inhibitors everolimus and temsirolimus, various inhibitors of IGF-1R and custirsen (OGX-011), an innovative antisense oligonucleotide directed against the cytoprotective chaperone, clusterin [18].

Histone deacetylase inhibitors such as panobinostat (LBH589) are currently evaluated and represent principally interesting approaches to target tumour-induced epigenetic aberrations believed to be critical for androgen receptor mediated signalling [19].

In addition to targeted therapies, the slowly-growing nature and high expression of tumour-associated antigens of prostate cancer have stimulated the development of several immunotherapeutic approaches. Just some months ago, FDA approved the first therapeutic vaccine ever, sipuleucel-T, for asymptomatic or minimally symptomatic metastatic castrate resistant prostate cancer [20,21]. Sipuleucel-T consists of autologous dendritic cells derived from a patient’s own peripheral blood mononuclear cells. The dendritic cells are collected through leukapheresis and exposed to a recombinant fusion protein composed of the tumour associated antigen PAP (prostatic acid antigen) linked to granulocyte-macrophage colony stimulating factor [20,21]. In the pivotal phase III trial, patients receiving sipuleucel-T had a median overall survival of 25.8 months compared to 21.7 for the patients receiving placebo, without any substantial side-effects related to the vaccine [21]. Other examples of vaccine approaches under evaluation are vector-based strategies (Prostvac) [22] or whole tumour cell vaccines (GVAX), the latter however with recently reported negative phase III trials [23,24]. Ipilimumab, an anti-CTLA4 monoclonal antibody, represents a principally different immunotherapeutic approach under evaluation since treatment with ipilimumab is supposed to enhance T-cell activation. Phase I and II data have indicated activity [25,26] and phase III studies are under way.

Several agents targeting the bone with different mechanisms of action are under intensive investigation. Denosumab, a fully human monoclonal antibody that specifically bind to the ligand of RANK has recently been approved by the EMA for the treatment of bone loss associated with hormone ablation in men with prostate cancer at increased risk of fractures [27]. Furthermore, denosumab recently was reported superiority over zoledronic acid in delaying or preventing SREs (skeletal related events) in patients with bone metastases from castrate resistant prostate cancer [28]. Radium-223 is a new promising alpha-emitting bone-seeking radiopharmaceutical which has proven well tolerated with demonstrated effects on bone-ALP concentrations and survival parameters in a randomised phase II study [29]. A subsequent phase III study is running and in late planning phase is a trial evaluating combined treatment with docetaxel. Finally, a number of endothelin A receptor (ETα) targeted agents are under evaluation, which rely on the importance of this receptor in the development of prostate cancer progression and formation of bone metastases. Atrasentan and ZD4054 are agents, proven to selectively inhibit ETα, with encouraging phase II data that are under extensive evaluation in several large, randomised phase III trials.

Considering the large number of compounds under evaluation, the next decade appears to be an exciting time for prostate cancer research and management of advanced disease. The emergence of therapies that rely on improved molecular understanding of biological processes behind prostate cancer progression.
and therapeutic resistance is promising and stimulate to further research efforts.

How to best combine and evaluate significantly different treatments modalities such as radionuclides, vaccines, hormonal-, cytotoxic-, targeted- and other immunomodulatory treatments in terms of sequence, dosages, etc are examples of important issues that remain unclear and obviously will require significant work and multidisciplinary collaboration. Another important challenge is to strive for biomarker driven trials with the aim to clarify and define if certain populations, based on their tumour biological and/or clinical characteristics, are particularly sensitive to any of these treatment alternatives. By use of such strategies, we can better tailor and optimise the treatment of the individual patient while improving the cost/benefit for the society as a whole.

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REVIEW ARTICLE

Broadening horizons in medical management of prostate cancer

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Abstract

Hormonal therapy. Testosterone suppression achieved either medically or surgically is the standard initial treatment for men with advanced prostate cancer. Most men respond but the disease progresses after a median of 1–2 years. Clinical trials suggest that intermittent androgen deprivation therapy (ADT) provides equal or longer time to castration-independence than continuous ADT, and is preferred, especially since there are subtle long-term toxicities associated with ADT. Further hormonal manipulations (including addition and withdrawal of peripheral antiandrogens, steroid synthesis inhibitors such as ketoconazole, and estrogens) can be transiently effective in selected patients with castration-resistant prostate cancer (CRPC). Androgen-dependent signalling pathways remain active in most men with CRPC and are associated with mutation, changes in expression or modulation of the androgen receptor (AR); abiraterone acetate and MDV3100 are promising drugs being evaluated in clinical trials that may lead to further hormonal response.

Chemotherapy. Eventually men who progress rapidly, are symptomatic, and/or develop metastasis to visceral organs require chemotherapy. Three-weekly docetaxel with prednisone has been shown to improve survival and relieve symptoms but eventually men develop progressive disease or become intolerant to docetaxel. Multiple trials are evaluating new drugs (mainly molecular targeted agents) either given first line with docetaxel chemotherapy, or to men who have progressive disease after receiving docetaxel. Cabazitaxel was shown recently to improve survival as compared to mitoxantrone when used second line and has been approved by the United States Food and Drug Administration (FDA).

Conclusion. Despite major advances, treatment of men with advanced CRPC remains a challenge both for the seeker and giver of care.

Dr. Charles Huggins was awarded the 1966 Nobel prize in physiology and medicine for his discovery in the 1940s that hormones (specifically estrogens that led to decreased testosterone) could control the spread of prostate cancer. Orchiectomy became a standard treatment (since estrogens were associated with cardiac toxicity) and in the 1980s Leutinising Hormone Releasing Hormone (LHRH) agonists were introduced as a medical alternative. Medical or surgical castration remains the standard initial treatment for men with advanced prostate cancer. Despite clinical and biochemical response in > 80% of patients, mean duration of response to initial androgen deprivation therapy (ADT) is around 18 to 24 months, but is highly variable depending on the biological behaviour of the tumour. Some men with castration-resistant prostate cancer (CRPC) may respond transiently to further hormonal manipulations but eventually become resistant. Current standards for managing these patients and evolving treatment strategies are described below.

Hormonal treatment

Two variants of initial ADT have been investigated in multiple randomised trials: combined androgen blockade (CAB) and intermittent hormonal therapy. Studies have shown that initial CAB (orchiectomy or LHRH agonist with a peripheral anti-androgen) adds cost and toxicity without meaningful benefit as compared to monotherapy or surgery alone [1] and this strategy should not be used. In contrast, emerging data support the use of intermittent ADT, which is based on preclinical observations that intermittent suppression of testosterone delays the time to castration independence in animal models [2]. Several trials have confirmed that intermittent ADT, in which therapy is interrupted when the serum PSA falls to a low value and is reintroduced when it rises above...
a certain level, provides equal or superior duration of disease control compared with continuous ADT [3]. Intermittent ADT can now be regarded as standard treatment for men with a good initial response to treatment, especially as there are evolving data showing subtle long-term toxicities associated with ADT, including bone loss, metabolic syndrome and cardiac events [3–5] (Table I).

Awareness of the chronic toxicities associated with ADT that are summarised in Table I should lead to caution in introducing such therapy, especially in men with predisposing factors, such as a history of diabetes or cardiovascular events. While men with metastatic disease who are symptomatic or have a rapidly rising PSA should be treated, there is no evidence to support initiation of ADT in men with slowly rising PSA after initial local therapy, or those with slowly progressive asymptomatic disease. All men on ADT should be evaluated for bone density, with routine co-administration of calcium and vitamin D, and selective use of bisphosphonates in those with continuing bone loss [4].

Some men whose disease is progressing after primary ADT may achieve further transient response by adding a peripheral anti-androgen. About 20% of those that respond to addition of an anti-androgen may respond to its withdrawal, because the classical agents (flutamide, bicalutamide and nilutamide) can initially inhibit but subsequently stimulate the androgen receptor (AR) [6]. Further responses to hormonal manipulations can be obtained in some men by treatment with agents that inhibit synthesis of adrenal androgens (e.g. ketoconazole used with hydrocortisone), estrogens, glucocorticoids, and by switching from one peripheral anti-androgen to another.

Androgen-dependent signalling in men with CRPC

Despite the high rate of initial response, men eventually develop resistance to the above forms of hormonal therapy via mechanisms that include persistent signalling through the AR, mutation or changes in the expression or modulation of the AR, and activity of alternate cellular pathways that stimulate proliferation of prostate cancer cells [7]. Even in the castrate state, substantial levels of androgens may be produced within prostate tumour tissue and can stimulate the AR [8]. Around one quarter of prostate cancers in men with CRPC have been reported to carry a point mutation, especially Thr-Ala877 [9], resulting in activation by commonly used hormonal agents such as peripheral anti-androgens and estrogens. The AR-dependent signalling pathways have also been shown to cross talk with other growth signalling pathways in prostate cancer cells including those dependent on epidermal growth factors, vascular endothelial growth factor, fibroblast growth factors and transforming growth factor, thereby leading to mechanisms of cell survival despite the castrate state [7]. Two novel agents that target AR-associated signalling in men who are resistant to current forms of hormonal manipulation, abiraterone acetate and MDV 3100, are being evaluated in Phase III clinical trials.

Abiraterone acetate. Abiraterone acetate (hereafter ‘abiraterone’) is an orally active compound that functions by irreversibly inhibiting enzymatic activity of 17a-mono-oxygenase (17 alpha-hydrolase/C17,20 lyase complex), a member of the cytochrome p450 family. This enzyme catalyses the 17-alpha hydroxylation of steroids, required in two key steps for the conversion of cholesterol to androgens. Hence, abiraterone suppresses androgen synthesis in the testis, the adrenal glands and in other sites, including prostatic tissue. Abiraterone is reported to be well tolerated with easily managed side effects such as hypertension and hypokalemia, which can be reduced by co-administration of low-dose prednisone. In a multinational Phase II trial [10], which recruited 58 men with CRPC after docetaxel chemotherapy, abiraterone was associated with PSA response (i.e. 50% or greater maximal decline) in 43% of men including 30% of those pretreated with ketoconazole and 55% who were ketoconazole-naïve. Another post-chemotherapy Phase II trial reported PSA response in 24 (51%) of 47 patients. In both these trials treatment with abiraterone was associated with reduction in circulating tumour cell (CTC) counts – a marker reported to be predictive of survival of men with metastatic CRPC [11]. High baseline serum levels of dehydroepiandrosterenedione (DHEA), DHEA-sulfate (DHEA-S) and estradiol have been recognised as markers predicting biochemical response and time to progression after abiraterone [12]. Large multinational Phase III studies have completed recruitment including a double-blind placebo-controlled trial of abiraterone and prednisone versus prednisone alone in men with CRPC who have progressed after receiving docetaxel, and a related trial for chemotherapy-naïve men with CRPC who were minimally symptomatic.

Table I. Toxicities associated with ADT reported in clinical trials.

- Decrease in bone mineral density, leading to increased risk of fracture
- Loss of muscle mass
- Increase in body fat, especially abdominal subcutaneous fat
- Gynecomastia
- Impotence
- Hot flashes and symptoms of male menopause
- Anaemia
- Increased risk of diabetes
- Increased risk of coronary artery disease
- Increased risk of sudden cardiac death
**Chemotherapy**

Mitoxantrone, used with low-dose prednisone, was the first chemotherapy drug approved for treatment of men with CRPC, on the basis of improvement in symptoms (especially pain) in randomised trials compared to prednisone alone [15,16]. Mitoxantrone has the advantage of low toxicity, provided the total dose is limited to prevent cardiac toxicity, and is well tolerated even by very old men. There were no trends to improved survival in the mitoxantrone trials, although they were too small to detect them. Subsequently the TAX-327 trial demonstrated that docetaxel, given every three weeks at 75 mg/m² with prednisone 5 mg bid prolonged overall survival of men with CRPC by about three months, as compared to mitoxantrone and prednisone, and improved the quality of life of symptomatic men [17]. Surprisingly weekly docetaxel was not as effective, and not less toxic, in the 3-arm TAX327 trial. The SWOG 99-16 trial [18] compared docetaxel and estramustine with mitoxantrone and prednisone: it also showed a small benefit for the docetaxel arm, but together these trials suggest that estramustine adds toxicity without benefit, and there is no basis for the continued use of this drug. Only about half of men with CRPC respond to docetaxel chemotherapy, and all of them eventually discontinue this treatment because of toxicity or disease progression.

There are multiple causes of resistance to taxanes including cellular causes related to drug uptake and retention in cells, and changes to its binding to and action to stabilise β-tubulin. The drug may also have limited distribution within solid tumour tissue. Preclinical evidence has suggested that taxanes exert their antineoplastic activity in prostate cancer partly by blocking AR signalling [19], so that overexpression, amplification and mutation of the AR may also play a role in the development of a chemo-resistant state [20].

New types of chemotherapy, and molecular targeted agents are being (or have been) evaluated with the goal of either enhancing the effectiveness of docetaxel when used in combination, or of providing further benefit when used in men whose disease has progressed during or after treatment with docetaxel. Some of these agents are described below.

**Second-line chemotherapy**

*Retreatment with docetaxel.* For men with CRPC who demonstrate clinical and/or biochemical response to docetaxel as first-line chemotherapy, followed by a period off-treatment, re-introducing docetaxel is a reasonable option if the treatment was tolerated well.

*Mitoxantrone.* About 15% of men with CRPC respond to mitoxantrone after progressing on docetaxel [21], and given the excellent tolerance of this agent, it is a reasonable choice of second-line chemotherapy in symptomatic men with acceptable cardiac function.

*Satraplatin.* Satraplatin is a third generation oral platinum compound which had shown activity in a Phase II trial. Consequently, a large (n = 950) randomised Phase III clinical trial compared satraplatin to placebo, each with low-dose prednisone in men with CRPC after first line chemotherapy (only about half of the participants had received docetaxel) [22]. Risk of disease progression was reduced by satraplatin (Hazard Ratio, HR = 0.67; p < 0.001) with significant reduction in time to pain progression but there was no difference in overall survival. The FDA and European Medicines Agency (EMEA) did not approve this drug for use in men with prostate cancer.

*Cabazitaxel.* Cabazitaxel is a semi-synthetic derivative of the natural taxoid 10-deacetylbaccatin III and like docetaxel acts by binding and stabilising tubulin, resulting in the inhibition of microtubule depolymerisation and cell division, cell cycle arrest in the G2/M phase, and the inhibition of tumour cell proliferation. It differs from other taxanes in that it is a poor substrate for the membrane-associated, multidrug resistance, P-glycoprotein efflux pump and crosses the blood-brain barrier. A recent randomised controlled trial compared cabazitaxel (25 mg/m²) with mitoxantrone (12 mg/m²), each given with prednisone (10 mg/day) as second line
chemotherapy in 755 men with CRPC who had received docetaxel [23]. There was a statistically significant difference in overall survival favouring cabazitaxel (HR = 0.70, p = 0.0001) with an increase in median survival of about 2.5 months. This led to approval of cabazitaxel by the FDA and the drug will probably also be approved by EMEA. However, this drug may exacerbate peripheral neuropathy, a common residual side effect in men post-docetaxel – so that careful selection of patients will be crucial. Also, there was a ∼5% rate of toxic death in the cabazitaxel arm of the randomised trial that might have been reduced by administering a lower dose. A full article describing this trial is awaited, and should address how long after treatment with docetaxel the patients were enrolled; as described above, many patients who respond initially to docetaxel and have a break from treatment may benefit from re-treatment with docetaxel.

Ixabepilone and patupilone. Ixabepilone and patupilone are water soluble epothilones with a related mechanism of action to taxanes. They exhibit their anti-neoepithelial activity primarily by inhibition of microtubule formation. A randomised Phase II trial comparing ixabepilone and mitoxantrone with prednisone in docetaxel pre-treated men demonstrated PSA response rates of 17% and 20% respectively and overall survival of 9.8 months in the entire cohort [24]. Given this disappointing result, it seems unlikely that ixabepilone will be studied further in men with CRPC.

A Phase II study of patupilone in 45 patients (55% had previous taxane therapy) was well-tolerated but only 13% of patients had a PSA response and the median overall survival was 13.4 months [25]. A multicentre Canadian study evaluating patupilone in CRPC after first line docetaxel is planned.

Molecular targeted agents

Multiple targeted agents have been evaluated in Phase II trials at various stages of disease in men with CRPC, and some of them have been (or are being) evaluated in Phase III randomised trials to determine whether they augment the activity of docetaxel. These trials are described briefly below. However, as yet no molecular targeted agent (other than those acting via hormonal mechanisms) has shown sufficient activity to warrant approval for treatment of men with prostate cancer.

Inhibitors of angiogenesis. Formation of blood vessels is a requirement for growth of solid tumours including prostate cancer, and agents targeting angiogenesis are being evaluated. Bevacizumab, a monoclonal antibody blocking Vascular Endothelial Growth Factor-A (VEGF-A) has been approved for use with chemotherapy in people with advanced colorectal and lung cancer, on the basis of small improvements in survival. A recent randomised controlled trial evaluated bevacizumab with docetaxel and prednisone for men with CRPC but did not show a significant survival difference as compared to docetaxel and prednisone alone [26].

A large trial comparing docetaxel and prednisone with or without aflibercept, a potent inhibitor of VEGF-A, VEGF-B and placental growth factor, has completed accrual. Other inhibitors of angiogenesis such as sunitinib, and thalidomide and its analogues (e.g. lenolidamide), have shown activity in Phase II clinical trials in men with CRPC [27,28]. Randomised Phase III clinical trials evaluating sunitinib in post-chemotherapy patients and lenalidomide in combination with docetaxel are underway. One problem with this approach might be the presence of redundant pathways that may stimulate angiogenesis, such that inhibitors of only one pathway may have limited effects.

Inhibitors of Endothelin-A. Endothelin-A antagonists are reported to exhibit antitumour properties mainly by inhibition of cell proliferation, induction of apoptosis, decrease in osteoclastic bone resorption and inhibition of VEGF. However a randomised trial evaluating atresantan, an orally available inhibitor of endothelin-A did not meet its endpoint of time to disease progression in men with CRPC [29]. ZD4054, another Endothelin-A antagonist, is being evaluated in a randomised Phase III clinical trial in non-metastatic CRPC with dual primary end-points of progression-free survival and overall survival.

Apoptotic pathways. Some agents that are known to stimulate apoptosis have been evaluated in combination with chemotherapy for men with CRPC. Custirsen (OGX-011), an antisense oligonucleotide targeting clusterin gave encouraging results in a Phase II clinical trial in combination with docetaxel [30] leading to an ongoing Phase III clinical trial. Gataparsen sodium, an antisense oligonucleotide targeting survivin, and AT-101, an oral, pan-Bcl-2 inhibitor are being evaluated in Phase II clinical trials in combination with docetaxel and prednisone.

Inhibitors of other pathways. Agents targeting cell-survival pathways that have cross-talk with AR-dependent pathways have been evaluated in early phase clinical trials but the results are generally
disappointing. Studies of inhibitors of epidermal growth factor receptors (EGFR1 and 2; e.g. erlotinib, gefitinib, trastuzumab and pertuzumab) alone or in combination with docetaxel have not been encouraging. Similarly, the anti-interleukin-6 monoclonal antibody siltuximab (CNTO 328) led to increased mortality when combined with mitoxantrone as compared with mitoxantrone alone and the trial was prematurely terminated [31]. Despite discouraging results using targeted monoclonal antibodies, others including cetuximab, cixutumumab and figitumumab are being evaluated in Phase II clinical trials. A study of TKI258, an inhibitor of fibroblast growth factor (FGF) is recruiting men with CRPC focused at evaluating markers of FGF signalling in bone marrow and plasma.

Additional strategies

Immunomodulation. Men diagnosed with prostate cancer are generally old and may have multiple comorbidities leading to poor tolerance of cytotoxic chemotherapy. Immuno-modulation might be a preferable treatment strategy for these men. Two Phase III clinical trials evaluated administration of a vaccine (GVAX) consisting of two allogeneic prostate cancer cell lines (PC3 and LNCaP) , which were genetically modified through adenoviral transfer to secrete granulocyte macrophage–colony-stimulating factor and lethally irradiated. Two randomised trials comparing GVAX to docetaxel/prednisone (VITAL1) and GVAX + docetaxel/prednisone to docetaxel/prednisone (VITAL2) failed to meet their primary end-point of an increase in survival and were terminated prematurely [32,33]. In a novel but complex approach, dendritic cells were taken from patients, transported to a centre where they were exposed to prostatic acid phosphatase (PAP), and transported back to be re-infused into the patient. A recent Phase III randomised clinical trial evaluating this approach (known as Sipuleucel-T) in men with metastatic CRPC demonstrated a significant survival advantage over placebo (median survival 25.8 versus 21.7 months; hazard ratio 0.78; p = 0.03) [34]. This led to approval of this vaccine by the FDA (not yet by EMEA). There may however be challenges in bringing such agents into clinical practice because of the cost and logistics associated with the production process.

Bisphosphonates and Denosomab. Bone metastasis in CRPC is associated with RANKL-mediated osteoclast activation [35]. The potent bisphosphonate, zoledronic acid has demonstrated an effect to delay the time to skeletal related events (SREs: defined by time to pathologic fracture of the bones, need for surgical/radiotherapeutic intervention or spinal cord compression) in men with CRPC and bone metastasis [36]. More recently, a fully human monoclonal antibody against RANKL, denosumab was compared with zoledronate in a Phase III randomised controlled clinical trial with 1901 patients. There was delayed time to first SRE in favour of denosumab [37], but overall survival and time to cancer progression were similar and more frequent adverse events such as osteonecrosis of the jaw and hypocalcemia were seen in the denosumab arm. It seems unlikely that this (undoubtedly expensive) agent will provide substantial benefit compared to standard bisphosphonates.

Analalogues of vitamin D. After a Phase II trial (ASCENT) [38] with favourable PSA-response and survival with high-dose calcitriol (DN-101) as compared to placebo in combination with docetaxel, a Phase III clinical trial (ASCENT-2) was designed. However interim analysis after randomisation of 953 patients revealed inferior survival in men receiving DN-101 with docetaxel than those receiving docetaxel alone [39]. Median overall survival was 16.8 months for DN-101 subjects and 19.9 months for controls (HR = 1.33; p = 0.019) and hence the trial was terminated prematurely.

Radioisotopes. The bone-seeking radioisotopes, 89Strontium and 153Samarium are useful for palliation of pain in men with CRPC and widespread bone metastases, although they may suppress bone marrow function and increase the toxicity of subsequent chemotherapy. 89Strontium was reported to show substantial survival benefit (27.7 months versus 16.6 months, p = 0.001) when given as consolidation treatment post-chemotherapy in a Phase II study of men with CRPC and bone metastases [40], and is being evaluated in a randomised Phase III clinical trial. Similarly, the combination of 153Samarium with docetaxel in a Phase II study for men with CRPC and bone metastases was well tolerated, and showed long-lasting pain control and favourable survival [41]. Encouraging results were also reported with 223Radium in a randomised Phase II study [42]; a randomised Phase III trial of radium-223 versus placebo in men with symptomatic bone metastases from prostate cancer is underway.

In summary, drug development aimed at improving survival and its quality for men with advanced prostate cancer is a very active area of preclinical and clinical research that includes strategies involving hormonal agents, classical chemotherapy, molecular targeted agents, bone seeking radio-isotopes and immune modulation.

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Declarations of interest: Dr Tannock has advised several companies about prostate cancer trials and has also chaired such trials. He has received contributions to his research fund but does not accept personal remuneration from companies.

References

New drugs for prostate cancer


Prostate cancer from the horizon of the patient

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¹Oncology Center, Antwerp, Belgium and ²Department of Urology, Erasmus MC, Rotterdam, The Netherlands

Abstract
The democratization of civil society and the development of modern medicine changed the sacrosanct doctor-patient relationship to a doctor-partner dialogue. Values and respect were lost in the process where common courtesy and empathy in trust were replaced by patient rights. Launch of Europa Uomo. Europa Uomo, the European prostate cancer coalition, represents 22 national, autonomous patient support groups. Its aim includes increasing the awareness of prostate diseases, support individualized treatment as a balance between optimal medical treatment and personalized care delivered by a multiprofessional team. We expect our information/education from dedicated professional societies while in return we share care for properly informed members as well as a fast, unbiased and cheap distribution of information/innovation across the European continent.

The role of a patient group.
Our advocacy role is focused on quality of life, tailored treatment, knowledge of risk factors, support for research and last but not least active partnerships. We believe that we can play a modest but basic role in common actions to overcome inequalities in treatment and care in Europe. Our responsibilities range from defining patient obligations to facilitating translational research and saving scarce health resources.

The horizon of the patient.
Our hope is to plead for a treatment policy on the man first and then on his cancer and to improve treatment outcomes by multiprofessional collaboration and the development of expert Prostate Units. Future expectations. A transparent, open communication line between the multiprofessional team and the patient is mandatory. The existing uncertainties should be discussed with common sense but always leave a factor of hope in survival or quality of life.

In the ascent of mankind disease and its consequences was a calamity for the individual and the population. This resulted in a deep respect towards the healers of the times as it was accepted that the Gods used disease to punish populations and individuals alike for their committed sins. The patient could only hope for divine support to survive and recover sometimes despite the treatments received.

We all honor the great Hippocrates of Kos as the father of modern, human health care especially for his golden rule “do not harm”. Still each one of us knows that we do have to harm the patient with cancer to restore his health and end up with a new post treatment quality of life. For most of the history of civilization the patient had to submit to the Latin roots of the word. He had to have patience and endure his misery.

The actual reality is not so much different as we blame the environment as the major cause of cancer and repropach the lifestyle of the patient in smoking, alcohol consumption, lack of exercise resulting in obesity as causes of cancer. Major changes as secularization and democratization coupled to the development of the information technology and relative, widespread wealth in our Western societies created a social health care where the major income of most health care professionals comes out of the collected taxes from the population [1].

The net result is a development towards the mantra of the French population: “Liberté, égalité, fraternité”. We are still recovering from the 1968 wave where titles and expertise were brought down to the lowest possible denominator. Our elected politicians contributed so much to the process that they receive in population polls the lowest figures of trust while fortunately physicians and firefighters still enjoy the trust of the great majority of the population.

It is clear that this trust is based on the merits of the previous generation and that this generation has to earn their own credit in trust.

All these changes opened the chance for the patients to claim rights in the organization and practice in
Prostate cancer: A patient’s view

Social health care. It is of course true that they are at the bottom of the hierarchy of established stakeholders in health care and that the overall atmosphere of doctor – patient relation and communication could and still can be improved (Table I) [2].

This was effectively achieved in the USA with women’s groups as the National Women’s Health Network exercising pressure in civil society to defend their viewpoints on a number of public health issues quickly followed by many.

The launch of Europa Uomo

The Europa Donna association was launched in 1995 to improve breast cancer management in Europe and they lobbied successfully into the European Parliament. As this movement was supported by the European School of Oncology (ESO) it was predictable that a patient coalition against prostate cancer was to follow. In effect at the end of 2002 the concept of Europa Uomo was discussed in the Italian government at the time that the prime minister Berlusconi was diagnosed and treated for prostate cancer. After a number of preparatory meetings, Europa Uomo was formally established in 2004 in Milan as its legal site with the support of ESO and the Oncologic Center Antwerp (OCA). The original confederation formed with 12 autonomous national organizations expanded rapidly to 23 groups representing their respective countries in 2010. It represents and supports patient groups focused on prostate diseases in general and prostate cancer in particular. The aims include increasing the awareness of prostate diseases, the support of individualized treatment based on optimal medical treatment and personalized care as well as patients’ advocacy as a priority focused on quality of life, based on solidarity and mutual respect.

These goals are clearly expressed in our 2004 Manifesto (Table II). The ten objectives speak for themselves where one should note the emphasis on quality of life as well as the promotion of prostate cancer research [3].

Our trust in optimal medical treatment which we consider the responsibility of the treating multiprofessional group brought us the sympathy and genuine support of the professional groups. Our updated and evidence based information/education standards are exclusively from top of the bill professional support. For Europe we have received first quality instructions from our multiprofessional scientific committee and the executive board of the European Association of Urology (EAU). Please note that in the European health care organization based on national health care systems our national membership groups have their own scientific committee to implement the updated European guidelines adapted to their culture and standards.

On the other hand we want to be involved in patient-centered care that we like to share with a broad support on all aspects of care including psycho-social,

Table I. Health care stakeholders.

<table>
<thead>
<tr>
<th>Health authorities</th>
<th>Public</th>
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<tr>
<td>Insurance agencies</td>
<td>Private</td>
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<tr>
<td>Professionals</td>
<td>Clinical</td>
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<td>Industry</td>
<td>Pharma</td>
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<tr>
<td>Cancer leagues</td>
<td>Technology</td>
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<td>Consumers</td>
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<td>Patients</td>
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Table II. Manifesto Europa Uomo.

1. To find ways and means to promote quality of life for prostate cancer patients and their families;
2. To promote the dissemination and exchange of evidence-based as well as factual and up-to-date information on prostate cancer;
3. To promote prostate awareness and appropriate diagnosis and prognosis;
4. To emphasize the need for appropriate early detection;
5. To campaign for provision of and access to optimum treatment;
6. To ensure quality, supportive care throughout and after treatment;
7. To promote multiprofessional quality care and appropriate medical infrastructure;
8. To acknowledge good clinical practice and promote its development;
9. To ensure that all men fully understand any proposed treatment options, including entry into clinical trials and their right to a second opinion;
10. To promote the advancement of prostate cancer research.

Table III. Europa Uomo’s view on cancer management.

1. Optimal medical treatment
   - Evidence/conscious based
   - Multiprofessional
2. Patient-centered care
   - Shared care with broad support
   - Holistic/reciprocal respect

Table IV. Proactive prostate cancer call out.

- Governments to be aware of prostate diseases
- Governments to support research biomarkers
- Remember the risk factors of prostate cancer
- Tailored treatment to the individual patient through appropriate use of PSA test
- Partnership building to reduce burden of disease, identify common actions and overcome inequalities in medical treatment and holistic care
This policy worked so well that we organized a proactive prostate cancer call out in 2009 with our European Association of Urology (EAU) and ESO partners [4]. The call out is presented in Table IV. This call proved to be an instant hit and was immediately endorsed by the main oncological and patient organizations (Figure 1).

This European network contains all the expertise represented by surgeons, radiation and medical oncologists, nursing, technicians and all health and social personnel needed for its ultimate goal to provide optimal treatment and holistic patient care emotional, spiritual and financial problems related to disease (Table III).

The C taboo word

Tsunami information (professionals, media, friends)

Outcomes
Statistics
Loss of personality
Evidence Based, Guidelines, Nomograms

The medical labyrinth

The word cancer is a taboo word for patients in the diagnostic process causing a vision of death by cancer and acute panic in many instances.
covering psychological, emotional and social needs of the patient.

The decision of the European Commission to invite patient support groups to participate in the European Partnership Action Against Cancer (EPAAC) program is a milestone in the integration of the patient as a partner in cancer clinical research in Europe.

The role of a patient group

And yet despite of all this hard work and dedication by many we still face the sad problem that a newly diagnosed unprepared cancer patients faces the spell of the great C taboo word that his life is at stake and his quality of life gone for the rest of his remaining days (Figure 2) [5].

We call it the “lost” patient syndrome. A state of mind that stays for a number of days or weeks despite concerted efforts of the general practitioner, the nursing or data manager. A well known moment in the disease history where the patient has an endless list of questions and the doctor has never time to answer them. Here starts the supporting role of the patient support groups united in a shared mission, to mobilize the solidarity of the survivors to provide correct, updated, clear and validated information in a stepwise manner on the medical pathway from diagnosis to beyond primary treatment [6]. Ideally public awareness on the history of the disease has been available for the general public before the diagnosis. Further education, a sympathetic ear and understanding psychological and emotional distress can be improved by the shared experience of being treated for cancer [7].

The pitfalls next to the need for the “perfect” information/education are the number of experts to be encountered in a less than smooth circuit, the inflexible guidelines or nomograms representing cohorts but

<table>
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<th>Table V. Typical patient complaints.</th>
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<tr>
<td>Improper, incorrect information</td>
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<tr>
<td>The doctor has no time</td>
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<tr>
<td>No choice in their own treatment</td>
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<tr>
<td>Lack of respect</td>
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of treatment, understanding the communication fail-ures that can happen in busy centers, see the staff as trained individuals and above all inform them on any kind of other treatment or advice that one receives.

The fact that many cancer diseases especially breast and prostate become long time chronic diseases by the disease or by the treatment should encourage both doctors and patients to engage in a mutual congenial partnership [8].

As a conclusion one sees the constructive role of the patient support organizations as effective to reach holistic care, organize cheap and efficient grass roots information, support basic and clinical research and by being partner save money for unnecessary examinations in follow-up, chronic care (Table VI) [9]. These efforts and outcome results in improving health care can be easily multiplied by expanding the network. Basic requirements are shared interests, similar objectives and a good transparency of the positive but different goals of the participating partners including all possible stakeholders. Each association or project has its own broad or targeted agenda. The last thing one needs is an hidden agenda as a successful partnership is based on win-win situations where the expertise and/or the means of the different partners are utilized to reach a common goal (Table VII).

The horizon of the patient

Coming back to the individual prostate cancer patient still his horizon is the treating urologist. It is understandable as the track record of the urological specialty involves the mortality reduction of benign prostatic hyperplasia (BPH) to zero following an orderly transition from surgery to medical treatment of lower urinary tract symptoms (LUTS) and still looking for less invasive treatments.

The track record of managing prostate cancer has been less spectacular and has been uneven in the balance of facts and remaining open questions. In a way we watched the importance of two Noble prizes to reward progress in the clinical treatment of prostate cancer. The first one bestowed on Charley Huggins 25 years after his fundamental studies on the physiology of the prostate resulting in the surgical castration as primary treatment of prostate cancer. The second one bestowed on Andrew Schally for his isolation and analysis of the natural LHRH decapetide leading to a tsunami of medical castration.

All patients with prostate cancer have been treated or threatened with some endocrine manipulation and most have enjoyed the palliative power of primary endocrine treatment in a far advanced, metastatic prostate cancer. It is only in the last decades and after the outcomes of randomized, clinical trials that it became clear that managing prostate cancer involves more than endocrine treatment [10].

As simple observers of clinical progress and translational/basic research in cancer we are sometimes surprised by the professional groups ignoring clean facts and unanswered questions alike in proposing clinical decisions to their patients. We prefer to balance facts and uncertainties according to old wisdom before we try to come to a shared decision on important crossroads in the treated history of dealing with one’s prostate cancer (Figure 3).

A patient diagnosed with prostate cancer goes through different phases of the disease (Table VIII).

<table>
<thead>
<tr>
<th>Table VIII. Phases in the treated history of prostate cancer.</th>
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<tbody>
<tr>
<td>1. Suspicion: Serendipity according to age</td>
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<tr>
<td>Risk factors</td>
</tr>
<tr>
<td>Positive marker(s) (PSA)</td>
</tr>
<tr>
<td>2. Confirmation: Biopsies</td>
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<tr>
<td>3. No diagnosis without prognosis</td>
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<td>4. Primary treatment: Overdiagnosis (AS)</td>
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<tr>
<td>Underdiagnosis</td>
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<tr>
<td>5. Secondary treatment (WW)</td>
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<tr>
<td>6. Castration Resistant Prostate Cancer (CRPC)</td>
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<th>Table IX. Active surveillance vs. watchful waiting.</th>
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<td>Active Surveillance</td>
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<td>Watchful Waiting</td>
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<tr>
<td>Fit patient</td>
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<tr>
<td>Low risk cancer</td>
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<tr>
<td>PSA dynamics define treatment (+ biopsies)</td>
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<tr>
<td>Option: cure</td>
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<tr>
<td>Co-morbidity/age</td>
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<tr>
<td>High risk cancer</td>
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<tr>
<td>Symptoms define treatment</td>
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<td>Option: palliation</td>
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<th>Table X. In a nutshell.</th>
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<tbody>
<tr>
<td>1. Prostate cancer is a heterogeneous disease with a long,</td>
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<tr>
<td>natural history.</td>
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<tr>
<td>2. This chronicity is specific for the disease but includes</td>
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<tr>
<td>treatment related illnesses.</td>
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<tr>
<td>3. Age is the most important risk factor increasing the burden</td>
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<td>in an ageing society.</td>
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| Table XI. Incontinence/erectile dysfunction by treatment or age. |
|---------------------------------|------------------|------------------|
| Incontinence urine              | vs.              | Normgroep        |
| 23–48%                          | vs.              | 4%               |
| Incontinence bowel              | vs.              | 2%               |
| 5–14%                           | vs.              | 18%              |
| Erectile Dysfunction            | vs.              | Thesis F. Mols, 2007 |
| 40–74%                          | vs.              | 18%              |
Prostate cancer: A patient’s view

First of course the serendipity of the cancer and an increased PSA number. Second confirmation of the suspicion and then going for a reliable prognosis. Once established it is time to discuss primary treatment among the available procedures including active surveillance.

The next phase, many times disappointing, is the outcome result of the primary treatment and its side-effects. Again another shared decision in secondary treatment is important and the outcome of this treatment and its side-effects.

Last of course is the recognition that our prostate cancer is progressive and ultimately lethal. Here the treatments look more invasive as they don’t carry the promise of cure. The choice should include watchful waiting. The latter is very different from active surveillance as here there is no chance to return to primary, curable treatment (Table IX) [11].

By this time the need for a multiprofessional team is so obvious that the urge to establish expert prostate units in our health system becomes more attractive to the patient and professional alike.

Despite the label of incurable cancer many people can and do enjoy good quality lives. It is usually in the last year of their lives that prostate cancer suffer from the disease progress expressed in back pain, bone fractures, anemia, fatigue and lower urinary tract obstruction.

Prostate cancer is a chronic, heterogeneous disease with high incidences in the seventh and eighth decade of life with a specific mortality in the ninth decade. The low mortality, meaning death by prostate cancer, is relative (2–4%) as the number of patients is so high and most patients still die by their concomitant lethal diseases (Table X).

Future expectations

What do patients and their respective groups expect in future, optimal treatment?

First and above all a good communication line between the different members of the multiprofessional team with a transparent, open information to the patient. It would start in choosing treatment, now based on nomograms and guidelines [12,13], with objective, reliable treatment results and side-effects. The side-effects of curative treatment are sometimes underestimated for the individual patient. The figures related to primary treatment are important (Table XI).

Treat the available diagnostic uncertainties with common sense to keep the confidence of the patient. These uncertainties include the PSA numbers, the biopsies, the nomograms, the Gleason score and the imaging procedures. Improvements are possible and expected in all domains. For screening, prevention and primary treatment results we wait for the ongoing clinical trials (Table XII) [14].

Last we have seen improvement in treatment results of castration resistant prostate cancer (CRPC). Here the patients expect access to these clinical trials or new treatments. If one looks for a bottom line we should advise to treat the man and his co-morbidities first and then his cancer (Table XIII).

All these factors are condensed in Table XIV where we express hope that the existing gap between the available knowledge and the practiced reality may close in the near or distant future.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References

COMMENTARY

WHO International Consultation on Prostate Cancer: A summary

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During the past three decades, prostate cancer has assumed an increasingly prominent role in health care policy and delivery in many western countries. Prostate cancer is now the most commonly diagnosed non-skin tumor and the second leading cause of death after lung cancer. The dramatic rise in the incidence of this disease has been directly linked to the dissemination of testing for prostate specific antigen (PSA) and is highest in regions where annual PSA testing has become commonplace.

The introduction of PSA testing has resulted in several significant changes concerning how scientists, clinicians and public health officials deal with prostate cancer. PSA testing has impacted how we identify prostate cancer, the type of cancers we diagnose, and how we stage and manage this disease. This manuscript summarizes the papers presented at the WHO International Consultation on Prostate Cancer concerning the advances in screening, prevention and therapy for prostate cancer and highlights the future challenges that lie ahead.

A) Early detection and screening

In 2009 two publications appeared in the New England Journal of Medicine that advanced our understanding of the impact of PSA testing on the early detection of prostate cancer. On first glance, the conclusions from these studies appeared to be contradictory. The Prostate, Lung, Colorectal and Ovarian (PLCO) Cancer Screening Trial demonstrated no benefit from PSA testing, whereas the European Randomized Study of Screening for Prostate Cancer (ERSPC) trial suggested that PSA testing reduced prostate cancer mortality by 20% but with the caveat that at least 48 men required treatment to prevent one prostate cancer death.

Closer inspection of the study designs of these two trials reveals that the PLCO trial evaluated the impact of intensive annual PSA screening against modest PSA testing, whereas the ERSPC trial evaluated the impact of PSA screening versus no screening. The ERSPC trial tested whether PSA testing is efficacious, while the PLCO trial explored whether PSA testing is effective from a population perspective. When considering broad public health policies, this distinction helps guide appropriate clinical application. PSA screening must be efficacious if it is to be effective, but it may be not effective from a public health perspective even when it is efficacious for an individual patient.

In the PLCO trial, men were recruited to study centers and randomly assigned to receive either annual PSA tests for six years and digital rectal examinations for four years or usual care. If a study participant was found to have an abnormal PSA test or digital rectal examination, study researchers referred him to his primary care physician or other health care provider for further evaluation, a possible prostate biopsy, and treatment if the biopsy specimen was positive for cancer cells. In the ERSPC trial, potential study participants were identified from population registries and invited to participate in the study. Men who were found to have an abnormal PSA level were offered a transrectal ultrasound and prostate biopsy. Men with positive biopsies were provided definitive treatment.

Both of these studies have been the subject of numerous analyses and editorials identifying many technical problems inherent to both studies. Both trials, however, have identified the major public health issue of over diagnosis of prostate cancer as a result of repeated PSA testing. In the ERSPC study 42 cancers had to be identified and managed in order to prevent
one prostate cancer death. Many men who would not otherwise have been diagnosed with prostate cancer are undergoing biopsy and treatment. Without more information concerning the impact of PSA testing on health care quality of life and the economic costs associated with widespread PSA screening and treatment, it is currently inappropriate to adopt population based PSA testing as public policy.

One promising method to manage the problem of over-diagnosis of prostate cancer is genetic testing. Available markers currently explain approximately 25% of the heritability of this disease and researchers anticipate that within the next two years, the number of identified markers will increase from 35 to over 100 and will have the potential of explaining almost 50% of the heritable factors associated with prostate cancer. Potential clinical applications of these genetic tests include: a) defining a sub-population of healthy men who have an excessive risk of developing clinically significant prostate cancer, and b) integrating the information provided by genetic factors to assess an individual patient’s risk of developing clinically significant disease. Unfortunately, there is no data presently available that can be used to differentiate men with clinically significant disease from those who have clinically insignificant disease.

B) Assessing the tumor

The past decade has witnessed an explosion in the number of tools that can be used to assess prostate cancer. Traditional analysis of histology remains the gold standard for diagnosing prostate cancer and assessing the aggressiveness of this disease. The Gleason scoring system has been adopted world wide, but the system is not static. Consensus conferences conducted within the last few years have continued to modify how the Gleason system is applied. Gleason patterns 1 and 2 are rarely used by contemporary pathologists. Pathologists have also modified the definition of Gleason pattern 3. Increasingly men who harbor cribriform glandular patterns are classified as having Gleason pattern 4 disease. Glandular differentiation has become a key factor for many pathologists. Glandular differentiation is preserved in specimens classified as Gleason pattern 3+3 disease. When glandular differentiation is only partially preserved the specimen is usually classified as Gleason pattern 3+4 or 4+3. When glandular differentiation is absent the specimen is classified as having Gleason patterns 4 and 5.

Other factors that continue to demonstrate important prognostic significance include the number of positive cores at biopsy and the extent of tumor in each core. Perineural invasion is often reported, but it is unclear whether this factor provides independent prognostic information. Important changes in staging include the classification of microscopic bladder neck invasion and the intraprostatic seminal vesicle invasion as T3a disease. The diameter of the largest node is also clinically relevant. Sub-classifications of T2 disease provide no valuable information.

Prostate specific antigen remains the most important and widely used biomarker to assess the presence and potential extent of prostate cancer. Unfortunately PSA, like all biomarkers, has significant problems with false negative and false positive values. No single biomarker is capable of distinguishing clinically significant disease from normal pathology or indolent disease. In the future panels of biomarkers will be combined and entered into prediction models. During the past few years several nomograms and risk calculators have been developed.

Researchers have explored the performance of PSA using multiple approaches including PSA kinetics. The inherent analytical and biological variability of total PSA levels affects the interpretation of any single result. Men who will eventually develop prostate cancer have increased total PSA levels years or decades before the cancer is diagnosed. PSA velocity marginally improves the specificity of total PSA, but has limited use for screening or prognostication. The combination of PSA molecular forms and other biomarkers promises to improve the detection of prostate cancer. Examples include the blood markers PHI (−2proPSA, %PSA, tPSA), a four kallikrein panel (tPSA, %PSA, intact PSA and hK2), tissue markers (uPA axis, TGF-bets 1, IL-6R, Endoglin, Ki-67) and urine markers (PCA3).

Panels of biomarkers that capture the biological potential of prostate cancer are in the process of being validated. For advanced prostate cancer, circulating tumor cells appear to offer the greatest promise for predicting and monitoring the response to therapy. Unfortunately, at the present time no single biomarker is clinically useful as a predictor of prostate cancer progression.

Imaging techniques have also undergone significant advances during the past decade. Magnetic resonance imaging in particular shows promise in the diagnosis and clinical assessment of tumor grade and local extent. Prostate cancer is more difficult to detect and localize in the central gland as compared with the peripheral zone. MR offers the potential to improve our ability to identify and biopsy these lesions when compared to transrectal ultrasound (TRUS) and biopsy. Multiparametric MR imaging combines T2 weighted imaging, diffusion weighted imaging and dynamic contrast enhanced imaging. The typical prostate cancer lesion has low signal intensity on T2 weighted imaging, a low diffusion coefficient value, a high choline and creatine/citrate
ratio, high contrast agent permeability and rapid washout. Multiparametric imaging is needed because not all prostate cancer lesions exhibit all of these features. Aggressive cancers appear to demonstrate a high diffusion coefficient and a high choline and creatine/citrate ratio.

MR offers the potential to improve prostate biopsies. Lesions that are often missed on TRUS can be imaged on MR. An experienced radiologist can perform an MR directed biopsy within 30 minutes. An endorectal coil is needed for prostate cancer staging if only a 1.5T magnet is available. If a 3T magnet is available, an endorectal coil is only required to identify minimal capsular penetration. MR imaging can be used for conventional nodal staging using standard criteria for size and shape. Novel contrast agents are still considered experimental and require FDA and EMEA approval. MR offers the potential of outperforming bone scintigraphy when evaluating the axial skeleton for bone metastases. Dynamic contrast-enhanced MR imaging may play an important role in detecting tumor recurrence after surgery or radiation.

Positron Emission Tomography (PET) combined with computerized tomography also shows promise as a technique to measure the extent of prostate cancer. Many new tracers are available. FDHT targets the androgen receptor and therefore can be used to either guide prostate biopsies to assess the presence of metastatic disease or to assess a patient’s response to various chemotherapeutic agents.

Technitium-99 based bone scans have been traditionally used to assess patients who are likely to harbor bone metastases. Recent data suggest that technetium bone scans can also be used as a secondary end point in clinical trials. Patients who develop more than two new lesions on bone scan when compared to their base line scan have clinical evidence of clinical progression.

C) Therapy with curative intent

Men with moderate or high grade prostate cancers (Gleason score ≥ 7) face a substantial risk of disease progression when compared to men with low grade disease. This is especially true for those men who present with clinically palpable disease (stage T2 and T3). Two randomized trials conducted in Scandinavia revealed a survival benefit for men who received either surgery or radiation. The SPCG-4 trial randomized patients between immediate surgical intervention and deferred endocrine treatment. A total of 695 men were enrolled during the period 1989–1999. A review of the study cohort reveals that 76% of the participants had T2 disease and only 11% of the participants had disease detected on the basis of PSA testing. The distribution of Gleason scores was as follows: 48% Gleason 5–6, 23% Gleason 7, and 5% Gleason 8–10.

After a median follow-up of 10.8 years, 39% of the men had died. There was a small absolute difference in overall survival of 7% favoring men undergoing radical prostatectomy, but the difference was not statistically different. After 12 years the overall mortality rate was 33% among men undergoing surgery and 40% among men receiving deferred androgen deprivation therapy. Interestingly the survival advantage only favored those men who were age 65 or less at the time of surgery. Men older than this had similar outcomes in each of the two arms of the study.

The SPCG-7/SFUO-3 trial randomized men to either immediate androgen deprivation alone or androgen deprivation plus radiation therapy. A total of 875 patients were enrolled from 1996 to 2002. As with the SPCG-4 trial, most of the men enrolled had more advanced disease and presented because of clinical findings rather than on the basis of PSA screening. A review of the study cohort shows that 98% of the patients had T2 disease or higher with the majority presenting with T3 disease. Unfortunately, tumors were not graded by Gleason score, but 85% of the patients had WHO grade II disease or higher and 23% had seminal vesicle invasion. A review of PSA values at entry showed that only 24% of patients had a PSA lower than 10.0 ng/ml. These patients had considerably more advanced disease when compared to the typical patient presenting with newly diagnosed localized prostate cancer in the United States during the past decade.

After a median follow-up of 7.6 years, 79 men in the androgen deprivation alone group and 37 men in the radiation plus androgen deprivation group had died of prostate cancer. The ten year overall mortality was 39.4% in the androgen deprivation group and 29.6% in the radiation plus androgen deprivation group. Ten year prostate cancer-specific mortality was 23.9% in the androgen deprivation alone group compared with 11.9% in the radiation therapy plus androgen deprivation group.

The findings in both of these trials are remarkably similar. Men with clinically advanced, localized prostate cancer have a survival advantage after receiving surgery or radiation therapy. Despite their relatively advanced disease, a majority of the men enrolled died from a cause unrelated to prostate cancer within ten years of diagnosis. Among those men who ultimately died from prostate cancer, radiation or surgery lowered this probability by about 50%. Twelve years after surgery and ten years after radiation 12.5% of men undergoing surgery and 11.9% of men receiving radiation died from their disease. For those men in the control arms 17.9% and 23.9% respectively died of prostate cancer after 12 years and 10 years.
Very few of the prostate cancers treated in the Scandinavian trials were diagnosed on the basis of PSA testing and therefore it is difficult to generalize these findings to a contemporary population of men in the United States. At minimum these findings reflect 15 or 20 year outcomes if the lead time introduced by PSA testing is added. Surgery and radiation therapy, however, come at a price. The side effects of surgery and radiation therapy are well known and often include problems with urinary function, bowel function and especially sexual function.

In 2010 several problems remain. Low risk prostate cancer is poorly stratified and suffers from under or overtreatment. Active surveillance protocols are inadequate. Intermediate risk disease for men with a ten year life expectancy can be treated equally well with either radiation therapy or surgery after careful counseling. Men with high risk disease are often undertreated with monotherapy and have poor outcomes. Surgery is often underutilized for these patients who often require combination therapies for optimal outcomes. Randomized clinical trials are urgently needed to refine active monitoring protocols, to define low risk disease and to evaluate minimally invasive therapies.

D) Natural history of prostate cancer and the role of active surveillance

During the past two decades there has been a dramatic increase in the incidence of prostate cancer in those countries that have adopted screening for prostate specific antigen (PSA). Prostate cancer is now diagnosed at a rate that is 2.5 times higher in the USA where PSA testing is commonplace when compared to the UK where PSA testing is much less frequent. The dramatic differences between prostate cancer incidence rates and mortality rates have led many researchers and clinicians to recognize that prostate cancer is increasingly being over-diagnosed in many countries. While all prostate cancers are likely to progress, many cancers progress at remarkably slow rates and therefore are not destined to become clinically significant.

Several key studies have shaped our understanding of the natural history of disease progression. Between 1989 and 2004, Johansson and colleagues published a series of four articles that documented the outcomes of untreated prostate cancer. Albertsen et al. also reported on the long-term outcomes of a competing risk analysis of men diagnosed with localized disease between 1971 and 1984. Together these studies have shown that men with low grade disease (Gleason score \( \geq 6 \)) can frequently survive up to 20 years without evidence of disease progression. Conversely men with high risk disease (Gleason score \( \geq 7 \)) often die of this disease within ten years of diagnosis.

Unfortunately these studies do not reflect the impact of widespread PSA testing that has advanced the date of diagnosis by as much as ten years. As a consequence, men diagnosed with low volume, low grade T1c disease are at very low risk of disease progression. For these men intervention with either surgery or radiation frequently carries higher risks than surveillance. Interest in active surveillance of prostate cancer reflects this greater understanding of the relative impact of intervention. These considerations have led clinicians at several academic medical centers to propose criteria that identify men who have a low risk of disease progression. They have relied on concepts originally developed by Epstein that were based on pathological analysis that correlated biopsy findings with those found following radical prostatectomy. The core criteria include: a) men who present with prostate cancer in two cores or less (< 25% of the total biopsy specimen), b) neither core has more than 50% involvement with disease, and c) tumor histology that contains no Gleason patterns 4 or 5.

These concepts have yet to be validated by clinical trials. To date, support for this approach comes from several case series. Klotz et al. have published the longest follow-up of over 450 men participating in an active surveillance program. After a median follow-up of seven years overall survival is 78.6% and prostate cancer specific survival is 97.2%. Death from competing risks has been 16 times greater than the number of deaths from prostate cancer. Unfortunately, active surveillance carries risks. In the Klotz cohort, over 30% of patients have undergone more aggressive treatment because their disease has been reclassified as higher risk. Of these men, half have evidence of biochemical failure on the basis of a rising PSA. Whether these patients will ultimately die from prostate cancer remains to be determined.

Most likely, the concept of repeated assessments of the tumor over time is here to stay. We know that active surveillance leads to reduced overtreatment, but the risk of missing the window of curability is unknown, as is the optimal parameters for assessing the tumor. Issues related to quality of life must also be evaluated thoroughly.

E) Chemoprevention of prostate cancer

The rising incidence of prostate cancer and the recognition that many cancers are not clinically significant has caused many clinicians to pursue the concept of chemoprevention as a way to prevent overdiagnosis and overtreatment of this disease. The term “chemoprevention” implies that prostate cancer can be prevented. While this is a noble goal, a more
accurate description of current efforts is “risk reduction”. Primary chemoprevention refers to the reduction of the risk of prostate cancer development while secondary chemoprevention refers to the reduction of the risk of prostate cancer progression.

The two principal targets of prostate cancer chemoprevention have been inflammation and hormonal stimulation of the prostate. Inflammation has been associated with the development of lung cancer in smokers, hepatic cancer in chronic hepatitis and bowel cancer in inflammatory bowel disease. Prostate cancer, inflammation is associated with prostate cancer precursor lesions such as proliferative inflammatory atrophy and is believed to increase genetic instability leading to prostate carcinogenesis. Unfortunately, several studies such as the SELECT trial have failed to show that anti-inflammatory agents such as the antioxidants selenium and vitamin E can reduce prostate cancer mortality.

To date, interest in chemoprevention of prostate cancer has centered on the five alpha reductase inhibitors. Two trials, the finasteride chemo prevention trial (PCPT) and the reduction by Dutasteride of prostate cancer events (REDUCE) trial, have both demonstrated that the incidence of prostate cancer can be lowered in men who take these medications. These two trials, however, were fundamentally different. The PCPT trial tested whether prostate cancer incidence could be lowered among men who had normal PSA levels at entry. The REDUCE trial tested whether prostate cancer incidence could be lowered in the subset of men who were previously identified as having elevated PSA levels and therefore were at higher risk of being diagnosed with prostate cancer. The PCPT trial followed men for an average of seven years; the REDUCE trial followed men for an average of four years.

Both trials demonstrated that prostate cancer incidence was lower in men taking 5 alpha reductase inhibitors. The incidence of prostate cancers of all Gleason grades was lowered in both studies, but the primary impact was in the number of low grade cancers. Controversy surrounds both of these trials. The incidence of prostate cancer was four times higher in both arms of the PCPT trial than originally anticipated. Therefore it is unclear whether 5 alpha reductase inhibitors actually prevent prostate cancer or simply decrease the probability that a man will undergo prostate biopsy. Furthermore, these trials have raised a concern that 5 alpha reductase inhibitors may induce high grade lesions. Follow-up in both of these trials have been insufficient to determine whether these agents ultimately lead to lower prostate cancer mortality. Information from the REDDEEM study should provide new data concerning the role of 5 alpha reductase inhibitors in men choosing active surveillance as a treatment alternative.

F) Management of advanced disease, new drugs

Treatment of advanced prostate cancer has posed challenges for both clinicians and research scientists. Many drugs have been tested, but few have shown much efficacy in altering the outcome of this disease. Docetaxel, approved in 2004, was the first agent that showed evidence of a survival advantage. Since then researchers have explored many drugs targeting different biological mechanisms. Currently androgen deprivation therapy by either surgical or chemical castration remains the cornerstone for the management of advanced prostate cancer. Unfortunately, the effect of this approach is transient. Many patients developed androgen independent disease that progresses despite low levels of testosterone.

Since data from the TAX 327 and the SWOG 9916 trials were presented in 2004, docetaxel administered every three weeks has become the standard treatment for men with androgen resistant disease. Unfortunately, treatment has improved survival only modestly with an average median survival increase of less than 20 months. Recently cabazitaxel, a novel taxane with a favorable low affinity to multidrug resistant P-glycoprotein, in combination with prednisone has been approved by the United States Federal Drug Administration. A phase III trial demonstrated an overall survival benefit of 2.4 months when compared to mitoxantrone in patients previously treated with docetaxel alone.

The recognition that the withdrawal of anti-androgens can lead to a clinical response has demonstrated the continued importance of the androgen receptor signaling pathway in castrate resistant prostate cancer. Several new drugs have been developed to exploit biological mechanisms of androgen receptor mutation, androgen receptor amplification, ligand-dependent androgen receptor activation or enhanced local production of androgens. MDV3100, a non-steroidal compound, and RD162 are examples of two new agents that target the androgen receptor with higher affinity than bicalutamide. EPI-001 is another new compound that binds to the N-terminal domain of the androgen receptor and targets the trans-activation of the androgen receptor regardless of the presence of androgens. Abiraterone acetate is another interesting compound. This agent inhibits cytochrome P17 which catalyzes two key reactions in androgen biosynthesis.

Our understanding of the complex molecular pathogenesis of prostate cancer has led researchers to explore several novel drugs that target specific
molecular pathways such as: epidermal growth factor (EGFR) signaling, vascular endothelial growth factor (VEGF) signaling pathways, phosphatidylinositol-3-kinase (PI3K)/Akt mammalian target of rapamycin (mTOR) pathway as well as the insulin-like growth factor pathway. Multiple tyrosine kinase inhibitors with a typical anti-angiogenic profile such as sunitinib and sorafenib have also been evaluated. Unfortunately data concerning long-term outcomes are limited. The anti-VEGF antibody bevacizumab has also been evaluated but the results from a recent phase III trial, CALGB 90401 have been disappointing.

Src and src-family kinases that are involved in multiple signaling pathways central to the development of prostate cancer and the pathogenesis of bone metastases are currently being tested in phase III trials. Other agents under investigation include the mTOR inhibitors everolimus and temsirolimus, various inhibitors of IGF-1R and custirsene (OGX-011), an innovative antisense oligonucleotide directed against the cytotoxic phosphoantigen, clusterin. Tumor-induced epigenetic aberrations believed to be critical for androgen receptor mediated signaling have been targeted with histone deacetylase inhibitors such as vorinostat and panobinostat (LHB589).

The slow progression and high expression of tumor associated antigens have stimulated the development of several immotherapeutic approaches. The FDA recently approved the first therapeutic vaccine, sipuleucel-T, for asymptomatic or minimally symptomatic metastatic androgen resistant prostate cancer. Sipuleucel-T consists of autologous dendritic cells derived from a patient’s own peripheral mononuclear cells. A recent phase III trial reported a survival advantage of four months among men receiving this agent versus those receiving placebo. Other examples of vaccine approaches under evaluation include vector based strategies (Prostvac) or whole tumor cell vaccines (GVAX). Ipilimumab, an anti-CTLA4 monoclonal antibody, represents another immunotherapeutic approach under evaluation.

Several agents targeting bone metastases include denosumab, a fully human monoclonal antibody that specifically binds to the ligand of RANK. This agent was recently reported to be superior to zoledronic acid in delaying or preventing bone fractures. Radium-223 is a new alpha-emitting bone-seeking radiopharmaceutical which shows promise against bone metastases. A number of endothelin A receptor targeted agents such as Atrasentan and ZD4054 are also under evaluation.

Considering the large number of compounds under evaluation the next decade shows promise for the treatment of advanced, androgen resistant prostate cancer. New biomarkers should drive clinical trials that will target specific subgroups of patients with varying biological characteristics.

G) Prostate cancer from the patient’s perspective

The past two decades have witnessed a changing role for patients in the diagnosis and management of prostate cancer. Patients are demanding a more active role in decision making and no longer rely exclusively on the treating physician’s advice. These changes are part cultural, but are also a consequence of changing technology. Internet search engines enable patients to access enormous amounts of information with minimal effort. The physician is now frequently challenged to keep up with medical literature so he can interpret findings accurately for his patients.

Prostate cancer patients are demanding more information concerning their personal prognosis and whether a specific intervention is likely to prolong their life. Patients are increasingly aware that all treatments carry side effects and are demanding more information concerning the frequency and severity of treatment mishaps. These demands have become quite personal. Patients now seek information on surgeons’ individual skills and the frequency of complications. This information is often not available, but the adoption of electronic medical record keeping will greatly facilitate this process in the future.

Patients have become more sophisticated consumers. They recognize the potential trade off between the risks of a disease and the risks of intervention. They are demanding more quantitative information to help them decide what treatments are best for them. They recognize that many treatments may fail and demand information on treatment alternatives and salvage therapies. They recognize that increased longevity may or may not be the primary goal. A high quality of life is more often the preferred outcome.

Historically the role of a physician has been one of a trusted advisor who can help guide a patient by helping them understand how their disease will unfold in the future. Some physicians have become blinded by their own technology and now appear as salesmen touting their wares. The more sophisticated patients will seek the advisor and shun the salesmen. Clinical trials are protected by an ethical code that states that investigators must provide sufficient information to patients to help them make an informed decision. Many patients assume that this also occurs in clinical practice. Patient advocacy groups are working to ensure that physicians perform at this higher standard. If physicians do not, they risk losing the professional respect and privilege that took centuries to build.